



**The 6<sup>th</sup> Annual International  
Conference (AIC 2016)**  
*in conjunction with*



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Applications (ICMSA 2016)**

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## WELCOME SPEECH FROM THE RECTOR

Assalamualaikum Wa Rahmatullahi Wa Barakatuh,

In the Name of Allah, the Most Beneficent, the Most Merciful

May the peace, the mercy, and the blessings of Allah be upon you.

Distinguished Participants, Ladies and Gentlemen,

On behalf of Syiah Kuala University, I would like to welcome all of you to the The 6<sup>th</sup> Annual International Conference Syiah Kuala University in conjunction with The 12<sup>th</sup> International Conference on Mathematics, Statistics and Their Application (ICMSA), 2016.

I sincerely hope this conference is inspiring and also the one to be anticipated in the next years to come. The organizing committee is committed to make this conference a success with its ready applications not only to the university but also to the government. No matter how much we can accomplish by ourselves, whether it be research or development, it is never sufficient in this world of knowledge. Therefore, the focal drive of this conference is to exchange ideas, and by participating in this exchange, it is hoped that all parties who may benefit from the conference can apply it in managing activities in their areas. It is pleasing to note that the agenda of this conference covers a wide range of interesting topics related to life sciences, sciences and engineering, social sciences, and special topics on mathematics and statistics sciences.

Last but not the least, my deepest gratitude goes to the Organizing Committee, institutions, and companies who have directly and indirectly supported the well-running of this seminar. The committee has organized a vibrant scientific program and is working hard to present highly respected and internationally notorious speakers to lead it. Although we try our finest to be professional, on behalf of the Rector of Syiah Kuala University, please accept our sincere apologies should there be inconveniences that occur before, during, or after the event.

I wish you a very productive conference with exciting and encouraging discussions and exchange of knowledge so that together we can anticipate a future of groundbreaking sciences, technologies and education. May God bless us all with good health to make this event a successful and enjoyable one!

Thank you.

Prof. Dr. Ir. Samsul Rizal, M.Eng

Rector of Syiah Kuala University



## MESSAGE FROM THE CHAIRMAN

Assalamualaikum Wr. Wb.

Honorable Guests, Presenters, and Participants,

As the Chairperson of the Organizing Committee, I take the privilege to warmly welcome our distinguished speakers and delegates who have come from all over Indonesia and overseas to our conference today. We are indeed honored to have you here with us.

The Annual International Conference (AIC) conference is a forum of information distribution, scientific discussion of literature, research, innovative and sustainable technology, industry product, etc. The AIC activity has been carried out regularly by Unsyiah since 2011. This year, the university will host The 6<sup>th</sup> Annual International Conference (AIC) in conjunction with The 12<sup>th</sup> International Conference on Mathematics, Statistics and Their Application (ICMSA). Furthermore, in this year The AIC program will also deliver an Innovation Expo and Industrial Forum event.

With many research activities that are conducted today on the global extent, it is important to share them to promote integrity in research at an international level. Accordingly, about 150 papers will be presented in this event, including those in the fields of Sciences and Engineering, Life Sciences, Social Sciences, and ICMSA topics. Therefore, to all participants, I would like to thank you for your valuable contributions to this conference.

I am also happy to inform that the committee is fortunate to have five keynote and invited speakers from Australia, Canada, Thailand, Malaysia and Indonesia, who have supported us from the very beginning with their capabilities to try and personally come and meet you all here at the conference.

At this juncture, I would like to take the opportunity to thank everyone who has made this event happen. It is a great pleasure for me to be a part of the organizing committee to coordinate such a remarkable conference. It does not only function as a platform to bring us who are academicians, researchers, students and others in sharing our research and experiences, but it also bridge us to further share ideas, concerns and constructive examples that we gain from this conference to build our society.

Finally, I hope that all participants will have memorable moments through this conference. The weather in Banda Aceh at the moment is at its best, so we hope that you enjoy your stay in Banda Aceh.

Thank you.

Sincerely,

Chairman of Committee

Dr. Syaifullah Muhammad, M. Eng

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# **Keynote and Invited Speaker**

**AAC Dayan Dawood, Darussalam-Banda Aceh, Indonesia  
October 4-6, 2016**

## **Ministry of Agriculture Strategic Research Chair Program: Advanced Synchrotron Technology for Livestock and Feed Research**

Peiqiang Yu

*Professor and Ministry of Agriculture Strategic Research Chair  
Department of Animal and Poultry Science, College of Agriculture and Bioresources,  
University of Saskatchewan, 51 Campus Drive, Saskatoon, S7N 5A8, Canada  
\*Corresponding author: Tel: +1 306 966 4132; E-mail: [peiqiang.yu@usask.ca](mailto:peiqiang.yu@usask.ca)*

### **Abstract**

Novel research ideas and novel research tools play a significant role in advances in feed science and animal nutrition research. In this presentation, a novel approach was introduced to show the potential of the advanced synchrotron-based analytical technology, which can be used to study feed molecular structure and structure changes induced by various processing and treatments (e.g. gene-transformation, bioethanol processing, and heat processing) in relation to nutrient utilization and availability in animal. Advanced synchrotron radiation-based technique has been developed as a rapid and non-destructive and bioanalytical technique, unlike conventional wet analytical methods which during processing for analysis often result in destruction or alteration of the intrinsic feed structures. This cutting-edge bioanalytical technique, taking advantages of synchrotron light brightness, is capable of exploring the molecular chemistry or molecular structure of a biological tissue without destruction inherent structures at ultra-high spatial resolutions. To date there has been little application of synchrotron radiation-based infrared microspectroscopy to the study of feed inherent structures in relation to nutrient availability in animal science community.

In our ministry of agriculture strategic research chair program: advanced synchrotron technology for livestock and feed research, we use the beam stations at various international synchrotron centers, including the National Synchrotron Light Source in Brookhaven National Laboratory (NSLS-BNL, New York, USA) and Advanced Light Source (ALS, California) which are supported by the U.S. Department of Energy and various founding agency and Canadian Light Source (CLS) at University of Saskatchewan (Saskatoon, Canada) which is supported by Canadian federal and provincial funds are main synchrotron sources for our study. The outline of my presentation include: I. what is Synchrotron? a) short definition; b) major components of synchrotron; II. synchrotron molecular spectroscopy techniques; a) principle and advantage of synchrotron radiation; b) synchrotron radiation infrared microspectroscopy; III: applications: synchrotron-based research programs; a) feed structure in relation to nutrient availability; b) feed molecular-chemical make-up; c) feed molecular chemistry imaging; d) effect of gene transformation on feed structure; e) heat-induced changes in structure and relation to nutrient availability; f) effect of bioethanol processing on feed structure and quality. In summary, the synchrotron-based technology is making contributions to advances in feed science and nutrition research.

**Keywords:** Feed Structure, Synchrotron Application, Molecular Nutrition, Feed Technology, Molecular Imaging Nutrient Utilization and Availability

## **The 3 Ps of Reproduction: Pheromones, Photons and Phood**

Graeme B. Martin

\*UWA Institute of Agriculture M082, The University of Western Australia, Crawley 6009 Australia

Corresponding author: [graeme.martin@uwa.edu.au](mailto:graeme.martin@uwa.edu.au).

For about 40 years, I have been wondering about how reproduction is affected by the environment. A lot of my basic research is relevant to human fertility but it is difficult to ask fundamental questions with experiments on people so, in biomedical research, we use animal models. My models include the emu, ostrich, marsupials, domestic dog, African wild dog, rhino, and pygmy hippo. However, in this lecture series, I focused on the humble sheep: its reproductive system is similar to ours, perhaps more than we would like to admit, and it has the added advantage of being an important industrial animal. The sheep brain takes in information about night length (photons), the odours of its flock mates (pheromones), and the availability of phood, and integrates this information with information about its own body status (energy stores; pregnancy; lactation). It then enacts a strategy that has been fine-tuned over evolutionary timescales to maximise reproductive success. As with most scientific journeys, unexpected discoveries about reproduction in sheep have offered new perspectives about mammalian biology. For example, we used to think that brain cells cannot divide, but now we know that they can do so in response to photons and pheromones from the outside world. We also used to view reproduction as a simple process in which the brain produces a hormone that stimulates the ovaries and testes, but now we know that brain-gonad communication is an intricate two-way exchange. Even within the gonad, there is a whole extra suite of communication channels, perhaps the most astonishing involving a massive group of molecules called small RNAs that are produced by DNA and interfere with the control of the cells by the genes. Gone is the simple traditional view that a gene produces RNA that produces a protein. What are the implications for these discoveries? First, we are increasingly optimistic about the possibility of regenerating and repairing brain tissue. Second, small RNAs offer a whole new suite of possibilities for dealing with problems in our tissues. Third, photons, pheromones and phood have led to new options for clean, green and ethical management of livestock. So, in this one small presentation about reproduction, offer you three revolutions.

Keyword : Animal reproduction, RNAs, DNA, brain tissues







# **THEME : CHEMISTRY AND CHEMICAL ENGINEERING**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
October 4-6, 2016**

## Biodiesel Production by Microwave Assisted Methanolysis of Refined Palm Oil in a Flow Reactor

Marwan\*, Muhammad Furqan, Amzar Arfa, Cut Meurah Rosnelly

Department of Chemical Engineering, Faculty of Engineering, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia.

\*Corresponding Author: marwan@unsyiah.ac.id

### Abstract

Biodiesel is one of the most promising alternative fuels to reduce or even replace petroleum based diesel fuel. It offers many significant benefits, including being renewable, less greenhouse gas effect, low pollution, and non-toxic, while for the engine, biodiesel requires no modification and has excellent lubrication properties. Its production is potentially enhanced by combining the processes with microwave irradiation. Microwave does not only provide heating effect on the reactions, but also stimulate intermolecular interaction of involving materials such as reactants, solvent, and catalyst which is expected contributing on reaction rate enhancement. The purpose of this study is to produce palm oil biodiesel at a maximum yield in a short time by utilizing a flow reactor and microwave heating. The methanolysis was catalyzed by sodium hydroxide. The glass pipe reactor was installed inside a household type microwave. Flow rate of the reacting solution significantly determined the biodiesel yield. The yield was increased as the flow rate was reduced from 90 ml/min to 30 ml/min, and the yield was significantly dropped at lower flow rate due to soap formation in the feed tank. The results showed that the optimal condition of methanolysis was found at a methanol to oil molar ratio of 5:1 and a flow rate of 30 ml/min which resulted in maximum yield of 93%. This flow rate was corresponding to residence time of 1.33 min which is much faster than the reaction time needed in a batch reactor to achieve similar yield. FTIR analysis showed that the product formed is biodiesel. It is characterized by the formation of absorption at a wave number of  $1435\text{ cm}^{-1}$ , which is evidence of the formation of methyl ester groups. The final biodiesel met EN 14214 and SNI standards. It shows a promising feature of flow type microwave reactor to produce biodiesel.

**Key words:** palm oil, methanolysis, biodiesel, microwave, flow reactor.

### Introduction

Biodiesel offers many benefits as an alternative energy resource including being derived from a renewable domestic resource, thereby reducing the dependence on petro-diesel, and being biodegradable and non-toxic nature (Datta and Mandal, 2016). The total annual petroleum demand in the world increased from 90.3 in 2012 to 120.9 (million barrels per day) in 2040 with an average annual increase of 1.0% between 2012 and 2040 (USEI Administration, 2016). Further, petroleum fuel combustion has been known as the main reason for climate change and global warming. Annual production of CO<sub>2</sub> emissions has increased significantly in recent years. According to projected data, world energy-related CO<sub>2</sub> emissions rise from 32.2 billion metric tons in 2012 to 35.6 billion metric tons in 2020 and to 43.2 billion metric tons in 2040 (USEI Administration, 2016). Therefore, the demanding needs for a clean-burning and sustainable fuel such as biodiesel is constantly growing to avoid future problem of energy supply.

In a typical method of biodiesel preparation, reaction between a plant based oil with an alcohol in the presence of a homogeneous catalyst takes place under conventional heating; heat is transferred to the reaction molecules through convection, conduction, and radiation from the surface of the reactor. Microwave irradiation has become a prospective energy source for many organic syntheses, wherein chemical conversions are accelerated because of selective absorption of microwave energy by polar molecules, non-polar molecules being inert to the microwave dielectric loss (Varma, 2001). Many researches in the recent years show the microwave-assisted synthesis of biodiesel is faster, takes less than 5–6 min, gives higher yields, and produces fewer byproducts (Vyas *et al.*, 2010; Motasemi and Ani, 2012; Marwan and Indarti, 2016). Separation of the glycerol layer is easy and fast (Refaat *et al.*,

2008). Since the mixture of plant based oil, alcohol, and homogeneous base catalyst contains both polar and ionic components, fast heating is observed upon microwave irradiation, and because the energy interacts with the reacting compounds on a molecular level, a very efficient heating can be acquired (Barnard *et al.*, 2007). Microwave heating shows superior performance over conventional methods, where heating can be relatively slow and inefficient due to lack of energy transfer rate by convection currents and the thermal conductivity of the reaction mixture (Koopmans, 2006).

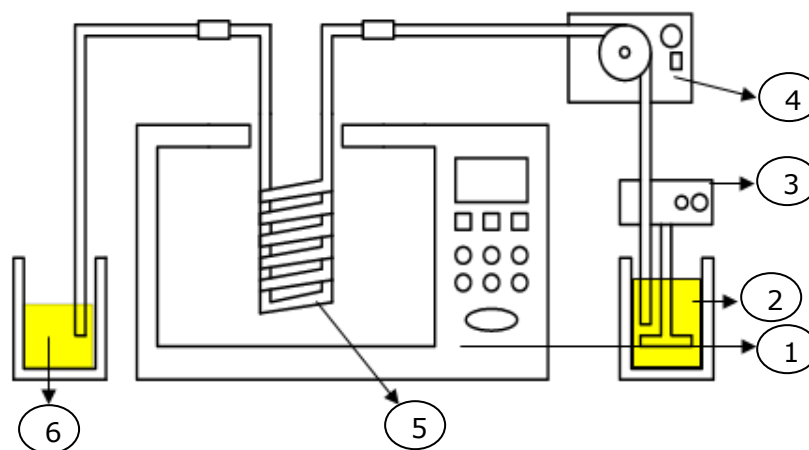
In the present work, preparation of palm oil biodiesel was studied by utilizing a flow reactor and microwave heating. Such flow system is much more suitable for large scale production. The methanolysis was catalyzed by sodium hydroxide and carried out at different flow rates and methanol to oil molar ratios. The resulted biodiesel was characterized for its functional groups, and some physical properties.

### Materials and Methods

The refined palm oil was purchased from a local store. The palm oil, methanol 99.8% (Aldrich), sodium hydroxide, and deionized water were used as received. Experiments were performed in a modified Panasonic's NN-ST 342M model microwave unit, working at frequency of 2.45 GHz and maximum power output 800 W. A coiled glass tube (made of Pyrex, 126 cm in length x 0.635 cm in inside diameter) was installed inside the microwave chamber. The reaction fluid was circulated by a peristaltic pump (MasterFlex).

Transesterification was carried out at fixed parameters for the oil amount of 250 g and catalyst loading of 1% (w/w of the oil). Different flow rates (10, 30, 60, and 90 ml/min) and molar ratios of oil and methanol (1:3, 1:4, and 1:5) were selected for the transesterification reactions. Fig. 1 shows arrangement of microwave reactor for the present experiments. The oil, methanol, and the catalyst were charged into a 1.0 L feed flask and stirred during the experiments. The mixture was flowed to the reactor inlet by a peristaltic pump at selected flow rate until the mixture in the feed flask was empty. The reactor outlet was connected to a product flask. During the reaction, the microwave oven was run with heating set at low-micro power. Thereafter, the reaction mixture was cooled to room temperature. The reaction mixture was settled in a separatory funnel overnight, and the biodiesel phase (upper layer) was obtained. Finally, the biodiesel was washed with warm water three times, and dried by adding sodium sulphate. The yield of biodiesel was evaluated by gravimetric method. As a comparison, the palm biodiesel was also prepared by conventional technique in a stirred reactor by water bath heating at 60°C for 15, 30 and 60 min. The agitation speed was kept constant at 200 rpm.

The resulted biodiesel was characterized to determine its density, viscosity, water content, acid number, and refraction index. The chemical changes were identified by Fourier Transform Infrared (FTIR) Spectrophotometer (Model 8400S, Shimadzu) equipped with Interferometer to exclude the effect of moisture and carbon dioxide in the surrounding atmosphere.

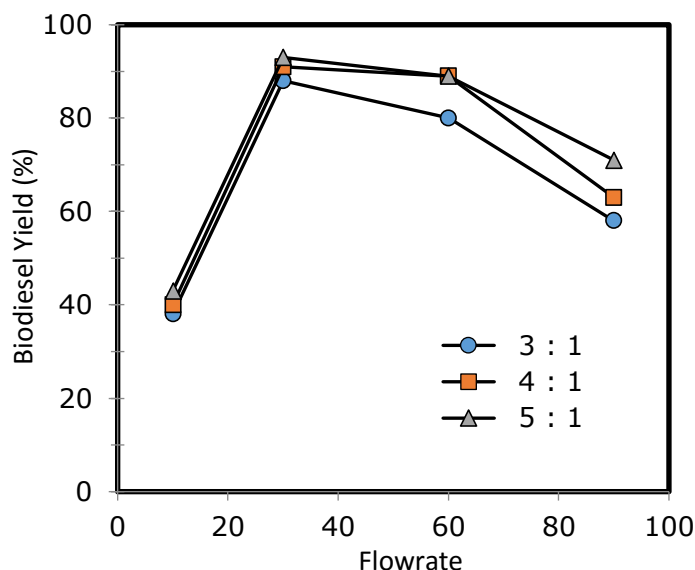


**Figure 1.** Schematic experimental setup (1. microwave oven, 2. feed flask, 3. stirring unit, 4. peristaltic pump, 5. coiled glass tube, 6. product flask)

### Results and Discussion

The reaction mixture was pumped through the coiled glass tube in a single pass. It is essential to determine a precise transesterification time to ensure completion of the reaction. For a flow system, the reaction time corresponds to its residence time, which is determined by the flow rate. Analysis of the

outlet flow indicates that biodiesel was being formed. Fig. 2 shows the biodiesel yield with flow rate for NaOH 1% at different methanol to oil molar ratios. The yield was increased as the flow rate was reduced from 90 ml/min to 30 ml/min, and then the yield was significantly dropped. At flow rate of 10 ml/min, soap formation in the feed tank was observed. It shows that the optimal condition of methanolysis was found at a methanol to oil molar ratio of 5:1 and a flow rate of 30 ml/min which resulted in maximum yield of 93%.

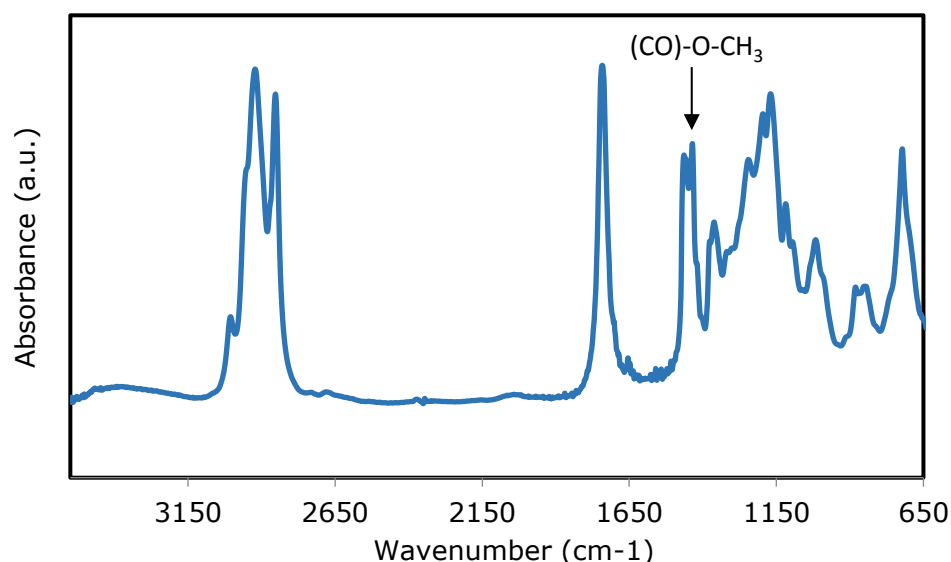


**Figure 2.** Biodiesel yield at different flow rates and methanol to oil molar ratios.

In the previous work (Marwan *et al.*, 2015), preparation of biodiesel in batch mode microwave reactor resulted in maximum yield of 94-96% for reaction time of 6-10 min, while the conventional heating method gave similar maximum biodiesel yield after 45 min. The maximum yield of 93% in the present work was observed at the flow rate of 30 ml/min. This flow rate was corresponding to residence time of 1.33 min which is much faster than reaction time needed in the batch mode to achieve similar yield. This result is comparable with the residence times of 1.75 min and 2 min found in Choedkiatsakul *et al.* (2015) and Encinar *et al.* (2012), respectively. Higher biodiesel yield at lower flow rate was due to long exposure time to microwave irradiation, and simultaneously causing an increase in thermal gradient during the reaction (Encinar *et al.*, 2012). Thermal microwave effects are revealed being dominant for homogenous-catalyzed reactions (Mazubert *et al.*, 2014).

Theoretical molar ratio of oil to methanol of 1:3 is required for the reaction, but higher than the stoichiometric value is necessary in practical production to enhance the degree of reaction completion. Moreover, effect of the molar ratio may be a key parameter due to high microwave absorption of methanol (Encinar *et al.*, 2012). Owing to its high dielectric constant ( $\epsilon = 33$ ) as compared to palm oil ( $\epsilon = 3$ ), methanol strongly absorbs microwave energy (Choedkiatsakul *et al.*, 2015). In this study, yield of biodiesel reached 88% for the reaction at stoichiometric composition and flow rate of 30 ml/min. Higher yields of 91% and 93% were obtained as the molar ratio was increased to 4:1 and 5:1, respectively. Effect of the molar ratio was more pronounced at higher flow rates or shorter residence times.

Fig. 3 shows FTIR spectra of the obtained biodiesel. Evidence of the formation of ester groups was characterized by a specific absorption band at  $1435\text{ cm}^{-1}$  arising from (CO)-O-CH<sub>3</sub>. The other strong peaks were related to carbonyl (C=O) at  $1737\text{ cm}^{-1}$  and C-O (antisymmetric axial stretching and asymmetric axial stretching) at  $1300\text{-}1000\text{ cm}^{-1}$ . In addition, the stretching vibrations of CH<sub>3</sub>, CH<sub>2</sub>, and C-H of the fatty acid chains appear at frequency around  $2916$ ,  $2854$ , and  $2999\text{ cm}^{-1}$ , whereas the bending vibrations ( $\nu_{\text{CH}_2}$ ) of these groups appear at  $1475\text{-}1350$ ,  $1350\text{-}1150$ , and  $719\text{ cm}^{-1}$  respectively. These facts are in good agreement with biodiesel spectra reported elsewhere (Naureen *et al.*, 2015; Rabelo *et al.*, 2015; Marwan *et al.*, 2015).



**Figure 3.** Infra-red spectra of the biodiesel produced in the microwave assisted flow reactor

Quality assessment was performed using physicochemical parameters such as density, viscosity, refraction index, acid number, and water content, and determined according to the EN14015 and ASTM D6751 standard methods. The results are listed in Table 1. The finally obtained biodiesel properties were within the mentioned range of biodiesel fuel standards.

**Table 1.** Quality assessment of biodiesel produced in the microwave assisted flow reactor

Properties	This Work	EN 14214	SNI
Density at 15°C	896 kg/m <sup>3</sup>	860-900 kg/m <sup>3</sup>	850-890 kg/m <sup>3</sup>
Kinematic Viscosity at 40°C	5,2 mm <sup>2</sup> /s	3,5-5,0 mm <sup>2</sup> /s	2,3-6 mm <sup>2</sup> /s
Refraction Index	1,44	-	1,45
Acid Number	0,07	max 0,5	max 0,8
Water Content	0,012%	-	max 0,05%

## Conclusions

A flow reactor system with heating by microwave irradiation was adopted for preparation of biodiesel. Refined palm oil was reacted with methanol, using sodium hydroxide as homogenous catalyst. The most influential variable was flow rate, which corresponds to residence time and also temperature gradient of the reaction. The optimal condition of methanolysis was observed at a methanol to oil molar ratio of 5:1 and a flow rate of 30 ml/min which resulted in maximum yield of 93%. This flow rate was corresponding to residence time of 1.33 min which is much faster than reaction time needed in a batch reactor to achieve similar yield. The study also showed that the quality of the produced biodiesel satisfies the European and Indonesian standards; hence, it can provide an alternative. Moreover, microwave heating offers a fast and easy route to this important biofuel with advantages of enhancing the reaction rate, and lowering production cost that making the biodiesel more economically feasible and being attractive to the consumers.

## Acknowledgements

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## In Situ Transesterification Of Screw Pine (*Pandanus tectorius*) Seed To Biodiesel Using Mechanical Stirrer

<sup>1,2\*</sup>Mahlinda, <sup>1</sup>M. Dani Supardan, <sup>1</sup>Husni Husin, <sup>1</sup>Medyan Riza

<sup>1</sup>School of Doctoral Engineering, Syiah Kuala University, Jl. Tgk. Syech Abdul Rauf 7 Darussalam, Banda Aceh 23111

<sup>2</sup>Institute for Research and Standardization of Industry, Banda Aceh, Jl. Cut Nyak Dhien No. 377 Lamteumen Timur Banda Aceh 23236

\*Corresponding Author: mahlinibr\_aceh@yahoo.com

### Abstract

This research aimed to produce biodiesel from screw pine (*Pandanus tectorius*) seeds through in situ transesterification using mechanical stirrer. The reaction time, reaction temperature, methanol to seed weight ratio and catalyst loading on the biodiesel yield had been observed. The experimental result showed that the maximum yield of biodiesel obtained was 86,38% at reaction time 240 minutes, reaction temperature 65 °C, mass ratio of methanol to seed 25:1, KOH catalyst loading 5% at 400 rpm of stirrer speed. Test result of physicochemical properties (viscosity, density, acid value) showed that these parameters conform to SNI 7182-2012. The analyses of fatty acid methyl ester component by Gas Chromatography–Mass Spectrometry identified five chemical compounds in biodiesel that were methyl palmitate (26,43%), methyl linoleate (25,57%), methyl octadec (30,57%), methyl oleate (2,73%) and methyl stearate (14,70%).

**Keywords:** screw pine seed, biodiesel, in situ transesterification, mechanical stirrer.

### Introduction

Biodiesel (Fatty acid Methyl Esters, FAME) is a new energy source that has grown in importance over recent years. Biodiesel name was initially introduced by *National Soy Diesel Development Board* (recently known as *National Biodiesel Board*) of United State of America in 1992, which was pioneer of biodiesel usage in motor vehicle (Singh and Singh, 2009). Compared with fossil fuel, biodiesel has many advantages such as renewable, biodegradable, produce lower emission, non-toxic and free from sulphur.. Biodiesel potentially reduces pollution level, environmental pollution and reduces toxic gas generated by motor vehicle (Martini and Shell, 1998; Knothe et al., 2005). Biodiesel usage as fossil alternative fuel could break two main problems, i.e. reducing energy crisis and reducing environmental damage (Refaat, 2010). Biodiesel can be produced from various natural materials such as vegetable oil and animal fat. Biodiesel can be used in compression-ignition (diesel) engines with little or no modifications because its properties are very close to petroleum diesel (Lee et al., 2011; Dermibas, 2009).

Transesterification of oils with short chain alcohol in the presence of a base catalyst is the most common way for biodiesel production process. Many new processes have been developed to get a more efficient biodiesel production process. Simultaneous processes of in-situ transesterification that combine reaction and separation operations in one unit have been investigated to simplify the biodiesel production process. This process eliminates the requirement of two separate processes of oil extraction and transesterification reaction, thus reducing processing time, cost, and the amount of solvent required (Shuit et al, 2010).

Meanwhile, screw pine (*Pandanus tectorius*) is one of the promising materials that has not been studied specifically for biodiesel production. Screw pine is one of the mangrove species categorized as a pseudo-mangrove species (Anonymous, 1987). This species has many benefits, not only from the ecological aspects, such as abrasion barrier, but also from a decorative aspect. This family has about 600 known species. These species vary in size and grow along mangroves and in local jungles. The leaves yield strong fibres that are used for making rope and weaving hats and mats (Sheltami et al, 2012; Aditya and Benyamin, 2008). The screw pine fruit is generally ovoid-shaped or globose with a diameter of 4–20 cm and a length of 8–30 cm. The fruit is made up of 38–200 wedge-like phalanges, which have an outer fibrous husk. Each phalange contains a minimum of two seeds and a maximum of eight seeds (Thomson et al, 2006).

In this study, the use of in situ transesterification using mechanical stirrer for biodiesel production from screw pine (*Pandanus tectorius*) seeds is presented. The main objective of this study is to examine the

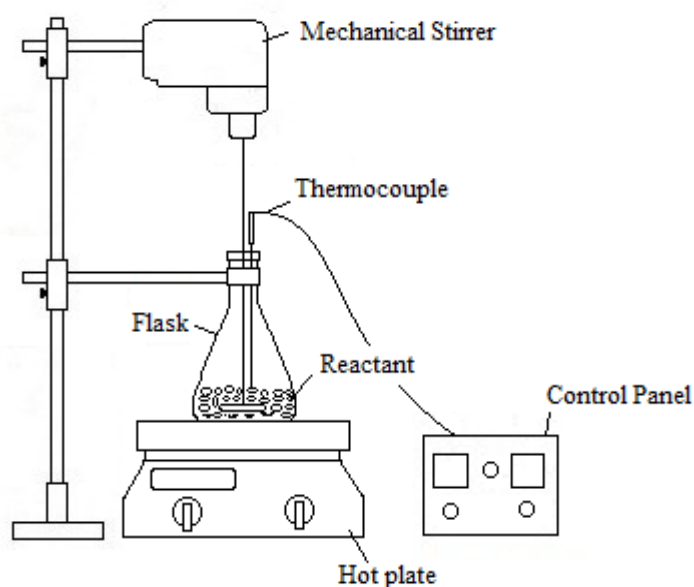
effect of process parameters, i.e. reaction time, reaction temperature, methanol to seed weight ratio and catalyst loading.

## Materials and Methods

### Material

The major feedstock used in this work was ripe screw pine. It was collected from pandanus trees in Ujung Batee (Aceh Besar district, Aceh Province, Indonesia). The reagent used in the study include methanol technical grade (Merck, 70%), chloroform (Merck, 99%) and KOH pellets (Merck, 85%). The apparatus for in situ transesterification process consisted of mechanical stirrer (SciLOGEX OS20-S), electric blender (Sharp SB-Til72), drying oven (Mettler), rotary evaporator (Laborota 4003), erlenmeyer (Pyrex) and separating funnel (Schott).

The experimental set-up of mechanical stirrer are shown in Figure 1.



**Figure 1.** Experimental Set-up

### Procedure

The seeds of *screw pine* were collected, cleaned and dried in an oven at 60 °C for about 48 h to remove the remaining moisture. The dried seeds were then crushed (particle size, 2–3 mm) in an electric blender. 10 g of crushed seeds was used as the starting material. It was mixed with mixture of methanol (mass ratio 15:1, 20:1, 25:1 and 30:1 w/w), chloroform (1:1 w/w) and KOH catalyst (3, 4, 5 and 6%). The in situ transesterification with mechanical stirrer was carried out at variable speed 200, 400 and 600 rpm. The heat is given by a hot plate at reaction temperature 50, 55, 60 and 65 °C with reaction time 120, 180, 240 and 300 minutes. The mixture was filtered and placed in a rotary evaporator to separate off the solvent and co-solvent. The oil fraction separates at 70 °C using a rotary evaporator. Then, the remaining mixture was allowed to stand for about two hours, while phase separation occurred by gravity settling with biodiesel on the top and glycerol at the bottom of the bottle. The liquid glycerol phase was removed and biodiesel was dried of the remaining water and impurities on a hot plate at 110 °C. The efficiency or yield of in-situ transesterification is defined as the percentage of biodiesel-rich phase over oil content in raw material which is determined by hexane soxhlet extraction. It can be calculated by Eq. (1)

$$\text{Biodiesel yield (\%)} = \frac{\text{Biodiesel mass (g)}}{\text{seed mass (g)} \times \text{oil content (\%)}} \times 100\% \quad (1)$$

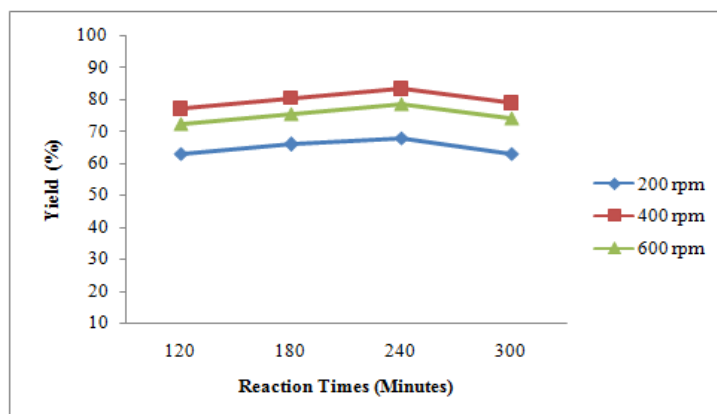
The biodiesel product is analysed to determine the physicochemical properties, such as viscosity, density and acid value with the accordance with the testing method of SNI 7182-2012. The composition of the biodiesel produced from in situ transesterification of screw pine seed was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

## Result and Discussion

### Effect of process variables toward biodiesel yield

The process variables such as reaction time, temperature, solvent mass ratio, amount of catalyst and stirrer speed directly affect to biodiesel conversion. These process variables affect both the yield and the biodiesel characteristics.

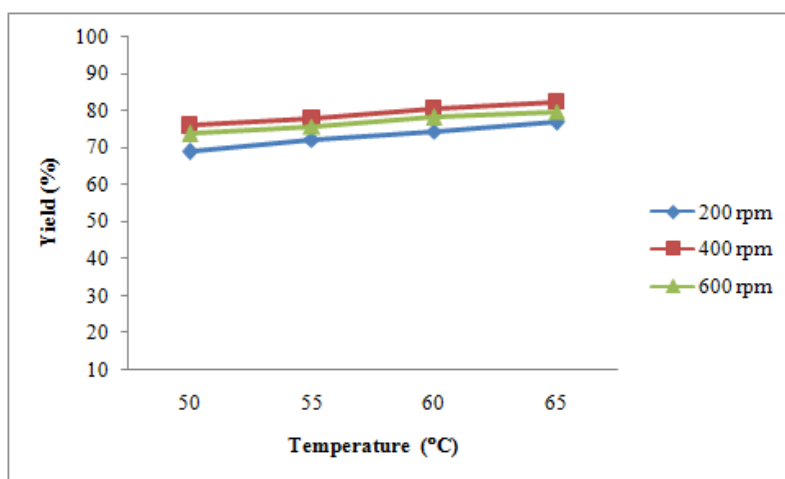
Reaction time is one of important factor that can influence the yield of biodiesel. As shown in Figure. 2, it can be observed that as reaction time increased, the yield of biodiesel increased, but at reaction times >240 min. no significant increase in biodiesel yield was observed.



**Figure. 2.** Effect of reaction times

The maximum yield of biodiesel is 83.38% achieved at 240 minutes of reaction time and stirrer speed 400 rpm. According to Liu and wang (2013) and Leung et al (2010), the reaction time will directly affect the yield of biodiesel. The biodiesel yield increase with increasing reaction time, but excess reaction time does not increase the conversion caused the backward reaction and soap formation, which resulted in reduction of biodiesel yield.

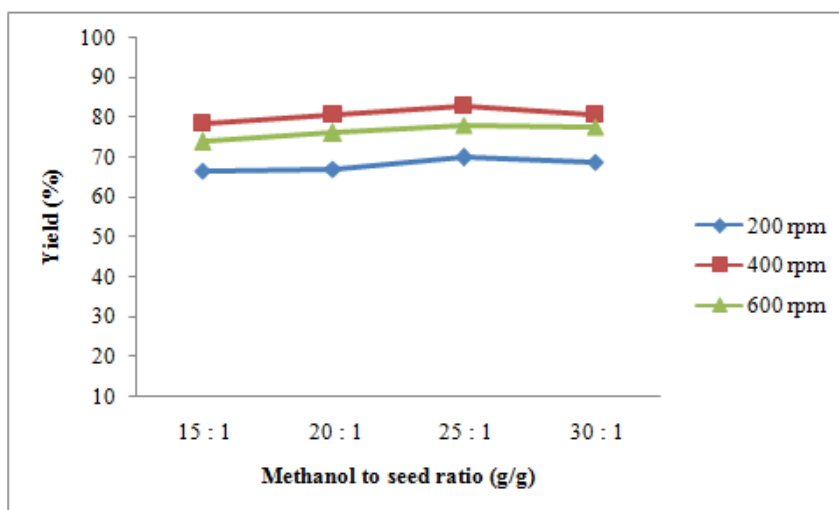
Reaction temperature also an important factor that can influence the biodiesel conversion. As shown in Figure 3, the reaction temperature effect on the biodiesel yield was studied in the temperature range 50 to 65 °C at atmospheric pressure. The maximum yield of biodiesel conversion from screw pine seed 82,5% was obtained at reaction temperature 65 °C. A decreased yield was observed when the reaction temperatures were above 65 °C. This can be as a result of higher solubility of reactants because higher temperature reduces the separation of methyl ester and glycerol phase. Also, a higher reaction temperature of 65 °C that was chosen is close to the boiling point of methanol which can lead to evaporation of some of the methanol during in situ transesterification process (Gude et al., 2012 and Umaru et al., 2014). Optimum reaction temperature usually varied between 50 to 65 °C depends on oil or fat type and method being used. Therefore based on various literature, it is suggested to use temperature near methanol boiling point to generate high biodiesel yield with shorter process time (Gashaw and Teshita., 2014).



**Figure. 3.** Effect of reaction temperature

Generally, in conventional transesterification requires three moles of methanol for each mole of oil (Meng et al., 2008). In situ transesterification differs from the conventional transesterification, where the extraction and transesterification proceed in one step, the methanol serves as extraction solvent and the esterification reagent, methanol is helpful in dissolving polar lipids and destroying the association between lipids and cell constituent. To study effect of methanol screw pine seed ratio, experiment was carried out with ratio weight of methanol to screw pine seed 15:1 to 30:1. The effect of methanol to seed ratio are show in Figure. 4.

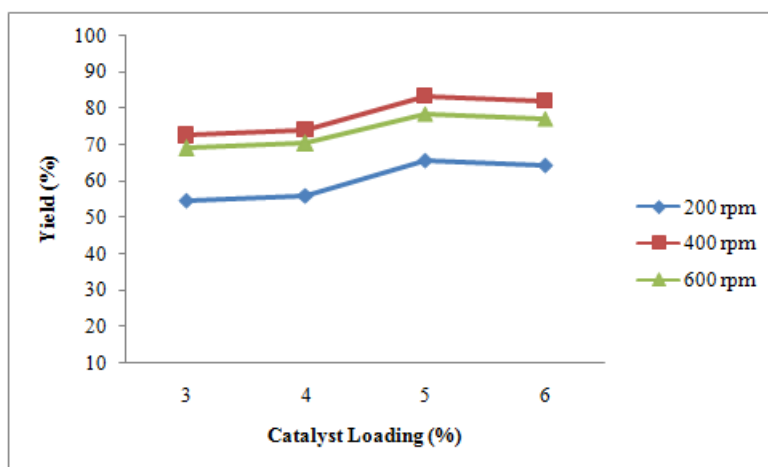
From the Figure 4, it was observed that biodiesel yield increased with increase in methanol ratio and maximum yield 82,91% was obtained at ratio of 25:1. When methanol mass ratio increased up 25:1, the biodiesel yield was decreased a little, but it still maintained on a relatively high conversion rate. In situ transesterification is an equilibrium reaction in which a large excess of methanol is required to drive the reaction to the forward direction. However, the excess methanol mass ratio to seed will slow down the separation process because of glycerin increased in solubility. When glycerin remain in solution, it also makes to drive the equilibrium back to the left, lowering the yield of biodiesel conversion (Komintarachat and Chuepeng., 2010).



**Figure. 4.** Effect of methanol to seed ratio

Commonly in biodiesel production, among the most commonly used alkaline catalysts are potassium hydroxide (KOH) and sodium hydroxide (NaOH) flakes, which have some advantages, such as being inexpensive, easy to handle in transportation and easy to store. In this experiment, the alkaline catalyst KOH was used. The effect of catalyst loading on biodiesel yield is shown in Figure 5.

It was found that the biodiesel yield was increased with increasing catalyst concentration and the maximum yield of biodiesel 83,31% is achieved at 5% of KOH catalyst. It was observed that low catalyst loading was insufficient to catalyse the reaction so that the conversion of biodiesel was lowest, but a catalyst loading >5% will decrease the biodiesel yield. Excess catalyst gave rise to formation of an emulsion that increased the viscosity and led to the formation of a gel, lowering the biodiesel production yield, which is possibly due the effect of saponification (Liu and Wang., 2013).



**Figure. 5.** Effect of catalyst loading

### Biodiesel Quality Analysis

The biodiesel obtained through the in situ transesterification was taken to analyzed the physico-chemical properties of biodiesel such as viscosity, density and acid value with the accordance with the testing methode of SNI 7182-2012. The result of biodiesel quality analysis are shown in Table 1.

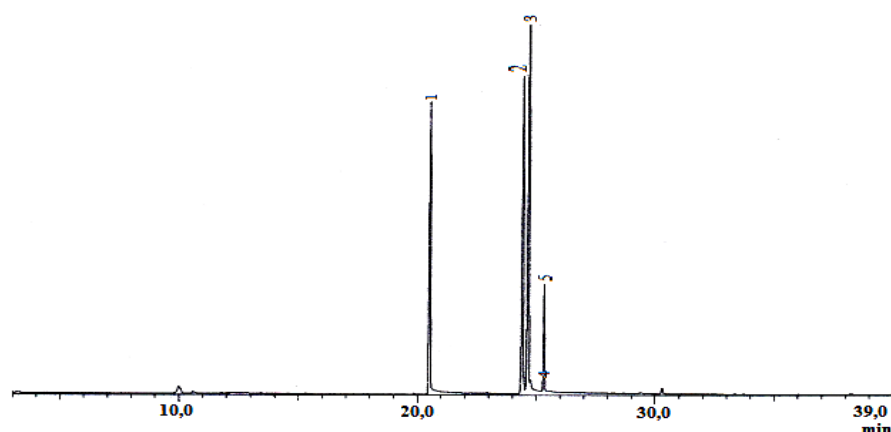
**Table 1.** Quality Test of Biodiesel from Screw Pine Seed

Property	Unit	SNI 7182-2012	Biodiesel
Viscosity (40 °C)	mm <sup>2</sup> /s	2,3 – 6,0	5,2
Density (40 °C)	kg/m <sup>3</sup>	850 – 890	888
Acid value	mg-KOH/g	Mak. 0,6	0,6

The viscosity of biodiesel is one of the most important properties that affect the mechanism of atomization in the fuel injection equipment. The viscosity is the resistance to flow of a fluid under gravity; high viscosity means high resistance to flow while low viscosity means low resistance to flow (Allen et al., 1999). Higher viscosity causes poor fuel atomization during spraying, increases carbon deposition on the fuel filter, demands more energy from the fuel pump and wears the fuel pumps and injectors. (Gutti et al., 2012; Meher et al., 2006). In situ transesterification is used to lower the viscosity of any kind of oil. From the result presented in Table 1, the viscosity of the produced biodiesel from screw pine seed was 5,2 mm<sup>2</sup>/s, this value is compliant with the SNI 7182-2012 standard.

The density of the biodiesel is also a very important parameter, the density values have been used to measure the amount of fuel in the fuel system by a volumetric method. The variation of the density affects the power and the fuel spray characteristics during fuel injection and combustion in the cylinder (Tesfa et al., 2010). Based on the data in Table 1, it can be seen that the density of the biodiesel produced from screw pine seed was 888 kg/m<sup>3</sup>. This value is in the range of SNI 7182-2012 standard.

Acid value is a measure of free fatty acids contained in a fresh fuel sample and free fatty acids from degradation in aged samples (Refaat., 2010). The acid value of biodiesel is influenced by the type of feedstock used for fuel production and by its degree of refinement. Acidity can also be generated during the production process, for instance, by mineral acids introduced as catalysts or by free fatty acids resulting from acid work-up of soaps. High fuel acidity will cause corrosion and formation of deposits within the engine, particularly in fuel injectors, by catalysing polymerization in hot recycling fuel loops (Refaat., 2009). The acid value of the present study was 0.6 mg-KOH/g. The acid value of the present study was 0,6 mg-KOH/g.



**Figure 6.** Chromatogram of Biodiesel

### Component Analysis of Biodiesel from Screw Pine Seed by GC-MS

Biodiesel samples at optimum conditions were analysed using GC-MS to identify the fatty acid methyl ester composition in screw pine oil sources with the help of the NIST library. The compositions of fatty acid methyl esters are shown in Figure 6. The result of GC-MS analysis shows that the major fatty acid esters from screw pine seed were methyl octadec (30.57%), methyl palmitate (26.43%), methyl linoleate (25.57%), methyl stearate (14.70%) and methyl oleate (2.73%). These five components are main components of biodiesel.

**Table 2.** Component of biodiesel from screw pine seed

Peak	R. Time	Area%	Name of Components
1	20.516	26,43	Methyl palmitate
2	24.422	25,57	Methyl linoleate
3	24.665	30,57	Methyl octadec
4	24.751	2,73	Methyl oleate
5	25.331	14,70	Methyl stearate

### Conclusion

In situ transesterification of screw pine seeds using mechanical stirrer has been performed. The highest yield of biodiesel from screw pine seeds was obtained of 83.38% in 240 minutes of reaction time, 65 °C of the reaction temperature, 400 rpm of stirring speed, 25:1 (w/w) of methanol to screw pine seed and 5% of catalyst concentration. The viscosity, density and acid value of biodiesel produced conform to the standards of SNI 7182-2012. The GC-MS analyses identified five chemical compounds in biodiesel from screw pine seeds, that were methyl palmitate (26.43%), methyl linoleate (25.57%), methyl octadec (30.57%), methyl oleate (2.73%) and methyl stearate (14.70%).

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## The Adsorption Process of Nitrite and Nitrate Content from Fertilizer Plant Liquid Waste of PT. PIM by Using Activated Carbon from Coffee Waste

<sup>1</sup>\*Mariana, <sup>1</sup>Mahidin, <sup>1</sup>Farid Mulana

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: mariana\_hasyim@yahoo.com

### Abstract

Waste from fertilizer plant that containing much nitrites and nitrates is very dangerous when discharge to aquatic ecosystems. The existence of excess nitrites and nitrates content in liquid waste can cause the death of aquatic organisms. Recently, the fertilizer industry is treating excessive amount of nitrites and nitrates by using a neutralization process and aeration (called as KPPL Unit). However the treatment is not considered effective and safe for the environment. Alternative methods that can be used to decrease nitrites and nitrates content is adsorption process by using biomaterial with some advantages including low cost, high adsorption capacity, utilization of waste cake and environmental friendly. The carbon content in coffee can be used as an bioadsorbent to decrease nitrites and nitrates contained in fertilizer waste. In this study, bioadsorbent was activated by using HCl where it is expected to increase the adsorption capacity. This study includes the preparation of bioadsorbent from coffee waste with a variety of process variables to form activated carbon that has a high reactivity and to be applied as biosorbent to treat the wastewater outlet of KPPL Unit of PT. PIM. This research is expected to contribute to the process of separation of nitrites and nitrates from liquid waste which is the most important issue in the plant that must be addressed. Process variable includes stirring time, pH, amount of adsorbent (g), and the concentration of the adsorbate. The results showed that the highest adsorption efficiency reached 88.9% and the adsorption capacity reached 28.5 mg/g. The process adsorption in this study followed the model of Isotherm Freundlich that assumes the physical adsorption process, with the largest  $R^2$  value for nitrite of 0.841 and for the largest  $R^2$  for nitrate of 0.862. While adsorption capacity for nitrite (n) as much as  $4,81 \times 10^{-3}$  and for nitrate (n)  $1,54 \times 10^{-3}$  and constant (Kf) for nitrite of  $2,42 \times 10^{-6}$  and for nitrate (Kf) of  $2,61 \times 10^{-4}$

**Keywords:** coffee grounds waste, activated carbon, nitrite, nitrate and freundlich isotherms

### Introduction

PT. Pupuk Iskandar Muda Aceh has been done wastewater management in their factory by using neutralization and aeration process. Some physical chemistry and biological processes have been used to remove nitrite and nitrate dissolved in drinking water and wastewater. However the current performed treatment is considered not sufficiently effective and safe for the environment, especially in the handling of high levels of urea in wastewater. Aeration was performed to release a number of ammonia contained in the effluent into the air. Although the content of ammonia in the water outlet of KPPL unit are below the permitted quality standards of KepMen LH No. 51 Year 2004 (nitrat < 0,008 ppm, nitrat < 10 ppm), but when discharged continuously it will accumulate and exceed the quality standards mainly on the outlet KPPL location just before it has spread. Therefore, it is required an appropriate advanced processing methods to reduce the content of ammonia, nitrites and nitrates from KPPL outlet of fertilizer industrial wastewater. But here the researchers focused on decreasing levels of nitrites and nitrates in the wastewater due to nitrate compounds cause several diseases, especially for babies, causing a condition known as methemoglobinemia, also called as blue baby syndrome (Ogata, et al., 2014). And nitrite excess will lead to a decreased ability of marine biota to bind  $O_2$ . Additionally, high nitrite compounds will also interfere with the process of spending nitrite compounds from the body of marine life (Trobos, 2007).

Coffee grounds are cheap and easily available materials and can be used to reduce levels of ammonia, nitrite and nitrate in the effluent. Coffee grounds including organic materials that can be made into activated carbon for use as an adsorbent or absorbent material (Sugiharto, 1987). Activated carbon is

a porous solids produced from carbonaceous material by heating at high temperatures. The previous study mentioned that the activated carbon from coffee grounds could adsorb up to 99.43% iron ions and able to adsorb the mercury reached 99% (Lubis and Nasution, 2002). In this study, the coffee grounds are used as materials for activated charcoal. Furthermore, activated carbon is used to lower levels of nitrites and nitrates in the wastewater of fertilizer industry outlet.

## Methods

Experiments was conducted as follows: (1) preparation of activated carbon biosorbent from coffee waste; and (2) the process of adsorption of nitrite and nitrate using a batch process.

### *Peperation of activated carbon*

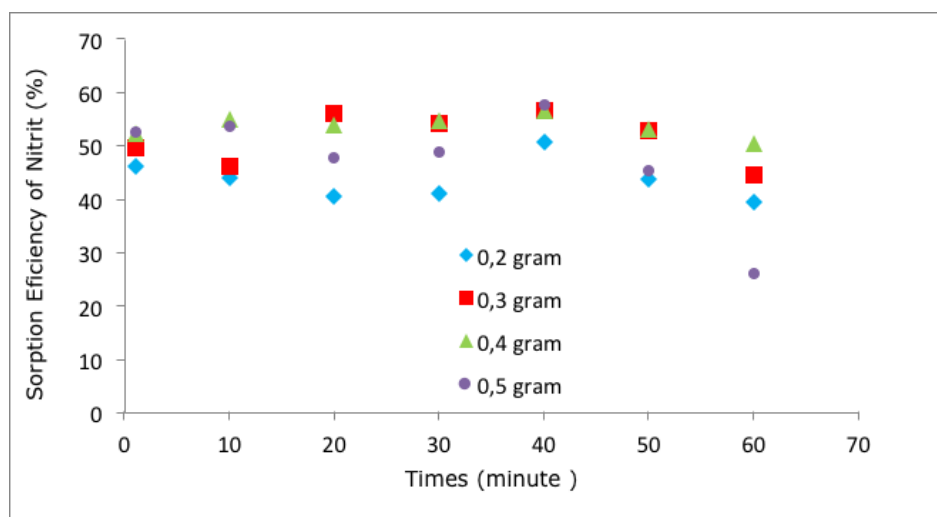
Coffee waste dried under the sun and then soaked in a solution of 0.1 M HCl for 48 hours. And drained, then washed with distilled water to pH neutral. Coffee waste that have been activated then put in oven to reduce the moisture content, then the material is inserted into muffle furnace at a temperature of 350°C for 3.5 hours. Sieved using a sieve 80-100 mesh and after the authoring process is finished the coffee grounds are stored in a desiccator.

### *Absorption of nitrate andnNitrite using batch process*

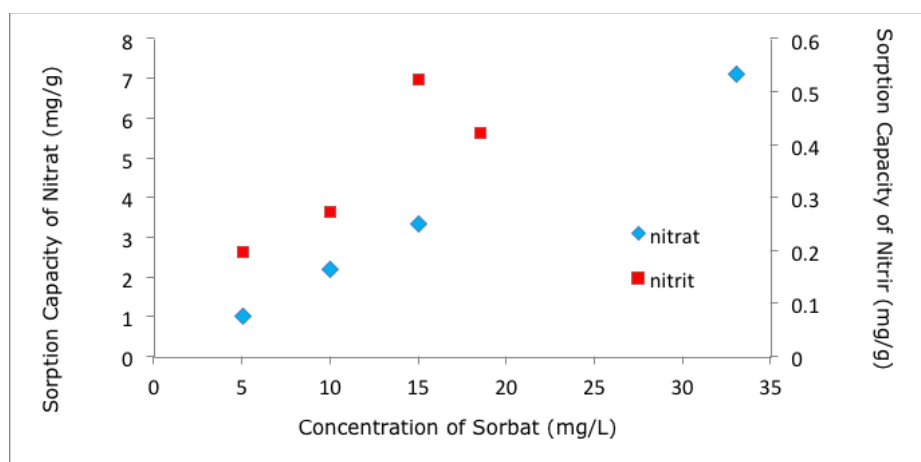
Liquid waste of fertilizer industry as much as 100 mL was contacted with activated carbon as much as 0.2 grams with size of 80-100 mesh at various stirring time. The wastewater before and after contacted with adsorbents was measured the nitrite nitrate levels by using spectrophotometry.

## Results and Discussion

The ability of activated carbon from the coffee dregs to adsorp and accumulate waste can be seen from the total concentration of nitrate and nitrite as well as ammonia contained in the waste water. Figure 1 shows that the longer the time, adsorption efficiency will be higher, but in this study it was apparent that the saturation point of the adsorbent in which the saturation point here is that activated carbon can no longer adsorb waste (pores of carbon binds nitrite nitrate and ammonia at the optimum concentration). With the increasing concentration of waste then the percentage removal of nitrite and ammonia nitrate will decrease. In this study, we obtained the saturation point of activated carbon adsorbent to adsorb the waste is at the time of 40 minutes.

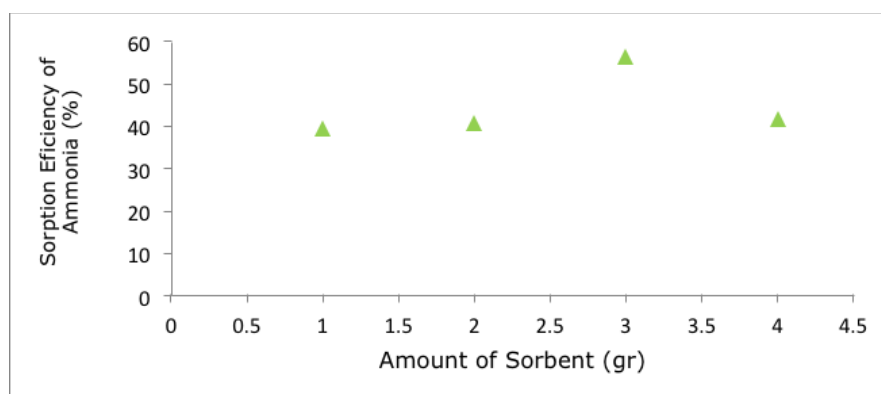


**Figure 1.** Effect of time toward adsorption efficiency of PT. PIM waste on various adsobent weight



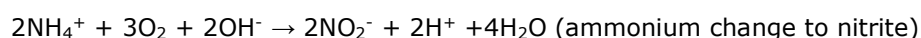
**Figure 2.** Effect of adsorbate concentration variations toward adsorption capacity of nitrite nitrate at 40 minutes and adsorbent weight of 0.4 gram

Figure 2 shows that the adsorption capacity of nitrate and nitrite increased with increasing concentrations of the adsorbate. This is because when the concentration of adsorbate become higher, then the more the number of substances that accumulate on the surface of the adsorbent, which is caused by a substance dissolved in a solvent which is spread more, so the possibility of contact between the adsorbate and adsorbent much greater (Syauqiah, 2011). Based on the experimental results, adsorption capacity for nitrite with a concentration of 5 mg/L, 10 mg/L, 15 mg/L and 18.5 mg/L respectively is 0.2 mg/g, 0.275 mg/g, 0.525 mg/g, and 0.425 mg/g while for nitrate at a concentration of 5 mg/L, 10 mg/L, 15 mg/L and 33 mg/L respectively is 1.025 mg/g, 2.225 mg/g, 3.375 mg/g, and 7.125 mg/g.



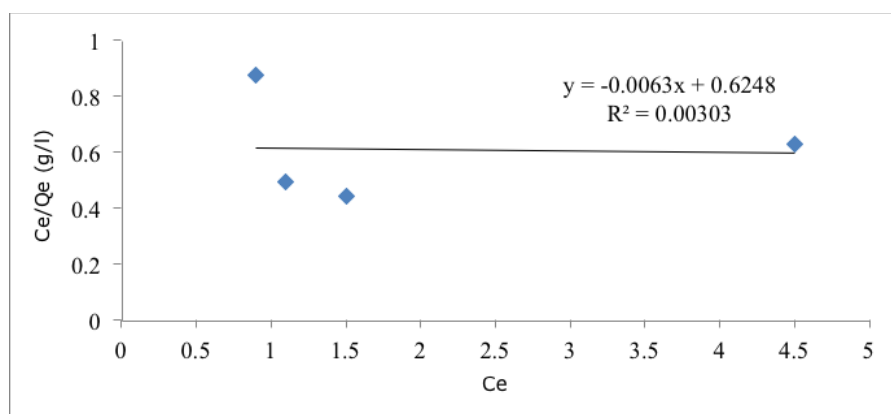
**Figure 3.** Adsorption efficient of ammonia from PT.PIM waste at 40 minutes

The more the adsorbent mass used, the adsorption efficiency of the waste also increases. The increasing weight of activated carbon is proportional to the increase in the number of particles and the surface area of activated carbon, thus causing a number of binding levels of nitrite, nitrate and ammonia on surface of adsorbents also increases and as a result the adsorption efficiency increases. From the above figure we can see that the adsorbent can not adsorb simultaneously all of three components from the waste, but adsorbent tends to adsorb more the ammonia component compare to nitrate nitrite. Most ammonia in nature and in wastewater is oxidized to nitrite and nitrate that are carried out by two kinds of autotroph bacteria on nitrification process, according to the reaction:

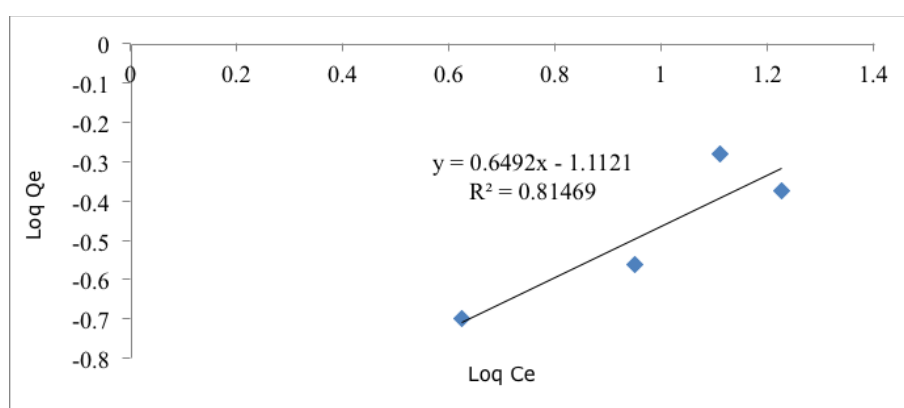


### Isoterm Adsorption

To determine the adsorption isotherm on nitrate and nitrite using bio sorbents of coffee grounds, this study use two adsorption models, namely Langmuir and Freundlich models. The models for nitrate adsorption are shown in Figures 3 and 4. Analogies are also made to the adsorption of nitrite.



**Figure 4.** Langmuir isotherm adsorption on nitrate adsorption by using activated carbon produced from coffee waste at a temperature of 27°C



**Figure 5.** Freundlich isotherm adsorption on nitrate adsorption by using activated carbon produced from coffee waste at a temperature of 27°C

Isoterm adsorption show relationship between some adsorbed components and adsorbate concentration at equilibrium (Apriliani, 2010). Value of adsorption capacity and its constant value of each adsorption isotherm model can be seen in Table. 1

**Table 1.** Langmuir and Freundlich isotherm adsorption

Component	Isotherm Langmuir			Isotherm Freundlich		
	$q_m$	$K_L$	$R^2$	$n$	$K_F$	$R^2$
nitrite	0,027	2,09	0,5614	4,81	2,42	0,841
nitrate	-2,56	1,61	0,003	1,54	2,61	0,862

Langmuir and Freundlich isotherm adsorption can be determined by understanding the value of  $R^2$ . Nitrite and nitrate adsorption isotherms using activated carbon produced from coffee grounds at 27°C followed the equation that has a value of  $R^2$  correlation close to 1. According to Table 1 it can be seen that the value of  $R^2$  on the adsorption of nitrate and nitrite that is approaching value of 1 is the Freundlich isotherm with  $R^2$  of 0.8421 for nitrite and 0,862 for nitrates. So it can be concluded that the adsorption of nitrite and nitrate at 27°C followed Freundlich isotherm equation.

Freundlich isotherm equation assumes that the active sites on the surface of the adsorbent is a heterogeneous, where the active sites, energy and the type of bonding that occurs is not the same. and multilayer adsorption occurs on the surface of the adsorbent. Freundlich isotherm assume that on all sides of the adsorbent, surface adsorption process will occur under the given conditions. Freundlich isotherm is not able to estimate their sides on the surface capable of preventing adsorption at equilibrium is reached and there are only a few active side are capable of adsorbing molecules dissolved.

Freundlich isotherm assume that interaction is in physics and Freundlich isotherm also explained that the adsorbent having a heterogeneous surface and each molecule has a different adsorption potential, where not all of the adsorbent surface adsorption have the same adsorption capacity (Ma'rifat, et al, 2014). So it can be described that the occurred adsorption between nitrite and nitrate on the surface of activated carbon from coffee grounds have a weak bonds and in form of heterogeneous due to less uniform of adsorbent surface.

Kf and n is obtained from the intercept point and slope from a straight line equation from above figure where each value obtained for nitrite to nitrate is 2.42 and 2.61 and n values that obtained for nitrite and nitrate is 4.81 and 1.54, respectively. If the n value between 1 and 10 is assumed as a good adsorbent (Dhanakumar, 2007). Therefore, activated carbon from coffee grounds which has a value of n = 4.81 and 1.54 show an effective adsorption process.

### Conclusions

Activated carbon produced from coffee grounds tend to adsorb more ammonia component compare to nitrite and nitrate components with adsorption efficiency as much as 56%. The highest adsorption capacity of nitrite was obtained at concentration of 15 ppm that is equal to 0.525 mg/g and of nitrate at a concentration of 33 ppm that is equal to 7.125 mg/g. The highest adsorption efficiency of nitrite was obtained at concentrations of 5 ppm that is equal to 16% and of nitrate at a concentration of 10 ppm that is equal to 88.9%. Nitrite and nitrate adsorption process using activated carbon produced from coffee grounds tend to follow the model of the Freundlich isotherm with nitrite R<sup>2</sup> values of 0.841 and nitrate R<sup>2</sup> value of 0.862.

### Acknowledgements

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## Simultaneous Adsorption of Trace Metal and SO<sub>2</sub> using Zeolite Adsorbent During Combustion of Brown Coal

<sup>1\*</sup>AsriGani, <sup>1</sup>Mahidin, <sup>2</sup>Khairil

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Mechanical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: asri.gani@che.unsyiah.ac.id.

### Abstract

The demand of cheap energy has made many industries turned to coal as an alternative energy source. The large deposit of brown coal in Indonesia makes its use economically viable even though it has low energy content compared to sub-bituminous or bituminous coal. Unfortunately, coal processing to produce energy particularly combustion processes create emissions in the form of trace elements and SO<sub>2</sub>, which are dangerous pollutants for health. Natural adsorbent zeolite can be used to adsorb trace element components and SO<sub>2</sub> simultaneously to reduce the negative effect of brown coal during combustion. This research observed the efficiency level of trace element adsorption such as Cd, Hg, Pb and SO<sub>2</sub> gas by natural zeolite during coal combustion. Combustions with various temperature and adsorbent ratio toward coal in briquette form were conducted for 30 minutes. The analysis towards trace metal in bottom ash such as Hg, Cd, and Pb were conducted using *Atomic Absorption Spectroscopy* (AAS) while SO<sub>2</sub> emission analysis was done using industrial gas combustion and emission analyzer. The results show that trace metal and SO<sub>2</sub> adsorption efficiency by zeolite increases as the adsorbent ratio increases but temperature decreases. High volatility level of Hg metal really influences the adsorption efficiency with increasing temperature. Zeolite performance tends to decrease at temperature 800°C because it started the desorption process. Optimum adsorbent capacity 10% for Hg adsorption was obtained at 6% adsorbent ratio and 600 deg C operating temperature and efficiency ratio for Pb and Cd were 36% and 35%, respectively.

**Key words:** Adsorption, SO<sub>2</sub>, trace metal, zeolite.

### Introduction

The use of coal as source of energy for industry has recently become the preference for many investors because it can reduce the operational cost and is widely available in Indonesia. About 70% of Indonesia's coal deposit consists of young coal. It is a source of energy with low economic value because it has high water content, low calorific value and cannot be exported. Its advantage on the other hand are low ash content and low mineral content.

Indonesia is still very dependent on the petroleum fuel at 47 % dependency level. The disadvantage of coal is because it is known as dirty and not friendly to the environment due to its sulfur, nitrogen content and ashes that contain traces of heavy metal (trace metal) that can cause air pollution. Sulphur oxides (SO<sub>x</sub>) emission to the environment are coming from both natural and anthropogenic sources. Power generator using fossil fuel and industrial activities are the main source of anthropogenic emissions (Bessone, 2006; Suriyawong et al., 2006). Acid rain is also generated by gasses from fossil fuel burning such as SO<sub>2</sub> and NO that lead to problems such as corrosion, disturbance to natural ecosystem and the loss of food chain such as small fish and other organisms (Deng and Lin, 1995). Reactions from SO<sub>2</sub> and NO in the atmosphere are also causing the formation of particles that cause health problems such as throat disturbance, eyes, nose irritation, cough and headache from short exposure to this polluted air especially for people with asthma. Several researches show that the use of zeolite in controlling SO<sub>2</sub> emission (Tantet et al., 1995; Gupta et al., 2004). Various zeolites with large volume and surface have been tested as adsorbents for SO<sub>2</sub> (Demirbas, 2006). Several advantages of using zeolite compared to carbon based adsorbent are its thermal tolerance and resistance to acid. It can also be regenerated and is low cost. Both natural and synthetic zeolite are much better than active carbon.

Coal fuelled electric power plants are the biggest mercury emission contributors to the air (Pavlish, 2006). Due to its high volatility and corrosion potential, Hg is the main impurity considered for coal burning with excess air (Bessone, 2006). Submicron mercury particles are formed during coal burning

(Suriyawong et al., 2006). There are very few research on traced elements. Some are on mercury classification (Kouvo and Backman, 2003; Gale, T.K. et al, 2005) modelling study of its characteristic and trace element modelling.

Some of the heavy metal (trace element) found in coal are mercury, plumbum, arsen, barium, berillium, boron, cadmium, chromium, thallium, selenium and molibdenum. Mojtehadi (1989) classified trace elements into three groups: (1) unvolatilized elements (2) element that are volatile during burning and found more in fly ash because of condensation (3) volatile elements that remain in gas form. Mercury belongs to group 3, while Pb and Cd are in group 2 (semi-metal), volatile and in gas phase in high temperature. Coal combustion with emission control during combustion is usually done in fluidized bed combustor. Heavy metal emissions are not dependent only on the amount of heavy metal content in the coal (Kouvo and Backman, 2003), but it is a complex combination of (a) the duration of bed use and fatigue condition of the metal (b) combustion temperature, (c) ash content and composition (d) fuel density and (e) chlorine and sulphur content related to trace metal volatility.

There are different characteristics of fly-ash resulting from coal combustion, depending on the main ingredient, type of emission control and type of storage. Sub micron trace metal is stated in *particulate matter* (PM). According to dynamic diameter, these particles can further be classified into total suspended particles (TSPS with less than 30  $\mu\text{m}$  or 100  $\mu\text{m}$ ), inhalable particle (PM<sub>10</sub> with <10  $\mu\text{m}$ ), and smooth particles (PM<sub>2.5</sub> with <2.5  $\mu\text{m}$ ).

This research aims to reduce the impact caused by coal combustion in the form of SO<sub>2</sub> and trace metal emission by adding adsorbent to contain the emission in bottom ash and not flying into the air. The type of adsorbent used is natural zeolite mixed into coal briquette. It is expected that during combustion this adsorbent will capture SO<sub>2</sub> and other heavy metal such as Hg, Pb and Cd from flying into the air and therefore minimize the impact of coal combustion. Chen et al, (1999) conducted study using sorbent kaoline, limestone and Al<sub>2</sub>O<sub>3</sub> to absorb heavy metal from waste. The best sorbent is limestone because the calcium and alkaline will react with metal chloride (Pb, Cd, Cu and Cr). The use of non-carbon adsorbent such as kaolin and lime powder to absorb Hg, Pb, Cd, Na as well as several volatile and non-volatile metal had been done by Wendt et al (2009). This research also investigates the morphology and chemical change of sorbent in high temperature. Adsorption processes are taking place in micropore while macropore roles are as transfer location for adsorbate from outer surface to the micropore (Ding and Bathia, 2003). Zeolite is a polar adsorbent and can generally be classified into two types which are natural and synthetic zeolite. Zeolite is usually alumina silica crystal with three dimensional structure formed by tetrahedral alumina and silica with pores that contained metal ion, usually alkali or earth alkaline and free moving water molecules. The chemical structure for zeolite molecule is  $M_{x/n} \cdot (AlO_2)_x \cdot (SiO_2)_y \cdot xH_2O$ . There are various zeolite structures. Natural zeolite usually contain K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup> or Mg<sup>2+</sup> cations while synthetic zeolite usually only consist of K<sup>+</sup> or Na<sup>+</sup> cations.

Natural zeolite has water molecules in the pores and free oxides on the surfaces such as Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, CaO, MgO, Na<sub>2</sub>O, K<sub>2</sub>O. The free oxides can cover the pores or active sites of the zeolite and can reduce the adsorption capacity and its catalyst nature.

## Materials and Methods

### Procedure

Submicron trace metal that can fly to the air are handled by adsorption using natural adsorbent friendly to the environment. SO<sub>2</sub> and trace metal need to be minimized because they can stick and piled up on industrial equipment such as boiler, incinerator, industrial furnaces and other burning/combustion material and reduce the equipment efficiency.

Coal used in this research are from Kaway XVI of West Aceh sub district in Aceh Province-Indonesia and the natural adsorbent (zeolite) is from West Java Province-Indonesia. The coal quality are low and consider as brown coal with sulfur content of 0.38% and 5.4% ash (d.b), calorific value 5904 cal/gr (Mahidin, 2009). The metal content in the ash and sulfur were analyzed especially for mercury (Hg), lead (Pb), and cadmium (Cd). Adsorbent was then added with various ratio and formed into briquette. The briquette was moulded in coal briquette moulding equipment pressed with pressure level of 10 ton/cm<sup>2</sup> (Permen ESDM No. 047, 2006). The briquette form is silinder with 1.5 in diameter and total weight 20 g.

Coal and zeolite as adsorbent were grounded using ball mill then screened until 60 mesh. Adsorbent compositions ratio are 2, 4, 6, 8 and 10% of sample weight briquetted and placed in sampling boat then burn in the furnace. Air flowrate was set based on sample weight and set at 40% excess with stoichiometric air ratio and control using flow meter calibrated by wet gas meter.



Combustions were conducted at temperature 600°C, 700°C and 800°C respectively for 30 minutes. Gases resulting from combustion were measured for its SO<sub>2</sub> concentration using *Industrial gas combustion and emission analyzer* (E4400, E instrument). SO<sub>2</sub> adsorption efficiencies were measured by comparing SO<sub>2</sub> released by the combustion with and without adsorbent addition. Heavy metal released and adsorbed by zeolite adsorbent were measured by analyzing its residual ashes from during combustion and left in the ceramic boat.

### Equipment

The experiments were conducted using electrically heated horizontal furnace, where reaction tube is made of stainless steel with 3 inch diameter and air flow 1.4 times stoichiometric air ratio ( $\lambda$ ) as shown in Figure 1. The reaction tube is 1 m length and the heat is maintained constant using controller and insulation.

The sample bricket was burned using excess air in various temperature and adsorbent ratio placed in *ceramic sampling boat*. The air flow rate was managed with calibrated flowmeter equipped with an analyzer to measure the resulting SO<sub>2</sub> concentration.

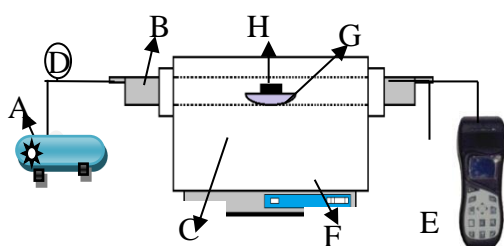


Figure 1. Experimental setup of electric furnace.

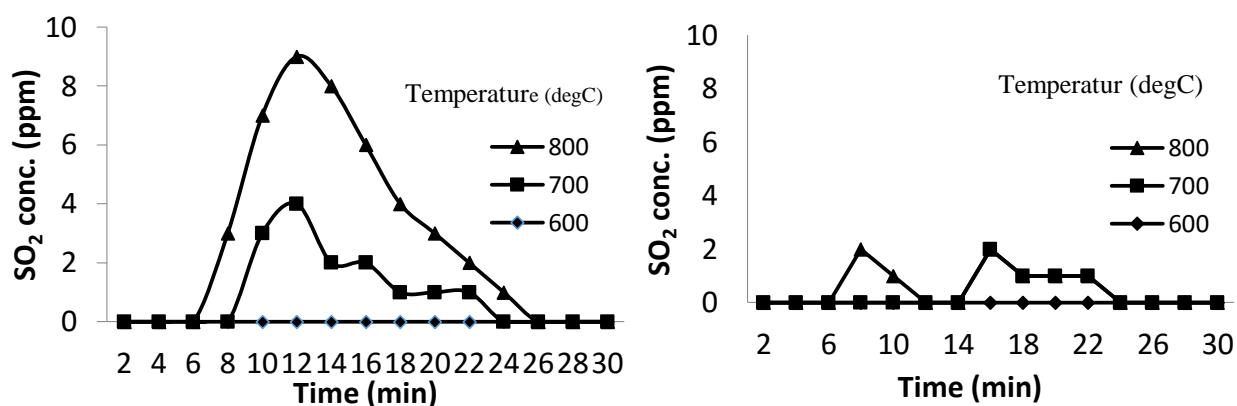
### Legend/Notes :

- A. Compressor (air supplier)
- B. Stainless steel Reaction Tube
- C. Electric Furnace
- D. Flow Meter
- E. Industrial gas combustion and emission analyzer
- F. Combustion set profile
- G. Ceramics Boat
- H. Coal bricket sample

### Results and Discussion

#### *Influence of combustion Duration towards SO<sub>2</sub> emission in coal Combustion*

SO<sub>2</sub> gas is emitted during the early phase of combustion as shown in Figure 2a and 2.b.



2a2b

Figure 2. Relationship between combustion temperature and SO<sub>2</sub> adsorption: (a) without; and (b) with 8% of zeolite adsorbent ratio.

The experiment also shows that increasing of SO<sub>2</sub> and earlier burn as combustion temperature increase without and with adsorbent addition. The results suggest increases of temperature affect on volatile matter release from coal and combustion process become earlier. Emission of SO<sub>2</sub> by addition of adsorbent was lower than without adsorbent. SO<sub>2</sub> emission was not emitted for combustion temperature 600 deg C due to the coal not decomposed at that temperature.

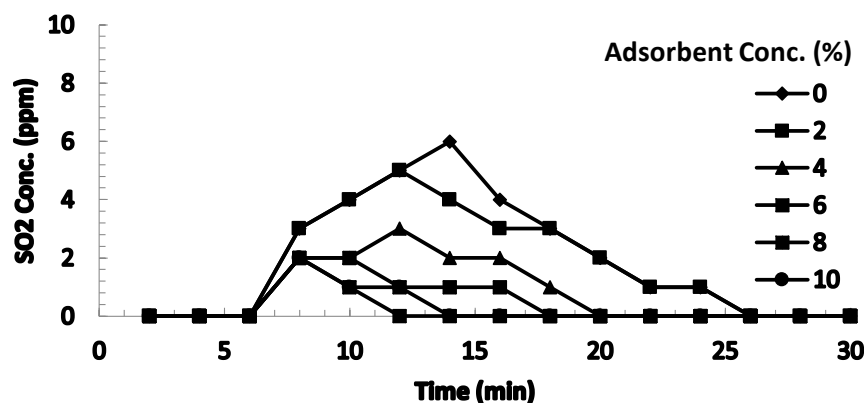
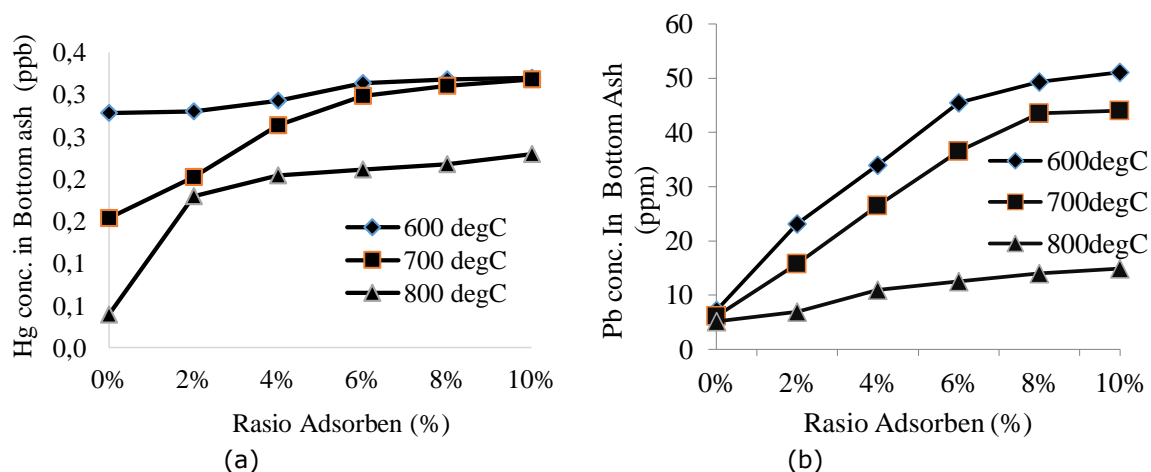


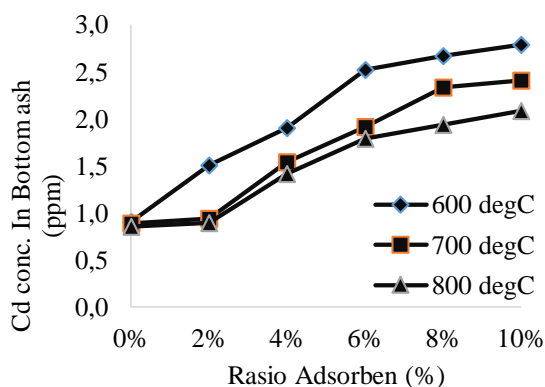
Figure 3 Relationship between adsorbent concentration and SO<sub>2</sub> adsorption at 800 °C.

There was no emission at temperature 600 °C. This is because the coal was not completely burned and SO<sub>2</sub> was not formed. Zeolite Calcination was only happened in at 600 °C. Calcination process was so important because it will make zeolite become more active. Calcination rate is influenced by mass and energy movement. Increased thermal conductivity will increase the rate of calcination (Caldwell et al, 1977). Figure 3 also shows that 10% adsorbent addition resulting in good adsorption of SO<sub>2</sub>. The figure suggests that adsorbent is capable of adsorbing SO<sub>2</sub> during combustion process.

#### Influence of Zeolite Adsorbent Ratio on Heavy Metal adsorption

Adsorption process of heavy metals such as Hg, Pb and Cd suspended in fly ash should be adsorbed by zeolite physically. Temperature has inverse influence over heavy metal content in bottom ash. The higher the temperature the less heavy metal content in bottom ash due to more volatile when temperature increases.





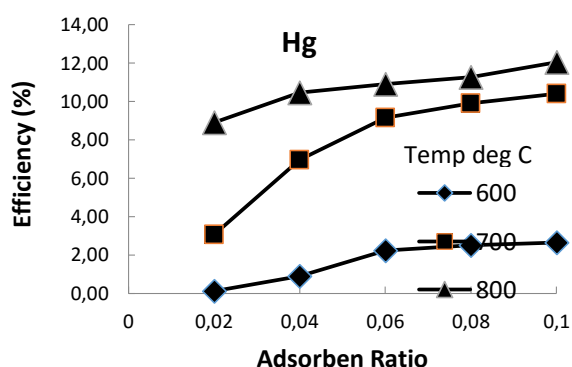
(c)

Figure4.Relationship between zeolite adsorbent ratio and Hg(a);Pb(b); and Cd(c)content in bottomash during combustion process.

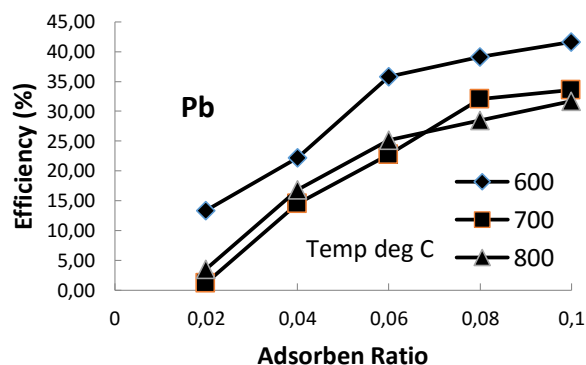
Volatile mercury can pollute the air and easily disperse and is very hard to capture. It can also be translocated in animal and plant. Zeolite adsorbent use is expected to control mercury content in bottom ash in *part per billion* (ppb) unit. The influence of adsorbent ratio toward mercury content in various combustion temperature is presented in Figure 4(a). It shows that zeolite adsorbent has a proportional influence in the adsorption of Hg. The higher zeolite adsorbent ratio the higher Hg content in bottom ash and the result suggest zeolite adsorbent affected on mercury adsorption. The figure also show mercury content in bottom ash is high at temperature 600 °C even without zeolite adsorbent is caused by unburnt coal. Adsorbent ability to absorb heavy metal such as mercury was observed at 8% to 10% ratio and give good result at temperature 700 °C.

Figure 4 (b) shows zeolite adsorbent influence towards Pb adsorption at different temperature and various adsorbent ratios. The same trend is shown for Pb adsorption which is the increase in adsorbent ratio will increase the amount of Pb absorbed. However, with the increase in temperature the adsorbent ability to adsorb Pb decreases. It is because the increase in temperature releases Pb in the volatile form and rate of diffusion volatile Pb to adsorbent is slower than devolatilization rate. The use of zeolite adsorbent for Pb is more effective at temperature 600 to 700°C. At temperature 800°C the effectiveness of zeolite to absorb Pb decreases. The optimum ratio of adsorbent in Pb adsorption is achieved at 8% adsorbent ratio for both 600 and 700 °C combustion temperature.

The same trend applies to adsorption ability of zeolite towards Cd is shown on Figure 4(c). Adsorption of Cd by zeolite adsorbent increase with the increase of adsorbent ratio. The optimum adsorption of Cd is at 600°C, while when the temperature higher (700 and 800°C) volatilization rate of the Cd higher than adsorption rate by zeolite. The differences in adsorption level between mercury (Hg) against Pb and Cd is due to the different boiling points. Mercury (Hg) can evaporate faster therefore making it less adsorbed by adsorbent. The concentration of Hg in bottom ash is within ppb while Pb and Cd are in ppm is due to the mercury content in the coal is smaller than Pb and Cd.



(a)



(b)

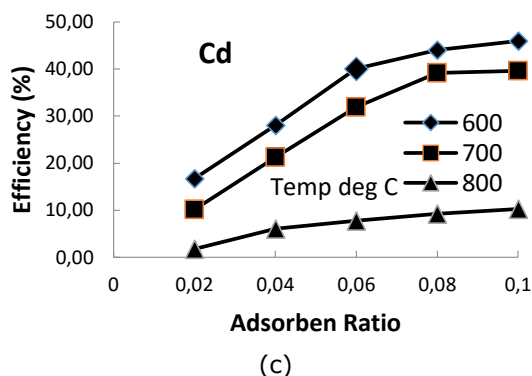


Figure 5. Influence of adsorbent ratio and temperature towards adsorption efficiency for: (a) Hg; (b) Pb; and (c) Cd.

#### Adsorption Efficiency

Zeolite adsorbent performance in capturing trace metal in coal combustion can also be assessed based on SO<sub>2</sub> and heavy metal adsorption in specific condition. The efficiency can be measured based on the following formula:

$$\eta = \frac{Ca - Ca_0}{Ca} \times 100\%$$

where  $\eta$  is efficiency,  $Ca$  concentration heavy metal in ash with adsorbent, and  $Ca_0$  concentration heavy metal in ash without adsorbent after combustion. The formula stated that adsorbate reduction is the difference of metal concentration with and without adsorbent addition.

The efficiency of Hg, Pb and Cd adsorption is presented in Figure 5 (a), (b) and (c), respectively. Generally, the efficiency will increase with the increase of adsorbent ratio. The result of adsorbent ratio toward efficiency for mercury shows different tendency compared to profile Pb and Cd as shown in Fig. 5(a). The result for mercury shows different tendency with efficiency increases along the increase of temperature. Mercury is more volatile compared to Pb and Cd. Mercury volatilizes at 600 °C, but at this temperature the calcinations process is not yet completed. Calcination process will increase with increasing of temperature, as results efficiency adsorption mercury at temperature 700 and 800 °C increases. This is because Hg is more volatile. The decrease of efficiency is caused by rate volatilization of trace metal (Pb and Cd) is faster than diffusion the metal to the adsorbent. Optimum efficiency for zeolite to adsorb mercury was observed at 8% adsorbent ratio for both temperature 700 and 800 °C. The efficiency for Pb and Cd will also decrease with the increase of temperature as presented in Figs. 5(b) and (c). This result suggests rate volatilization of metal Pb and Cd at higher temperature is faster than adsorption to zeolite adsorbent. Rate of adsorption of volatile metal to solid adsorbent is controlled by diffusion (Akhtar et al., 2014). The decrease efficiency performance tends to decrease at higher temperature suggest desorption process.

#### Conclusions

Natural zeolite can adsorb SO<sub>2</sub> and trace metal simultaneously during coal combustion. SO<sub>2</sub> emission tends to increase during the beginning of combustion. Adsorption efficiency of Hg is influenced by its high volatility. The efficiency tends to increase along with the increase of temperature. The influence of diffusion for Pb and Cd adsorption by adsorbent decreases the efficiency with the increase of temperature. The rate of adsorption is controlled by volatile metal diffusion to the adsorbent (zeolite). Zeolite performance tends to decrease at 800 °C because it starts to desorb.

#### Acknowledgements

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## Chitosan-Rhodamine B Probe As A Simple Colorimetric Naked-Eye Sensor For Hg<sup>2+</sup> Ions In Aqueous Solution

<sup>1</sup>\*Zarlaida Fitri, <sup>2</sup>Della Kharisma, <sup>1</sup>M. Adlim

<sup>1</sup>Department of Chemical Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup> Undergraduate Student of Department of Chemical Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: zarlaida.fitri@unsyiah.ac.id.

### Abstract

Chitosan-rhodamine based chemical sensor for colorimetric detection of mercury (II) ion was formulated by mixing chitosan, rhodamine B, acetic acid, and potassium iodide. This probe exhibited selectivity over other metal ions. Color change from pink to purple was observed during the determination of Hg<sup>2+</sup> ion and the limit of detection by naked-eye was found to be 10  $\mu$ M which is lower than the permitted level of Hg (II) in drinking water (30  $\mu$ M) as defined by World Health Organization (WHO). The chemical sensor can be applied to detect Hg<sup>2+</sup> in river and well water sample.

**Keywords:** chitosan, rhodamine B, mercury (II) ion, chemical sensor

### Introduction

Mercury is a toxic heavy metal with the chemical symbol Hg (Hydrargyrum). In nature, mercury contained in organic and inorganic forms. Organic mercury include those in which mercury is bound to the structure containing carbon atoms such as methyl, ethyl and phenyl, while inorganic mercury include mercury, mercury vapor (Hg<sup>0</sup>) and salts of mercury (I) (Hg<sub>2</sub><sup>2+</sup>) or mercury (II) (Hg<sup>2+</sup>) (Wang 2012). Among the forms of mercury, the mercury ion (II) is the most common source of pollution and stable in nature because it is soluble in water. Therefore, Hg<sup>2+</sup> ions easily enter the human body through the food chain (Bernhoft, 2012). These ions easily pass through biological membranes and harmful for human health, especially in organs such as brain, digestion, kidneys, heart, nervous and endocrine systems. In addition, mercury (II) ion can interfere fetal development in the womb (Oskoei, 2015 and WHO: Exposure to Mercury, 2007).

Pollution of mercury (II) ions is usually sourced from human activities, such as burning of fossil fuels (mainly of coal combustion), industrial effluents and most come from sewage purification gold mining output. Disposal of waste mercury arbitrarily prohibited and regulated in the Law of the Republic of Indonesia Number 32 Year 2009 on Environmental Management Articles 58 and 59, but there is still lack of awareness and lack of government supervision of mercury waste disposal. This has led to social fidgetiness because of the difficulty to detect mercury (II) ions in the water.

The detection of mercury (II) ion can be performed using instruments Atomic Absorption / Emission Spectrometry (AAS / AES), Inductive Coupled Plasma-Mass Spectrometry (ICP-MS), and a Fluorescence Spectrometry AFS) (Rastogi, 2012). Nonetheless, alternative detection methods are easier to use also has attracted the attention of many researchers because of technical instrumentation is costly and a long time to analyze it so that needed a faster way and practical to detect mercury (II) ions in water, one of which is using the color reagent rhodamine B.

Rhodamine B can be used to detect mercury (II) ions in water with a variety of methods, such as chemiluminescence method composed of permanganate, rhodamine B and gold nanoprism conducted by Oskoei (2015). In addition, Zheng et al (2008) determines the mercury (II) ions in water using rhodamine B which is coated with gold nanoparticles as fluorescence sensor. Both of these methods are relatively complex, takes a long time, can only be done in the laboratory and is costly due to using gold as the base material of the sensor. Therefore, it need a way of detecting mercury (II) ions in the water that are not only sensitive to mercury, but also practical and economical for use by public. The present study was focused to develop chitosan-rhodamine B chemical sensor to be able to detect Hg<sup>2+</sup> below 30  $\mu$ M by naked eye.

## Materials and Methods

### Procedure

#### Preparation of chitosan-rhodamine B based chemical sensor

A stock solution of chitosan was prepared by dissolving 0.66 grams of chitosan in 100 mL acetic acid 5%. Chitosan-rhodamine B based chemical sensor solution was formulated by mixing 2 mL of the chitosan stock solution with 1 mL sulfuric acid 10.8 M, 1 mL potassium iodide 0.15 M, and 1 mL of rhodamine B  $5 \times 10^{-4}$  M. The mixture was stirred vigorously for 30 minutes. The same procedure is done to make the solution of another sensor but the sulfuric acid was replaced with acetic acid.

#### Colorimetric detection of $\text{Hg}^{2+}$ ions

A typical  $\text{Hg}^{2+}$  ions detection procedure was conducted as follows.  $\text{Hg}^{2+}$  ions solution with different concentration were obtained by serial dilution of the stock solution. Typically, 1.0 mL of  $\text{Hg}^{2+}$  ions solutions with various concentration were added to 0.3 mL of the prepared chitosan-rhodamine B sensor and the visual color changes was recorded and analyzed. To assess the selectivity of  $\text{Hg}^{2+}$  ions detection, samples containing various metallic ions were also analyzed with the same procedure.

#### Evaluation of $\text{Hg}^{2+}$ in real water samples

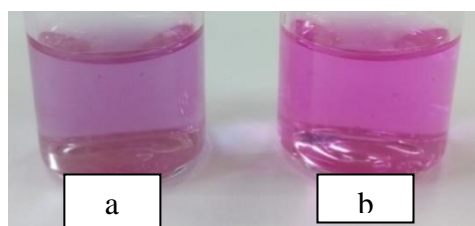
In order to assess the usefulness of the proposed method for the determination of  $\text{Hg}^{2+}$  ions, it was applied to actual samples of water. The bottled water sample (Aqua) was purchased from local supermarket in Banda Aceh and the tap water sample was collected from domestic drinking water supply in Banda Aceh. Besides, well water sample of chemistry laboratory FKIP Syiah Kuala University and Lamnyong Banda Aceh river water were also investigated.

## Results and Discussion

### Composition of Sensor

The chitosan-rhodamine-B based chemical sensor which was prepared by acetic acid compared to sulfuric acid gave different color when using in detection of  $\text{Hg}^{2+}$  ions as depicted in Figure 1. The reaction between rhodamine B with  $\text{Hg}^{2+}$  ions only occurs under acidic conditions. Using sulfuric acid in composition of the sensor give pH 1 but sulfuric acid caused chitosan precipitated so that it make the color uneven (clotting). This is consistent with the statement of Adlim (2003) that chitosan is a compound that is not soluble in water, strong alkaline solution, slightly soluble in nitric acid and hydrochloride acid and insoluble in sulfuric acid. Therefore, it is then tested in a solution of acetic acid as a replacement for sulfuric acid. Acetic acid can dissolve chitosan through protonation of free amino group ( $\text{NH}_2 \rightarrow \text{NH}_3^+$ ) at a pH of less than 6.5 (Dash, 2011).

The sensitivity of the sensor was also decrease when the sensor was prepared using acetic acid compare to sulfuric acid, probably because the sensor less acidic (pH 3).



**Figure 1.** Comparison of color change of the sensor after addition of  $\text{Hg}^{2+}$  ion. (a) Sulfuric acid and (b) Acetic acid

The mechanism of mercury (II) ions detection using the sensor possibly through complex formation  $[(\text{HgI}_4)^{2-}][(\text{Rhodamine B})^+]$  in water due to reaction of potassium iodide with mercury and rhodamine B. (Loo, 2012)

### Response Time

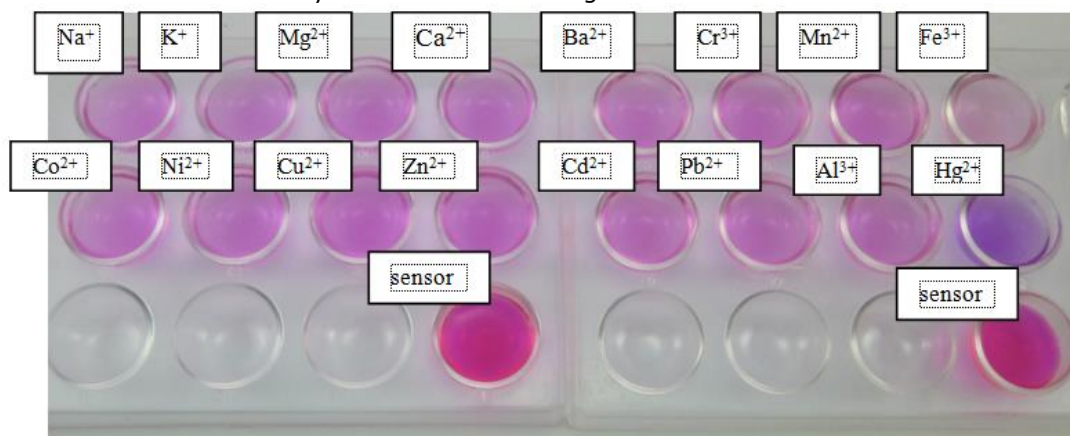
It is well known that the response time is an essential matter for an excellent chemical sensor. The chitosan-rhodamine B sensor that has been prepared when interact with  $\text{Hg}^{2+}$  ions give respond almost instantly (less than 1 minute) which is shown by color change from pink to purple.

### Selectivity

Experiments were carried out to assess the changes in the color of the chitosan-rhodamine B sensor that occurred 2 minutes after mixing 100  $\mu\text{M}$  of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Fe}^{3+}$ ,  $\text{Pb}^{2+}$  and others ions with the sensor. As



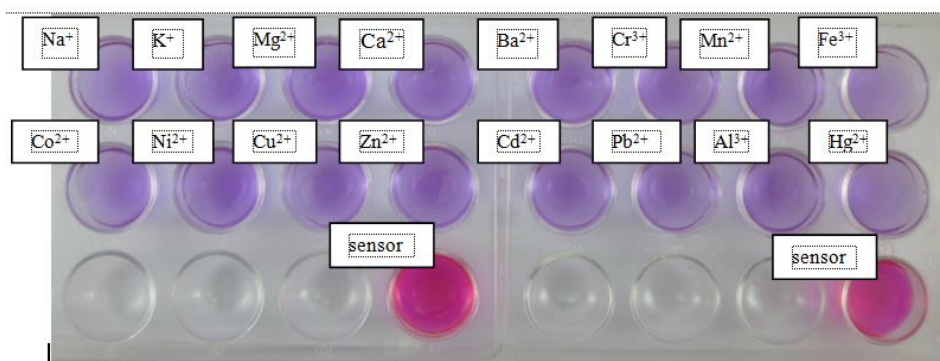
presented in Figure 2, only interaction of the sensor with  $\text{Hg}^{2+}$  ions change color from pink to purple. It means that the sensor only selective to detect  $\text{Hg}^{2+}$  ions.



**Figure 2.** Color change of the chitosan-rhodamine B sensor in the presence of various representative metal ions (100  $\mu\text{M}$ )

#### Effect of interfering ions

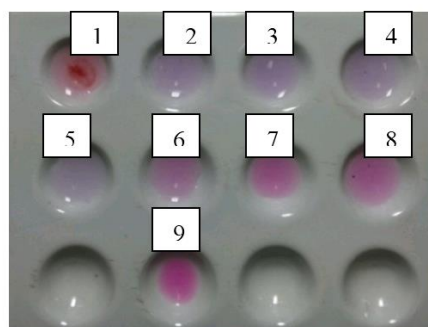
The colorimetric response of the chitosan-rhodamine B in the presence of various environmentally relevant and potentially competing ion at concentration of 100  $\mu\text{M}$  were individually examined under the same condition after addition of  $\text{Hg}^{2+}$  ions. In term of the visual observation, the color of the chitosan-rhodamine B probe changed from pink to purple only in the presence of  $\text{Hg}^{2+}$  ions, the color change was not affected if there were other interfering ions as shown in Figure 3.



**Figure 3.** Color change of the chitosan-rhodamine B probe in the presence of various representative metal ions (100  $\mu\text{M}$ ) and addition of  $\text{Hg}^{2+}$ .

#### Sensitivity (Limit of Detection, LOD) of $\text{Hg}^{2+}$ detection

The sensitivity of the chitosan-rhodamine B sensor was investigated at room temperature. A series of different concentration of  $\text{Hg}^{2+}$  ions when interacted with the sensor were observed by naked eye, photographed and analyzed.



Notes: concentration of  $\text{Hg}^{2+}$  ions

1 = 0.01 M

2 = 1000  $\mu\text{M}$

3 = 600  $\mu\text{M}$

4 = 300  $\mu\text{M}$

5 = 100  $\mu\text{M}$

6 = 10  $\mu\text{M}$

7 = 1  $\mu\text{M}$

8 = 1  $\mu\text{M}$

9 = control (Chitosan-Rhodamine probe)

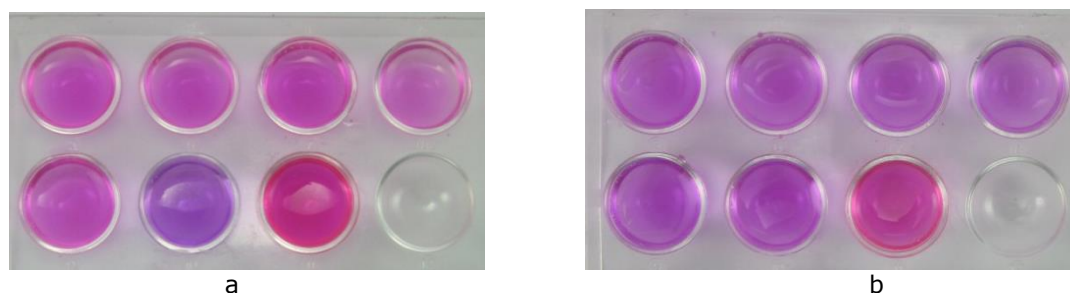
**Figure 4.** Sensitivity of the chitosan-rhodamine B toward  $\text{Hg}^{2+}$  ions (sensor: sample = 1:4)



As shown in Figure 4, with the increase of  $\text{Hg}^{2+}$  concentration, the color change from pink to purple. Based on the observation by naked eye, it can be said that the sensor still can detect  $\text{Hg}^{2+}$  as lower as 10  $\mu\text{M}$ . This sensor detection limit is lower than the minimum concentration permitted by the WHO standard of 30  $\mu\text{M}$  for drinking water (Chen, 2015).

#### Determination of $\text{Hg}^{2+}$ in real water samples

Detections were also performed in different sample water such as Lamnyong river water, bottled water sample (Aqua), tap water, and well water. Neither sample induced any visible color change in the chitosan-Rhodamine B probe, indicating that the  $\text{Hg}^{2+}$  ions contents of these water samples were below 10  $\mu\text{M}$  (Fig. 5a). However, when the water samples spike with  $\text{Hg}^{2+}$  ions 100  $\mu\text{M}$ , the color of the water change to purple (Fig. 5b). These results thereby confirmed that the detection method employed and reveal the prospective of the method developed herein for the detection of  $\text{Hg}^{2+}$  ions in real water samples.



**Figure 5.** a) Color of water samples after addition of the sensor b) Color of water samples that spiked with  $\text{Hg}^{2+}$  after addition of the sensor. (Top line from left to right: local water refills, well water, tap water, Lamnyong river water; Bottom line from left to right: bottled water (Aqua), merkuri (II) ions solution and the sensor solution)

#### Conclusions

A colorimetric  $\text{Hg}$  (II) ions detection using chitosan-Rhodamine B probe has been developed. The method has demonstrated good selectivity for  $\text{Hg}$  (II) ions against other metal ions and the presence of interfering ions does not affecting detection. The limit of detection of  $\text{Hg}^{2+}$  ions by naked eye is 10  $\mu\text{M}$ . Finally, the probe is capable of detecting  $\text{Hg}^{2+}$  ions in real water samples.

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## PI Control of a Continuous Bio-Reactor

Rudy Agustriyanto

Department of Chemical Engineering, Faculty of Engineering, University of Surabaya (UBAYA), Surabaya, Indonesia;

\*Corresponding Author: rudy.agustriyanto@staff.ubaya.ac.id.

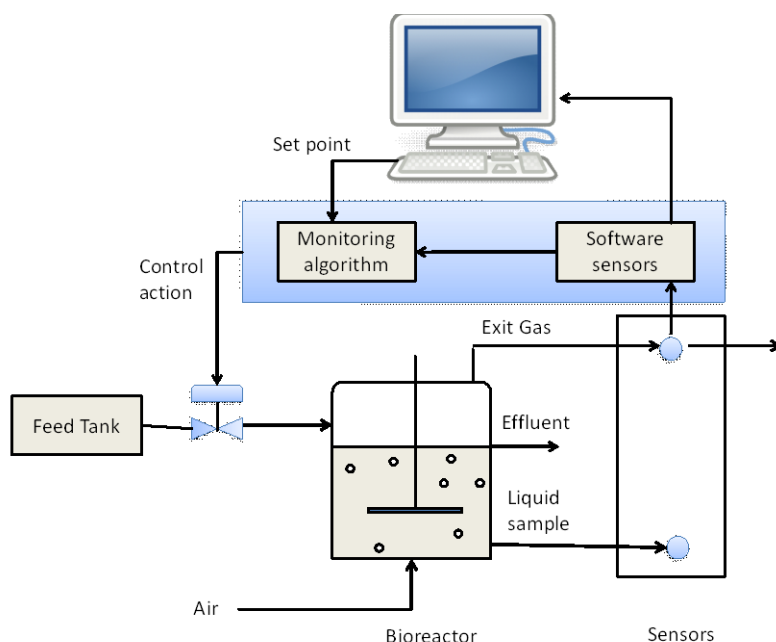
### Abstract

A bio-reactor is a vessel in which chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. On the basis of mode of operation, a bio-reactor may be classified as batch, fed batch or continuous (e.g. a continuous stirred-tank reactor model). An example of a continuous bio-reactor is the chemostat. This paper investigates a PI (Proportional Integral) control of a continuous bio-reactor which is tuned by direct synthesis method. Process performance for servo problem were presented in this paper.

**Key words:** Proportional Integral control, simulation, bioreactor, direct synthesis method.

### Introduction

The use of a control system for monitoring and controlling a biological process is shown in Figure 1 (Dochain, 2008). The central element of this scheme is the process. On this process, a number of measurements were carried out, either in the liquid or gas medium. On the basis of the knowledge available about the process and the control objectives, control algorithm can be developed.



**Figure 1.** Schematic representation of bioprocess control system

In industrial bioprocess, the main objective is usually to maximise microbial growth or microbial production of some compound produced by microorganisms (Johnsson, et.al., 2015). Therefore, it is necessary to maintain a suitable environment for microorganisms at all times.

Dynamic study of the biological process, as in any processing industry, is important. Modeling biochemical processes is also a delicate exercise. It is different from physical process where there are laws that have been known for centuries. The majority of the models in biology depend on empirical laws.

Riggs and Karim (2006) presented the dynamic model of the bio-reactor system. Based on the differential equation provided, Agustriyanto (2015) obtained the Laplace transfer functions of the

bioreactor which were first order. These transfer function models are very useful for design of the automatic control of the bio-reactor system.

PI (Proportional Integral) or PID (Proportional Integral Derivative) controllers have been the most extensively used process control technique in the process industries for many decades because of their simplicity, robustness, and wide ranges of applicability. The direct synthesis approach is a popular design method for PI/PID controllers because explicit tuning formulas can be analytically derived by using process models.

The aim of this research is to implement PI (Proportional Integral) control algorithm to the bio-reactor system and to determine controller settings based on simple direct synthesis method (Seborg, 2010). Performance of the controller for servo problem will be presented.

## Methods

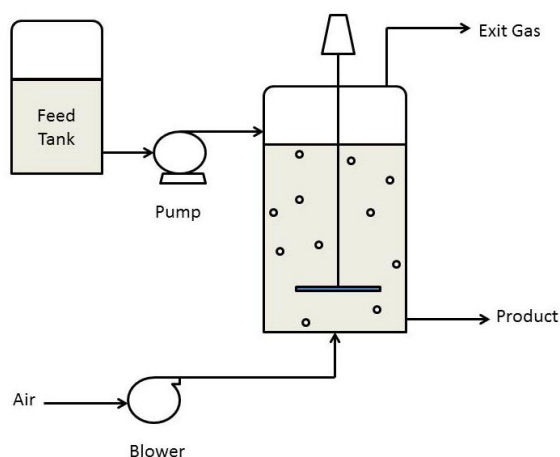
### The Bio-Reactor System

The bio-reactor system studied here (Riggs and Karim, 2006) is shown in Figure 2. A mechanistic model for this process is presented in the following:

$$\frac{dx}{dt} = -\frac{F_V}{V}x + \mu_{\max}x \quad (1)$$

$$\frac{dS}{dt} = \frac{F_V}{V}S_F - \frac{F_V}{V}S - \frac{1}{Y_{xS}}\mu_{\max}x \quad (2)$$

$$\frac{dP}{dt} = -\frac{F_V}{V}P + \frac{1}{Y_{xP}}\mu_{\max}x \quad (3)$$



**Figure 2.** Continuous bio-reactor system

The Monod kinetics is assumed for cell growth and that most of the substrate is consumed by the cells. The process parameters and variables for this model is given in Table 1.

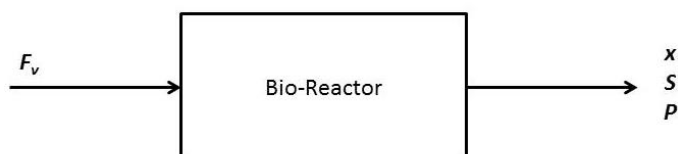
Feed contains sugar as a substrate ( $S$ ) from corn or other grains (such as wheat, rice, barley etc) and nutritional salts to support for cell ( $x$ ) growth. The cells ( $x$ ) consume the substrate ( $S$ ) and produce the product ( $P$ ) and  $CO_2$ . An air blower provides oxygen to the cells. The exit gas is primarily composed of  $N_2$  from the air, the unconsumed  $O_2$ , and  $CO_2$  produced by the cells from the consumption of sugar. The cell concentration is measured by a turbidity meter, the substrate concentration is measured by an on-line HPLC analyzer. In industrial bio-process, filters are usually used for all streams entering and leaving the reactor to maintain sterile conditions although they are not shown in the Figure.

**Table 1.** Process parameters and steady state values

Symbol	Parameters and Variables	Values and units
$F_v$	Feed rate to the reactor	1000 L/h
$K_s$	Monod's saturation constant	0.1 g/L
$P$	Product concentration in the reactor	1.25 g/L
$S$	Substrate concentration in the reactor	25 g/L
$S_F$	Substrate concentration in the feed to the reactor	50 g/L
$t$	Time	h
$V$	Volume of the reactor	5000 L
$x$	Cell concentration in the reactor	0.25 g/L
$Y_{xP}$	Yield factor	0.2 g-cells/g-product
$Y_{xS}$	Yield coefficient	0.01 g-cells/g-substrate
$\mu_{max}$	Maximum specific growth rate	0.2/h

### Open Loop Transfer Function

The open loop transfer function for Bio-Reactor System (Figure 3) has been found (Agustriyanto, 2015) by solving those model equations (i.e Eq.(1) to (3)) subject to the parameters and steady state values given in Table 1 using Differential Equation Editor (DEE) in Matlab. The results were then identified using System Identification Toolbox. The method were also explained in Agustriyanto and Fatmawati (2013) and Agustriyanto (2014).



**Figure 3.** Open loop bio-reactor

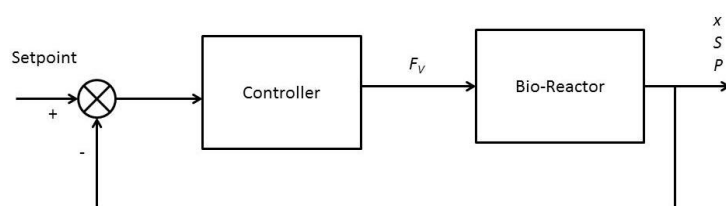
system

The results are as follows, where bar sign show that the variable are in deviation form.

$$\begin{bmatrix} \bar{x} \\ \bar{S} \\ \bar{P} \end{bmatrix} = \begin{bmatrix} \frac{-0.005}{100s+1} \\ \frac{0.54813}{96.663s+1} \\ \frac{-0.025}{100s+1} \end{bmatrix} [\bar{F}_v] \quad (4)$$

### Control of Bio-Reactor System

The product ( $P$ ) was selected as controlled variable and feed rate to the reactor ( $F_v$ ) as manipulated variable. The closed loop system for Bio-reactor is shown in Figure 4. It is assumed that the transfer function for sensor and final control element (control valve) are 1, therefore they can be neglected in the figure.



**Figure 4.** Closed loop of the bio-reactor system

PI (Proportional Integral) mode was selected for the controller and as the transfer function of the system are first order, then direct synthesis method of tuning (Seborg, 2010) can be applied.

### Direct Synthesis Tuning Method

Direct synthesis method for a first order process:  $G_p(s) = \frac{K_p}{\tau_p s + 1}$  are as follows:

Let  $G_p(s)$  = transfer function of the process  
 $G_c(s)$  = transfer function of the controller

If all the dynamics of the other elements in the closed loop system were neglected, the following overall transfer functions was obtained:

$$\frac{cv}{SP} = \frac{G_c \cdot G_p}{1 + G_c \cdot G_p} \quad (5)$$

Where  $cv$  = controlled variable  
 $SP$  = Setpoint

The above equation can be re-arranged to get the equation for feedback control law as follows:

$$G_c = \frac{1}{G_p} \left[ \frac{\frac{cv}{SP}}{1 - \frac{cv}{SP}} \right] \quad (6)$$

In other words, controller consists of the inverse of the process model and the specification of the characteristic response of closed loop.  $\frac{cv}{SP}$ . The process model can be obtained from plant identification,

while characteristic response of the closed loop ( $\frac{cv}{SP}$ ) must be specified. A form of simple specification are as follows:

$$\frac{cv}{SP} = \frac{1}{\lambda s + 1} \quad (7)$$

Where:  $\lambda$  is the closed loop time constant specified by the user.

Substituting the above expression into the control law:

$$G_c = \frac{1}{G_p} \left[ \frac{\frac{cv}{SP}}{1 - \frac{cv}{SP}} \right] = \frac{1}{G_p} \left[ \frac{1}{\lambda s} \right] = \frac{\tau_p s + 1}{K_p \cdot \lambda s} = \frac{\tau_p}{K_p \lambda} \left[ 1 + \frac{1}{\tau_p s} \right] \quad (8)$$

Based on the description of the process, then we can get ideal form of the PI controller:

$$k_c = \frac{\tau_p}{K_p \lambda} \quad (9)$$

$$\tau_I = \tau_p \quad (10)$$

This direct synthesis method can be used for servo problem of first order process only. Chen and Seborg (2002) also presented PI controller design based on disturbance rejection. For other process which are not first order, direct synthesis method are also available (Rao et.al., 2008; Anil and Sree, 2015).

### Results and Discussion

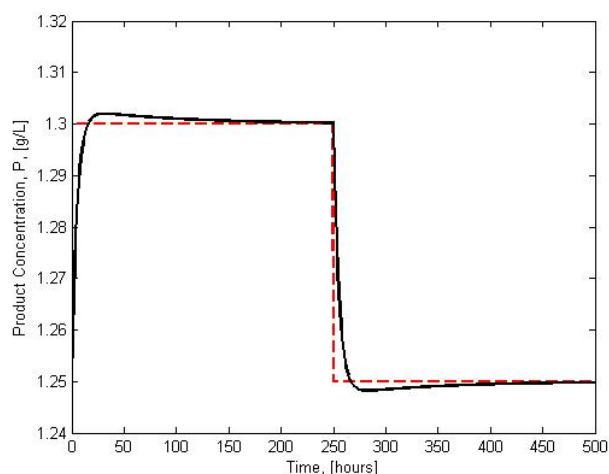
A closed loop system of a Bio-Reactor were simulated by using Simulink. Table 2 shows controller parameter obtained by Direct Synthesis method. Here, the value of  $\lambda = 5$  was chosen, while  $K_c$  and  $\tau_I$  were calculated using Eq.(9) and (10).

**Table 2.** Controller parameters

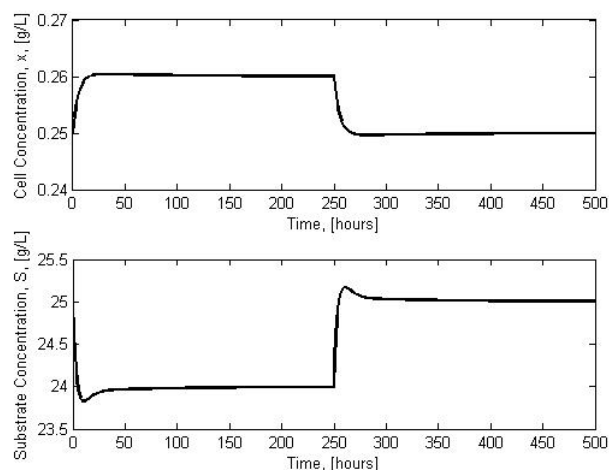
Loop	Kc	$\tau_i$	$\lambda$
(P-Fv)	-800	100	5

As can be seen in Figure 5, product concentration follow the changes in set point. The performance is quite good. The performance for this servo problem (trajectory tracking) can be increased if we use smaller value of  $\lambda$ .

Figure 6 shows the dynamic of two other variables (cell and substrate concentration) which were not controlled. When the set point changed from 1.25 to 1.3 g/L, it will affect cell and substrate concentrations. In the new steady state values, cell concentration become 0.26 g/L while substrate concentration become 24 g/L from the initial values of 0.25 and 25 g/L respectively.



**Figure 5.** Plot of product concentration (P) vs time (h) and its set point (--)



**Figure 6.** Plot of cell (x) and substrate concentration (S) vs time

## Conclusions

Closed loop simulation results for servo problem of a continuous bio-reactor system have been presented. The direct synthesis tuning method was used in this simulation and gives good performance for set point changes as shown in Figure 5. The set point changes also affect cell and substrate concentration as presented in Figure 6.

## Acknowledgements

This work was supported by the University of Surabaya.

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## Activation of Palm Midrib by Using Mixed Citric Acid and Tartaric Acid and its Application for Adsorption of Zn (II) Heavy Metals from Wastewater

\*<sup>1</sup>Farid Mulana, <sup>1</sup>Mariana, <sup>1</sup>Pocut Nurul Alam and <sup>1</sup>Abrar Muslim

Department of Chemical Engineering, Faculty of Engineering, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: faridmln@yahoo.com

### Abstract

Adsorption experiments by activated palm midrib by using mixed citric acid and tartaric acid of zinc (II) metal ions were carried out in this study. Zn(II) heavy metal ion from wastewater as by-product of industrial activity is one of problem in environment. Palm midrib that containing lignin, cellulose, and hemicellulose are one of low cost potential candidates to be utilized as adsorbent. The purpose of this research is to activate midrib of palm by using a mixed citric acid and tartaric acid and to know adsorption efficiency of activated midrib of palm adsorbent on Zn (II) metal uptake from wastewater. To determine adsorbed amount of Zn (II) intoactivated adsorbent an Atomic Adsorption Spectroscopy Method was conducted and to identify substituted groups, analysis samples by using Fourier Transform Infrared was performed. The result showed that the adsorption process was relatively not so fast and equilibrium was reached after about 120 min of contact time. The optimum adsorption efficiency was 89.7 %. These results were obtained on 20 ppm of Zn(II) adsorbate concentration for 120 minutes of contact time and concentration of 1.6 M mixed citric acid and tartaric acid for activation process.

**Keywords:** adsorption, citric acid, tartaric acid and Zinc (II)

### Introduction

Heavy metal in industrial aqueous wastes can be produced both from physical and chemical industry such as smelting, alloy industries, metal plating, mining operations, tanneries, chloralkali, radiator manufacturing and storage batteries industries (Kadirvelu *et al.*, 2001).

Those excessive heavy metals aqueous wastes in environment due to industrialization and urbanization have induced many problems for human being and its environment. Heavy metal ions could not be degraded into harmless end products (Gupta *et al.*, 2001) as occurring for organic pollutants in which could be degraded by biological process. Therefore, heavy metal has to be treated before discharging to environment because the presence of heavy metals ions would be problems due to their toxicity to many life forms. There are a lot of treatment processes for heavy metal removal from wastewater such as membrane filtration, ion exchange, precipitation, adsorption and coprecipitation/adsorption. Adsorption process is considered to be a highly effective technique for removal of heavy metal from waste stream (Sharma *et al.*, 2011). Activated carbon has been used extensively as adsorbents for wastewater treatment industries. However activated carbon remains as an expensive adsorbent. Therefore, the searching of inexpensive adsorbents from agricultural solid waste could be an alternative method to reduce pollution (Grassi *et al.*, 2012).

Homeland of Indonesia generated a lot of solid wastes from agricultural products that can still be used for the adsorbent more economically valuable than just used traditionally. As an example, the utilization of midrib of palm was only be partly used as cattle feed and wasted largely in nature. In addition to the use of traditional above the midrib of palm actually have a pore size varies that a very important aspect in adsorption process. With a proper activation process, pores size can be enlarged and adapted to the size of molecules of liquid waste of heavy metal so that adsorbate can be absorbed easily into pore of adsorbent. So far, some researchers have used other biomaterials to absorb heavy metals including coffee waste (Rossner *et al.*, 2009), paddy straw (Kumar *et al.*, 2006), tea waste (Amarasinghe *et al.*, 2007) and other adsorbents (Bhattacharya *et al.*, 2008). A low-cost adsorbent such as midrib of palm can be chemically activated with an oxidator to increase the adsorption capacity of heavy metals due to the increase of surface area and surface charge after activation process. Adsorption capacity can be increased through interaction with the metal oxidator in solution (Marshall *et al.*, 1999, 2000, 2001).



The processing of solid waste materials with some acids such as citric acid, tartaric acid and phosphoric acid at a rather high temperature can increase the adsorption of heavy metal ions (Wong *et al.*, 2003; Marshall *et al.*, 1999, 2000, 2001; Wafwoyo *et al.*, 1999; McSweeny, 2006). With this activation, some acid groups can occur and form ester groups (Marshall *et al.*, 2001). Chemical activation of midrib of palm with citric acid together with tartaric acid can enhance the adsorption capacity of heavy metals liquid waste where this happens because during the activation process not only increases the amount of carbonyl/hydroxyl group and enlarge the surface area of the adsorbent but also create more negative sites on the surface of midrib of palm. This research aims is to study the ability of activated midrib of palm to adsorb zinc (II) metal ions toward adsorption efficiency by varying the initial concentration of adsorbate and to know the effect of adsorbent activation by using citric acid and tartaric acid.

## **Materials and Methods**

### **Equipment and Materials**

The equipments and tools used in this study were hot plate, glassware, desiccator, digital scales, oven dryer, Atomic Absorption Spectrometry (AAS) type AA 7000 (Shimadzu), FTIR IR Prestige 21 (Shimadzu) and sieve shaker. On the other hand materials used were midrib of palm with particle size of 40-60 mesh, sodium hydroxide, citric acid 99.9% (Merck), tartaric acid 97.5% (Merck), a solution of  $Zn(NO_3)_2$  (Merck) and distilled water.

### **Research Procedure**

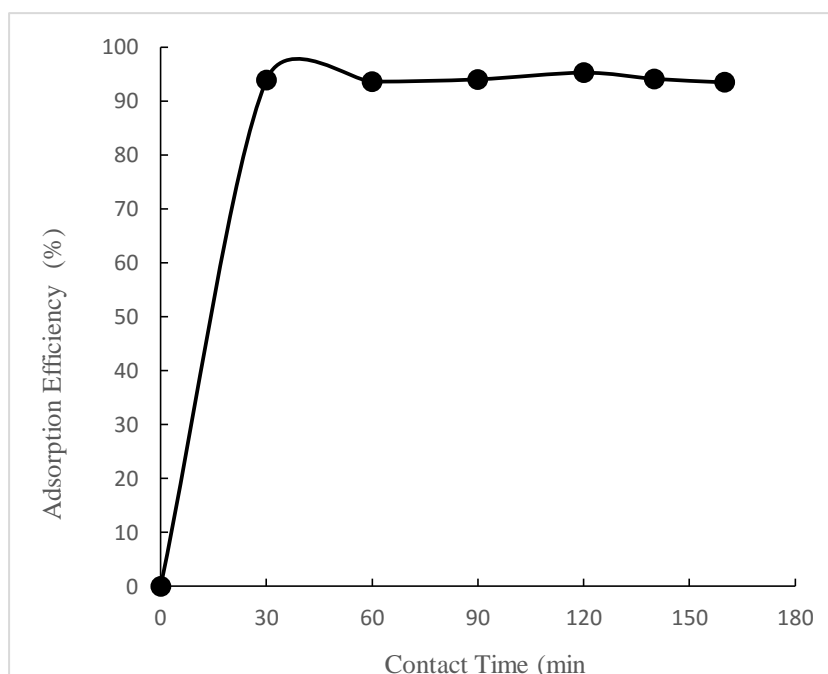
First, a small size of midrib of palm washed several times with distilled water to remove dust and dirt and later dried under sunlight. Having dried, midrib of palm was soaked in hot water for 1 hr, then dried again under sunlight for 4 hr and later dried in oven dryer at 110 °C for 1 hr. Dried materials were then cut using a mixer and sieved on the size of 40-60 mesh. Finally, midrib of palm were dried again at 110 °C for 3 hours and then stored in a desiccator. A few grams of midrib of palm were dissolved into a solution of KOH 0.5 M. The addition of KOH during activation will form a few new sites on the surface of the adsorbent so that will increase adsorption capacity. The mixture of midrib of palm and KOH is then stirred at 80 rpm for 30 min at room temperature. Then washed by distilled water repeatedly until a pH of  $\pm 7$ . Finally dried at 50 °C for 2 hr.

1 gram of netralized adsorbent then was mixed with 100 ml of 0.5 M citric acid and 0.5 M tartaric acid on the same ratio for 2 hr at 80 rpm, then dried for 3 hr at 50 °C. The same procedure was repeated for citric acid/tartaric acid concentrations of 0.8; 1.2, and 1.6 M. Modified adsorbent was then washed with distilled water to remove the excess of citric acid and tartaric acid until a pH of  $\pm 7$ , followed by re-drying at 50 °C for 24 hr (Marshall *et al.*, 2000). For adsorption process, 1 gr of activated adsorbent was mixed with 100 ml of  $Zn(NO_3)_2$  of 20 ppm at speed of 80 rpm for different time (0 to 150 minutes) at room temperature. After the adsorption process was completed, sample solution was taken to be analyzed by AAS. The above treatment was repeated for concentrations of 40, 60, and 80 ppm (15, 44, 54 and 73 ppm of AAS results).

### **Results and Discussion**

#### **Effect of Contact Time Toward Adsorption Efficiency**

To determine the adsorption efficiency of metal Zn (II) we have performed the adsorption process at some contact time and the results can be seen in Figure 1. The test was conducted on the initial adsorbate concentration of 20 ppm with a concentration of citric and tartaric acid activator each are 0.4 M.



**Figure 1** The relationship between contact time (minutes) and the adsorption efficiency (%) at Zn (II) initial concentration of 20 ppm and the concentration of citric acid and tartaric of 0.4 M.

From Figure 1, adsorption efficiency that is obtained at 0, 30, 60, 90, 120, 140 and 160 minute are 87.54; 93.60; 93.88; 95.24; 94.08; 94.68%, respectively. Equilibrium time is obtained at 120 minute, which at that time the percentage of metal adsorption of Zn (II) reached 95.24% on the initial adsorbate concentration of 20 ppm. With a longer of stirring time, the ability of adsorbent to bind to each other will be even greater. This is because with a long contact time between adsorbent and adsorbate is enabling more and more bonds to be formed between adsorbent particles with heavy metal ions. Citric and tartaric acid as activator solution plays an important role on adsorbent in the adsorption process. The presence of citric acid and tartaric during the activation process resulted in degradation of material that will form the more pore. By increasing the concentration of activator, then the metal ion adsorption efficiency of Zn (II) would be higher. The increased concentrations of citric acid and tartaric might be proportional to the increase in the pore distribution and surface area of the adsorbent causing a number of binding metal ions of Zn increases and as well as adsorption ability also increases.

Other researcher Pratama (2015), informed that the amount of Zn (II) adsorbed on the first 40 minutes increase significantly. After contact time 60 minutes it still remains increasing but become flat after 120 minute. From this study, it can be concluded that the optimum contact time is at 120 minute. The quite fast elimination of metal ions in the early minutes of adsorption process may be due to at the beginning of the adsorption process many sites and pore of adsorbent is still empty so that the tendency of the solution to be absorbed into the adsorbent is much higher with increasing contact time and slow the rate of adsorption began to decline until they reach equilibrium time. Okafor (2012) suggested that some natural adsorbent contains cellulose and lignin which is a material with huge potential to be used as bioadsorbent. Lignin and cellulose have a pore structure which is a major requirement of the adsorbent.

#### **Effect of Citric Acid and Tartaric Acid Concentration on Adsorption Efficiency**

Citric and tartaric acid concentration also affects the adsorption efficiency of the adsorbate solution on adsorbent. This phenomenon can be seen from Figure 2 that states the relationship between the increase of activators concentration toward adsorption efficiency.

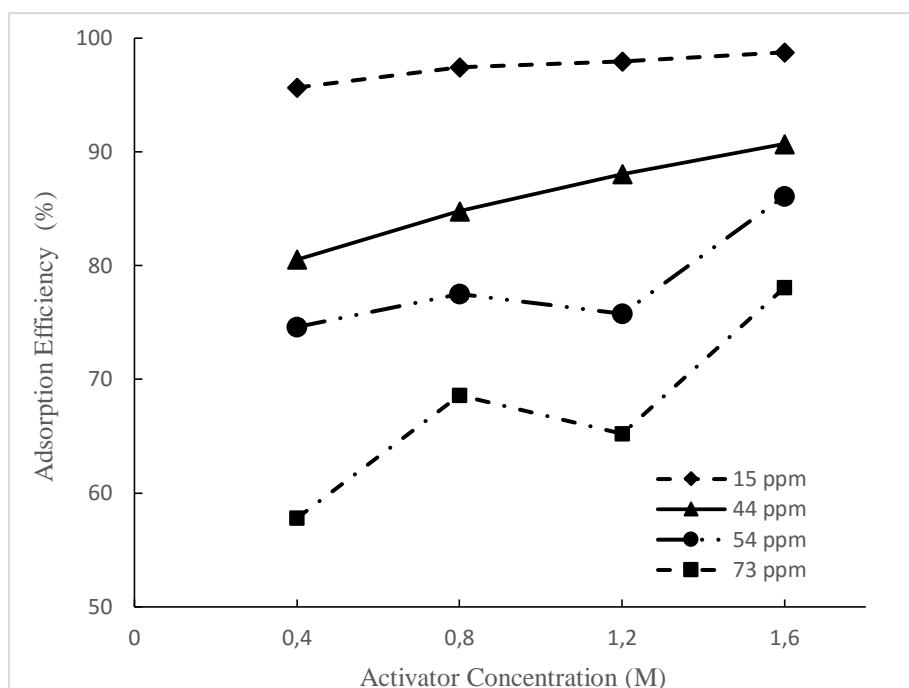


Figure 2. Effect of citric and tartaric acid concentration (M) and the initial concentration of adsorbate (ppm) on adsorption efficiency (mg/g) at the contact time of 120 minutes.

The increase of citric and tartaric acid concentration on activation process of adsorbent affect metal ion adsorption efficiency of Zn (II). The highest adsorption efficiency of 98.7 % was obtained at citric acid and tartaric concentrations of 1.6 M with Zn (II) initial concentration of 20 ppm.

#### Activation Effect of Adsorbent by Using Citric Acid and Tartaric Acid

FTIR analysis was conducted to understand the effect of adsorbent activation by using citric acid and tartaric acid on various concentrations. This analysis also to see the groups that exist in the sample and their absorbance at certain wavenumber and to see the changes of groups after their reaction with other compounds. FTIR data presented is FTIR data of adsorbent prior to activation, after activation with KOH, after activation with citric and tartaric acid at various concentration of 0.4 M; 0.8 M; 1.2 M and 1.4 M and adsorbent after adsorbed metal ion of Zn (II) with a concentration of 80 ppm. Graph wavelength infrared spectra was shown in Figure 3 below:

From Figure 3 it can be seen that the midrib of palm adsorbent (without activation) has a hydroxyl bond with the changes of absorption spectra at wavenumber of  $3570\text{ cm}^{-1}$ . After the activation process by using concentration of citric acid and tartaric of 0.4 M it already apparent formation of a carboxyl group at a wavelength of  $1600\text{ cm}^{-1}$  indicating the presence of COH group where this group comes from the absorption spectra of the activator solution that were added. The same phenomena can be observed in the spectra after activation with citric and tartaric acid concentration of 0.8; 1.2 and 1.4 M, where the broadest absorption spectra can be seen on activation with citric and tartaric acid of 1.4 M. At a concentration of 0.4 M, carboxyl groups have narrow peak. However, with increasing concentrations of citric and tartaric acid it can be seen that the absorption area (peak) become wider. The same spectra also occurred after activation with concentrations of 0.8, 1.2 and 1.4 M where at a concentration of 1.4 M was wider that indicating that more of carbonyl groups was formed with the increasing concentration of citric and tartaric acid.

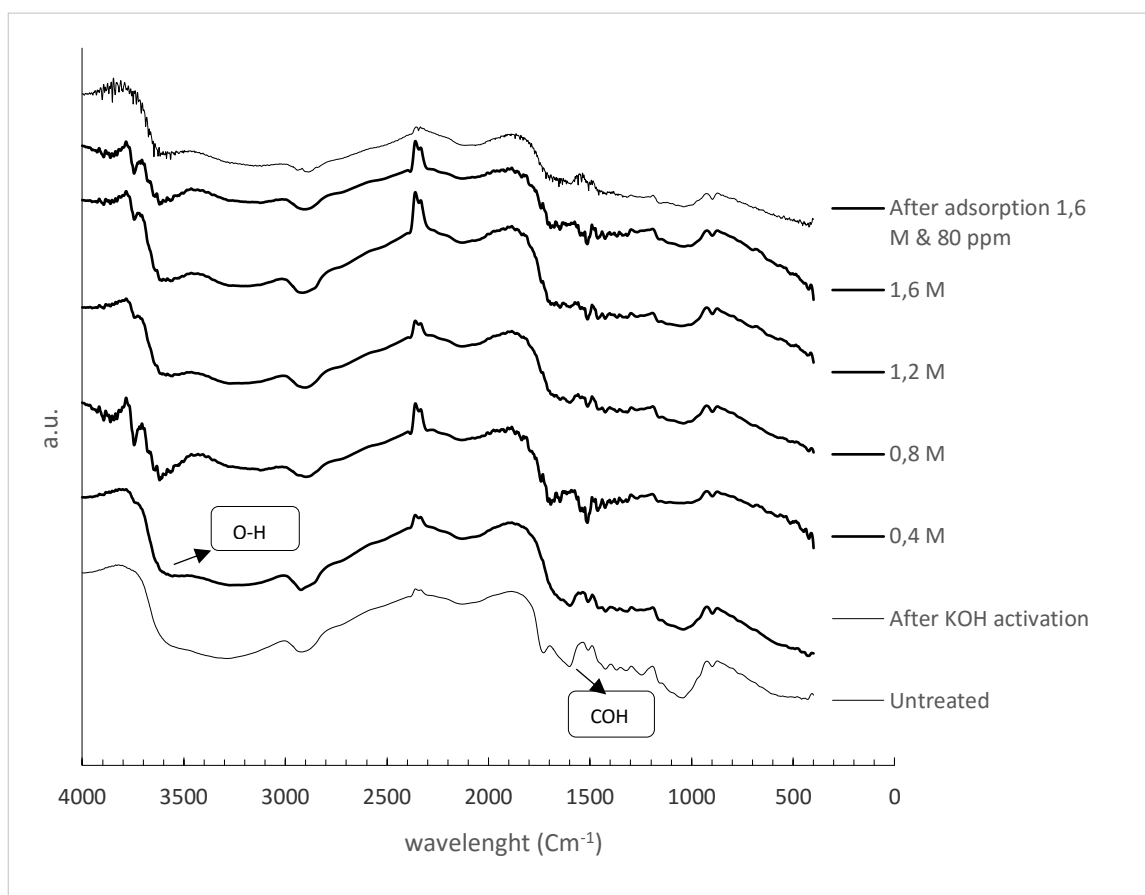


Figure 3 The infrared spectra of midrib of palm adsorbent on various conditions

At a wavelength of  $3600\text{ cm}^{-1}$  also appeared the absorption spectra that indicates the O-H group of carboxylic acids, namely at the activation of 0.4 M. This happens because the effect of citric and tartaric acid activation as well. On activation of citric and tartaric acid from 0.4 M to 1.4 M, it can be seen that there was a shift of absorption spectra from right to left (larger wavelength), which is also influenced by the increase of citric and tartaric acid concentration. Spectra of adsorbent after adsorption process it appears that spectra was not much different from the adsorbent without adsorption process. Based on the performed research, in the presence of hydroxyl groups on the adsorbent, the metal ion of Zn (II) will be easier to interact with the hydroxyl group (O-H) which is a hard base (Hayyu, 2012).

### Conclusions

The adsorption process of Zn (II) metal ions onto activated midrib of palm reached the highest adsorption efficiency of 98.7%. These adsorption processes were conducted at initial zinc (II) concentration of 20 mg/L and 1.6 M of mixed citric and tartaric acid concentration with contact time of 120 minutes. The adsorption process was influenced significantly by addition of citric and tartaric acid as mixed activator. With the increase of mixed citric and tartaric acid concentration, the adsorption efficiency would also increase significantly. The presence of carboxyl group and hydroxyl group was due to the influence of citric acid and tartaric acid activation. On activation of the adsorbent with only small concentration of citric acid and tartaric acid provided an appearance of carboxyl groups in the absorption wavelength spectra of  $3600\text{ cm}^{-1}$  which indicate a OH group.

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## Synthesis And Characterization Of Bioplastic Based On Cassava Starch-PLA For Food Packaging Application

<sup>1</sup>\*Harunsyah, <sup>1</sup>Ridwan, <sup>1</sup>Salahuddin

Department of Chemical Engineering, State Polytechnic Lhokseumawe, Buketrata, Lhokseumawe 24300, Indonesia;

\*Corresponding Author: aroensyah@gmail.com

### Abstract

Nowadays, conventional plastics are extensively used in almost daily activities such as the plastic packaging bags produced from polyethylene and polypropylene. However, the products from these polymers cause environmental problem. To replace the conventional plastics with bioplastic can solve this problem. The applications of bioplastic base on cassava starch-polylactic acid as a potential alternative choice. Synthesis of bioplastics made from a mixture of cassava starch and polylactic acid on variation of concentration plasticizer glycerol and polylactic acid has been studied. The general aims of this research are the synthesis and characteristic of bioplastic base on cassava starch with glycerol and polylactic acid modification. The bioplastic characteristic consist of, tensile strength, elongation, spectra of molecule structure by FTIR. The research used 5 combination of glycerol and polylactic acid. The results of this research showed that added of polylactic acid and glycerol was improved the physic and mechanical of bioplastic. The optimum tensile strength was showed at increasing plastilizer glicerol 2 mL and polylacted acid 5 mL was value 5.10 MPa and elongation 84.96% . Base on data of FTIR, the film plastic that produced did not happen change function group so we can know that interaction in film plastic thta produced just physical interaction. Cassava starch add polylacted acid based films plasticized with glycerol showed interesting mechanical properties being transparent, clear, homogeneous, flexible, and easily handled.

**Key words:** cassava starch, biodegradable plastics, mechanical propeties, plasticizer

### Introduction

Over the last decades, the use of conventional plastics as food packaging material has increased considerably. Conventional plastics are widely used for packaging and other applications because of their several advantages compared to other materials. For example, plastics are inexpensive, light weight and chemically inert. Moreover, they are heat-sealable, easy to print on and offer the flexibility of fabricating into various shapes. Unfortunately, conventional plastics have their origin in petrochemical industry making them non-biodegradable and non-renewable (Janssen. L, 2009 and Q.X. Zhang, 2007). The non-biodegradable and non-renewable nature of plastics has been a serious disadvantage to their application leading to huge municipal wastes and environmental degradation.

So the use of conventional plastic as food packaging material facing various environmental problems, which cannot be recycled and cannot decompose naturally by microbes in the soil, resulting in the accumulation of plastic waste that causes pollution and damage to the environment. Therefore, to replace the conventional plastics with biodegradable plastic base on starch can solve this problem.

Starch is a natural polymer, inexpensive, readily available, and often used as a filler for the replacement of petroleum-derived synthetic polymers to decrease environmental pollution. However, starch has severe limitations because of its solubility and poor water-resistance, making starch products very sensitive to the relative humidity at which they are stored and used. Starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barrier in packaging materials. Starch often used in industrial foods. They have been used to produce biodegradable plastics to partially or entirely replace conventional plastics (plastic polymers) because of its low cost and renewability, and it has good mechanical properties (Xu *et al.*, 2005).

Starch has been considered as the most promising raw material to develop new environmentally friendly materials especially for packaging and disposable applications because of its low density, its renewable character and its complete biodegradability, and its availability worldwide under different shapes at relatively low cost (Garcia *et al.*, 2011; Wu *et al.*, 2009; Wilhelm *et al.*, 2003a; Almasi *et al.*, 2010).

The main disadvantages of biodegradable plastic base on starch, compared to conventional plastics, are their hydrophilic character and their poor mechanical properties which lead to low stability (Li *et al.*, 2011; Lopez *et al.*, 2011). In order to replace conventional polymer by biopolymers, these drawbacks have to be circumvented. Indeed, depending on the targeted applications, one may need specific properties such as stiffness, flexibility, and strength.

And also, there are some strong limitations for developing starch based products, since they present poor tensile properties and high water vapor permeability when compared to conventional films derived from crude oil (Souza *et al.*, 2010) on account of their hydrophilic nature and their sensitivity to moisture content, a factor that is difficult to control (Wilhelm *et al.*, 2003b).

Numerous studies have been conducted to optimize the properties of biodegradable plastic base on starch. The most important properties in bioplastic materials include mechanical and thermoforming properties, gas and water vapor permeability, transparency and availability (Janssen. L, 2009). The main objective of this study is to the synthesis and characteristic of bioplastic base on casavva starch with glycerol and polylactic acid (PLA) modification. To be selected of PLA due to PLA has attracted both industries and research institutions. It is one of the biopolymer whose properties are comparable with the commercial plastic such as poly(ethylene terephthalate) (PET) (Huneault *et al.*, 2007). PLA production is derived from annually renewable resources such as corn starch, cassava starch or sugarcane. PLA exhibits good properties such as biodegradability, heat resistance, transparency, good mechanical properties and processability (Yang *et al.*, 2009; Lemmouchi *et al.*, 2009), causing it to be used in many packaging applications. The important requirement for packaging materials is high tensile strength, ductility, flexibility, transparency and good barrier properties (Ljungberg and Wesslen, 2003). However, PLA is still limited for its application because of its price (expensive because of the complicated synthesis), brittleness, rigidity and low crystallization rate (Huneault and Li, 2007; Yang *et al.*, 2009). Therefore, plasticizers are used to increase the flexibility of PLA for packaging applications such as, packaging films, wrap films, stretch films and agricultural mulch films.

## Materials and Methods

### Procedure

#### Film preparation

The method of preparation was adapted from Cyras *et al.* (2008), Müller *et al.* (2008) and Araujo-Farro *et al.* (2010). Cassava starch film solutions (concentration 10% w/v) was stirred at room temperature for 15 minutes on a magnetic stirrer (250 rpm). The solutions was added glycerol (Merck Millipore, 85%) and polylacted acid (PLA) that polylacted acid produced by polymerization of lactic acid (L (+) Sigma-Aldrich, 98%) to polilaktat acid (APL) by heating 200 ml of lactic acid in the temperature of 70-75 °C for 10 minutes then added 2 grams of catalyst SnCl<sub>2</sub> evenly stirred while heated in the same temperature for 15 minutes and then cooled and the viscous liquid obtained polylacted acid. The mixture was then heated at 80±2 °C in a thermal bath under constant stirring for 30 minutes. Then the films were obtained by casting, pouring the hot suspension into rectangular moulds. These moulds were left at room temperature for at least 4 hour to allow bubbles to dissipate and then dried in an oven with air circulation at 30 °C for 24 hours. The dry films were removed from the moulds and stored at controlled conditions (25 °C and 75% of relative humidity) for at least 48 hours before measurements.

Table 1. Composition of glycerol-PLA /cassava starch in 100 mL film solution

Film Formulas	Composition (gram/100 mL water)		
	Cassava Starch (gram)	Glycerols	Polylacted Acid
Formula-A	10	1.0 mL	1.0 mL
Formula-B	10	1.5 mL	2.0 mL
Formula-C	10	2.0 mL	3.0 mL
Formula-D	10	2.5 mL	4.0 mL
Formula-E	10	3.0 mL	5.0 mL

#### Film Testing Method

In general, the physical and electrical properties of plastics are influenced by temperature and relative humidity in a manner that materially affects test results. In order to make reliable comparisons between different materials and between different laboratories, it is necessary to standardize the humidity conditions, as well as the temperature, to which specimens of these materials are subjected prior to and during testing. Therefore, all films were conditioned prior to subjecting them to permeability and mechanical tests according to Standard method, ASTM-D618-61. Films used for testing Water Vapor Permeability (WVP), Tensile Strength (TS) and Elongation (E) were conditioned at 75% relative humidity

and 25 °C by placing them in a desiccators over a saturated solution of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  for 24 hours or more. For other tests, film samples were transferred to plastic bags after peeling and placed in desiccators. The tensile strength and elongation at break of the films were measured using a computer type universal testing machine (HUNG TA, TH-8503) according to the ASTM D 882-02 method.

## Characterization

### a) Thickness of the films biodegradable plastics

The thickness of the films was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 ( $\pm 5\%$ ) at five random locations on the film. Mean thickness values for each sample were calculated and used in water vapor permeability (WVP) and tensile strength (TS) calculations.

### b) Scanning Electron Microscopy (SEM)

Scanning elektron microscopy film surface morphology was examined using scanning electron microscopy. The samples were mounted on stub with double-sided adhesive tape and coated with a thin layer of gold (JEOL JFC-1600 auto fine coater). Images were taken using a JEOL JSM-6510-LA Japan with an accelerating voltage of 0.5 to 30 kV. TEM images were recorded with a JEOL model transmission electron microscope, operating at 200 kV, with a point-topoint resolution of 0.3 nm.

## Results and Discussion

### Effect of plasticizer on Tensile Strength (TS) and Elongation at Break (E%)

The effect of the addition of glycerol (1.00; 1.50; 2.00; 2.50; and 3.00 ml of cassava starch film solutions) to cassava starch films to test the mechanical properties was studied. The mechanical properties of films plasticized by glycerol was assessed by measuring their tensile strength (TS) and elongation at break (E%) for five plasticizer concentrations.

**Table 2.** Effect of glycerol and PLA concentration on tensile strength and elongation

No	Glycerol (mL)	Tensile Strength (Mpa)					Elongation (%)				
		Polylacted Acid, PLA (mL)					Polylacted Acid, PLA (mL)				
		1.0	2.0	3.0	4.0	5.0	1.0	2.0	3.0	4.0	5.0
1	1.0	3.33	3.82	2.94	3.43	4.31	35.52	16.12	55.00	33.88	16.40
2	1.5	3.65	4.80	4.61	4.71	5.00	43.60	103.44	74.04	80.76	93.72
3	2.0	4.31	4.84	4.12	5.00	5.10	51.56	101.40	50.76	48.32	84.96
4	2.5	3.33	4.61	3.55	4.70	4.61	29.56	49.00	36.56	27.44	31.00
5	3.0	3.43	4.41	3.14	4.21	4.70	83.28	41.24	40.52	50.08	39.40

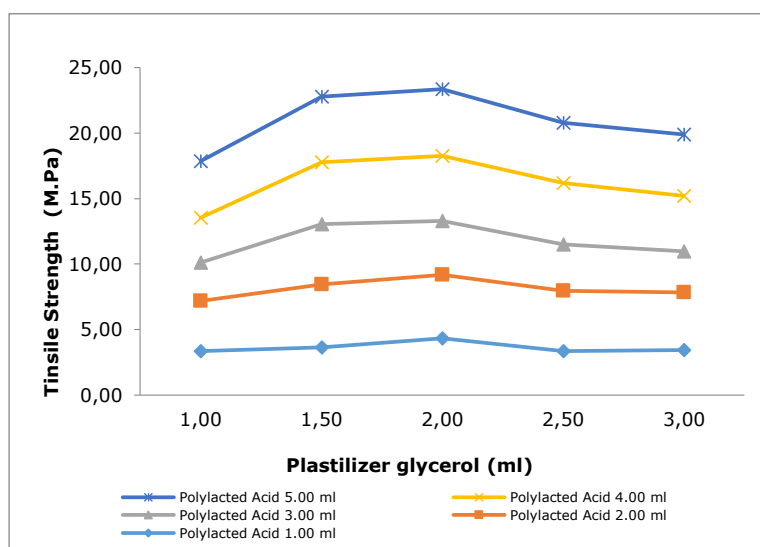
Elongation at break is the extendibility of film length from initial length to the point of break or can be defined elongation at break (E%) as the ability of films to deform before finally breaking. Elongation helps to determine the flexibility and stretchability of films. In general, the film appearance was transparent. The addition of glycerol from 1.0 to 2.0 mL improved the flexibility of the cassava starch films (Figure 1). However, films which had more than 2.0 mL of added glycerol were hard to peel since the film was so sticky it would tend to and stick to itself.





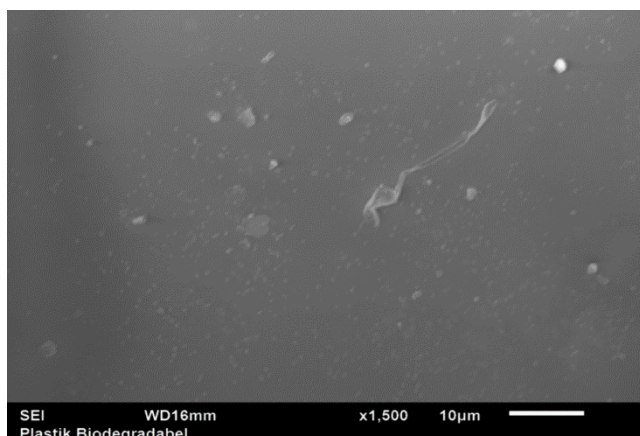
**Figure 1.** Cassava starch film at maksimum and minimum condision

Figure 2 show the changes in tensile strength (TS), elongation at break (E%) as well as Young's modulus (E) of cassava starch films with different amounts of concentration of glycerol and polyacted acid. The results showed that an increase in the concentration of plasticizers yielded a increasee in TS and elongation (E). Glycerol is added in the composition of the plastic compiler will insert into the structure of the starch and then the hydrogen bonds in the starch is reduced in the presence of glycerol. Plasticizer has the ability to reduce internal hydrogen bonding intermolecular bonds. The addition of the plasticizer lowers intermolecular force, increase the flexibility of the film and the film barrier properties.



**Figure 2.** Effect of plastilizer on tensile strength

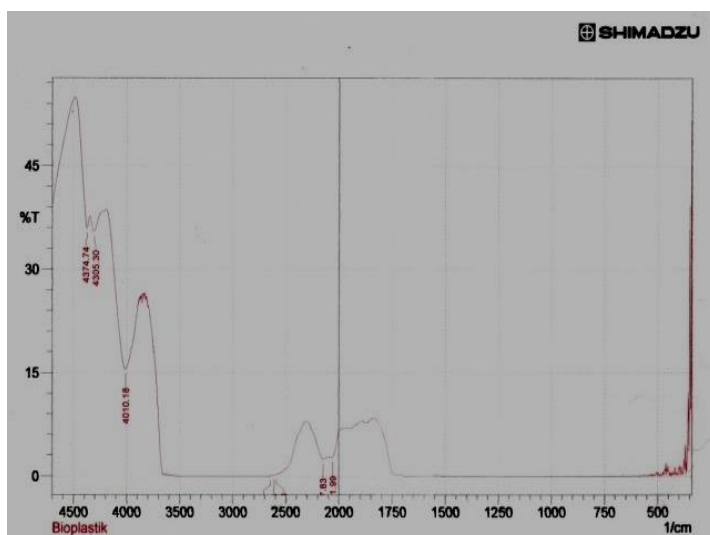
The changes in mechanical properties of characterized by the plasticizers weakening the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant. Thus the increase in the plasticizer concentration causes a reduction of the TS due to the decrease in the intermolecular interactions. Besides, the increase in the plasticizer concentration increases the moisture content of the film because of its high hygroscopic character, which also contributes to the reduction of the forces.



**Figure 3.** Scanning electron microscopy cassava starch film

### Film Plastic Surface Morphology

Morphology, hygroscopicity and mechanical characteristics of the film plastic produced is closely related to the concentration of glycerol and APL that were added in synthesizing of the film plastic produced. Effect of glycerol concentration and APL were added to the on morphology films can be seen in Figure 3. Visually one of the results of scanning electron microscopy film plastic produced for optimum conduction (plastilizer glycerol 2 mL and APL 5 mL) is not porous and its looks smoother, no cracks or air bubbles and In general, the film appearance was transparent (Figure 1). By using SEM (figure 3), the morphology of the resulting film packaging there are a number of starches that this clumping occurs because starch insoluble depleted due to the effects of complaining and temperature conditions are not uniform when the plastic film is made.



**Figure 4.** Spectrum FTIR cassava starch film

According to the spectral data in Figure 4, the spectrum of the plastic film produced is almost similar to the constituent components of starch, constituent components of starch. There is no happen change and there only is the process of blending physics. There are the group C = O and CO ester carbonyl is indicated that film plastic is environmentally friendly.

### Conclusions

- Cassava starch based films plasticized with glycerol showed interesting mechanical properties being transparent, clear, homogeneous, flexible, and easily handled.
- The results establish that films plastic based on cassava starch mixture with polylacted acid and plasticized with glycerol can be considered as an interesting biodegradable alternative packaging material.

- Plastic film made from cassava starch by using glycerol and the addition of plasticizers polilaktat acid (PLA) has mechanical properties such as transparent white, clean, homogeneous, easily bent and easily handled.
- The optimum value of tensile strength occurs upon the addition of plasticizers glycerol 2 mL and 5 mL polilaktat acid additions is to 5.10 MPa.
- Plastic film hydrophilic or not resistant to water and the resulting functional groups together with the constituent components of starch.
- In the synthesis of plastic film that is happening is the process of blending physics and the group C = O and CO ester carbonyl make environmentally friendly plastic.
- further research needs to be conducted to see the effect of temperature variation on mechanical properties of bioplastics.

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## Utilization of Crude Extract Papain from Papaya Latex as A Coagulant in The Tofu Production

<sup>1\*</sup>Faridah, <sup>1</sup>Fachraniah, <sup>1</sup>Ariefin, <sup>1</sup>Ayu Ardhia Rizqi, <sup>1</sup>Cut Meutia Sari

Chemical Engineering Department, State of Polytechnic Lhokseumawe, Km 280 Buketrata, Lhokseumawe, 24301, Indonesia

\*Corresponding Author: [ida4699@yahoo.com](mailto:ida4699@yahoo.com)

### Abstract

Papain was made from the papaya latex fruit. It was called crude papain has been used in the process of making tofu. Tofu is made from soy beans. Soy beans first made into soy milk. After that, tofu was produced by using soy milk with the addition of crude papain from papaya latex. The purpose of this research is to utilize crude papain from papaya latex as a coagulant in the tofu production. The process of making tofu is done by varying the concentration of the crude papain and operating temperature. From the research results in the optimum conditions produce tofu occur at the optimum temperature and pH of the enzyme papain. The concentration of 5% crude papain was a good tofu production. Whereas the pH at 5.8 and the temperature 60 °C. And then, tofu was determined the organoleptic of tofu by panelists.

**Keywords:** Tofu, crude papain, concentration, temperature

### Introduction

Tofu is a food product derived protein from soy protein coagulation process is carried out by using coagulant protein. During this time the use of coagulant in the process of making tofu-based chemicals such as GDL, acetic acid (vinegar), calcium sulfate, iekulohsira (the remaining solution of making salt from sea water) and CaCl<sub>2</sub>. The use of coagulant in the process of making out will affect the level of protein in tofu, resulting in out different characteristics, such as tofu sour taste (Lampert, 1970), slightly bitter (Sri, 2011), and produces very crumbly texture.

In addition to using chemicals, tofu can also be made by using enzymes. Enzymes are proteins that function as catalysts for chemical reactions in biological systems. Enzymes have high catalytic power. Enzymes can increase reaction speed of up to one million times faster than without the enzyme reaction. Enzyme molecules also have a certain degree of specificity to the substrate of the reaction (Stryer, 1988). Today, utilizing enzymes increasingly used in producing the food product. One enzyme that is widely used is papain. Papain is a protease (proteolytic) that can break down proteins. Papain is able to agglomerate protein, resistant to temperature, and in low concentrations can function well (Nurhidayati, 2003). Papain has some characteristic like papain free of chemicals, can make your own, non-toxic, and no side reactions (Winarno, 1987). Papain can be found in papaya latex fruit.

Papain can be extracted from papaya latex (Nitsawang, S. et. al., 2006). Crude papaya latex called papain and used for the first time in 1911 for beer chill-proofing, representing the first industrial application of enzymes. Nowadays, Enzyme papain has been applied in food industry such as making wine (Nitsawang et al., 2006; Benucciet al., 2011). In addition, crude papain can be used as a coagulant in making tofu from soy milk (Metyet al., 2013) and manufacture of cottage cheese (Egrina and Titin, 2010). Crude papain derived from the papaya latex fruit has been applied in the manufacture of tofu from soy milk. Expected from this research can provide information about the use of the enzyme papain from papaya latex as coagulant in the manufacture of tofu from soybeans and getting the optimum concentration and the optimum temperature in the tofu production with the addition of crude papain as a coagulant.

### Materials and Methods

#### Production Crude Papain

Crude papain was obtained from fresh papaya latex with directly picked from trees. Three or four vertical incisions were made in the fruits with a sharp to a depth of 2 to 3 mm (Stryer, L., 1988). Papaya latex mixed with a solution of 0.1% v/v (NaHSO<sub>3</sub>: NaCl, 1: 1). Papaya latex solution mixture stirring until homogen and then add 100 ml phosphate buffer pH 7 (Stryer, L., 1988). The solution is left for 1 hour

at 4 °C. After that the solution was centrifugated at 1500 rpm for 20 minutes and separated supernatant and sediment. The supernatant is as crude papain and stored at 4 °C.

### **Making the Soy Milk**

Soy beans soaked with water (Soy beans:Water, 1: 5) overnight. Soy beans soaked weighed 500 gram sand add water as much as 5 liters (Soy beans:water, 1: 3). Then crushed and filtered. The supernatant is as soy milk.

### **Manufacture of Tofu**

Research using soy milk as a protein source, soy milk is heated by varying the operating temperature is 60°C, 70 °C, 80 °C and 90 °C with a heating process 25 minutes and added to the enzyme papain concentrations varied 5%, 10%, 15%, 20 % and 25% v/v. Soy milk is stirred and then printed and analyzed.

### **D. Determination of Water Content**

The method used in the determination of water content is a method using the mid standards by using the equipment Moisture content determination MX-50. This analyzer equipment based on thermo gravimetric analysis of drying the sample using a halogen lamp. A total of 5 grams of tofu heated to a temperature of 160 ° C for ± 8.5 minutes and the resulting moisture content in percent.

### **E. Determination of Chemical Composition of Tofu**

Chemical analysis carried out on tofu to determine its composition. The parameters tested such as pH and dissolved protein content. Tofu pH was measured by using a pH meter while soluble protein content was measured using the *Lowry* method.

## **Results and Discussion**

### **A. Tofu production**

Tofu is a traditional food for the people of Indonesia as a high-protein food source. Due to tofu contains amino acids essential. Tofu is produced from soybeans. In this study, soybeans before it are used as a raw material for making tofu. Soybeans first converted into soy milk. Soy milk is used as raw material in tofu production. Tofu was obtained from soy milk with adding coagulant. Coagulant will change the protein in soy milk into peptides and amino acids. Tofu produced from soy milk is used papain as coagulant. Papain which is a crude extract or so-called crude papain derived from papaya latex fruit. Papaya latex fruit extracted from papaya fruit, then extracted with a solution of sulfite ( $\text{NaHSO}_3$ : NaCl, 1: 1) and a buffer solution. From the extraction of this solution became crude papain. Crude papain is used as an ingredient in the process of making tofu as coagulant. The research looked at the effect of the addition of crude papain as coagulant material by varying the concentration of crude papain is 5%, 10%, 15%, 20% and 25% v/v. In addition, tofu will also be reviewed when the operating temperature of the addition of crude papain.

**Table1.** Effect of Temperature and Concentration of crude papain as coagulant in tofu production

Temperature (°C)	Concentration of crude papain (%)	Weight (g)	pH
60	5	118.6	5.8
	10	87.24	5.8
	15	82.02	5.81
	20	74.18	5.81
	25	75.78	5.82
70	5	113.61	6
	10	81.56	6.12
	15	87.87	5.97
	20	73.86	6.05
	25	76.12	6.08
80	5	65	5.07
	10	52.4	5.16
	15	60	5.3
	20	75	5.05

	25	29	5.06
90	5	53	5.1
	10	45	4.99
	15	48.22	5.08
	20	45.17	5.08
	25	35.87	5.06

Tofu is the result of precipitation of soy protein that cannot be dissolved back. If the pH of soymilk made at the isoelectric pH of the protein will have precipitation then clots. It can be seen that the protein clotting occurs at pH above pH isoelectric tofu (Table 1). This is because pH resulting from tofu is the isoelectric pH of the pH of the enzyme papain. Crude papain as papain enzyme has a pH optimum. The optimum pH of the enzyme is pH upon enzyme activity reached the highest value. From the results of previous studies Papain as crude papain has a pH optimum between pH 4-6. Papain activity reached the highest value at pH 6. At the optimum pH, enzymes are the ionization level is most appropriate for binding to the substrate. Conformation of the enzyme in the form of a very stable, so that the effectiveness of the binding of the enzyme-substrate high (Stryer, 1988). This shows that the crude papain can use as a coagulant material in the production of tofu. Tofu was obtained a good results at pH 5.8- 6 and less produced tofu at pH below 5.8. Changes in pH around pH optimum load causes changes in amino acid residues, especially those that make up the active center of the enzyme, and also cargo residues constituent amino acid substrates [casein]. This causes a decline in the effectiveness of the binding of the enzyme-substrate, so that the change in pH around pH 6 causes a decrease in enzyme activity. So that upon the formation of clotting protein tofu diminishing. This is due to the pH below optimum pH decreased activity of the enzyme papain.

In addition to pH tofu, operating temperature also affects the performance of crude papain protein to build up in the process of making tofu (Table 1). A higher operating temperature of the heavy eating tofu produced diminishing returns. This is because the papain active works at the optimum temperature at temperature 50-60°C. The warming above the optimum temperature of the enzyme papain will reduce the activity of the enzyme papain as a coagulant. So that tofu produced above the optimum temperature of the papain generates diminishing weight. Heating above the optimum temperature of the enzyme causing a conformational change in the enzyme that leads to any changes destructive. Covalent bonds which maintain secondary and tertiary structures of enzymes break up, resulting in damage to the enzyme molecule (Stryer, 1988). This has resulted in damage to the decline in the activity of the enzyme, so that the weight of tofu produced diminishing returns. Concentration also affects the weight of the resulting tofu (Table 1). A higher concentration of crude papain is used the less severe the resulting tofu. The concentration of crude papain affect the tofu produced. This study was conducted using 300 mL of soy milk with the addition of enzymes with concentrations varying from 5% by mass to 25% v/v with the addition of the enzyme papain carried out at a temperature varying from 60 °C-90 °C.

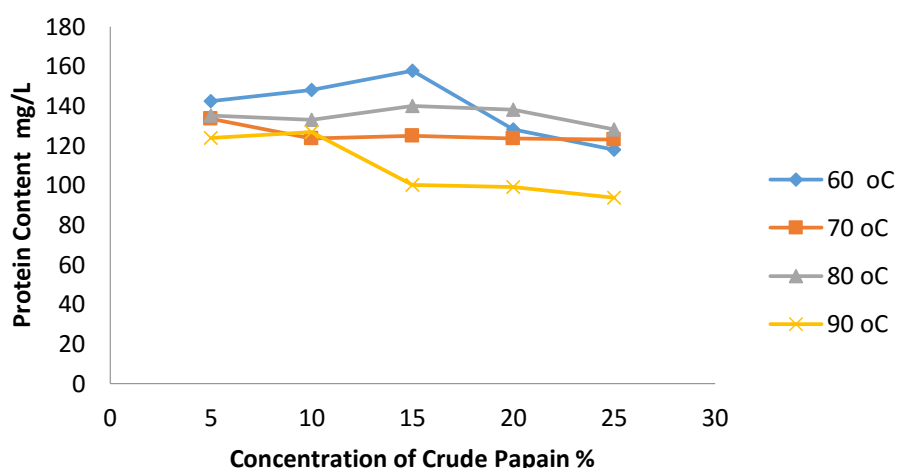
It can be seen that the addition of the enzyme with small concentrations produce the quantity/weight of larger products. The number of products due to the results obtained in plenty of water are difficult to separate from the solid product due to the coagulation process is less than perfect. This is caused occurrence of protein denaturation. The resulting tofu had a nice texture (mushy). On the addition of enzyme and optimal operating temperature at the optimum temperature rang enzymes and pH optimum enzyme produces greater weight than others. The good clotting protein at concentration crude papain 5% v/v, at a concentration of 5% addition of enzyme activity in the coagulation process is good enough, this happens when the amount of enzyme sufficient for such reactions and parameters for enzyme activity sufficient. If the amount is less than the enzyme activity of the enzyme is less for the clotting reaction, otherwise if too much enzyme is added, the possibility of media available is not adequate to the needs of existing enzyme activity, then the tofu produced is also reduced, because at least occur coagulation. Besides the addition of enzymes that too much will lead to flavor are less preferred by consumers. It seemed to be a bit bitter due to the influence of crude papain derived from papaya latex fruit. If the concentration increases, the enzymatic reaction rate will go up to a certain point and finally the constant (Winarno, 1980). From studies with variable concentrations of the enzyme can be concluded that the optimal enzyme concentration in the manufacturing process is 5% and the temperature operation of crude papain at 60 °C and pH 5.8.

#### **B. Protein concentration of dissolved**

The effect of adding the enzyme papain concentration and the operating temperature at the time of adding the enzyme to the levels of soluble protein produced. Papain enzyme concentration and

temperature of the addition of the enzyme affects the levels of soluble protein in tofu products produced. Where the enzyme papain concentration the higher the value the more soluble protein is reduced, it is influenced by the operating temperature at the time of adding the enzyme papain. Where the higher the temperature the lower the levels of soluble protein found in tofu. Soluble proteins were analyzed by using the method of Lowry.

In Figure 1, it can be seen that the crude papain is added at a temperature below and above the optimal temperature, the crude papain its enzyme activity in the protein clotting reaction is not optimal. Research to determine the optimal temperature of the enzyme is done by making the addition of tofu using soy milk 300 ml with the addition of the crude papain and varying temperatures. Results of the analysis showed that the addition of the enzyme papain temperature effect on the levels of soluble protein in tofu produced. Where the value of the dissolved protein is the best at a temperature of 60 °C with the addition of the crude papain concentration of 15% v/v, the higher the concentration will result in the action of the enzyme papain is not supported by the maximum operating temperature. At higher temperatures increased the number of products and the content of soluble protein ketch getting smaller. This is because temperature affects the activity of the enzyme, where the higher the temperature the higher the reaction rate, but the higher the temperature, the faster the enzyme damage (denature enzyme). From these results it can be concluded that the best results can be obtained with the addition of the enzyme papain 15% v/v enzyme papain with the addition of enzyme operating temperature of 60 °C.

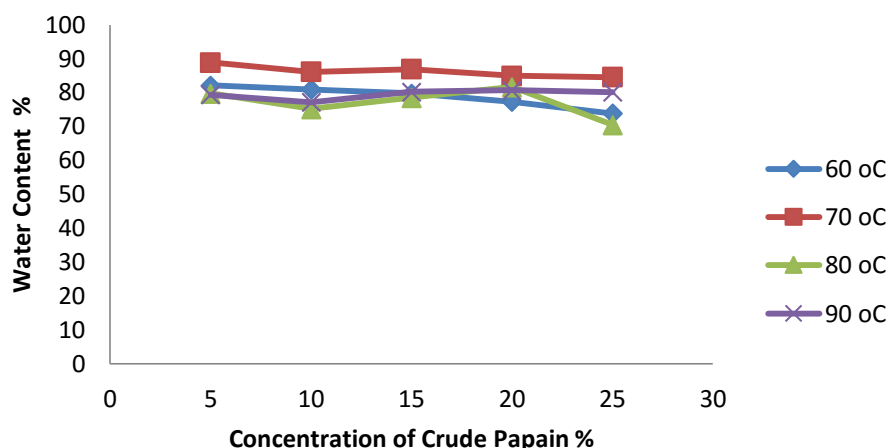


**Figure 1.** Effect of temperature and concentration of enzyme papain in tofuproduction towards solubleprotein

### C. Analysis of Water Content

The water content in the production of tofu influence on the resistance tofu was determined. From the results of research conducted that the water content contained in the lowest operating temperature of 60 °C with crude papain concentration of 25% v/v. It can be seen from Figure 2 that the water content above the optimum temperature of the enzyme papain generates high water content. This causes the resulting know is not durable. Due to the high water content was found. The less water content contained in the tofu, the more resistant and durable the product, due to the high water content causing microorganisms.





**Figure. 2** Effect of concentration of enzyme papain and temperature to water content in tofu production.

### Conclusions

Papain enzyme derived from the papaya fruit sap can be used as a coagulant in the manufacture of tofu. Concentration of 5% and operating temperature of 60 °C is a good condition for the enzyme papain in the process of making tofu. The water content of tofu to show that tofu can be long lasting with little water content. From the organoleptic test can be concluded that with the addition of enzyme papain and operating temperatures are affecting not organoleptic test criteria. Where tofu produced the panelists liked although not significant.

### Acknowledgment

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## Synthesis of $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub> Core/Shell Nanocomposite and Catalytic Oxidation of Phenolic Contaminants in Aqueous Solutions

<sup>1,2\*</sup>Edy Saputra, <sup>2</sup>Jhon Armedi Pinem, <sup>3</sup>Syaiful Bahri, <sup>1</sup>Shaobin Wang

<sup>1</sup>Department of Chemical Engineering, <sup>1</sup>Department of Chemical Engineering, Curtin University, GPO Box U1987, Perth, WA 6845, Australia.

<sup>2</sup>Department of Chemical Engineering, Riau University, Pekanbaru 28293, Indonesia

\*Corresponding Author: edysaputra@unri.ac.id.

### Abstract

$\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub>core/shell catalyst was prepared by hydrothermal and test for adsorption and catalytic advanced oxidation of phenol in aqueous solution using oxone®. Their properties were characterized by several techniques such as X-ray diffraction (XRD). The catalyst is highly effective in heterogeneous activation of oxone® to produce sulfate radicals for phenol degradation. The catalytic activity depends on catalyst loading, oxone® concentration, phenol concentration.  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub>core/shell can achieve 100% phenol degradation efficiency in 50 min at the condition of 25 ppm phenol, 0.4 g/L catalyst, 2 g/L oxone®, and 25 °C.

**Key words:** Catalytic, core/shell, nanocomposite, water treatment.

### Introduction

Worldwide attention to the problem of clean water is increasing day by day. This is because of the need for clean water increased along with the increase in population and economy, as a result demands for fresh water also continuously growing (Jackson, R.B., *et al.*, 2001). The main concern in this case is not due to depletion of fresh water, but the damage done by industrial and human activities. Thus, water is the most pressing challenges such as health, agriculture, energy and urbanization [2]. Water sources are available today are not as abundant as it seems, because only a very limited amount of available water resources can be used without any treatment. Only a small portion of the total amount of fresh water is about 0.26% which can be used for human purposes, located in lakes, reservoirs and rivers [3]. Based on the use of fresh water, agriculture is the biggest user at around 70% of the total fresh water, followed by industry 25% and 5% domestic [4]. In most cases, the main contaminants originating from agriculture is the increased use of pesticides (insecticides and herbicides) [5], while the industry as a by-product of their activities [6]. Since, only a small amount of water available for human needs, therefore it is important to preserve water resources for future generations by way of advance process in an appropriate manner before being discharged into water bodies. In order to manage environmental pollution, many countries have designed environmental regulations containing guidelines regarding the maximum threshold allowed for a variety of toxic organics that can be disposed of in the environment, for instance 0.5 mg/L of phenol in water stream for Australia wastewaters [7, 8]. However, even with the strict regulations of the water resources are still a major problem. Therefore, it is important to develop a waste treatment technology that can destroy pollutants into non-toxic compound or biodegradable end products. Recently, many investigations have reported that sulphate radicals are superior to hydroxyl radicals in oxidation for water treatment. In most previous investigations, Co<sup>2+</sup>/peroxymonosulphate (oxone®, HSO<sub>5</sub><sup>-</sup>) has been found to be an effective route for sulphate radical generation [9]. However, a major issue in using Co metal ions is the toxicity and secondary pollution. Therefore, alternative catalysts with less toxicity and environmental benign for peroxymonosulfate activation are highly required.

### Experimental section

#### Material synthesis and Characterization

A  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub> was obtained with two-step preparation. First, MnCO<sub>3</sub> was synthesized by a hydrothermal method reported by Li *et al.* [10]. Typically, potassium permanganate (3 mmol) and an equal amount of glucose were put into 60 mL distilled water at room temperature, and then 0.1 M tartaric acid was added to the above mixture and continually stirred to form a homogeneous solution, which was then transferred into a 125 mL Teflon-lined autoclave. The autoclave was sealed and maintained at 150 °C for 10 h, and then cooled down to room temperature naturally. The resulted solid product (MnCO<sub>3</sub>) was filtered, washed with distilled and dried in air at 100 °C overnight. Later on,  $\alpha$ -

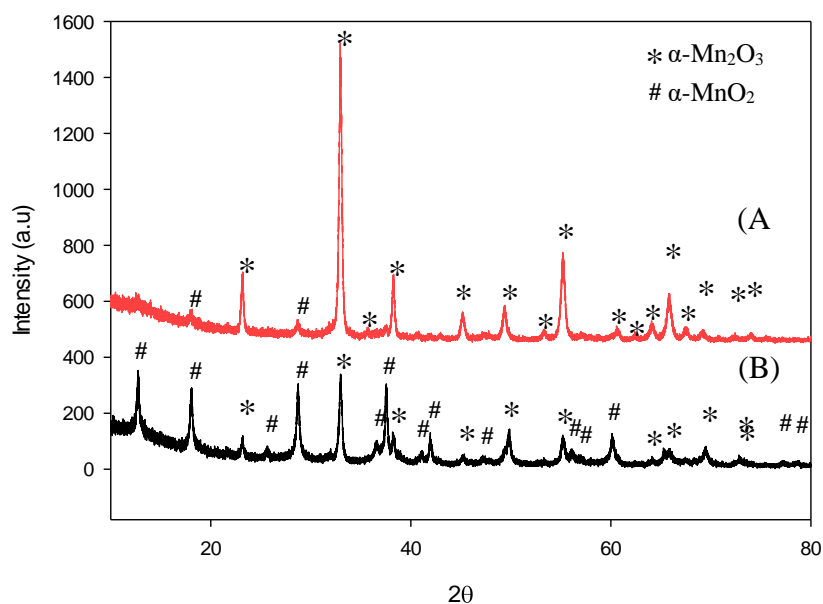
$\text{Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$  was prepared using a modified method from Cao et al. [11]. The as-synthesized  $\text{MnCO}_3$  (0.1 g) were put into distilled water (50 mL) at room temperature, and then  $\text{KMnO}_4$  (0.05 g) was added to the mixture and sealed. The mixture was stirred vigorously for 15 min and then allowed to stand for 17 h at room temperature. The resulted solid product ( $\text{MnCO}_3@ \text{MnO}_x$ ) was filtered, washed with distilled and dried in air at 100 °C overnight. After that, the product was calcined at 400 °C in air for 2 h to form  $\alpha\text{-Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$ . Catalysts were characterized by XRD, XRD patterns were obtained on a Bruker D8 (Bruker-AXS, Karlsruhe, Germany) diffractometer using filtered Cu K $\alpha$  radiation source ( $\lambda = 1.54178$  Å), with accelerating voltage 40 kV, current 30 mA and scanned at  $2\theta$  from 10 to 80°.

### Kinetic study of phenol degradation

Phenol degradation tests were carried out in a 1-L glass beaker with 500 mL containing 25 mg/L of phenolic solutions. The reaction mixture was stirred constantly at 400 rpm. Firstly, a fixed amount of peroxymonosulfate (oxone Sigma-Aldrich) was added into the solution for a while, then a catalyst was added into the solution to start the oxidation reaction of phenol. At certain time, 0.5 mL of water sample was withdrawn from the mixture using a syringe filter of 0.45  $\mu\text{m}$  and then mixed with 0.5 mL of pure methanol to quench the reaction. The concentration of phenol was analysed using a Varian HPLC with a UV detector at  $\lambda = 270$  nm. The column used was C-18 with mobile phase of 30 %  $\text{CH}_3\text{CN}$  and 70% ultrapure water. For comparison, a homogeneous oxidation with manganese ions and oxone was also conducted by addition  $\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  of the chemicals into the phenol solution simultaneously. The  $\text{Mn}^{2+}$  concentration was kept as the same as the catalyst loaded in solution.

### Results and Discussion

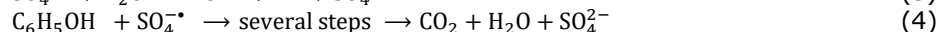
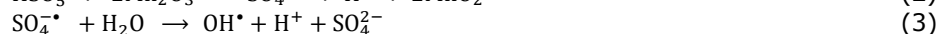
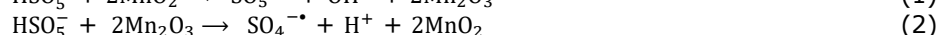
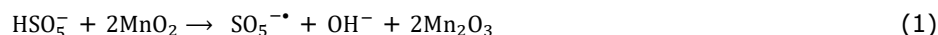
**Figure 1** shows XRD pattern of  $\alpha\text{-Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$ , including thermal treatment of  $\text{MnCO}_3$  precursor at 400 °C in air for 2 h. The two samples present different crystalline peaks. In **Figure 1A**, the diffraction peaks occurred at 23.13°, 32.95°, 35.68°, 38.23°, 45.17°, 49.34°, 53.27°, 55.18°, 64.07°, and 65.08°, confirming the formation of  $\alpha\text{-Mn}_2\text{O}_3$  (JCPDS No. 41-1442). A small amount of  $\alpha\text{-MnO}_2$  could also be detected. The diffraction peaks in **Figure 1B** occurred at 12.78°, 18.10°, 25.71°, 28.84°, 36.69°, 37.52°, 41.25°, 41.96°, 49.86°, and 60.27°, confirms the formation of  $\alpha\text{-MnO}_2$  (JCPDS No. 44-0141). **Figure 1B** also shows the diffraction peaks of  $\alpha\text{-Mn}_2\text{O}_3$ , suggesting the presence of mixed two phases of  $\alpha\text{-MnO}_2$  and  $\alpha\text{-Mn}_2\text{O}_3$ .



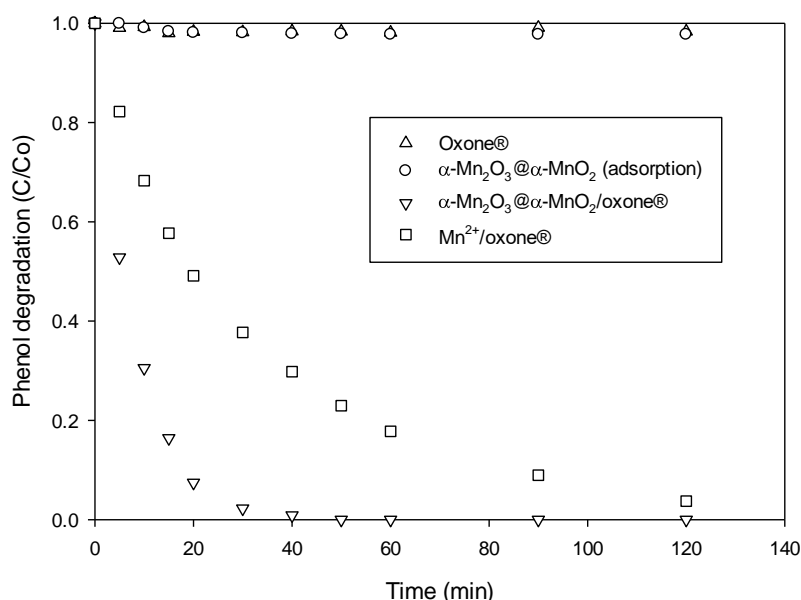
**Figure 1.** XRD patterns of manganese oxide catalysts. (A)  $\alpha\text{-Mn}_2\text{O}_3$  (direct calcination of  $\text{MnCO}_3$  precursor), (B)  $\alpha\text{-Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$ .

**Figure 2** shows the preliminary tests of adsorption and catalytic oxidation of phenol using  $\alpha\text{-Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$  core/shell catalyst. The  $\alpha\text{-Mn}_2\text{O}_3@ \alpha\text{-MnO}_2$  showed little adsorption of phenol at less than 5% removal efficiency. From the figure, it is also seen that phenol removal would not change significantly

in the presence of oxone® without a catalyst, indicating that oxone® itself could not produce sulfate radicals to induce phenol oxidation. Phenol degradation would occur when catalyst and oxidant were simultaneously present in the solution.  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub> core/shell catalyst could degrade phenol at 100% in 30 min in the presence of oxone®. The high degradation rate was due to the activation of oxone® by  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub> core/shell catalyst to produce sulfate radicals. Compared with the efficiency of homogeneous oxidation, Mn<sup>2+</sup>/oxone® system, the heterogeneous system exhibited much higher activity. The homogeneous system could result in removal of phenol at 97% in 120 min. In addition, TOC measurements indicated that TOC removal in  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub>/oxone® system was about 78% within 60 min. The reaction mechanism for heterogeneous phenol oxidation can be proposed as below.



Several investigations have been reported for heterogeneous core/shell catalysts for organic decomposition using different oxidants. Xing et al. [12] studied Fe<sub>3</sub>O<sub>4</sub>/FeMnO<sub>x</sub> core/shell nanoparticles for methylene blue discoloration with H<sub>2</sub>O<sub>2</sub>. The Fe<sub>3</sub>O<sub>4</sub>/FeMnO<sub>x</sub> core/shell nanoparticles could achieve 90% colour removal at dye (methylene blue) concentration of 25 mg/L within 240 min. Park et al. [13] used silica-Mn core-shell structured nano-composites with H<sub>2</sub>O<sub>2</sub> for methylene blue degradation at 5 mg/L. The silica-Mn core-shell composite could achieved 82% methylene blue degradation within 120 min. Xing et al. [14] studied the magnetic core-shell nano-composite (Fe<sub>3</sub>O<sub>4</sub>@MnO<sub>x</sub>) for heterogeneous activation of H<sub>2</sub>O<sub>2</sub> to generate hydroxyl radicals (OH•), targeting the decomposition of 2,4-dichlorophenoxyacetic acid. They found that Fe<sub>3</sub>O<sub>4</sub>@MnO<sub>x</sub> could activate H<sub>2</sub>O<sub>2</sub> to achieve 86% removal at 2,4-dichlorophenoxyacetic acid concentration of 20 mg/L in 150 min. Thus, it appears that  $\alpha$ -MnO<sub>2</sub>@Mn<sub>2</sub>O<sub>3</sub> core/shell catalyst presented higher activity in the degradation of phenol in the presence of oxone®.



**Figure 2.** Phenol degradation with time in adsorption and catalytic oxidation. Reaction conditions: = 25 ppm, catalyst = 0.4 g/L, oxone = 2 g/L, and T = 25 °C.

## Conclusions

A  $\alpha$ -Mn<sub>2</sub>O<sub>3</sub>@ $\alpha$ -MnO<sub>2</sub> core/shell composite was successfully synthesized by a shape-controlled method. The particle exhibits high activity in heterogeneous activation of oxone® for oxidation of phenol in water.

## Acknowledgements

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## Application Of Water Hyacinth (*Eichhornia Crassipes*) For Treatment Of Wastewater From Chicken Farm

<sup>1</sup>\*Suhendrayatna, <sup>1</sup>Marwan, <sup>1</sup>Putri Nurwahyuni, <sup>1</sup>Ria Susanti, <sup>2</sup>Elvitriana

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Environmental Engineering, Faculty of Engineering, University of Serambi Mekkah, Batoh, Banda Aceh 23246, Indonesia;

\*Corresponding Author: suhendrayatna@unsyiah.ac.id.

### Abstract

This study occurred to find out the level of effectiveness *Eichhornia crassipes* plants to reduce organic content of the wastewater from chicken farm. Research carried out by phytoremediation method with different variations of the residence time (HRT) 2 days, 4 days and 6 days, respectively with each variation of HRT and effluent was taken and analyzed for 5 times by a predetermined time. Results showed that *Eichhornia crassipes* grew well in chicken farm wastewater and the application of water hyacinth *Eichhornia crassipes* on a chicken farm wastewater could reduce 93.98% COD and 71.43% BOD, respectively at HRT of 6 days. These results reached to the conclusion that water hyacinth plant, *Eichhornia crassipes* has a potential for organics content reduction in wastewater from chicken farm by phytoremediation application.

**Keywords:** *Eichhornia Crassipes*, Chicken farm wastewater, phytoremediation.

### Introduction

*Eichhornia Crassipes* is an aquatic plant that grows well in tropical and subtropical. Based on its ability growing on contaminated environmental conditions and its ability absorbing pollutants, this plant is widely used as a medium of phytoremediation for wastewater treatment. IBW Group (1998) reported that *Eichhornia crassipes* has good assimilation ability to degrade COD, BOD, ammonia, nitrogen, and phosphate in the root tissue. Maine *et al.* (2007) describes *Eichhornia crassipes* able to absorb heavy metals (Cr, Ni, and Zn) from waste tooling factory. Lagos *et al.* (2009) also reported that *Eichhornia crassipes* has the ability to reduce dye in pulp mill effluent. Furthermore, Suhendrayatna *et al.* (2008) reported that *Eichhornia crassipes* able to reduce 78% of ammonia from water phase.

Biosorption of pollutants by aquatic plants such as *Eichhornia Crassipes* can occur through three stages, namely metal biosorption by roots, translocation of contaminants from roots to other parts of plant, and the localization of these substances on the part of certain cells to maintain not inhibit the metabolism of plants (Wendy *et al.*, 2005). The absorption of pollutants by plants begins with bringing the substance into the media around the roots (rhizosphere) in several ways depending on the species of plant. Substance then taken into the root cells, and transported through xylem and phloem transport network to the other plants. The transport efficiency increased when contaminants are bound by the chelate molecule. To prevent poisoning of cells, plants detoxify pollutants by accumulating it in certain parts, such as roots and latex. Biosorption of pollution elements contained in waste water followed by the degradation of carbon, nitrogen, and complex chemicals, and also accompanied by the decreasing of organic content in media as an indication as an indication that there has been a reduction in pollutant content.

Research involving aquatic plants such as *Eichhornia Crassipes* in an effort to reduce contaminants in water and waste media has always been an interest current subject (Fodor *et al.*, 2003; Erdei *et al.*, 2005, Suhendrayatna *et al.*, 2008). This subject is in line with the development of research towards finding new methods for reducing pollutants in the environment. Results of this study describes the ability of *Eichhornia Crassipes* to reduce the organic content from chicken farm wastewater.

## Materials and Methods

### Preparation of water hyacinth

*Eichornia crassipes* collected from the area in Banda Aceh City with 15 – 20 cm length was cultivated in pond for a couple months to reach acclimatization phase. The study was conducted at an outdoor laboratory as a quality control step to control the factors of temperature, water supply, diseases, and pest.

### Experiment

*Eichornia crassipes* was exposed to wastewater from chicken farm for six days under controlled condition ponds. Each ponds contained ten stems of *Eichornia crassipes* and conducted in series continue process. Tap water also prepared as control media. The study started by flowing wastewater in the ponds planted with water hyacinth plants, *Eichornia crassipes* continuously at HRT 2, 4, and 6 days. During six days of the experiment, water temperature ( $29 \pm 4$  °C) and atmospheric air were maintained and the growth of *Eichornia crassipes* was observed by measuring the growth of plant in cm scale in every day for six days. Water phase from outlet port were analyzed by standard methods to determine the reduction of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), pH, turbidity, and Total Suspended Solids (TSS) under standard procedure.

## Results and Discussion

### Characteristic of chicken farm wastewater

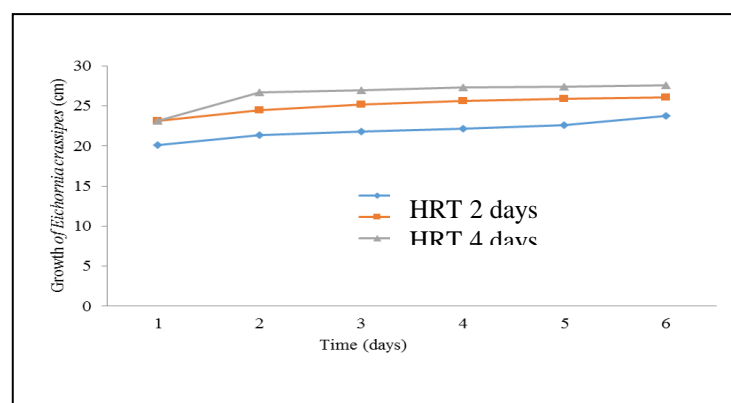
Wastewater used in this study was obtained from the chicken farm in Blang Bintang, Aceh Besar sub district with the characteristics as tabulated in Table 1.

**Table 1.** Characteristic of chicken farm wastewater

No	Parameter	Value
1	Chemical Oxygen Demand (COD)	765 mg/L
2	Biological Oxygen Demand (BOD)	16,8 mg/L
3	Total Suspended Solid (TSS)	278 mg/L
4	pH	7,8
5	Turbidity	463,7 NTU
6	Temperature	26,4 °C

### Growth of *Eichornia crassipes* in chicken farm wastewater

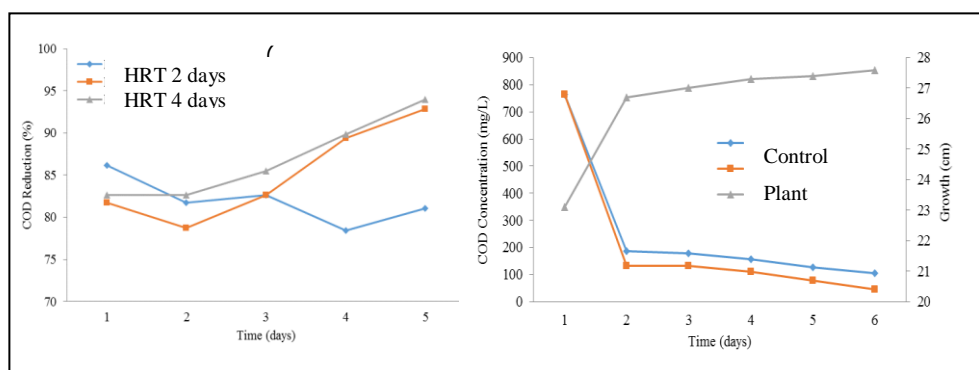
After *Eichornia crassipes* were exposed to the wastewater, the growths of water hyacinth were observed by measuring the increase of shoots at specified interval times and the results are illustrated in Figure 1. Figure 1 shows that plants grew well proving that chicken farm wastewater did not inhibit the growth of water hyacinth plants. However, at HRT 6 days a change in physical plant, the tip of plant leaf withering.



**Figure 1.** Effect of chicken farm wastewater on the Growth of *Eichornia crassipes* (COD: 765 mg/L; BOD: 16.8 mg/L; TSS: 278 mg/L; and T:  $29 \pm 4$  °C)

### COD Reduction in chicken farm wastewater

Figure 2 (A) shows the increasing of BOD reduction in chicken farm wastewater by water hyacinth, *Eichornia crassipes* during for phytoremediation process. Initial COD concentration on wastewater was 765 mg/L and during treatment, COD concentration in each treatment of HRT 2 days, 4 days, and 6 days were 145 mg/L, 55 mg/L, and 46 mg/L, respectively with COD removal efficiency 81.04%; 92.81%, and 93.9%, respectively. COD removal efficiency conducted best for HRT 6 days and reached 93%.

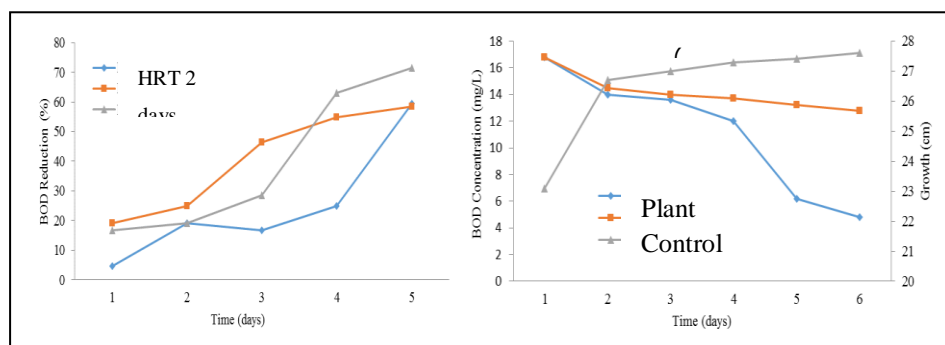


**Figure 2.** COD reduction in chicken farm wastewater by water hyacinth, *Eichornia crassipes* (A). COD removal efficiency (B). COD reduction (COD: 765 mg/L; BOD: 16.8 mg/L; TSS: 278 mg/L; HRT: 6 days; T: 29±4 °C)

Figure 2 (B) shows that decreasing of COD concentration by water hyacinth on HRT 6 days was 133 mg/L, 133 mg/L, 111 mg/L, 78 mg/L, and 46 mg/L, respectively while in control was 186 mg/L, 178 mg/L, 157 mg/L, 126 mg/L and 106 mg/L. This result lead a conclusion that COD concentration decreased due to the presence of water hyacinth in water phase and other activity of microorganisms. Similar results were also reported by other researcher (Prabu and Udayasoorian, 2007) showed that reduction of COD concentration of pulp mill wastewater by aquatic plants *Typha latifolia* and *Cyperus pangorei* were 62.55% and 49%, respectively. Suhendrayatna e al. (2011) also reported that aquatic plants, *Typha latifolia* and *Saccharum spontaneum* were able to reduce COD to 67.17% and 60.74%, respectively with HRT 24 hours for 9 days. Akhirruliawati and Amal (2010) also reported that longer operating time can lead to a large reduction of COD concentration and greater concentrations of *Degra simba* to treat waste causing greater reduction in concentrations of COD.

### BOD Reduction in chicken farm wastewater

Figure 3 (A) shows the increasing of BOD reduction in chicken farm wastewater by water hyacinth, *Eichornia crassipes* during for phytoremediation process. Initial BOD concentration on wastewater was 16.8 mg/L and during treatment, BOD concentration in each treatment of HRT 2 days, 4 days, and 6 days were 6.8 mg/L, 7.0 mg/L and 4.8 mg/L, respectively with BOD removal efficiency 59.52 %, 58.33 %, and 71.43 %, respectively. BOD removal efficiency conducted best for HRT 6 days and reached 71.3%.



**Figure 3.** COD reduction in chicken farm wastewater by water hyacinth, *Eichornia crassipes* (A). COD removal efficiency (B). COD reduction (COD: 765 mg/L; BOD: 16.8 mg/L; TSS: 278 mg/L; HRT: 6 days; T: 29±4 °C)



Figure 3 (B) shows that decreasing of BOD concentration by water hyacinth on HRT 6 days was 14.0 mg/L, 13.6 mg/L, 12.0 mg/L, 6.2 mg/L, and 4.8 mg/L, respectively while in control was 14.5 mg/L, 14.0 mg/L, 13.7 mg/L, 13.2 mg/L, and 12.8 mg/L. These results suggest that a decrease in BOD concentration was not only influenced by water hyacinth plant, but also involved activity of microorganisms that can degrade organic compounds in the process of phytoremediation. Zimmels, et al (2006) suggested that longer treatment time causes more organic compounds degraded and BOD concentration in water phase will be increased. The application of water hyacinth, *Eichornia crassipes* and *Pistia stratiotes* in domestic wastewater treatment in Israel could be reduce BOD at the operating time of 8 days at 19 mg/L and at operating time of 14 days, BOD value decreased to 3.5 mg/L. Suhendrayatna e al. also reported that aquatic plants, *Typha latifolia* and *Saccharum spontaneum* were able to reduce BOD to 56.72%, and 37.31%, respectively with HRT 24 hours for 9 days.

## Conclusions

This study has been described the ability of *Eichhornia Crassipe* to reduce the organic content from chicken farm wastewater and leads the following conclusions.

1. After *Eichornia crassipes* were exposed to the chicken farm wastewater, the growths of water hyacinth grew well and proving that wastewater did not inhibit the growth of water hyacinth plants;
2. The application of water hyacinth *Eichornia crassipes* on a chicken farm wastewater could reduce 93.98% COD and 71.43% BOD, respectively at HRT of 6 days;
3. These results reached to the conclusion that water hyacinth plant, *Eichornia crassipes* has a potential for organics content reduction in wastewater from chicken farm by phytoremediation application.

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## Identification of Mineral of Jades from Nagan Raya Aceh, Indonesia by using XRD and SEM-EDX Techniques

<sup>1</sup>\*Julinawati, <sup>1</sup>Surya Lubis, <sup>1</sup>Irfan Mustafa,

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Science, University of Syiah Kuala, Darussalam, Banda Aceh, 23111, Indonesia

\*Corresponding Author: juli\_fuadi@yahoo.com.

### Abstract

XRD and SEM-EDX techniques can be used to identify of mineral in a variety of stones including the jades. The samples of the jades was obtained from Nagan Raya Aceh. Based on data of XRD and SEM-EDX, they showed that Jades Nagan raya Aceh belonged to a mineral of silicate. The types of minerals in the Jade of Nagan Raya respectively is grossular ( $\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$  from the Garnet group, which is a mineral of nesosilicate, the other type mineral is a calcium magnesium ferro silicate,  $(\text{Ca}_{10}(\text{Mg},\text{Fe})_2\text{Al}_4(\text{SiO}_4)_5(\text{Si}_2\text{O}_7)_2(\text{OH},\text{F})_4)$ , from the vesuvianite group, which is a mineral of sorosilicate and the actinolite,  $(\text{Ca}_2(\text{Mg},\text{Fe})_5(\text{Si}_8\text{O}_{22})(\text{OH})_2)$ , from the amphibole group which is a mineral of inosilicate. XRD and SEM-EDX are the analysis techniques that can identify the types of mineral and the results obtained more quickly and accurately.

**Key words:** XRD, SEM-EDX, Jades, Mineral, Nagan Raya, Aceh.

### Introduction

Nagan Raya is one of area in western of Aceh which is about 300 Km west of Banda Aceh. Geologically, Nagan Raya has traces of highly complex tectonics. It is characterized by the discovery of various types of rock in the area Beutong, Nagan Raya (Djarot, S, 2014). One of the rock types of the most popular of the area is Jades.

Jade is one of the precious stones with the trade name given to a type of ornamental stone, which generally have the physical properties of the color variations of green, ranging from light green to dark green. These rocks are arranged by the type of mineral or a combination of various minerals and crystals formed by one or a combination of several crystals. In Asia, the traditional term used for all kinds of jade carving that is currently divided into different minerals such as jadeite, nephrite, omphacite, serpentine, naming it depends on the content of the main mineral, (Krzemnicki, M.S., 2008).

Determining the quality of precious stones is mostly based on its physical properties. The physical properties is often used is the crystalline form, luster, color, streak, hardness, cleavage, fracture and the specific gravity (Wahyudi, T., 2014). Determination in this way cannot be explained in more detail on the content of mineral types. While in gemology, the chemical composition is necessary to determine the quality of the precious stones, (Herman, D. Z., 2009).

Classification of mineral of Jade Nagan Raya of Aceh has been done and only based on the classification of color, hardness and the other physical properties of the rocks (Aflah, N., et al., 2004). Determination in this way does not describe the mineral content of rocks more detail, therefore, this study will identify the mineral Jades of Nagan Raya Aceh using the techniques of XRD and SEM-EDX.

Methods of X-ray diffraction (XRD) is one potential method to identify chemicals in some fields of science. The identification results will be obtained in the form of the diffraction pattern and this pattern is generated based on the distance between atoms and intermolecular of the material being examined. In addition, XRD is a method that has selectivity and high efficiency (Srivastava, A, 2012; Li, W., et al, 2011).

SEM-EDX is a technique that can be used for quantitative and qualitative analysis of sample elements. SEM-EDX technique based on the analysis of the spectral characteristics of X-ray radiation emitted from the sample atoms for electron beam irradiation (Martinez, M., 2010). Identification using SEM-EDX can provide more accurate results. In addition, this method is simpler and has a shorter analysis time (Srivastava, A., et al., 2006). Currently, SEM-EDX technique has been used for the identification and interpretation systems in mineral and rock porosity distribution.

## **Materials and Methods**

### **Procedure**

The sample used in this study are Jades from Beutong Nagan Raya of Aceh. The tools used are X-Ray Diffraction (XRD) PANanalytical Empyrean and SEM Type JOEL JSM-6701F integrated with test Energy Dispersive X-ray Spectroscopy (EDX).

### **Techniques of XRD**

XRD is operated using a voltage source Cu at 40 KV and 40 mA as a radiation source. The use of scanning angle starts from 10° to 100°. Samples were crushed it first in mortal pestle or using a grinding mill, then the sample is inserted into the sample holder. Samples were subjected to an X-ray emitted from the voltage source. The result obtained in accordance with the diffractogram of typical crystal lattice of each compound.

### **Techniques of SEM-EDX**

Samples of Jades crushed and pulverized prior to use a mortar or grinding mill, then placed in a sample that has been attached to the carbon tape. Furthermore cleared the rest of the sample which was not attached and where the samples are inserted into the SEM sample holder. The results will be obtained in the form of SEM images samples at the surface and form a graph or chart on the EDX shows the percentage of the elements of the sample being analyzed.

## **Results and Discussion**

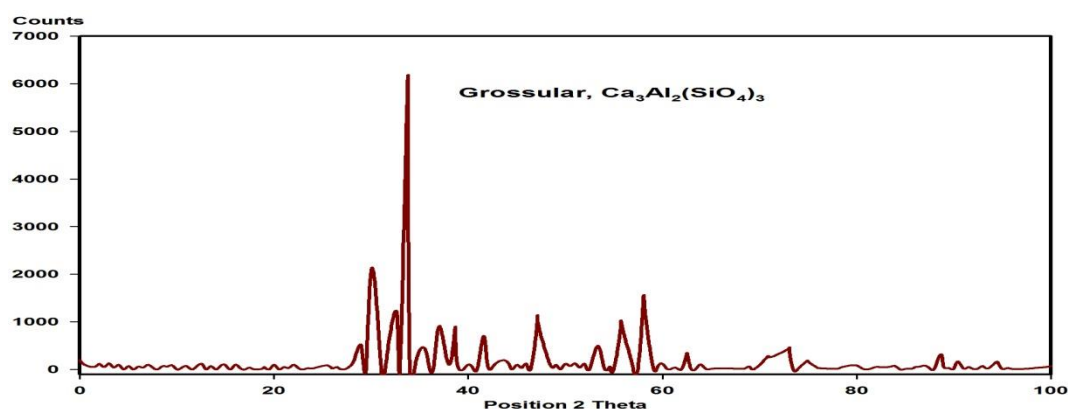
Minerals are inorganic materials that occur naturally and have a regular atomic arrangement with a specific chemical composition and provide specific physical properties. In nature, there are more than 2000 types of minerals have been known, but only a few minerals are found as rock-forming minerals. To identify the type of minerals in the Jade Nagan Raya of Aceh, this study used a techniques of XRD and SEM-EDX.

XRD technique provides results in the form of diffraction data and the quantization of the intensity of diffraction of the corners of a material. The data provided on this technique is X-ray diffraction intensity of the diffracted and angles  $2\theta$ . Each pattern appearing in an XRD pattern represents a crystal plane that has a particular orientation, so that is very typical pattern for each mineral (Widyawati, 2012).

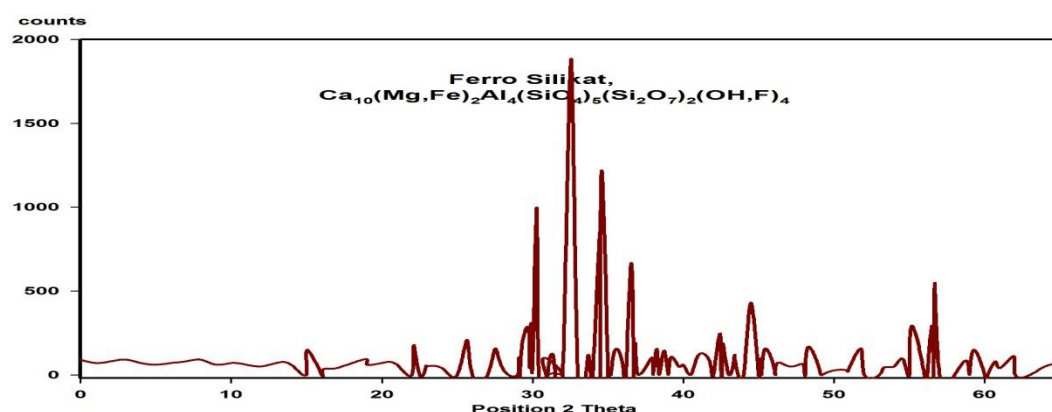
SEM-EDX is a technique that can be used to get information about the types of minerals in the sample, such as the content of elements and oxides of the rock. Each sample will be analyzed using the area. Electron beam generated from area gun transferred to the sample. The flow is then focused electron beam using an optical electron Columb before the electron beam on the sample. After the electron beam on the sample, there will be some interaction in samples irradiated. Interactions that occur will then be detected and converted into an image by the SEM analysis and in graph form by EDX analysis (Yurugi, T., et al., 2001, Prasad, Y., 2011). This tool is commonly used for a variety of applications including the interpretation of the existence of mineral and rock porosity distribution system so that it can be used to distinguish mineral content in different types of rocks from various regions in Indonesia (Taufiq, A., 2008).

The results obtained from the analysis of the Jade of Nagan Raya of Aceh using a Scanning Electron Microscope - Energy Dispersive X-ray (SEM-EDX) is data on the percentage of elements and oxides of the rock. Differences in the content and the percentage of elements and oxides depending on the geological processes that occur in these rocks, which is influenced by the process of dissolution of different minerals and hydrothermal alteration during the its displacement of the earth to break through surfaced that result from tectonic events or volcanic (Herman, DZ, 2008).

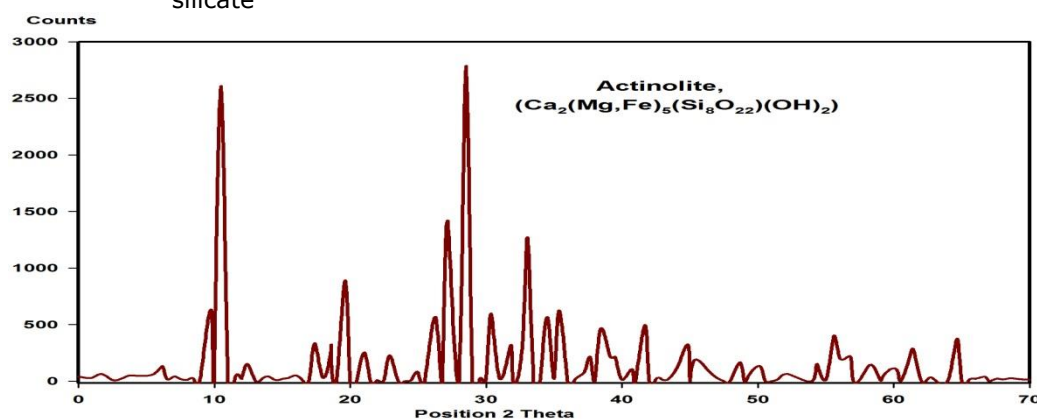
Based on the XRD data shown in Figure 1 and Table 1, shown that the first sample of jade containing mineral of grossular ( $\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$ ) of the garnet group which is a mineral of nesosilicate. Chemical elements of Fe and Al in a small amount in the Jades is as impurity (Srivastava, A., Vinod KJ, 2012), these results be proved from the results of SEM in Figure 4, Table 2 and Table 3. Grossular have hexoctahedral isometric crystal structure and has a hardness 6.5-7 scale of Mohs. Additionally, grossular have various colors from yellow to green (Nassau, 1992) and it is an early form of the mineral of omphacite. Omphacite has the chemical formula  $(\text{Ca},\text{Na})(\text{Mg},\text{Fe}^{2+},\text{Fe}^{3+},\text{Al})\text{Si}_2\text{O}_6$  and is known as one of type of jade (Krzemnicki, M.S., 2008).



**Figure 1.** Diffractogram of the first sample of jade containing minerals of grossular



**Figure 2.** Diffractogram of the second sample of Jade containing minerals of calcium magnesium ferro silicate



**Figure 3.** Diffractograms of the third sample of Jade containing minerals of actinolite.

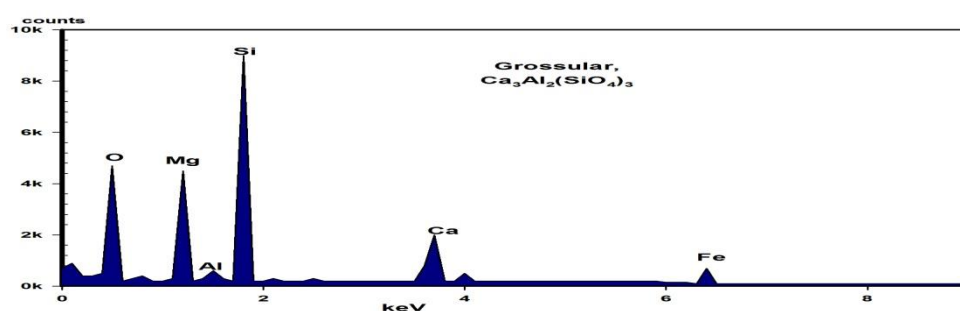
The second sample of the jade containing of mineral calcium magnesium ferro silicate,  $(\text{Ca}_{10}(\text{Mg,Fe})_2\text{Al}_4(\text{SiO}_4)_5(\text{Si}_2\text{O}_7)_2(\text{OH,F})_4)$ , are shown in Figure 2 and Table 1. Mineral of calcium magnesium ferro silicate including vesuvianite group, which is a mineral of sorosilicate. Chemical composition of mineral vesuvianite similar to grossular, is evident also from the XRD results in Figure 1 and Figure 2 and the results of SEM are shown in Figure 4 and Figure 5. This similarity is caused mineral of vesuvianite formed from the metamorphosis of mineral of grossular (Ito, J. and Arem, JE, 1970). Mineral of vesuvianite which is known by idocrase formed from the magma with a high of chemical element of Mg. Vesuvianite formed as a tetragonal crystal with a hardness of 6-7 scale of Mohs and have variations in color from green, brown, yellow, or blue, depending on the element impurities.

Based on the XRD results in Figure 3, Table 1 and the results of SEM in Figure 6, Table 2 and Table 3, the third sample of Jade contains minerals of actinolite including minerals amphibole group which is a mineral inosilicate. Actinolite has a prism system or needle-shaped crystals of the formula  $(\text{Ca}_2(\text{Mg,Fe})_5(\text{Si}_8\text{O}_{22})(\text{OH})_2)$ . These minerals are rich in the chemicals element magnesium or ferrum

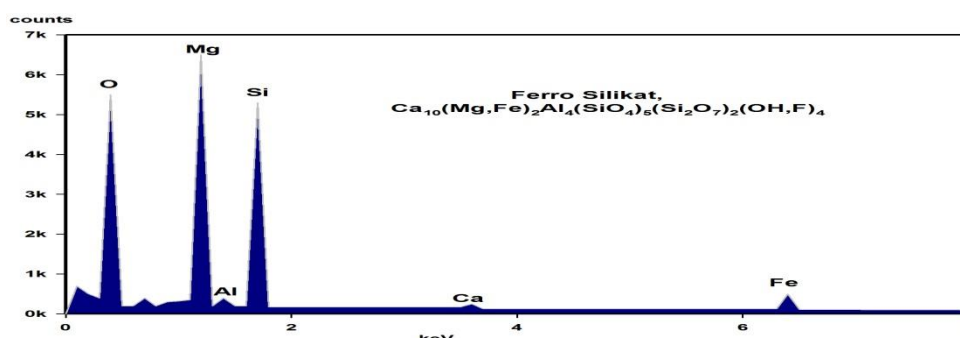
(iron) that affect the appearance of translucent to opaque. Actinolite color ranges from dark green to yellowish green with a hardness 5.5 to 6 Mohs scale. The varieties of actinolite is better known is nephrite jade.

**Table 1.** Angle 2 $\theta$  of Jade Nagan Raya of Aceh

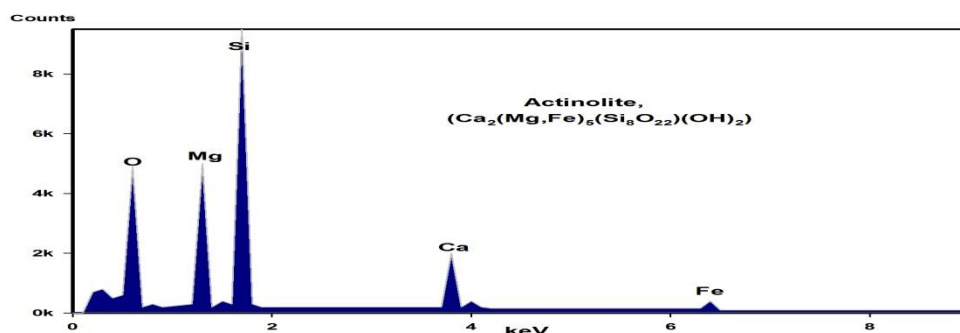
Samples	Angle 2 $\theta$	Type of mineral
Sample 1	30,1; 33,7; 33,8; 37,0; 38,6; 47,1; 47,2; 55,7; 55,8; 58,0; 58,5	Grossular, (Ca <sub>3</sub> Al <sub>2</sub> (SiO <sub>4</sub> ) <sub>3</sub> )
Sample 2	25,7; 29,8; 29,9; 30,3; 32,6; 34,5; 34,6; 36,6; 44,5; 55,6; 56,5; 56,7	Calcium magnesium ferro silicate, (Ca <sub>10</sub> (Mg,Fe) <sub>2</sub> Al <sub>4</sub> (SiO <sub>4</sub> ) <sub>5</sub> (Si <sub>2</sub> O <sub>7</sub> ) <sub>2</sub> (OH,F) <sub>4</sub> )
Sample 3	9,8; 10,5; 19,6; 26,3; 27,2; 28,5; 30,4; 33,0; 34,5; 35,4; 38,4; 41,7	Actinolite, (Ca <sub>2</sub> (Mg,Fe) <sub>5</sub> (Si <sub>8</sub> O <sub>22</sub> )(OH) <sub>2</sub> )



**Figure 4.** SEM-EDX results of the first sample of Jade containing mineral of grossular.



**Figure 5.** SEM-EDX results of the second sample of Jade containing mineral of calcium magnesium ferro silicate.



**Figure 6.** SEM-EDX result of the third sample of Jade containing minerals of actinolite.

**Table 2.** Percentage (%) of element of Jade stone Nagan Raya of Aceh from SEM-EDX result.

Samples	Percentage of element (% Wt)					
	O	Mg	Al	Si	Ca	Fe
Sample 1	40.56	14.56	0.59	32.51	8.32	3.10
Sample 2	42.63	27.03	0,47	26.31	0,62	4.04
Sample 3	35.55	12.15	-	24.42	6.97	2.43

**Table 3.** Percentage (%) of oxides of Jade Nagan Raya of Aceh from SEM-EDX result.

Samples	Percentage of oxides (% Wt)				
	MgO	Al <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>	CaO	Fe <sub>2</sub> O <sub>3</sub>
Sample 1	22.76	1.00	62.00	10.29	3.95
Sample 2	42.63	0.89	52.01	5,40	5.36
Sample 3	23.71	-	61.41	11.02	3.86

## Conclusions

Based on data of XRD and SEM-EDX, they showed that the Jades Nagan Raya of Aceh belonged to a mineral of silicate. The types of minerals in the Jade of Nagan Raya respectively is grossular ( $\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$  from the Garnet group, which is a mineral of nesosilicate, the other type mineral is a calcium magnesium ferro silicate,  $(\text{Ca}_{10}(\text{Mg},\text{Fe})_2\text{Al}_4(\text{SiO}_4)_5(\text{Si}_2\text{O}_7)_2(\text{OH},\text{F})_4)$ , from the vesuvianite group, which is a mineral of sorosilicate and the actinolite,  $(\text{Ca}_2(\text{Mg},\text{Fe})_5(\text{Si}_8\text{O}_{22})(\text{OH})_2)$ , from the amphibole group which is a mineral of inosilicate. XRD and SEM-EDX are the analysis techniques that can identify the types of mineral and the results obtained more quickly and accurately.

## Acknowledgements

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# **THEME : ARCHITECTURE, CIVIL AND MECHANICAL ENGINEERING**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
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## Development and Performance Test of Furrower Model Blade to Paddlewheel Aerator

<sup>1</sup>\*Samsul Bahri, <sup>2</sup>Radite Praeko Agus Setiawan, <sup>3</sup>Wawan Hermawan, <sup>4</sup>Muhammad Zairin Junior

<sup>1</sup>Mechanical Engineering Department, State Polytechnic of Lhokseumawe, Buketrata, Lhokseumawe 24301, Indonesia;

<sup>2</sup>Department of Mechanical and Biosystem Engineering, Bogor Agricultural University, Dramaga, Bogor 16680, Indonesia;

<sup>3</sup>Department of Mechanical and Biosystem Engineering, Bogor Agricultural University, Dramaga, Bogor 16680, Indonesia;

<sup>4</sup>Department of Aquaculture, Bogor Agricultural University, Dramaga, Bogor 16680, Indonesia;

\*Corresponding Author: soel\_73@yahoo.com

### Abstract

The successful of intensive aquaculture is strongly influenced by the ability of the farmers to overcome the deterioration of water quality. The problem is low dissolved oxygen through aeration process. The aerator device which widely used in pond farming is paddle wheel aerator because it is the best aerator in aeration mechanism and usable driven power. However, this aerator still has a low performance of aeration, so that the cost of aerator operational for aquaculture is still high. Up to now, the effort to improve the performance of aeration was done by two dimensional blade design. Obviously, it does not provide the optimum result due to the power requirements for aeration is directly proportional to the increase of aeration rate. The aim of this research is to develop three dimensional model furrower blades. The design parameters will be focused on the geometry of blade such as shape, horizontal angle of blade, vertical angle of blade and holes diameter. The operational condition was carried out at the submerged blade 6, 9, and 12 cm with a wheel speed 154 rpm. The performance test was performed to get the torque that occurs, the electric power consumption and the splash coverage volume, which produced as the similarity of oxygen transfer rate occurs. The geometry design of furrower mode blades was 1.6 cm diameter hole. The blade position vertical angle direction and horizontal angle was 45° and 30°. The experimental result showed that the torque occurs for the blade 6, 9, and 12 cm were 45.85 N·m, 47.13 N·m, and 48.28 N·m. The electrical power consumptions were 552.19 Watt, 567.54 Watt, and 581.42 Watt. The splash coverage volume were 3.754 m<sup>3</sup>, 4.322 m<sup>3</sup>, and 4.095 m<sup>3</sup>. It was found that the optimum performance paddlewheel of furrower model blades operated on the submerged blade is 9 cm. In this condition, it showed that the lowest electrical power consumption and the largest splash coverage volume achieved.

**Keywords:** paddlewheel aerator, furrower model blade, design parameters, electric power consumption, splash coverage volume.

### Introduction

Aeration is a mechanism of adding some amount of oxygen into water to provide sufficient amount of oxygen. Aeration is carried out by increasing water and air contact using aerator device. One type of aerator device which widely used in pond farming is paddle wheel aerator (Laksitanonta 2003). Paddlewheel aerator is considered as the most appropriate aerator device due to aeration mechanism and wide usable driven power (Romaine & Merry 2007). According Lekang (2007) standardized aeration efficiency is directly proportional to the standard oxygen transfer rate and inversely proportional to the power consumption.

Aeration rate is influenced by water and air surface contact, differential oxygen concentration, film surface coefficient and turbulence (Boyd 1998). Geometry, size and wheel speed affect aeration performance (Moulick *et al.* 2002). Largest size tends to have higher aeration which coincide followed by higher driven power needs due to higher drag force. This condition causes certain problem in utilizing paddle wheel aerator as it causes increasing operational cost including electrical and fuel consumption. Electric power consumption is an important parameter in utilization paddlewheel aerator because effected on operating costs. In addition, to provide the value of the energy consumption needs in the factual, the measurement of electrical power is also a reference for the farmers in selecting the aerator (Kumar *et al.* 2013).

Up to now, the effort to improve the performance of aeration was done by two dimensional blade design. Obviously, it does not provide optimal result because of the power requirements for aeration is directly proportional to the increase of aeration rate. Therefore, the aim of this research is to develop model of

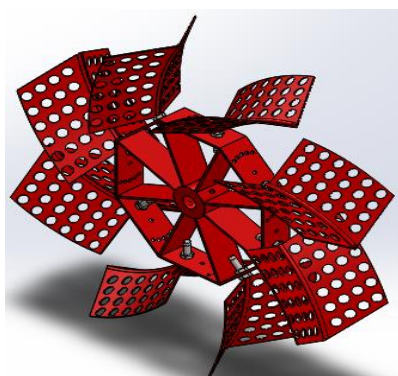
three dimensional furrower model blade. The design parameters will be focused on geometry of the blade; shape, horizontal angle of blade, vertical angle of blade and holes diameter.

## Materials and Methods

### Materials

#### Furrower model blades

Furrower model blades are based on the geometry of the arch blade wheel optimization results that have been done before (Bahri 2015), the hole diameter was 16 mm, 45° of vertical angle blade position and 30° of horizontal position as shown in Figure 1. The blade consist of eight pieces with the outer width was 174 mm, the length 176 mm and 300 mm for radius of curvature of the blade.



#### Paddlewheel test unit

Structural design paddlewheel consists of frame, reduction rotation transmission systems, paddlewheel blades and laying the instrument. The frame using the steel angle with the dimensions, shape and strength customized with support and load other components. Motor used is 1 phase AC electric motor with a power of 1 HP at 1440 rpm rotational speed.

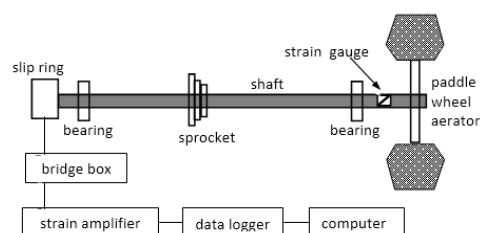
### Procedure

#### Treatments variations

The tests was conducted on the freshwater pool with dimensions of lenght 350 cm, 200 cm width with a water depth of 40 cm. Submerged variations blade is done by changing the height position paddlewheel support. The tests was conducted at a rotational speed of 154 rpm with the blade submerged 6, 9, and 12 cm.

#### Torque measurement

Torque measurement was done by using a strain gauge mounted on the wheel shaft. The sensor was connected to the strain amplifier (DAS-406B DC Strain Amp) through the slip ring and the bridge box were recorded by using data loggers (minilab 1008) and stored in the computer (Figure 2). Measurement data in the form of voltage (mVolt) was converted to strain ( $\mu\text{st}$ ) and torque measurement values ( $\text{N}\cdot\text{m}$ ) with calibration values that have been done before.



**Figure 2.** Torque measurement scheme

#### Power measurement

Paddlewheel power measurement was done by measuring the electrical power consumption of electric motors using Ammeter (DO2A) which was connected to an electrical outlet. Reading the power measurement value (Watt) was done by using a digital video camera recording on display Ammeter.

Rated power was taken on the average value that often was showed from the reading video playback recording for each treatment testing.

### Splash coverage Volume Measurement

Splash coverage volume was done by taking the recorded images used a digital camera from the front side and side the wheel at the time of testing. Then, the digital image was processed by using a CAD programs to create segments (grid), which the splash coverage volume was the number of multiply results of segments area at the front side water splash with the side of water splash.

### Results and Discussion

The water splash produced visible from the front and side the wheel of the submerged blade 12 cm as shown in Figure 3.

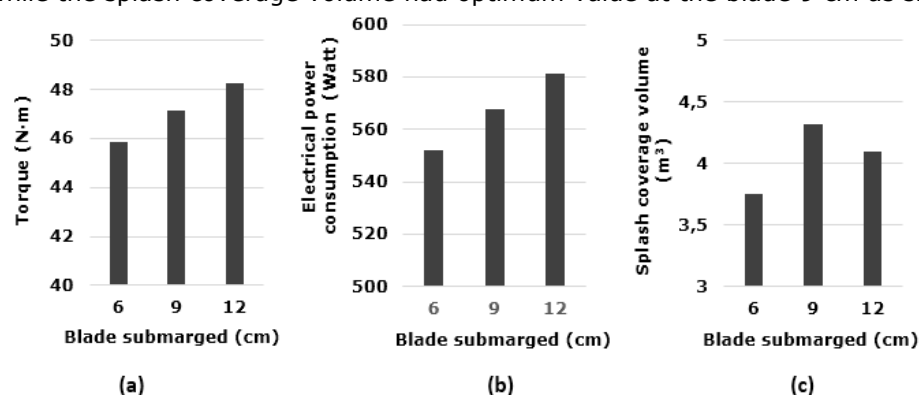


**Figure 3.** The image of water splash and calculation segment of splash coverage volume  
Torque occurred, electrical power consumption and splash coverage volume produced at different blade submerged shown in Table 1.

**Table 1.** Performance test results

Blade Submerged (cm)	Torque (N·m)	Power consumption (Watt)	Splash covered volume (m <sup>3</sup> )
6	45.85	552.19	3.754
9	47.13	567.54	4.322
12	48.28	581.42	4.095

The increasing of blade submerged resulted in greater torque and increasing the power consumption, while the splash coverage volume had optimum value at the blade 9 cm as shown in Figure 4.



**Figure 4.** The relation of submerged blade with torque (a), power consumption (b) and splash coverage volume (c).

The increase in torque due to changes blades submerged of 6 cm to 9 cm and 12 cm respectively 2.79 % and 5.30%, while the electrical power consumption were 2.78% and 5.29%. This is caused by the increase of water mass that driven by paddlewheel, thus increasing the drag force of the blade wheel as revealed by Kang (2004). While the difference between the increase in torque with the electric power is due to loss in the reduction system.

Splash coverage volume maximum occurred in submerged blade 9 cm was 4.322 m<sup>3</sup> (up 15.13%) with 567.54 Watt power consumption, whereas in 12 cm increased by 9.08%. Submerged blade 6 cm have little mass of water to be splashed, whereas in 12 cm of displaced water mass was too large, thus slowing rotation due to the great drag force of blade. The coverage volume is obtained approaching splash coverage volume of Taiwan paddlewheel models (two-wheel) 4.356 m<sup>3</sup> with 852 Watt power consumption (Bahri *et al.* 2015).

Based on the ratio of the electric power consumption, showed that furrower model blades has a good effect on reducing the drag force of the blades. This is as mentioned by Munson *et al.* (2006) that the drag coefficient is determined by the geometry and dimensions of an object. While the increase in volume is obtained by the spread of water splash due to furrower blades. Reducing of water collision momentum due to the tilt angle of the blade overcome by a rotational speed of paddlewheel was 154 rpm compared to Taiwan paddlewheel models was 120 rpm. This is as stated by Park *et al.* (2014) and Liu and Peymani (2015) that the power consumption and the flow rate increased directly proportional to the depth of submerged blades and increasing paddlewheel rotation.

Standardized aeration efficiency is directly proportional to the standard oxygen transfer rate and inversely proportional to the electrical power consumption (Lekang 2007). Based on the assuming which of splash coverage volume as standard oxygen transfer rate, so it's gained the splash coverage volume and power consumption of paddlewheel furrower model blade can improve standardized aeration efficiency.

## Conclusions

From the experiment, it can be concluded:

1. Paddlewheel of Furrower model blade consumed less electrical power but produced greater splash coverage volume.
2. Design of Furrower model blades was 1.6 cm diameter hole, 45° of vertical angle blade position and 30° of horizontal position.
3. The optimum performance paddlewheel of furrower model blades operated on the submerged blade 9 cm with 567.54 Watt of electrical power consumption and 4.322 m<sup>3</sup> of splash coverage volume.
4. The furrower model blades can improve the performance of paddlewheel.

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## The Priorities of Selection Suppliers

<sup>1</sup>\*Ikhsan Siregar, <sup>1</sup>Dewi Surya

<sup>1</sup>Department of Industrial Engineering, Faculty of Engineering, University of Sumatera Utara, Medan 20155, Indonesia;

\*Corresponding Author: [ikhsiregar@gmail.com](mailto:ikhsiregar@gmail.com), [ikhsan.siregar@usu.ac.id](mailto:ikhsan.siregar@usu.ac.id)

### Abstract

Procurement of raw materials is the main activity in ensuring the smooth production. Delivery delays by suppliers of raw materials affect the smooth production so the impact on the delivery of orders to customers. XYZ delayed delivery of raw materials foam and wire by each supplier with a frequency of 66% of the total delivery made. Therefore, supplier evaluation needs to be done so that the company can take appropriate decisions based on the priority suppliers. Integration of Fuzzy Analytical Network Process (FANP) and Complex Proportional Assessment with Grey Theory (COPRAS-G) is used in the evaluation of the supplier where the scale of influence among criteria and assessment of suppliers of the criteria obtained using a questionnaire distributed to five managers to gain weight - the weight criteria which serve as inputs for priority suppliers. Results priorities foam raw material suppliers HI, PF, MF, and DCI while the priority sequence obtained raw materials supplier wire are BUWI, AJ, and GGS.

**Keywords:** Supplier Evaluation, Criteria, Priority, Fuzzy ANP, COPRAS-G

### Introduction

Delay delivery raw materials by supplier who reached 66 % encourage company to do rush order so as to cause the emergence of the additional costs for companies are the cost of save, the cost of a message and opportunity cost (Lwiki,2013) .In addition schedule production will also is disturbed, the delivery cause delays in order to the consumer .That this will induce belief and customer satisfaction .Hence , evaluation supplier to do so as to be firm judge exactly .

Zhang et.al.(2015) maybe select a little bit supplier in solving similar problems in company considering five supplier. Excess of methods used is that uncertainty information can be dealt with good and this method able to process interrelation relations between criteria -- evaluation criteria supplier. Mobin (2015) uses the method COPRAS. For an election supplier in Iran company manufacturing. The research involving five people expert opinions to decide supplier who could potentially used as an alternative considering 4 criteria the supplier, the quality, flexibility, technical abilities. Profit model this is the way calculation simple and consideration criteria and non-beneficial beneficial. In addition COPRAS G can solve problems with the number of alternative many and consider maximum and minimum attribute through intervals.

Suppliers priority is expected to become the foundation firm on taking a decision in evaluate supplier with the integration fuzzy analytical network (FANP) and complex proportional assessment with grey theory (COPRAS-G).

### Methodology

Research object being observed is supplier foam and wire. Variable contained in this research are:

1. The independent variable, namely for variables affecting the dependent variable for both a positive or negative .An independent variable in this research is the criteria for selecting the supplier namely the quality of, the price of, the reliability of delivery, service, capacity, the flexibility and responsiveness, the human factor, technology and innovation.
2. Dependent variable, a variable whose values or values influenced or determined by other variables. Dependent variable to research this, namely the scale of priority supplier.

Data collection in this report is written with use of the instruments questioner consisting of questioner ANP about the relationship between the influence of the criteria and questioner COPRAS G regarding the provision of the value of alternative intervals for each criterion. Respondent's questionnaire is parties

that deal with obtaining raw materials that is quality manager, financial manager, logistic manager, production manager and purchasing.

The research phase consisting of two stages namely:

1. Preliminary research;
  - a. Introduction study to identify problems that there are companies, production process and information.
  - b. Literature study and the theory of supporters so as to get solutions to solving a problem.
  - c. the collection of primary data early research by interviewing and FGD's ( focus group discussion of manager logistics and purchasing based on a list criteria of the theory JINTUKAR to determine of understanding criteria sub criteria and relations network while secondary data in the form of suppliers and data delay delivery raw materials every supplier.
2. Advanced research
  - a. Data collection for advanced research with questionnaire matrix pairwise to have ranks of interest and questionnaire COPRAS to get set value alternative.
  - b. Data processing with the methods Fuzzy ANP and COPRAS-G.
  - c. conducted the results of the data processing
  - d. drawn conclusions and feedback to research conducted

## **Results and Discussion**

The results of criteria and sub criteria supplier the result of FGD's among others.

1. C1: Quality
  - K1: Conformity against desired specification
  - K2: The percentage of rejection goods entrance
  - K3: The reliability of safe guarding quality standards
  - K4: Certification quality products
2. C2: Price
  - H1: Product price
  - H2: Cost of Shipment
  - H3: Discount percentage
  - H4: Payment
3. C3: The reliability of delivery
  - KP1: The accuracy of the shipping period
  - KP2: Flexibility transformation the shipping period
  - KP3: long lead time
4. C4 : Services
  - P-1: There was a policy warranty and the claims
  - P-2: Period of completion claim or complaint
  - P-3: ease communication
  - P-4: quality improvement
  - P-5: technical support
5. Flexibility and responsiveness
  - FK-1: fulfillment of procedure in dealing with complaints
  - FK-2: speed in response to protest
  - FK-3: response to the complaint
  - FK-4: flexibility against customization
6. Profile supplier
  - PS-1: reputation supplier
  - PS-2: capacity and production facilities
  - PS-3: location supplier

The results of the relationship between criteria and sub criteria.

After criteria and sub criteria determined next done the determination of the relationship between criteria and sub criteria through the FGD results .The results of relations criteria and sub criteria be input on the software Superdecision that is shown on figure 1.

Figure 1 show the connection interrelation (feedback) between sub criteria with other cluster. Between the two will be assessed in matrix pairwise to be counted each weight.

The results of the weighting of sub criteria

Fuzzy ANP used to obtain weight each -- each criteria suppliers and counted by adopting Chang's extent analysis. The results of weight sub criteria can be seen in table 1.

**Table 1 The results of sub criteria.**

criteria	weight	criteria	weight
K-1	0,0958	P-2	0,0333
K-2	0,0443	P-3	0,0737
K-3	0,0701	P-4	0,0173
K-4	0,0394	P-5	0,0711
H-1	0,0812	FK-1	0,0504
H-2	0,0279	FK-2	0,0340
H-3	0,0361	FK-3	0,0129
H-4	0,0275	FK-4	0,0327
KP-1	0,0843	PS-1	0,0006
KP-2	0,0373	PS-2	0,0434
KP-3	0,0514	PS-3	0,0079
P-1	0,0275		

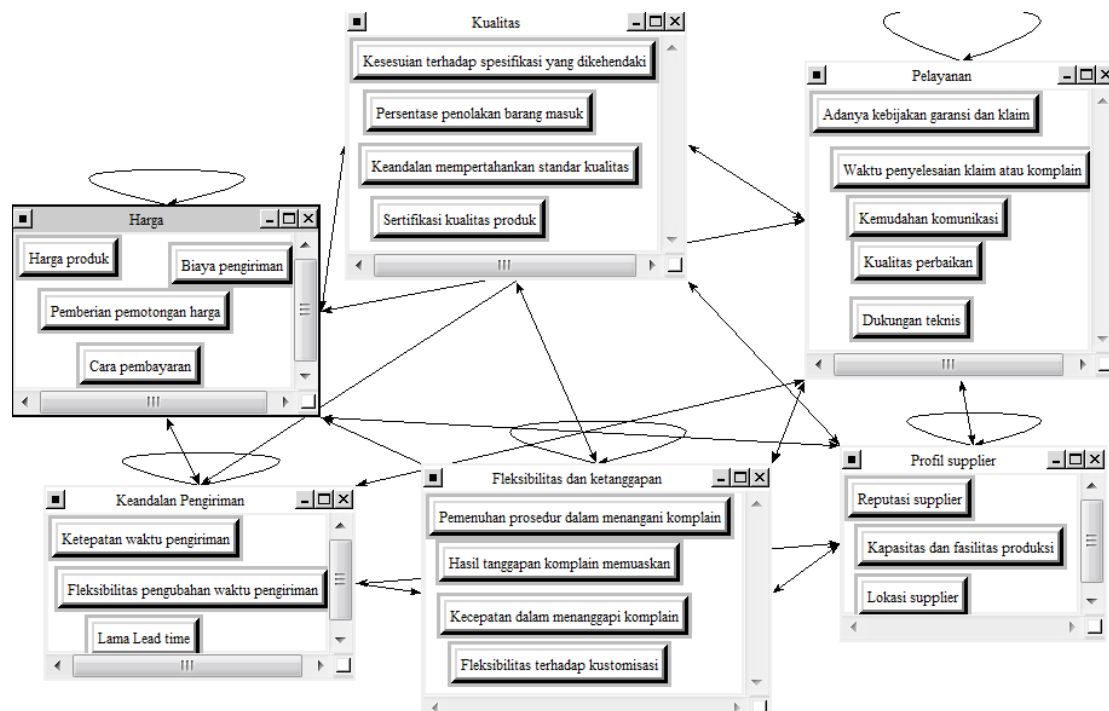


Figure 1. Network between criteria

Table 1 shows that conformity to specification considered to be very important because has weight highest while reputation having weight the lowest in evaluation supplier.

The results of priority suppliers

COPRAS will produce priority supplier foam based on degrees utility .Table 2 shows the calculation on weights and the order supplier foam and table 3. The calculation on weights and the order supplier wire.

**Table 2 .The results of COPRAS supplier foam**

Description	HI	PF	DCI	MF
Relative weight (Qi)	0,2681	0,2477	0,2417	0,2427
Degrees utility (Ni)	100,00%	92,38%	90,16%	90,53%

Table 2 show results degrees utility for the order supplier foam namely HI (100%), PF(92,38%), DCI(90,16%) and MF (90,53).

**Table 3.The results of COPRAS supplier wire**

Description	GGs	BUWI	AJS
Relative weight (Qi)	0,3303	0,3353	0,3345
Degrees utility (Ni)	98,49%	100,00%	99,74%

Table 3 show results degrees utility for the order supplier wire namely GGS (98,49%), BUWI ( 100 % ) and AJS ( 99,74 % ).

Based on priority suppliers foam, DCI and MF are placed two bottoms as the company it is necessary to consider replace DCI or mf with other supplier. The application of the results of the study can have an impact on the cost savings on the buy raw materials foam that can be seen in table 4.

**Table 4 cost savings**

The raw materials foam 1700 kg /month			
<b>Supplier(Alternative 1)</b>	<b>HI</b>	<b>PF</b>	<b>MF</b>
Order Quantity (kg)	567	567	567
Purchase price / kg	Rp 18.600	Rp 19.200	Rp 20.800
Seller price	Rp10.546.200	Rp 10.886.400	Rp 11.793.600
<b>Total</b>	<b>Rp 33.226.200</b>		
<b>Savings</b>	<b>Rp 33.405.000-Rp 33.226.200=<b>Rp 178.800</b></b>		
<b>Supplier( Alternative 2)</b>	<b>HI</b>	<b>PF</b>	<b>MF</b>
Order Quantity (kg)	567	567	567
Purchase price / kg	Rp 18.600	Rp 19.200	Rp 20.800
Seller price	Rp10.546.200	Rp 10.886.400	Rp 11.793.600
<b>Total</b>	<b>Rp 33.226.200</b>		
<b>Savings</b>	<b>Rp 33.405.000-Rp 33.226.200 = <b>Rp 178.800</b></b>		

Table 4 shows alternative 1 using three suppliers with supplier DCI not chosen produce thrift Rp. 178.800 alternative 2 with supplier MF persons not elected produce thrift Rp. 632.400. While reductions delay delivery raw materials can be seen in table 5.



**Table 5 The reduction of delays in raw materials**

<b>Supplier (Alternative 1)</b>	<b>HI</b>	<b>PF</b>	<b>MF</b>
Delay raw materials	2 day	3 day	4 day
The delays in raw materials	9 day		
<b>Supplier (Alternative 2)</b>	<b>HI</b>	<b>PF</b>	<b>DCI</b>
Delays Raw Material	2 day	3 day	5 day
The delays in raw materials	10 day		

Table 5 shows alternative 1 produce reduction delay delivery better than alternative 2 in which delay early as many as 14 days reduced to nine days.

## Conclusions

The criteria most influential in evaluation supplier is conformity to specification is intended , the accuracy of the shipping period , product price ease communication and technical support.

The results of priority suppliers raw materials foam is HI, PF,MF, DCI, while the ranking priority suppliers raw materials wire obtained BUWI, AJ, GGS.

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## **Vehicles Potholes Detection Based Blob Detection Method and Neural Network Backpropagation Model**

<sup>1\*</sup>Dewiani Djamaluddin, <sup>1</sup>Andani Achmad, <sup>2</sup>Rivanto Parung

<sup>1,2</sup>Department of Telecommunication and Information Engineering, Faculty of Engineering, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia;

<sup>2</sup>College Students of Telecommunication and Information Engineering, Faculty of Engineering, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia;

\*Corresponding Author: dewiani@unhas.ac.id.

### **Abstract**

In Indonesia, the number of potholes is quite high. The situation is exacerbated that until now the data of location point of pothole is still done manually by field personnel the Department of Transportation or other related services, which would require time and cost. This study aimed to produce a prototype of detection system and location point data of pothole automatically that attached on public transport so that it can be a solution to collect data on locations of potholes. Detection system with vision-based methods use blob detection and neural network backpropagation. Blob detection is used to detect potholes based on convexity shape and neural network backpropagation to detect potholes by the texture of inside the pothole. The training data of neural network using a segment pattern grayscale value that obtained from parts of pothole 25 x 25 in size. The prototype was built using Raspberry Pi and OpenCV library. Results showed quite low accuracy and still need to be improved.

**Key words:** Backpropagation, Blop Detection, Neural Network, Potholes, Raspberry Pi, OpenCV.

### **Introduction**

According to the traffic Act No. 22 of 2009 article 229, paragraph 5 there are three factors that cause traffic accidents, namely the negligence of road users, unworthy vehicles, and also unworthy road. One of unworthy road is potholes. In 2014, from 95.906 the number of accidents recorded, there were at least 1.000 incidents or 18% was caused by the pothole. The situation is further exacerbated because until now the location point data of pothole is still done manually by field personnel or technician the Department of Transportation or related agencies of other highway maintenance, or still waiting for reports from people who certainly takes time and costs.

According to the traffic Act No. 22 of 2009 article 229, paragraph 5 there are three factors that cause traffic accidents, namely the negligence of road users, unworthy vehicles, and also unworthy road. One of unworthy road is potholes. In 2014, from 95.906 the number of accidents recorded, there were at least 1.000 incidents or 18% was caused by the pothole. The situation is further exacerbated because until now the location point data of pothole is still done manually by field personnel or technician the Department of Transportation or related agencies of other highway maintenance, or still waiting for reports from people who certainly takes time and costs.

Artificial Neural Network is an information processing paradigm that is inspired by biological neural systems, like the brain that processes the information which consists of smallest processor units called neurons (Syafuruddin et.al., 2012). Neural network was formed to solve a problem such as pattern recognition, classification, or detection due to the learning process. Backpropagation is a supervised learning training techniques that are widely used because it is able to deal with the introduction of complex patterns. In backpropagation, each unit that is in the input layer is connected to every unit that is in the hidden layer.

Raspberry Pi is commonly abbreviated Raspi is a Single Board Computer that has a modest size (Halfacree & Upton, 2012). Raspberry Pi is an Linux-based open source so easily modified as needed. The main systems of Raspberry Pi using Debian GNU/Linux and Python programming languages. OpenCV, or Open Computer Vision is an open source library that is devoted to image processing based C/C++ is widely used in computer vision. OpenCV is designed for computational efficiency and provide the infrastructure of computer vision that is easy to use.

The purpose of this research is to produce a prototype of pothole detection attached on the public transport. If the prototype is successfully detects the presence of potholes, the system automatically

records the location point of vehicle pothole with google maps API integration. The prototype was built with the Raspberry Pi and OpenCV aimed to producing a prototype that is cost-effective. Hopefully, the presence of prototype can facilitate the department of Transportation or the relevant office of highway maintenance to conduct location point data of pothole in creating conditions Zero Hole or Free Potholes on the road in Indonesia or in other places.

### Related Work

As the visual form, potholes have the characteristics to distinguish it from other objects on the highway. In general, potholes has a circle-ellipse shape depending on the driver's viewpoint, is darker than the surrounding area, and the inner surface of the pothole is generally coarser than the surrounding road surface (Koch & Brilakis, 2011a).

Based on the physical form, the method used to detect pothole can be divided into vibration-based methods, 3D reconstruction-based methods, and vision-based methods (Taehyeong & Seung-Ki, 2014). 3D reconstruction-based methods are able to detect potholes until detects the volume of pothole, but it has a high cost to produce a system with this method (Koch & Brilakis, 2011a). Vibration-based methods can detect potholes without influenced by conditions surrounding it, but to be able to detect potholes, the system must first contact with potholes, which can certainly endanger the driver or the vehicle. In addition, the shock of speed bumps, bridge expansion joints, and sunken manhole can be detected as pothole (Eriksson et al., 2008). The use of other physical media such as InfraRed can also be undertaken, but not so effective on sunny day due to interference from sunlight (Nienaber et al., 2015).

Nienaber et al. (2015) using a simple image processing techniques to detect potholes. Image processing techniques are used such as canny filter and contour detection. The result has a precision of up to 81.8% but it still needs development in detecting pothole which its side clogged with dirt or sand. Pawade et.al. (2015) use Field Programmable Gate Arrays module to detect pothole. The system uses detection variation of Sobel, Prewitt and Canny edge. The results are able to provide the number of clear pothole, but has a relatively long process because the paralel algorithm process. Koch and Ioannis (2011a) classify the detection area based on the defect and non-defect. The use of shape-based thresholding algorithm histogram, morphological thinning, and elliptic regression are able to produce accuracy up to 85.9%, but has inefficient computing Koch and Brilakis (2011b).

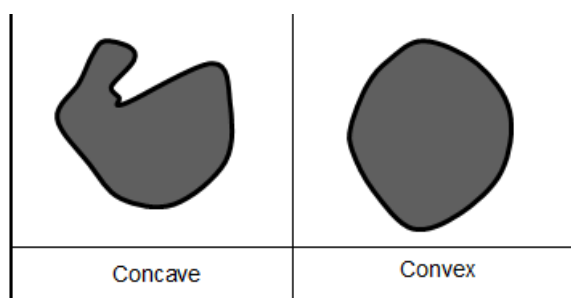
### Methods

#### Thresholding

*Thresholding is the simplest method of image segmentation. Thresholding is used to segment an image by setting all pixels intensity whose values are above a threshold to a foreground value and all the remaining pixels to a background value. Thesholding process is undertaken to further process efficiencies.*

#### Blop Detection

Blob detection parameter used is convexity parameter. This parameter is used for pothole is generally circular with a different shape from the viewpoint of the driver. In OpenCV, the higher of convexity parameter value, the higher of convexity form level that can be detected, whereas if the value of convexity parameter is lower, then blob detection can detect until concave shape. Examples of the different forms based on the convexity level can be seen in Figure 1. All of object being detected as a blob and have parameter values as the given value will be regarded as a pothole. Then, the output of blob detection is final selection by using Neural Network Backpropagation. Neural Network Backpropagation is used to classify output of blob detection by dividing the asphalt with potholes by grayscale degree pattern of the inside of pothole.



**Figure 1.** The differences of a blop based on convexity level

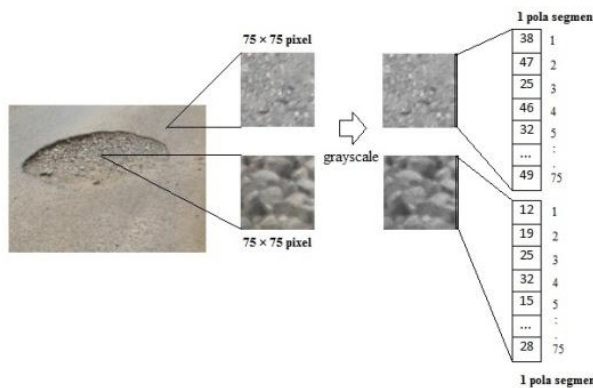
#### Preprocessing

Image acquisition for training data through the cropping process in size 75 x 75 pixels and converted to

grayscale image for later classified as training data for potholes and for asphalt. Cropping image will be training data by taking a grayscale value vertically, so that the cropping image will have 75-degree pattern of grayscale value  $1 \times 75$  pixel. Thus, for a sample size of  $75 \times 75$ , there are 75 patterns that can be taken, where:

- Conventional segmentation matrix models  $1 \times 75$  pixel.
- Horizontal conventional segmentation with one group of pixels horizontally
- Vertical conventional segmentation with 75 groups of pixels vertically

Untuk proses preprocessing dapat dilihat pada Figure 2.



**Figure 2.** The acquisition process of training data for neural network backpropagation

### Neural Network Training

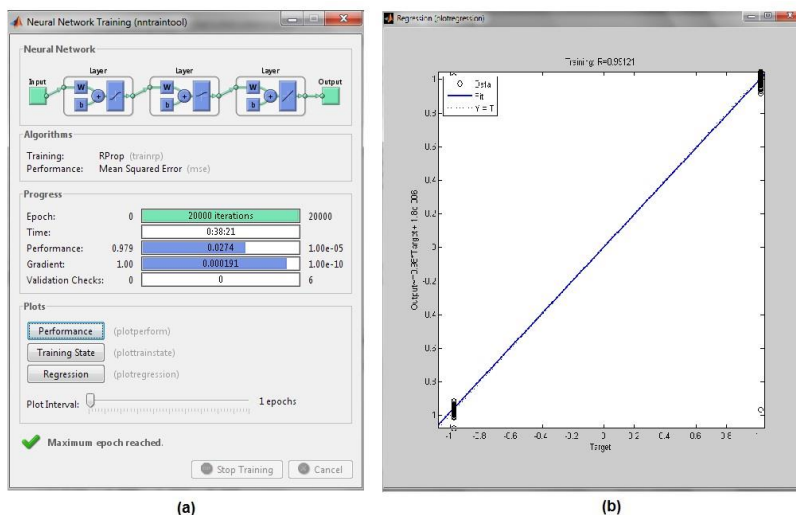
The process of neural network using a Matlab 2014b application. For training data of cropping and trees areas will be saved in a file format .mat. In making the target data, will be loaded on the training data that has been stored previously to be a target value according to the class are:

- Target rated 1 for training data of potholes
- Target rated 0 for training data of asphalt

The process of neural network training to do as much as 20000 epoch. Epoch is one cycle of neural network training. Error is error level to be achieved in the system. The system will stop to do training process if the number of epoch has been reached or error value has been as expected. MSE (Mean Square Error) is the mean square error (error). The smaller of MSE, the better of obtained result.

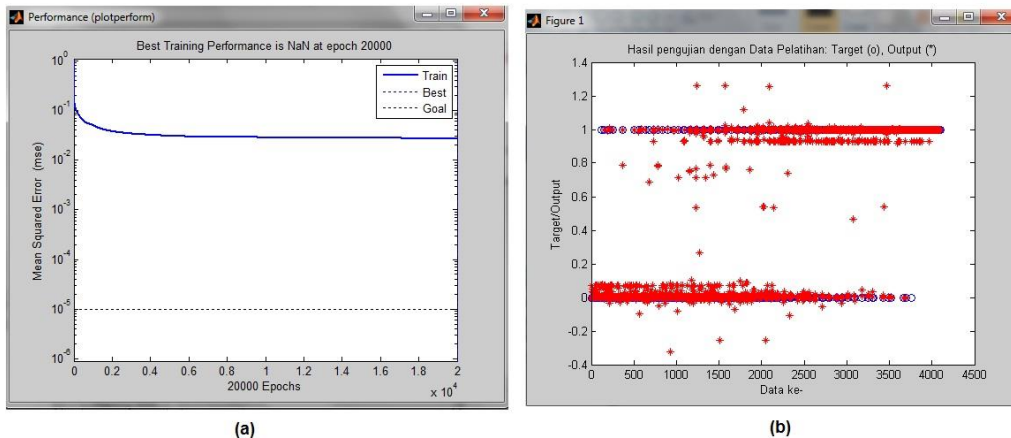
Figure 3(a) shows when the epoch maximum has been reached or training has been completed. The length of training with 20000 epoch during 38:21 minutes to achieve performance at 0.0274. Figure 3 (b) shows chart the relationship of target with output. R is a linear gradient as results of linear regression. If the output of network is exactly equal to its target, then the gradient will be worth 1. The closer of data with Fit line, the closer of trained pattern.

In this 20000 epoch, most of data is almost exactly with the data target, as shown by the farthest data from Fit line is not more than 0.2.



**Figure 3.** (a) The process of Neural Network training. (b) the relationship of target with targets

In Figure 4 (a), X-axis is the number of epochs and Y-axis is the root mean squared error (RMSE) of the training data set for each epoch. The closer of Train line with Goal line, then mistake during the training is closer to the target to be achieved. RMSE value used to be one of criteria for a learning system to stop learning. In addition RMSE values can also be used as a criterion for epoch for the system to stop learning. RMSE is an error rate of output value of the learning system to the pairs value of training data output, in other words RMSE is a difference occurs between the desired output with the actual output. Based on the graph, it appears that the greater number of epoch, then error will decrease. After reaching epoch 2000, a decrease in the error is smaller and constant before epoch 18000. Target desired error at epoch 20000 is still far from the desired target.



**Figure 4.** (a) Performance of neural network training process. (b) Testing results with training data. As seen in Figure 4 (b) most of training data segment pattern has approached target/output given, although there is still some small segment pattern that is far from the desired target. For target 1 (potholes), the highest segment pattern is 1.28 and the lowest is 0.58. As for the target of 0 (asphalt), the highest segment pattern is 0.43 and the lowest is -0.38.

### Prototype System

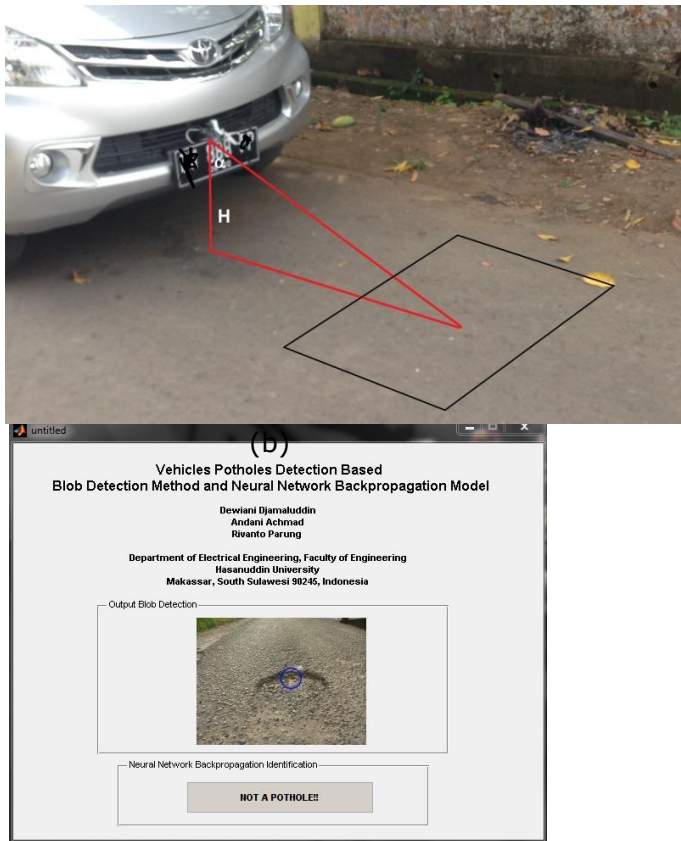
Raspberry Pi is used as the main media and the main processor of resulted prototype. A camera module of Raspberry Pi is used to capture the image of highway continuously. Micro SD used as storage media and power is obtained from a powerbank. GPS and 3G modem modules is used to lock the position of the prototype when a pothole is detected. If a pothole is detected, the position of location point of potholes can be displayed on a web with Google maps API integration. The resulting prototype is shown in Figure 5.



**Figure 5.** Prototype of potholes detection

### Testing

Testing is done with a prototype direction angle  $\alpha \approx 45$  and a height  $H \approx 0.5$  feet from the asphalt surface as shown in Figure 6(a), aimed for the camera is always facing toward the highway. Several scenarios carried out to test the reliability of the prototype as the position of the sun is in front of the driver, the sun is in the right or left side of the driver, and the weather was sunny and cloudy. The shape, size, condition of potholes tested is varied. Testing is conducted in Tamalanrea, Makassar, Indonesia as many as 30 locations of potholes.



**Figure 6.** (a) The testing process. (b) Examples of system output, blob detection successfully detect the potholes, but Neural Network classify into Not a Potholes Object

## Results and Discussions

Testing showed the detection results still indicate a failure of detection is high enough. In many cases, blob detection has been able to detect the presence of potholes based on the pattern of convexity shape. But in the process of classification of asphalt and potholes of Neural Network Backpropagation considers output of blob detection as not potholes (asphalt). The process of neural network as determiner of potholes detection classifies detection result of Blob Detection that are appropriate to be a non-potholes (not detected). The sample of output of the system is shown in Figure 6(b).

The number of detection error of Neural Network Backpropagation is assumed because the selection of training data is inappropriate. With varying conditions of asphalt, the possibility of similarity in the degree of grayscale pattern between the inside of pothole with asphalt becomes higher. This causes the detection process of potholes; especially on low-quality asphalt is fail. On the condition of pothole covered trees shadow or buildings, detection error becomes greater. This is due to the pattern of grayscale pattern of asphalt and the inside of pothole to be same.

## Conclusions

The presence of pothole gives a negative impact to the people. Potholes can endanger the driver or the vehicle, this condition becomes more difficult because in collecting data of location point of potholes from the related agencies or the government is still done manually. For that a detection system and automatic data collection of potholes that can be attached on public transport can help the task of relevant agencies in conducting highway maintenance.

The results of these studies still need to be improved. By focusing on the detection process of blob detection methods based on its shape or by making the training data from the more precise Neural Network, it is expected to improve the accuracy and reliability of the prototype. The use of other

intelligent methods such as Neuro-Fuzzy or add adaptive filtering process can also be another alternative.

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## Performance of Network Mobile Multi Node Wireless Sensor For Application to Landslide Movements

<sup>1\*</sup>Hafsah Nirwana, <sup>1</sup>Eddy T, <sup>1</sup>Muh.Ahyar, <sup>1</sup>Ibrahim Abduh, <sup>2</sup>Zahir

<sup>1</sup>Teacher of Electro Engineering Department, Politeknik Negeri Ujung Pandang

<sup>2</sup>Teacher of Electro Engineering Departement, Hasanuddin University

\*Corresponding Author: yayeng555@yahoo.co.id / Hanir@poliupg.ac.id

### Abstract

Landslides are the movement of slope-forming materials such as rocks, debris materials, soil, or a mixture of these materials, moving down or off slopes. In connection with an early warning of landslides, a very important thing to note is the process of collecting information or data on the occurrence of landslides (landslide). The more points of node sensors are installed on landslide-prone areas, the more accurate the quality of an early warning system is. The research was aimed at creating a model of landslide movement and creating and analyzing models of multi-sensor data communication based on ZigBee IEEE 802.15.4. The research was to create a model of landslide movement based on the speed rate of soil movement by using a mobile Wireless Sensor Network/WSN and make the laying model of some WSNs (multi nodes) for an area coverage  $\pm 300\text{m}^2$ . This modeling used the software of Network Simulator version 2 or NS-2 with a range of scenarios. The results revealed that based on the network quality, the delay of some scenarios were very erratic; the packet delivery ratio value decreased when the number of clusters and/or nodes increased; and when the number of clusters and/or nodes increased, the throughput got bigger.

**Key words:** Landslide, WSN, Mobile, Zigbee, Delay, PDR, Throughput

### Introduction

Ground motion is the movement of land mass to a lower place due to gravitational force (Magetsari, 2001 in Ira, 2013). Landslides are defined as the movement of materials or soil from the surface of slopes downward slopes of the earth that is caused by gravity force (Sulistianto, 2001 in Ira, 2013). They are also defined as the movement of slope-forming materials such as rocks, debris materials, soil, or a mixture of these materials that move down or off slopes. The occurrence of landslides can be explained as follows. Water that seeps into the ground increases soil weight. If water penetrates soil until it is impermeable and turns to be slip plane, it becomes slippery and the weathering soil on it will move following and off the slope (VSI, 2011). In general, a landslide has a sliding area.

Early warning (Early Warning System) is a series of act of giving an immediate warning by an authorized agency to the public about the possibility of a disaster occurrence in an area. The early warning system for landslides can reduce or prevent the loss of lives and properties when landslides happen. In connection with the early warning of landslides, a very important thing to note is the process of collecting information or data on the incidence of landslides (landslide). The more points the sensor nodes mounted on landslide-prone areas, the more accurate the quality of an early warning system will be.

The research was aimed to create a model of landslide movement and create and analyze the models of multi-sensor data communication based on ZigBee IEEE 802.15.4.

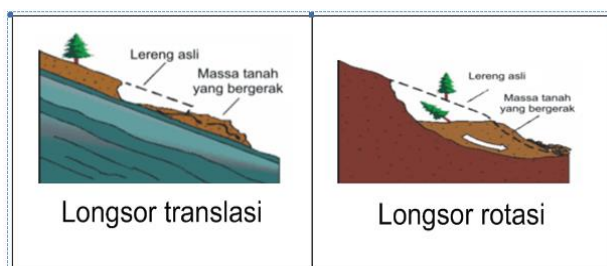


Figure 1. Landslide Condition

Wireless sensor network (Wireless Sensor Network/WSN) is a smart sensor in which each point on the sensor has the ability to sense its surroundings (sensing), and processes the data obtained as well



ascommunicates, but it is developed in a larger scale and can be connected to one another. Accordingly, it will be able to performan oversight function (monitoring) continuously (real time) on an environment that will be sensed by the wireless sensor network collectively. Wireless sensor network is a new generation of sensor systems(sensory system), althoughit is still limited to data processing ability only and has a limited bandwidth for communication(Ata E2005&GeorgeA, 2009).

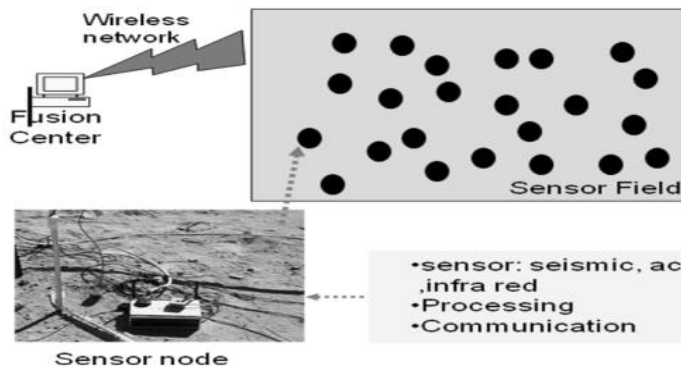


Figure 2. Wireless Sensor NetworkSystem (Abdul H dkk,2009)

In a *Wireless Sensor Network*there are 2 kinds of topology (Sri A,2011), namely:

1. *Hierarchical*
2. *Flat- type*

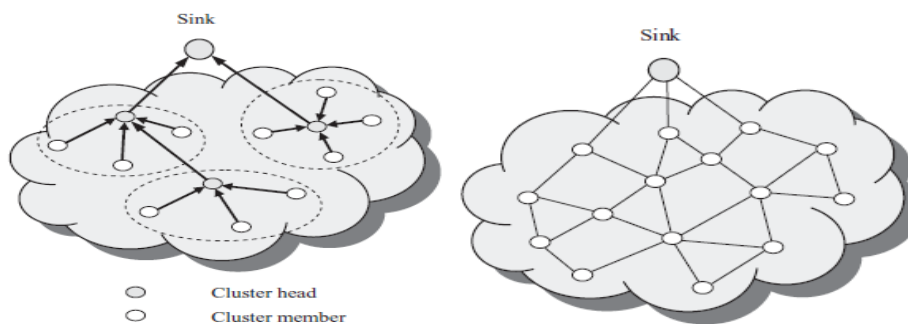


Figure 3. Single-hop cluster typeFigure4 Topologyflat-type

The hierarchical architecture can be seen in Figure 3 and 4 above. In this topology, the sensor nodes are grouped in clusters. Sensor nodes are arranged in a hierarchical arrangement so that there are 3 kinds of nodes, i.e. the sink node, the cluster head, and cluster members. Sink node functions as a regulator of multiple cluster head in its application. Several cluster members become the members of a cluster head.

### Research Methodology

The research was to create a model of landslide movement by using Wireless Sensor Network/WSN that is mobile and make the laying model of some WSNs(multi-node) for an area coverage  $\pm 300\text{m}^2$ . This modelling used the software Network Simulator version2 or NS-2.

### Data Communication Simulation Designing of Mobile Multi Node Wireless Sensor

For modelling the data communication path from the cluster node to the head node using ZigBee IEEE 802.15,the cluster head is positioned in the middle of the cluster nodes with a typology form of ad hoc type where one of the nodes will be assigned as a proxy to perform coordination among nodes in a group.

The concept of data communication path modeling consists of two nodes:

- a. Node point of lanslide data of WSN; in this location several wireless sensor nodes are installed, which are divided in multiple cluster nodes. And each cluster node is equipped with a local alarm.
- b. Head node; this point serves as the data centre of avalanche that originate from some node clusters. This section is also equipped with an alarm and automatic stop signs for road users who will cross the road in the area.

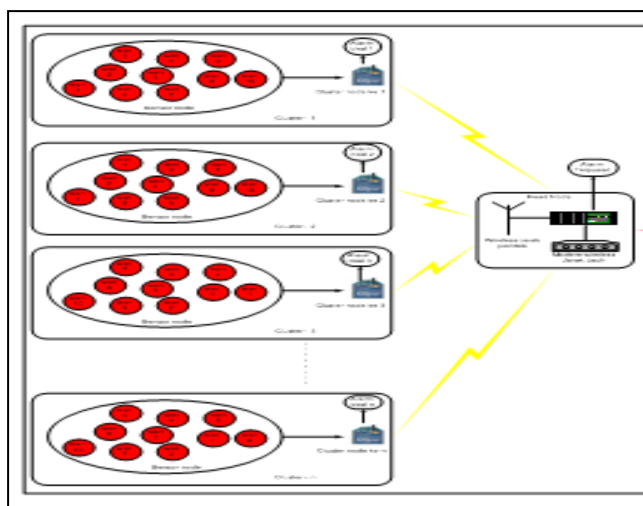


Figure 5. Model of data communication path of WSN *Multi Node* (Hafsah N, 2014)

The scenario rules of the research are as follows:

- The condition of WSN is mobile.
- The position of WSN is fixed
- The number of clusters ranges from 2 to 10 clusters.
- The number of nodes/WSN in each cluster varies, i.e. 3, 5, 7, and 10 nodes.
- The performance calculated is Delay, Packet Delivery Ratio and Throughput.

So, if scenario F1 has 10 clusters, it means that there is a WSN with 30 nodes. There is a situation where scenarios have the same number of nodes but the nodes are placed in different positions, for example scenario F1 with 10 clusters and scenario F4 with 3 clusters. Both scenarios have WSN with 30 nodes.

The designing of the mobile WSN consists of several stages: determining the parameters of communication devices, the position of the sensor nodes and their topology, and the number of clusters and nodes. Then the simulation was constructed with the simulator of Network Simulator 2 (NS-2) version 2:34 with a modification of PHY and MAC QoS support, language programming C++ and Tcl. The result of the simulation was file traces, which were displayed in the form of data and graphs using AWK scripts and Gnuplot application.

## Result And Analysis

A mobile condition is the visualization of condition of sensor that has a movement, which means that land movement takes place. The land movement is observed in terms of distance so that the condition can be calculated if it is still secure, unsecure, or a landslide is taking place. Making a decision on the condition is based on the speed rate of the land movement.

### 1. Results of mobile Zigbee WSN network performance

The parameters used for measuring the results of mobile Zigbee WSN network performance were Packet Delivery Ratio (in %), Throughput (in bps), and Delay (in milliseconds). Each table of the measurement results includes the three parameters for comparing the results with a variety of scenarios. The performance measurement results in Table 1 and the graph shapes for the results of performance measurement of the data communication systems from the table are displayed in some images below.

Table 1. WSN mobile Zigbee

Amount of Cluster (WSN)	PDR (%)				Traoughput ( bps )				Delay (mili detik)			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
2	96.55	98.69	93.44	70.12	4.97	8.29	10.87	10.60	25.51	74.11	118.99	46.76
3	100	99.19	85.28	60	7.52	12.09	12.87	13.33	46.76	34.02	46.11	444.93
4	99.41	89.49	77.77	46.74	9.52	13.62	14.87	13.49	21.69	91.06	52.17	134.63
5	90.94	75.68	61.06	38.97	11.66	15.01	16.31	15.16	94.71	121.74	122.79	203.08
6	83.39	62.03	48.32	30.73	11.43	14.91	16.29	14.08	141.5	248.29	230.87	115.35
7	85.48	61.41	50.86	32.76	13.11	15.95	18.44	17.75	229.94	135.46	309.17	221.69
8	75.06	52.67	48.36	30.40	13.67	16.12	19.83	18.24	218.02	463.52	158.15	163.75
9	74.47	49.02	37.55	24.59	15.61	17.30	18.34	16.00	527.11	215.93	323.68	220.16
10	66.59	47.76	37.31	22.52	15.33	18.13	19.05	17.01	398.48	244.85	185.63	301.67

After having conducted the simulation with NS-2, one of the outputs was in the form *file Namtrace* that was used as an input for graphical display of simulation called *Network Animator* (nam). The result was a form of WSN positioning condition in landslide-prone locations. Below is a form of namtrace outputs from the scenario of fixed position of 10 clusters and 10 nodes/clusters, which was scenario F4.

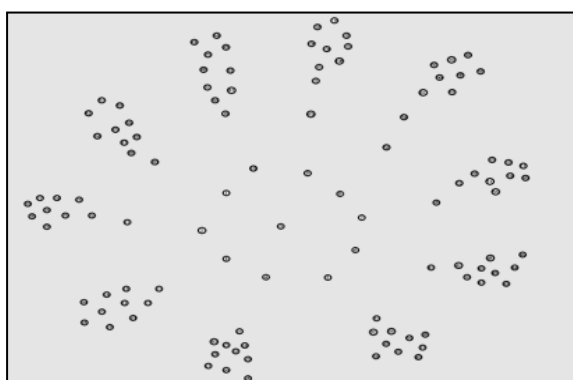


Figure 8. Wireless Sensor Network (WSN) positioning

## 2. QOS Measurement of Networks

### a. Delay performance

- ✓ For all scenarios F1, F2, F3 and F4, the delay values varied when the number of clusters or WSN increased. Sometimes the delay values increased when the number of clusters or WSN increased, but sometimes reduced as well.
- ✓ The shortest delay, which was 21:49 ms, happened to scenario F1 with a number of 4 clusters, while the longest delay occurred to scenario F1 with 9 clusters.

Based on the results obtained, the delay performance graph is shown in Figure 9.

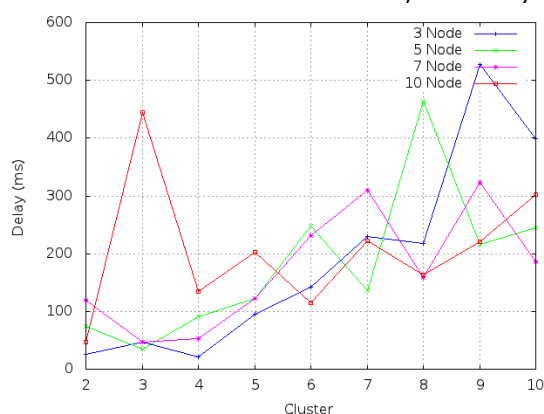


Figure 9. Delay graph of Zigbee Mobile WSN with a fixed position of WSN

## b. Packet Delivery Ratio performance

PDR performance measurement is a parameter to determine the ratio of the number of packets received by the receiving node to the total packets sent in a certain time period. The results are shown in Table 1.

The bigger the number of nodes was, the smaller the PDR was; and the bigger the number of clusters was, the smaller the PDR was. This means that more data sent will be received successfully if the number of nodes or clusters is small. For more details, this is visualized in graphical form in Figure 10.

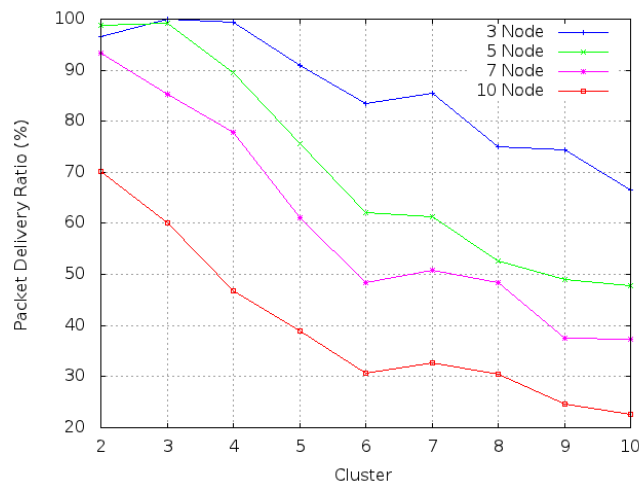


Figure 10. PDR graph of Zigbee Mobile WSN for fixed position of WSN

## c. Throughput performance

Throughput is the total number of success full packet arrivals at the destination observed during a specific time interval divided by the duration of the time interval. The comparison of throughput of each scenario shows the amount of data that can be transmitted in a time unit.

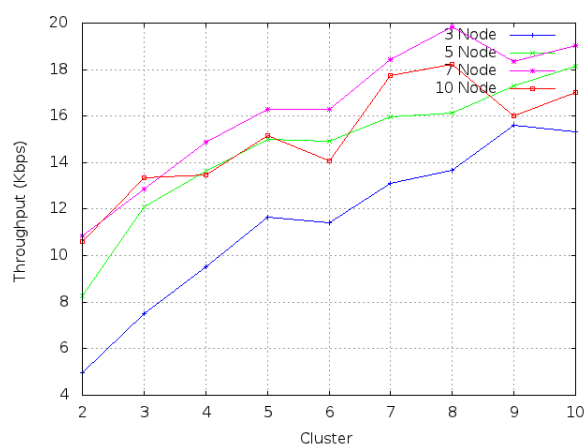


Figure 11. Throughput graph of Zigbee Mobile WSN for a fixed position of WSN

The bigger the number of cluster nodes and/or nodes was, the greater the throughput would be. However, although scenarios had the same number of nodes, they would result in different values of throughput when they had different topologies; for example, the results of scenario F1 with 10 clusters and scenario F4 with 3 clusters. Even though these two scenarios had the same node number of 30 WSNs, they resulted in different throughput, that is, 15.33 bps and 13.33 bps respectively.

## Conclusions

1. Based on its quality, the Wireless Sensor Network using ZigBee could be used as a Landslide Early Warning.
2. The delivery time of information (delay) of the network was uncertain for a various numbers of clusters and nodes.
3. The Packet Delivery Ratio will decrease in value if the number of clusters and/or nodes increases.

4. Increased number of cluster nodes and/or nodes will increase throughput.
5. NS-2 can only model the soil movement speed rate at Grade/Category 5, 6, and 7, that is, big landslides, landslides, and landslides will occur.

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## CFD Simulation of LPG Combustion in Annular Combustion Chamber of Micro Gas Turbine

<sup>1,2\*</sup>Asyari Daryus, <sup>1</sup>Ahmad Indra Siswantara, <sup>1</sup>Budiarso, <sup>1,3</sup>Gun Gun R. Gunadi, <sup>1</sup>Rovida Camalia

<sup>1</sup>Department of Mechanical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, Indonesia;

<sup>2</sup>Department of Mechanical Engineering, Faculty of Engineering, Universitas Darma Persada, Jakarta Timur 13450, Indonesia;

<sup>3</sup>Department of Mechanical Engineering, Politeknik Negeri Jakarta, Depok 16424, Indonesia;

\*Corresponding Author: asyari@yahoo.com.

### Abstract

This paper presents the CFD analysis of combustion in annular micro gas turbine combustion chamber for three types of LPG available in the market to find their combustion characteristics for micro gas turbine system. The simulations had been done using CFD-SOF(r) software, two dimension geometry model, finite rate and eddy dissipation model for combustions and  $k-\epsilon$  turbulence model for fluid flow. Three types of LPG available on the market used for simulations are: propane, butane, and mixed LPG. Energy released by the fuel assumed 100 kJ/s. The maximum temperature found for propane, butane, and mixed LPG are 1661 K, 1728 K, and 1725 K respectively while the temperature at the exit of chamber for the same fuels are 1441 K, 1447 K, and 1554 K respectively. Since the maximum gas temperature and velocity of gas are found on mixed LPG combustion, the combustion of mixed LPG would give better results for micro gas turbine performances.

**Key words:** CFD, turbulence model,  $k-\epsilon$ , LPG, combustion chamber, micro gas turbine

### Introduction

Micro Gas Turbine (MGT) is a gas turbine system that usually generates power between 25-500 kW (Daryus et al., 2016; Paepe et al., 2014). It has many advantages, such as high power density, environmental friendly, low operational and maintenance cost and flexible for many kinds of fuels (Basrawi et al., 2013; Daryus et al., 2016; Siswantara et al., 2016). Its primary applications are electrical power and/or heat generation and widely used in various area, such as buildings, homes, hotels, restaurants, convenient stores, fast food restaurants and even in offshore oil and gas industry (Capstone, 2008; Latcovich et al., 2003).

One of the advantages of micro gas turbine is flexible for many kinds of fuels, such as solar, ethanol, biomass, CNG, and LPG. The LPG is a gas fuel that easier to obtain in the Indonesia's market and has high flame temperature (around 2270 °C) (Turns, 2000), that is attractive to be used for micro gas turbine. There are three types of LPG available, i.e., propane LPG, butane LPG and mixed LPG. The gas content in accordance with its name, so the propane LPG means that its main content is propane, and so on. The minimum content of these gases have been regulated by government regulations which not less than 95 vol% (Bumi, 2009).

Understanding the combustion process will help an engineer to design the optimum combustion chamber or to decide the right fuel to be used. Investigating the phenomena of gas inside the combustion chamber can be done through experiments or simulations or both. Experiment investigations is usually difficult to be conducted due to the complex chemical process, heavy turbulence, and high temperature and pressure (Bulat et al., 2011) and furthermore, it will need expensive and complex laser diagnostics and modified combustion chamber (Bicsak et al., 2012). Simulation is an option in describing the combustion process in a combustion chamber that is easy, cheap and faster to be done. Computational Fluid Dynamics (CFD) is one method used in simulations where the mathematical formulas, such as turbulent flow and combustion process are calculated in numerical way, and the complex mathematical equations are modeled so that the calculations more easily resolved.

Developing the Proto X-3 Bioenergy Micro Gas Turbine, a prototype of micro gas turbine, the usage the fuel that easy to obtain is primary concern. LPG, used by many sectors like industries and homes, is widely available in the market. Since there are 3 types of LPG available, the investigation of their

characteristics need to be performed to determine which one is the best for this prototype of micro gas turbine. The investigation has been done using CFD simulations.

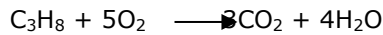
The aim of this research is to investigate the characteristics of combustion of three types of LPG. The results will determine the type of LPG to be used for "Proto X-3 Bioenergy Micro Gas Turbine".

## Methods

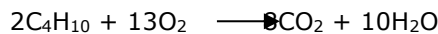
### Combustion Model

Westbrook-Dryer one-step model is used for combustion. This model gives a good estimation of indicator of the expected temperature levels. The turbulent non-premixed combustion process is simulated using Finite Rate and Eddy Dissipation model. In this model, the reaction rates are assumed to be controlled by the turbulence, so the complex Arrhenius chemical kinetic calculations can be avoided.

The chemical reaction for the propane LPG is:



And for butane LPG is:



### Turbulence Model

For turbulence calculations, the  $k$ - $\varepsilon$  turbulence model is used. This model has two extra transport equations, i.e., transport of kinetic energy,  $k$ , and transport of dissipation,  $\varepsilon$ . The transport equation of kinetic energy is formulated as (Versteeg & Malalasekara, 2007):

$$\frac{\partial(\rho k)}{\partial t} + \text{div}(\rho k \mathbf{U}) = \text{div} \left[ \frac{\mu_t}{\sigma_k} \text{grad } k \right] + 2\mu_t E_{ij} \cdot E_{ij} - \rho \varepsilon \quad (1)$$

and the transport equation of dissipation is:

$$\frac{\partial(\rho \varepsilon)}{\partial t} + \text{div}(\rho \varepsilon \mathbf{U}) = \text{div} \left[ \frac{\mu_t}{\sigma_\varepsilon} \text{grad } \varepsilon \right] + C_{1\varepsilon} \frac{\varepsilon}{k} 2\mu_t E_{ij} \cdot E_{ij} - C_{2\varepsilon} \rho \frac{\varepsilon^2}{k} \quad (2)$$

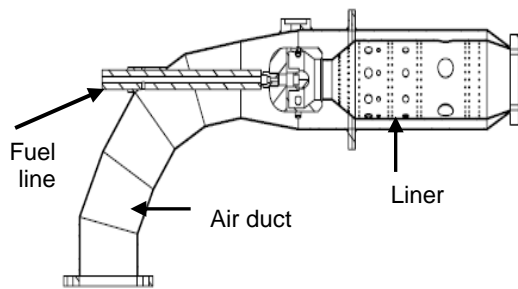
where:

$$\mu_t = \rho C_\mu \frac{k^2}{\varepsilon} \quad (3)$$

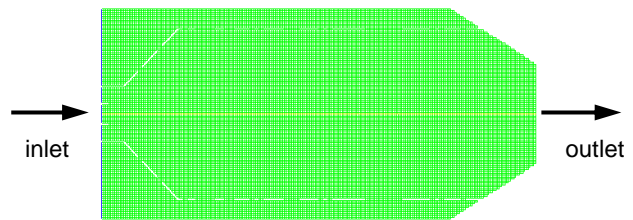
$\rho$  is a density,  $\mathbf{U}$  is a velocity vector,  $\mu_t$  is a eddy viscosity,  $E_{ij}$  is a mean rate of deformation, if  $i$  or  $j = 1$  corresponds to the  $x$ -direction,  $i$  or  $j = 2$  the  $y$ -direction and  $i$  or  $j = 3$  the  $z$ -direction,  $C_\mu$ ,  $\sigma_k$ ,  $\sigma_\varepsilon$ ,  $C_{1\varepsilon}$ , and  $C_{2\varepsilon}$  are constants.

### Geometry

The longitudinal view of combustion chamber is shown on Figure 1. The combustion chamber is annular type with 112 mm inside diameter and 253 mm length. The inlet inside pipe diameter is 30 mm for primary air supply and 10 mm for fuel injector. The fuel injector is located inside the primary air pipe. The secondary air is injected using 3 rows of holes with 9 holes in a row and diameter of 10 mm, 5 mm, and 8 mm for each row sequentially. There are 5 dilution air holes with diameter of 20 mm in the liner.



**Figure 1.** Combustion Chamber



**Figure 2.** 2D mesh model of combustion chamber

### **Meshing**

2D structured mesh model is chosen for the simulations because the purpose of the study not to find the precision results but to compare between various type of LPGs, so the processes could use the medium specification of computational devices and faster. It consists of 13794 cells of 1 mm element-size (Figure 2). It is generated by the CFDSOF(r), the same software for the calculations. The further refinement in either direction does not change the temperature and scalar variables at any point in the combustion chamber considerably.

### **Boundary Conditions**

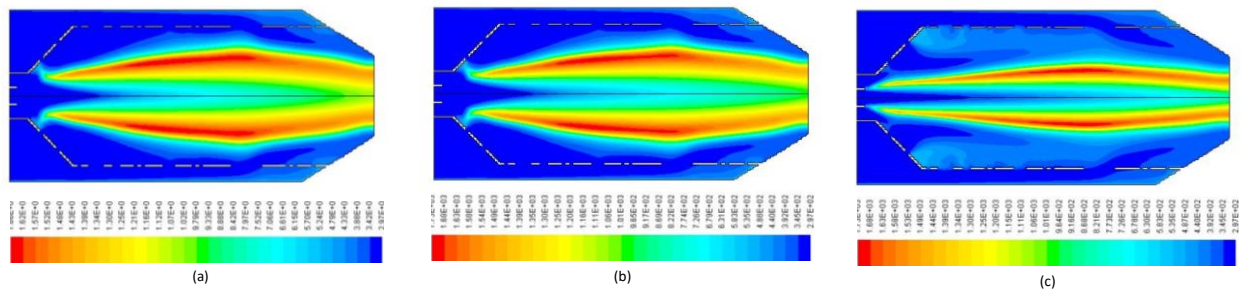
LPG is used for combustion. There are 3 types of LPG available in the market, i.e., propane LPG, butane LPG and mixed LPG. As the name implies, the main content of propane, butane and mixed LPG are propane ( $C_3H_8$ ), butane ( $C_4H_{10}$ ) and a mixture of both respectively. The simulations has used these three LPGs for the combustion. The experiment data of micro gas turbine system, such as temperature, pressure and fuel mass flow, are used for boundary conditions. Some previous simulations with various air mass flow has been conducted to get proper air mass flow for the combustion. Only the proper air mass flow is analyzed in this study. The general boundary conditions of fuel are: heat generated from the combustion assumed to be 100 kW equivalent to 0.0022 kg/s of mass, the inlet temperature is 297 K, propane mass fraction is 1 for propane LPG, butane mass fraction is 1 for butane LPG, the mass fraction of propane and methane both are 0.5 for the mixed LPG, and turbulence intensity assumed to be 10%. The boundary conditions of the inlet air are: the temperature is 312 K, and the turbulence intensity is 10%. The boundary condition of the outlet of combustion chamber is defined by providing relative pressure value, which is zero Pascal.

### **Results and Discussion**

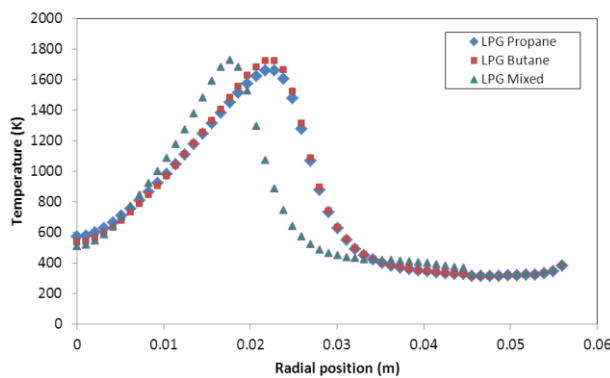
After conducting some simulations, the proper air mass flow for the optimum results are found to be 0.02 kg/s for propane and butane LPG and 0.04 kg/s for mixed LPG. The lower air mass flow will cause the hot gas to spread near the wall while the higher air mass flow will shift the combustion process to downstream.

Figure 3 shows the simulation results of the temperature distributions along the axial length of liner for the three LPGs. The contour for propane and butane LPG seems not many differences, but the mixed LPG has the thinner flame than the other two. This is possibly due to the higher air mass flow that push the hot air to the outlet direction. Higher temperature is visible in the primary to secondary zone. The highest temperature for propane LPG found 1661 K, while for butane and mixed LPG are 1728 K and 1725 K respectively. Figure 4 shows the plot of temperature in radial (y-axis) direction from centerline in the hottest section, 102 mm from the left side for propane and butane LPG and 146 mm for mixed LPG. It can be seen that the hottest gases exist between 17 mm to 23 mm in the radial direction or in the middle between the centerline and the liner wall, leave the wall cool, while at the centerline (y=0 mm) the temperature found around 500 K.

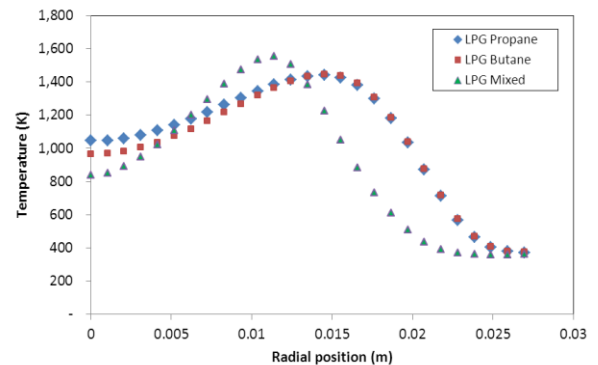




**Figure 3.** Contour of temperature. (a) Propane LPG, (b) Butane LPG, (c) Mixed LPG.



**Figure 4.** Plot of maximum temperature in radial direction

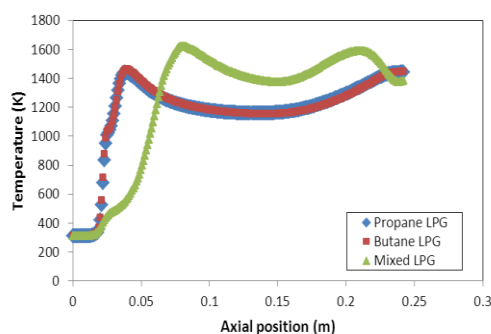


**Figure 5.** Plot of temperature at outlet in axial direction

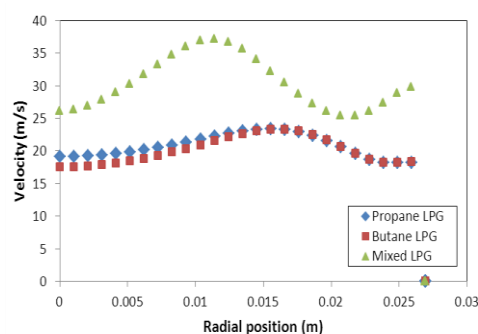
Figure 5 is a plot temperature in radial (y-axis) direction at outlet. The highest value found in middle between centerline and liner wall or between the distance of 11 mm to 15 mm. i.e., 1441 K, 1447 K and 1554 K for propane, butane and mixed LPG respectively and go on decreasing towards the wall. The mixed LPG is the highest among the three. Although the mixed LPG has the highest temperature but it decrease more rapidly than the others so it might has the lower average temperature and keep the wall cooler.

The temperature distribution in axial direction at distance of 14 mm from centerline or in the middle of the outlet section is shown on figure 6. The temperature rises in the primary zone steeply and comes out at outlet relatively at the same value. There is a slight decrease in the middle because at this range the hot gas moves upward as seen on Figure 3.

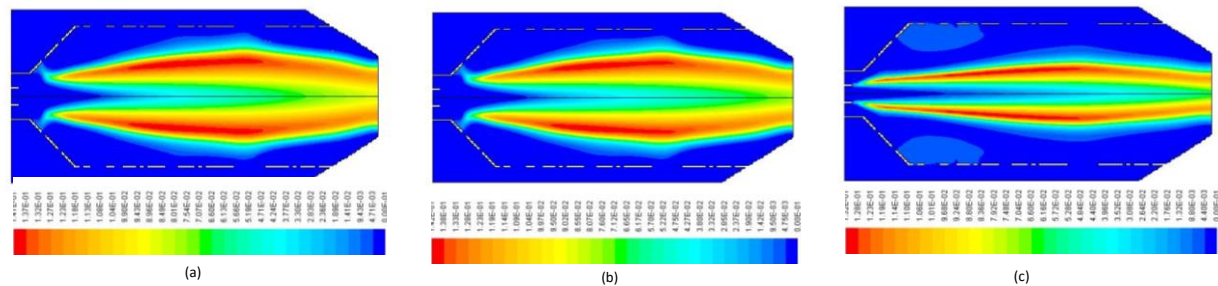
Figure 7 shows the velocity of gas at outlet of combustion chamber. The mixed LPG has the highest value while the others are almost the same. This is possibly due to the higher air mass flow in mixed LPG than others. It means that the mixed LPG has the higher kinetic energy.



**Figure 6.** Plot of temperature in axial direction



**Figure 7.** Plot of velocity in radial direction at outlet



**Figure 8.** Contour of CO<sub>2</sub>. (a) Propane LPG, (b) Butane LPG, (c) Mixed LPG.

The CO<sub>2</sub> distribution is shown on Figure 8. CO<sub>2</sub> is the product of combustion together with H<sub>2</sub>O. It is visible in the combustion zone and diffusing through the outlet. The CO<sub>2</sub> behave in a manner similar to the temperature (Figure 3) means that the combustion results the carbon dioxide.

### Conclusions

The Proto X-3 Bioenergy Micro Gas Turbine, the prototype of micro gas turbine system, will be run using LPG as its fuel. Since there are three kind of LPG available in the market, i.e., propane LPG, butane LPG and mixed LPG (mix of propane and butane), then the CFD simulations have been done for the gas flow in an annular combustion chamber of micro gas turbine system for all of these LPG using CFDSOF(r) software to see their characteristics against combustion. The combustion model used for simulations was finite rate and eddy dissipation model and the turbulence model is  $k-\varepsilon$  model.

The temperature distributions are almost the same between propane and butane LPG but a little bit different for mixed LPG where its contour is thinner. This is possibly caused by more air mass flow consumed by this type of LPG than the others. The highest temperature for propane, butane and mixed LPG are 1661 K, 1728 K and 1725 K respectively, meanwhile, the maximum temperatures at the outlet are 1441 K, 1447 K and 1554 K for propane, butane and mixed LPG respectively.

The highest velocity of gas at outlet is found in mixed LPG, while the propane and butane LPG have the almost similar velocity. This phenomenon might be caused by its higher air mass flow. From the CO<sub>2</sub> distribution, it is visible that CO<sub>2</sub> behave in a manner similar to the temperature. From the results of simulations, the mixed LPG is chosen to be used for the micro gas turbine because it has higher temperature and kinetic energy.

### Acknowledgements

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# **THEME : AGRICUTURAL SCIENCE AND PLANT BIOLOGY**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
October 4-6, 2016**

## Antimicrobial Activity of Chitosan Enriched with Lemongrass Essential Oil Against *Phomopsis vexans* of Eggplant

\*Nurul Faziha Ibrahim, Eleoni Rikan Marten

School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu;

\*Corresponding Author: nurulfaziha@umt.edu.my

### Abstract

Chitosan (CH) and essential oil (EO) have great potential as edible coatings due to their unique physiological and biological properties as food coating, preservative, antimicrobial and antioxidant. In this study, application of these compounds to inhibit the growth of *Phomopsis vexans* on eggplants was evaluated. The effectiveness of CH, EO and their combination in controlling phomopsis fruit rot were examined throughout an *in vitro* study. Results of the present study showed that combination of CH and EO at four different concentrations; 0.5% CH + 0.5% EO, 0.5% CH + 1.0% EO, 1.0% CH + 0.5% EO and 1.0% CH + 1.0% EO were significantly effective in inhibiting the growth of *P. vexans in vitro*. Therefore, combination of CH and EO has potential as an edible fruit coating that can reduce the use of chemical fungicides which in turn improve the food safety for consumers.

**Keywords:** chitosan, essential oil, phomopsis fruit rot, *P. vexans*.

### Introduction

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop that widely planted all over the world. The fruit can grow throughout the years and becomes important agriculture commodity, especially in Malaysia. However, the fruits are susceptible to a wide range of pests and pathogens which cause severe loss in all stages of both growth and development. Phomopsis blight caused by *Phomopsis vexans* is the most significant and widespread fungal disease that reduces the yield and marketable value of the crop. Occurrence of the disease can cause losses in yield production which could result in significant economic loss, especially in the fruit marketing chain (Prusky, 2011).

To overcome the occurrence of post-harvest disease, chemical treatments are usually applied to protect fruits and vegetables during transit, storage and marketing. However, numerous negative effects on the environment and human health have been attributed to the persistent application of chemical fungicides (Ranasinghe *et al.*, 2003). The use of fungicides is increasingly restricted due to public concern over food safety and demand on healthier food products. For this purpose, both chitosan and essential oil have potential as alternative treatments for chemical fungicides because they have antimicrobial properties, particularly applicable as edible coatings to prolong shelf-life, can preserve quality of fresh foods and have less environmental effects (Cheah *et al.*, 1997; Isman, 2000; Burt, 2004; Assis and Pessoa, 2004). Application of chitosan, lemongrass or combination of these two naturally occurring antimicrobial components, chitosan and lemongrass essential oil, could give a unique system that improved the antimicrobial properties of the existing treatments. The combination of lemongrass essential oils into the chitosan perhaps may reduce losses of active compounds due to evaporation and develop possibilities for prolonged antimicrobial action and improved food safety. Therefore, the objective of this study was to evaluate the antimicrobial effects of chitosan (CH), lemongrass essential oil (EO) and their combination against *P. vexans* isolated from eggplants (*Solanum melongena* var. *serpentium*).

### Materials and Methods

#### Isolation of fungal isolate

Eggplants with phomopsis blight symptoms were collected from commercial markets in Kuala Terengganu, Malaysia. Prior to isolation, the infected tissues were cut into 5 mm<sup>3</sup> pieces, and surface-sterilized by quick dipping in sodium hypochlorite and rinsing in sterile distilled water three times. Then, the tissues were blotted dry before plating on Potato Dextrose Agar (PDA). The plates were incubated at 27±2°C until mycelia grow. The growing colonies were single spore and identified based on morphological characteristic.

### Pathogenicity test

For pathogenicity test, healthy eggplants with uniform size were purchased from commercial market in Kuala Terengganu. All fruits were washed with tap water and surface-sterilized with NaOCl, before being rinsed with distilled water. All eggplants were air-dried prior to inoculation with fungal isolate of *P. vexans*. For this purpose, pure isolate of *P. vexans* was subcultured onto PDA and incubated for 7-days at 27±2°C. Then, the healthy eggplants were artificially wounded, before inoculated with 5 mm plug agar of *P. vexans* culture. Control fruits were inoculated with water agar (WA) without *P. vexans* isolate. The inoculated eggplants were placed in a plastic tray covered with plastic wrap and incubated at 27±2°C for 15 days. Appearance of brown lesions on eggplant fruits were evaluated every three days interval and scored using disease scales (Table 1) based on **Wokocho** (1990) with some modifications.

**Table 1.** Disease severity scales

Scale	Severity (%)
0	No visible disease symptoms
1	Infection less than 10%
2	Infection between 10% - 20%
3	Infection between 20% - 30%
4	Infection between 30% - 50%
5	Infection more than 50%
6	The whole fruit is infected

After scoring, disease severity (DS) was calculated using the formula as follows:

$$\text{Disease Severity (\%)} = \frac{\sum(n \times \text{score of disease scale})}{d \max \times \sum n} \times 100$$

(n=no of infected replicates, d= maximum disease scale)

### Preparation of EO, CH and CH+EO media

Essential oil of lemongrass (EO) was purchased from Essfa Essential Oil Company, Malaysia. Then, the EO was diluted with distilled water to prepare 0.5% and 1.0% solutions. Chitosan solutions, 0.5% and 1.0%, were prepared by dissolving chitosan powder in distilled water and acetic acid according to El Ghaouth *et al.* (1991). Then, the solutions were adjusted to pH 5.5-pH 6.0 before preparing the media. Each solution was mixed with PDA before poured into petri dish. For combination of CH+EO medium, solution of CH and EO were prepared separately before mix with Tween 20 and PDA.

### In vitro antifungal assay

Antifungal activity of *P. vexans* was evaluated on eight different treatments; T1: 0.5% CH, T2: 1.0% CH, T3: 0.5% EO, T4: 1.0% EO, T5: 0.5% CH + 0.5% EO, T6: 1.0% CH + 0.5% EO, T7: 0.5% CH + 1.0% EO, T8: 1.0% CH + 1.0% EO. Agar plug of 7-days old *P. vexans* cultures (5 mm diameter) was applied in the centre of petri dish for all the treatments. All plates were incubated at 27±2 °C for 21 days. Diameter of mycelial growth was measured and percentage of mycelial growth inhibition was determined according to the following formula:

$$IP = dc - dt \times 100/dc$$

(IP = Inhibitory percent, dc = mycelium growth diameter in control, dt = mycelium growth diameter in treated petri dish)

### Results and Discussion

An isolate of *P. vexans* was isolated from phomopsis blight symptoms of eggplant, and has been identified based on morphological characteristics. Based on colony appearance, *P. vexans* isolate grew as cottony layer with white to dark brown in colour. It produced many black spot fruiting bodies called pycnidia. Pathogenicity test results showed that the isolate was pathogenic to eggplant. After 15 days of inoculation, the inoculated area of eggplant appeared as dark brownish zones developed with yellow concentric rings. The symptoms on the eggplant were severe as the storage day increased (Table 2). After re-isolation, the same isolate of *P. vexans* was obtained, thus fulfilled the Koch's postulate. Control did not show any symptom.

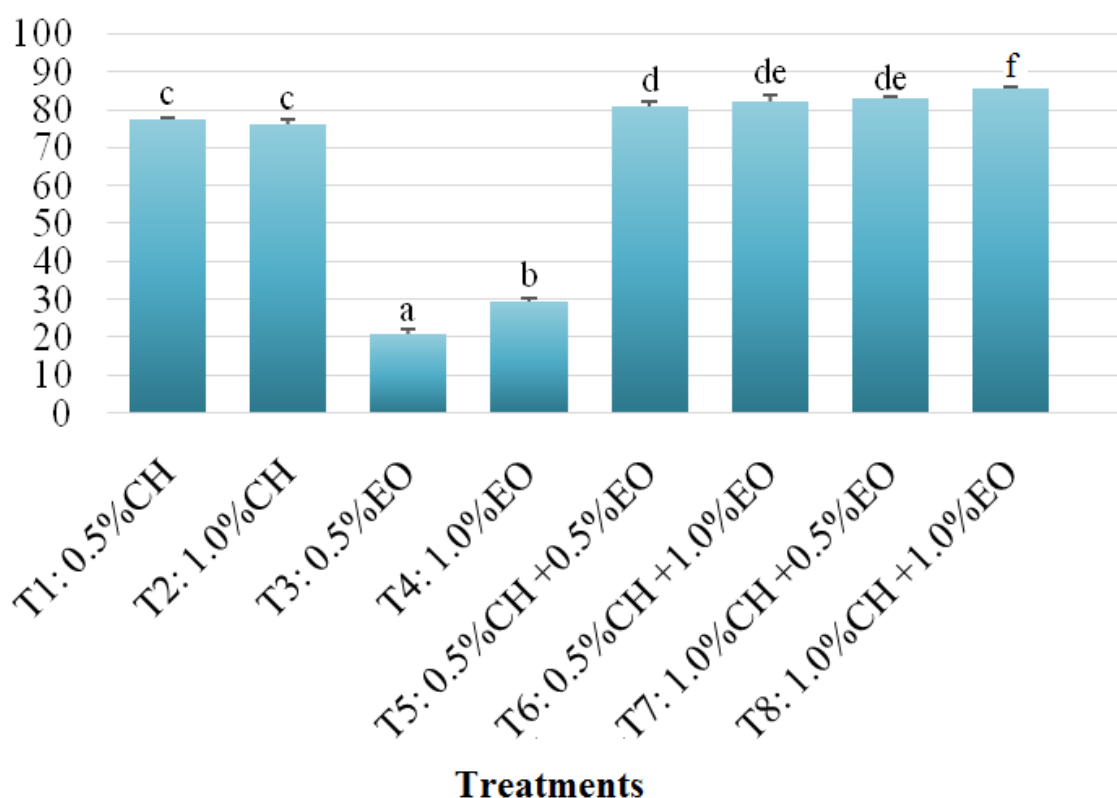
**Table 2.** Disease severity of eggplant inoculated with *P. vexans*

Days interval	Average DS (%)
3	13.9
6	47.2
9	61.1
12	69.5
15	86.1

Phomopsis blight or phomopsis fruit rot is a destructive disease caused by *P. vexans*. The fungal isolate has been considered to be restricted to *Solanum melongena* (Sherf and McNab, 1986). Occurrence of the disease has been reported in many warmer areas worldwide which reduce yield production of eggplant that lead economic loss (Smith *et al.*, 1988; Prusky, 2011). It can be easily transmitted throughout the seed as well as in harvested fruits. Seedlings from infected seed will show pre-emergence and post-emergence damping-offs which later become stem blight on the young eggplants (Pan and Acharya, 1995).

In this study, there were eight different treatments which were; T1: 0.5% CH, T2: 1.0% CH, T3: 0.5% EO, T4: 1.0% EO, T5: 0.5% CH + 0.5% EO, T6: 0.5% CH + 1.0% EO, T7: 1.0% CH + 0.5% EO and T8: 1.0% CH + 1.0% EO. Figure 1 shows the inhibitory percentage for each treatment against *P. vexans* isolate. From *in vitro* experiment, T5, T6, T7 and T8 were significantly effective ( $p < 0.05$ ) in inhibiting the growth of *P. vexans* compared to T1, T2, T3 and T4. Among all treatments, T8 shows the highest percentage (85%) which considered as the best treatment in inhibiting mycelial growth of *P. vexans*.

### Inhibitory (%)



**Figure 1.** Inhibitory percentage each treatment against *P. vexans* isolate

Based on the inhibition percentage of mycelial growth was significantly ( $p < 0.05$ ) influenced by different treatments and concentrations. Combination of CH and EO in T5, T6, T7 and T8 could effectively control the growth of *P. vexans* on PDA compared to single treatment using only chitosan or essential oil. However, combination of CH (1.0%) and EO (1.0%) in T8 was the best treatment in inhibiting the growth

of *P. vexans*. It demonstrated that T8 was the highest natural protective agent with some antimicrobial. Incorporation of chitosan and essential oil increase the potential of both compounds as antifungal agents. Lemongrass essential oil is widely used as edible coating since it has bactericidal & fungicidal properties against microorganism (Maqbool *et al.*, 2010).

Single treatment with CH or EO only showed that T1 and T2 were significantly better in inhibiting the *P. vexans* growth than T3 and T4. Treatment 3 (T3) with 0.5% EO showed the lowest inhibitory percentage and could be indicated as less effective treatment. Antimicrobial study against *Aspergillus niger* showed that the mycelial growth was reduced with increasing concentration of lemongrass essential oil (Sessou *et al.*, 2012). In real food system, essential oil was required at high concentration which could be attributed to reduce diseases in fruit (Abd-Alla *et al.*, 2011). It is similar to chitosan which showed higher antimicrobial activity as the dose increased (Edirisinghe *et al.*, 2012).

## Conclusions

Present study shows that chitosan (CH) enriched with lemongrass essential oil (EO) was effective to inhibit the growth of *P. vexans* isolate. This suggests that combination of CH and EO could be effective natural antimicrobial agents compare to synthetic fungicides to manage postharvest diseases of vegetable.

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## Fig Wasps Emergence Sequence and the Number of Nematodes Carried Out of *Ficus racemosa* Figs

<sup>1\*</sup>Jauharlina, <sup>1</sup>Eka Putra, <sup>2,3</sup>Stephen Compton

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>School of Biology, Faculty of Biological Sciences, University of Leeds, United Kingdom;

<sup>3</sup>Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa;

\*Corresponding Author: ljauharlina@unsyiah.ac.id.

### Abstract

Emergence of female pollinating fig wasps from their natal figs occurs in a sequence because of the small size of the hole(s) cut through the fig wall by the male fig wasps. Nematodes often develop inside figs, using the female pollinators to transport them between figs. The effect of position in the female emergence sequence on nematode loads was investigated using *Ceratosolen fusciceps* Mayr, a pollinator of *Ficus racemosa* figs. The study was conducted on several fig trees grown along the road in mountainous areas of Leupung and Lhoong Districts, Aceh Besar Regency, about 25 km to 40 km from the provincial capital, Banda Aceh. Although there was a variation between crops in nematodes carried, there were usually more nematodes on the first fig wasps to emerge. The first female wasps to emerge from figs might suffer from increased predation by ants that were waiting on the fig surface during the emergence period and the rapid emergence of most females might be an adaptation to reduce the predation

**Keywords:** Agaonidae, mutualism, non-mutualism

### Introduction

The interaction between fig trees (Moraceae) and their pollinating wasps is a striking example of an obligate mutualism that has been evolving for more than 60 million years (Ronsted *et al.*, 2005; Cook and Segar, 2010). Each *Ficus* species is pollinated by a single (or few) species of fig wasp (Hymenoptera: Agaonidae). The agaonid female wasps are the only pollen vectors for the figs, while the wasp offsprings feed and develop inside the figs.

Fig trees have a unique enclosed inflorescence (syconium or the fig), an urn-shaped structure which forms a hollow ball and is lined with numerous tiny flowers. The female wasps (foundresses), loaded with pollen, enter the receptive figs through a narrow often bract-lined ostiole. Inside the figs the foundresses either actively or passively pollinate flowers and lay eggs in some of them, and the foundresses then die (Weiblen 2002; Herre *et al.*, 2008). The wasp offsprings and the seeds develop over a period of a few weeks. The wingless male wasps emerge first from the galls and search for the galls containing female wasps to mate by inserting their genitalia through the hole that is made by the males. The female fig wasps then emerge into fig cavity, and at the same time the male flowers mature and are releasing pollens. The female wasps collect pollen before they come out from the fig through an exit hole (or holes) chewed by the male wasps through the fig wall. The male wasps generally die inside the fig or on its outer surface. The winged, mated, pollen-bearing female wasps disperse and search for new receptive figs to enter. The pollens brought by the wasps will be deposited deliberately onto the female flowers when pollination is active (Kjellberg *et al.*, 2001).

Several species of nematodes have been reported to be transferred into receptive figs by female pollinating wasps when they enter through the ostiole. The nematodes develop and reproduce inside the figs along with the development of seeds and wasp offsprings. The new generation of nematodes attach themselves to the female wasp offsprings when they are ready to exit from the natal fig and disperse in search for new receptive figs (Kanzaki *et al.*, 2009; Jauharlina *et al.*, 2012). The figs where the nematodes and wasps develop provide an ideal situation for the development of a 'passenger-vehicle' (nematodes and mite-fig wasps) relationship, since the nematodes need to disperse to enter another suitable fig as well as the fig wasps (Krishnan *et al.*, 2010). The nematodes typically attach themselves onto the offsprings of pollinator females to transport them from natal figs (Kanzaki *et al.*, 2009; Jauharlina *et al.*, 2015). Often their feeding behaviour is poorly known.

The timing of fig wasps emergence varies between species, particularly between species of fig wasps that disperse during the day or at night (Zachariades *et al.*, 2010). Ants (Hymenoptera: Formicidae)

are regularly found to prey on the pollinating wasp *Ceratosolen capensis* Grandi, and other fig wasps as they are exiting from the figs of the monoecious fig tree *F. sur* in Southern Africa (Cushman *et al.*, 1998). The male pollinators that emerge first from the fig are ten times more likely to be captured and killed by the ants that are waiting on the fig surface. Predation on male pollinators helps decrease predation on the female pollinators that emerge later (Zachariades *et al.*, 2010).

Earlier studies found that earlier female wasps that emerged together rapidly as a group were less likely to be taken by ants than later-emerging stragglers. The earliest female pollinators to emerge from the figs may however also have a higher risk of being captured by ants because not all the waiting ants will be occupied by other fig wasps (Ranganathan *et al.*, 2010; Zachariades *et al.*, 2010). The emergence sequence of female pollinators may also influence the number of nematodes carried by them, as nematodes need to be transported outside the figs by the females to search for other suitable figs. This research was conducted to examine whether the emergence sequence of female pollinating wasps is correlated with the number of nematodes carried.

## Materials and Methods

All presentations and discussions will be carried out in a forum. Two types of oral presentations are available, i.e. a ten-minute presentation with a five minute of discussion, and a three-minute presentation accompanied with a poster. More discussion is expected in the poster session. The use of MS PowerPoint® is recommended for all oral presentations.

The study was focused on *Ficus racemosa* L., a monoecious fig tree belongs to the subgenus Sycomorus. *Ficus racemosa* is the only monoecious Sycomorus species occurs outside Africa, and grows commonly along rivers in Southeast Asia (Corner, 1965). The figs produce in large clusters on short and leafless branches emerging from the trunk and main branches. During development, they change from small, thin-walled figs in A-phase to large, thick walled figs in later phases. The figs grow from 1 cm in diameter during A-phase up to more than 3 cm at maturity in E-phase (Pers. Obs). The color changes from green in younger figs becoming bright red when they are ripe (Paarakh, 2009). The figs of *F. racemosa* are pollinated by the fig wasp *Ceratosolen fusciceps* Mayr (Wang and Sun, 2009).

The study sites of the fig trees were located in the northern part of Sumatra Island in Aceh Province, Indonesia. The study was undertaken from June 2013 until February 2014. The trees grew along the road in mountainous areas of Leupung and Lhoong Districts, about 25 km to 40 km from the provincial capital, Banda Aceh. The region had a tropical climate that supported rainforest vegetation, with fairly constant average temperatures throughout the year and little diurnal variation. The annual average temperature was 27° Celsius. Monthly minimum and maximum temperatures were quite stable, with only slight variation between months. The average minimum temperature ranged between 22.1 and 23.9 °C, while maximum temperatures were between 30.2°C and 34.0° C (Data obtained from Blang Bintang Station, the closest Meteorological Station under the Indonesian Meteorological and Geophysical Agency).

Six mature D-phase figs from a crop were removed and placed separately inside net bags of 12 cm x 9 cm in size and tightened with a string to prevent the emerging wasps flying out. All fig samples were analyzed in the Plant Disease Laboratory, Department of Agrotechnology, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia. Any fig that released wasps before arriving in the laboratory was excluded from further observation. Each bag containing a fig was labeled and placed onto the laboratory bench, so that it could easily be recognized when the first wasp emerged. The 'foundresses' emergence sequence was divided into three categories: (1) the females that emerged during the first five minutes after the emergence of the first male, (2) the females that emerged from 5 to 10 minutes after the first emergence, and (3) the females that emerged more than 10 minutes after the first emergence.

The three categories of emergence were chosen based on preliminary observations of the emerging process of the fig wasps from their natal figs. The female fig wasps emerged as soon as the male pollinators exited the figs from an exit hole made by the males. During the first five minutes, the female fig wasps emerged at a high rate leaving almost no time lag between emergences of each female. In the next five minutes, the emergence was a bit slower with lags between two consecutive emerging fig wasps. After 10 minutes the emergence of female wasps was much slower than those in the first one. All fig wasps that will emerge have usually left the figs one hour after the first emergence of male pollinators.

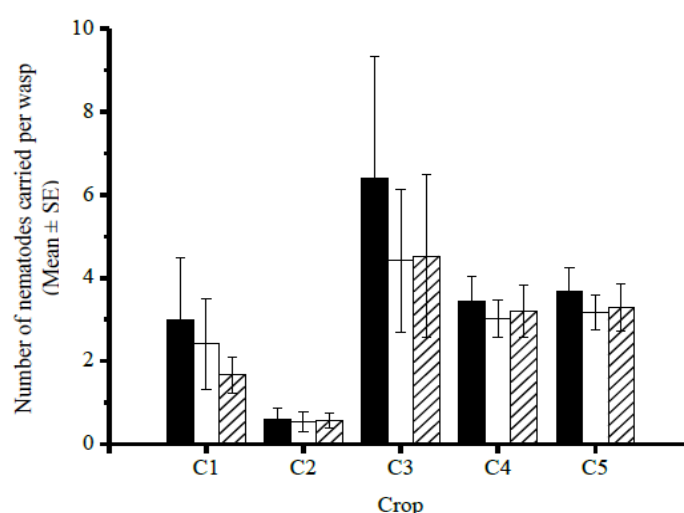
The female pollinator fig wasps that emerged from the figs were monitored during the first five minutes. Then the fig was quickly removed from the initial bag and placed in the second bag. Again, using a stopwatch the fig was left in the second bag for another five minutes. Each fig was then removed from the second net bag and placed inside the third bag where the fig was left for one hour to let all wasps emerge. Ten female pollinators were sub-sampled from each bag. Each wasp was placed individually onto glass slide with a drop of sugar water solution (40%). Emerging nematodes from the wasps were counted within one hour. Figs from five crops which came from five different trees were monitored.

Data were analyzed using the lme4 package in R programme (Ihaka and Gentleman, 1996) version 2.14.12. Poisson distribution was used as error in the models. Over dispersion in each model was corrected by including fig identity as individual level random effect. The response variables were the numbers of nematodes carried per female pollinator in each category of emergence

## Results and Discussion

All adult fig wasps (pollinators and non-pollinators) that were going to emerge (some fail to leave their galls) had emerged from the figs within one hour after the first wasp emerged. The wasps mostly emerged in the first ten minutes, the remaining wasps left the figs slowly. Out of the five crops, one (Crop 2) showed relatively lower numbers of nematodes attached per female pollinator at emergence compared to those of the other four crops (Figure 1). Not all of the sampled female fig wasps had nematodes with them, but there were four figs that had nematodes in all the sampled individuals. The highest proportion of female fig wasps with nematodes was found on the first females to emerge (emergence within the first five minutes), ranging between 50 and 100 % in each fig. The second emergence period had nematodes in between 30 and 80 % of the females and the last emergence group had nematodes in between 20 and 60 % in different figs.

The mean numbers of nematodes carried per female ranged between 0.60-6.40 for the first five minutes of female emergence, between 0.53-4.42 for the second period, and 0.57-4.53 for the last period, across five crops (Table 1). The first five minutes of emergence had nematode numbers that ranged from 0-51 per female, the second period (between 5 and 10 minutes) had 0-55 nematodes per female, and last females to emerge (after 10 minutes) had 0-33 nematodes per female. Position in the emergence sequence showed a significant effect on the number of nematodes carried by female pollinators (lmer,  $z = -3.441$ ,  $P = 0.0006$ ). The first females to emerge carried significantly more nematodes than those that emerged later, while both the second and last groups of females to emerge carried nematodes in similar numbers (Figure 1).



**Figure 1.** The numbers of nematodes carried by each female pollinator *Ceratosolen fusciceps* in relation to emergence times (female pollinators that carried no nematodes were included in calculations). The emergence sequence of female pollinating wasps was divided into three phases: (1) the wasps that emerged during the first five minutes (solid bars), (2) the wasps that emerged after 5 to 10 minutes from the first emergence (open bars), and (3) the wasps that emerged more than 10 minutes after the first emergence (hatched bars). (N = 30 wasps per fig, 6 figs per crop).

**Table 1.** The numbers of nematodes carried by female pollinators as they emerged from their natal figs in relation to their position in the emergence sequence

Crop	N1	N2	Number of nematodes carried per emerging female pollinator in relation to their emergence times (Mean $\pm$ SE)		
			< 5 minutes	5-10 minutes	> 10 minutes
C1	6	30	3.00 $\pm$ 1.48	2.42 $\pm$ 1.10	1.67 $\pm$ 0.44
C2	6	30	0.60 $\pm$ 0.28	0.53 $\pm$ 0.25	0.57 $\pm$ 0.18
C3	6	30	6.40 $\pm$ 2.92	4.42 $\pm$ 1.72	4.53 $\pm$ 1.95
C4	6	30	3.45 $\pm$ 0.60	3.02 $\pm$ 0.46	3.20 $\pm$ 0.63
C5	6	30	3.68 $\pm$ 0.58	3.17 $\pm$ 0.42	3.28 $\pm$ 0.58

N1 = numbers of figs sampled. N2 = number of wasps sampled in each fig. Number of wasps sampled in each crop was 180, and in total there were 900 wasps sampled across five crops observed

The emergence sequence of female pollinators in this study was similar to that described by Zachariades, Schatz and Compton (2010) for an African species, with a rapid exit of female pollinators immediately after the exit hole was created by the males. Whether the first females to emergence from the figs come from the galls located toward the centre of figs or from peripheral galls toward the fig wall was still unknown. This study found that although there was a great variation between crops in nematode loads on each female pollinator, there were generally more nematodes carried on the first females to emerge. Reflecting this, the maximum mean numbers of nematodes carried per female was 6.40 for the early female emergence period, 4.42 for the next five minutes of emergence, and 4.53 for the last emergence period.

Ants can be a danger for pollinators because they are frequently found to capture pollinating wasps as they enter or emerge from figs (Ranganathan, Ghara and Borges 2010). The presence of predatory ants during the emergence of fig wasps from D-phase figs of *F. racemosa* is stimulated by the volatiles released by D-phase figs or by the scent of the emerging fig wasps themselves (Ranganathan and Borges 2009). The predatory ants mostly found on *F. racemosa* figs in India are *Oecophylla smaragdina* (Fabricius) and *Technomyrmex albipes* (Smith) (Ranganathan, Ghara and Borges 2010). Several other predators also attack pollinators at these times, such as dragon flies, staphylinid beetles, phorid flies, and birds (Bronstein, 1988). Earlier in their development, the presence of ants can be beneficial for the pollinating fig wasps because they reduce attacks by non-pollinating fig wasps that lay eggs in younger figs (Cushman *et al.* 1998). The first female wasps to emerge from figs may suffer from increased predation by ants that are waiting on the fig surface during the emergence period (if the ants are not satiated by earlier-emerging male fig wasps) and the rapid emergence of most females may be an adaptation to reduce ant predation (Zachariades *et al.*, 2010).

## Conclusions

There was a variation between crops in nematodes carried by female fig pollinating wasps, however there were usually more nematodes on the first fig wasps to emerge. Most female wasps emerged in the last five minutes after the first female appeared from the exit hole

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## Study on Fermented Complete Feed by Using Sago Residues as Main Sources Diet on Performance and Internal Organ of Sheep

\*Samadi, Sitti Wajizah, Yunasri Usman

<sup>1</sup>Department of Animal Husbandry, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia.

\*Corresponding Author: samadi177@yahoo.de

### Abstract

Enriched animal feed by fermentation has been widely used to improve feed digestibility and animal production. One of the agroindustry by products that can be utilized as animal feed is sago residue which is abundant and readily available in Indonesia. The objectives of this study were to evaluate performance and internal organ of sheep which were fed fermented complete feed by using agro-residues from sago starch processing industries as main source diet. Feed was formulated on the basis of 40% sago residue and mixed with other ingredients (rice brand, coconut meal, bread by product, soybean meal and soybean hulls) to fulfill the requirement of sheep with 16.10% of CP, 2.80 Mcal ME/kg and TDN 60.88% based on calculation. Mineral and Urea were added to the complete feed to reach mineral and CP requirements of sheep. Complete feed formulation was fermented using 3 commercial fermentation products (Saus Burger Pakan® (SBP), Probion® and EM4®) for 21 days. Totally 16 sheep weight of 14.55±0.33 kg were used in this study. The model used for statistical analysis was a randomized complete block design (RCBD) with 4 treatments (control and 3 different commercial fermentation products) and 4 replications. Parameters measured were animal performances (body weight gain, feed consumption, FCR and final body weight) and internal organs. The results of this study indicated that administration of different commercial fermentation products into fermented complete feed based on sago residues were not significantly influenced ( $P>0.05$ ) on sheep performance. In this study, internal organs were not statistically calculated and presented in describing information.

**Keywords:** sheep, sago residues, complete feed, fermentation, performance, and internal organ

### Introduction

It is well recognized that crop and agro-industrial residues are of low nutritive value and relatively low digestibility. It makes crop residues and agro-industrial by products to be unpalatable, thus their consumption is also low. Several methods have been introduced to modify nutrient contents and digestibility of feed from crop residues and agro-industrial by products (Rahman *et al.*, 2011; Shrivastava *et al.*, 2011; Wajizah *et al.*, 2015; Samadi *et al.*, 2015).

Sago residues from sago starch processing industries are abundant and readily available in Indonesia. However, sago residue contains low protein and high crude fiber. According to Tampoebolon (2009) sago waste contains 28.30% crude fiber and only 1.36% crude protein. In addition, Linggang *et al.* (2012) informed that sago residues consisted of starch (58%), cellulose (23%), hemicellulose (9.2%) and lignin (3.9%). As consequences, sago residue is slow and limited ruminant degradation of the carbohydrate compound in the rumen. Furthermore, Van Soest (2006) stated that because of low content of nitrogen, it makes main deficiencies from agro residues resulting in the low value of residues as feed for ruminant animals. Studies to improve low quality residues as animal feed have been conducted by using various methods such as physics (Samadi and Yu 2011; Samadi *et al.*, 2013), chemistry (Bata, 2008) and biology (Pandey *et al.*, 2000; Wajizah *et al.*, 2015; Samadi *et al.*, 2015). Feed technology fermentation is practical and promising alternative to improve nutritional value of agro residues. Research conducted by Wulandari *et al.* (2014) indicated that there was not negative effect on application cocoa pod on animal feed after fermentation with the best in vivo treatments was fermented complete feed. The objectives of this study were to evaluate performance and internal organ of sheep fed fermented complete feed by using sago residues as main sources diet.

### Materials and Methods

#### **Sago residues and diet formulation**

In this study, feed was formulated based on 40% sago residue and mixed with other feed ingredients such as rice brand, coconut meal, bread by product, soybean meal and soybean hulls to reach nutritional sheep requirements (16.10% of CP, 2.80 Mcal ME/kg and TDN 60.88%) (SNI and NRC, 2007). Mineral

and Urea were added in the complete feed to fulfill mineral and CP requirements of sheep. Complete feed formulation was fermented by administration three commercial fermentation products (Saus Burger Pakan® (SBP), Probion® and EM4®). These commercial fermentation products like SBP consists various microorganisms such as cellulolytic, lactic acid, amylolytic microbes (Wulandari *et al.*, 2014b). Microorganisms from commercial products were activated in the 2 % of molasses solution for 2 hours, before complete feed was fermented (Wulandari *et al.*, 2014b). Samples were maintained for anaerobe fermentation at room temperature for 21 days. Composition of feed formulation for this study is shown in Table 1.

**Table 1.** Composition of experimental diet based on sago residues as main ingredients (% as fed)

Ingredients	Treatment*			
	F <sub>0</sub> (Control)	F <sub>1</sub> (SBP)	F <sub>2</sub> (Probion)	F <sub>3</sub> (EM-4)
1. Sago residue	40	40	40	40
2. Rice brand	18.5	18.2	18.2	18.2
3. Coconut meal	18	18	18	18
4. Bread by product	2.5	2.5	2.5	2.5
5. Soybean meal	8	8	8	8
6. Soybean hulls	8	8	8	8
7. Urea	2	2	2	2
8. Molasses	1.5	1.5	1.5	1.5
9. NaCl	0.5	0.5	0.5	0.5
10. Mineral*	1	1	1	1
11. Commercial Fermentation Product**	0	0.3	0.3	0.3
TOTAL	100	100	100	100

\*F<sub>0</sub>= Control (without fermentation); F<sub>1</sub>= Fermentation with SBP; F<sub>2</sub>=Fermentation with Probion and F<sub>3</sub>= Fermentation with EM-4

### Animals and research procedures

Totally 16 male local sheep with the weight of 14.55±0.33 kg (average ± SEM) were used in this study. Before conducting the experiments, sheep were injected hematodin (Romindo Primavetcom) with the dosage of 1.5 ml (taurine: 0.2 g; ammonium ferric citrate: 2 g; methionine: 1 g; histidine-HCL: 0.5 g; tryptophane: 0.25 g; cobalt acetate: 0.05 g; cynocobalamin: 0.001 g dan Excipient: 100 ml). Sheep were also administrated anthelmintic Invervet (Vetanco, Argentina) with the dosage of 0.5 ml each. Before data collection, all sheep were weighted and randomly allocated at the cages (0.5 x 1.0 m). Each cages was equipped with individual feeding and drinking systems. One week before doing research, cages were disinfected by using Rodalon (Pyridam) with the dosage of 15 ml per 10 liter water to reduce pathogen micro-organisms and insects. Feed adaptation was for a week to adapt the animals with the feeding and research environment and controlled for 24 hours, Performance data (body weight gain, feed consumption and Feed Conversion Ratio) were collected for 60 days. Feed and water were provided ad libitum. At the end of experiment, all animals were weighted. Animals used for carcass and internal organ data were fasted for 24 hours and slaughtered thereafter.

### Statistical analysis

Statistical analyses ( $p < 0.05$ ) was run with the program Statistical Software Package SPSS. The model used for the analysis was randomized complete block design (RBD) with 4 treatments (control and 3 different commercial fermentation products) and 4 replications. The following is formula applied for statistical analysis:  $Y_{ij} = \mu + T_i + \beta_j + e_{ij}$ , where,  $Y_{ij}$  was an observation of the dependent variable  $ij$ ;  $\mu$  was the population mean for the variable;  $T_i$  was the effect of the fermentation, as a fixed effect,  $\beta_j$  represented the effect block and  $e_{ij}$  was the random error associated with the observation  $ij$ . Differences between variables were compared by a one-way analysis of variance (ANOVA). Verification of variance homogeneity and identification of statistical significance was applied by duncan multiple range test. Observations with ( $p < 0.05$ ) were considered to be statistical significant and trends were declared at  $P < 0.10$ .

## Results and Discussion

### Animal performances

Body weight gain was a reflection of consumption accumulation, fermentation, metabolism and absorption of nutrition into body. Excesses of feed from body maintenance will be utilized to increase body weight gain and it could be reflection of quality and bioavailability of feed (Anotonius, 2009). The average of body weight gain from this study was between 84-98 g/day. The result of study indicated

that complete feed fermented by SBP, Probion and EM4 did not significantly influence ( $P>0,05$ ) on body weight gain (Table 2). However, body weight gain of sheep fed complete feed fermented by SBP was higher 16.67%, 15.29% and 11.36% to control, Probion and EM4 respectively.

**Table 2.** Performances of sheep fed fermented complete feed based on sago residues by using different starters.

Parameters	Treatment			
	K	SBP	P	EM4
BWG (g/d)	84.00±7.00	98.00±10.00	85.00±9.00	88.00± 8.00
FI (g/e/h)	834.33±48.83	978.13±34.34	1017.63±50.71	1020.71±35.87
FCR	10.18±0.35	10.09±0.01	11.89±1.10	12.06±0.66

Note: K (Control); SBP (Saus Burger Pakan); P (Probion); EM4 (Effective microorganism-4)

In accordance with Wijaya and Utomo (2001) feeding sheep with palm oil by product fermented with 1% EM based on body weight gain (BWG) of local sheep increased 83 g/day body weight compared to non-fermented palm oil by product with only 63 g/day. This result was better compared to other researches in which Haryanto *et al.* (2002) reported that administration of probion (0.5-1.5%) from concentrate with the basal diet of elephant grass (*ad libitum*) and 1% of commercial concentrate resulting in body weight of 46.4-49.3 g/day.

In this study, there was no significantly difference on BWG ( $P>0,05$ ) due to no significantly difference on dry matter feed intake. Parakkasi (1999) stated that body weight gain was influenced by feed intake. In addition, feed consumption was effected by coefficient digestibility, feed quality, fermentation, physiological status, physical and chemical characteristics and palatability in the rumen (Leng, 1991). Increase of BWG was also supported by improvement of crude fiber ferment ability,  $\text{NH}_3$  utilization, and crude protein utilization. Crude protein utilization was related to utilization of nitrogen, where each gram of nitrogen consumed to be used as body weight gain (Kardaya, 2010). Wulandari *et al.* (2014) also reported that sheep fed complete feed by using pod cacao as basal diet with and without fermentation (SBP) was not significantly ( $P>0.05$ ) on BWG, dry matter consumption and feed conversion ratio (FCR).

Difference from research conducted by Kamalidin *et al.* (2012) fermented pod cacao with Biofti, there was a significant increase of feed consumption at fermented pod cacao compared to non-fermented cacao. In this experiment, feed was formulated by using 40% of pod cacao. However, increase of feed consumption was not followed by improvement of BWG and FCR, although fermented pod cacao was higher of BWG compared to control. Feeding of fermented putak with *Trichoderma reesei* and *Aspergillus niger* by using local male goat as experimental animals increased feed consumption with the increase of fermented putak consumption. This is positive indication between fermented putak consumption and nutrient consumption since protein organic content such as single cell protein (SCP) was increased by fermentation of putak. Feeding of 40% fermented putak in the concentrate had no negative effect of growth and digestibility (Hilakore, 2008).

Dry matter consumption was not significantly difference ( $p>0.05$ ) between control and fermented complete feed (SBP, Probion and EM4) with the amount of 978.12; 1017.63 and 1020.71 g/day for SBP, Probion and EM4 respectively (Table 2). Although it was not significantly difference, fermented complete feed tended to give positive results with higher consumption compared to control. The amount of feed consumption was already fulfilled the feed requirement of sheep with the 100 g BWG namely 840-950 g/day (Haryanto dan Djajanegara, 1993). It was assumed that fermented feed improved dry and organic matter consumption and create good rumen condition to stimulate acceleration of microbe leave rumen for further digestibility process. Besides, it was effected by dry matter consumption and feed quality, BWG was also effected by nutrient utilization efficiency in the metabolic process in the body relating to FCR value.

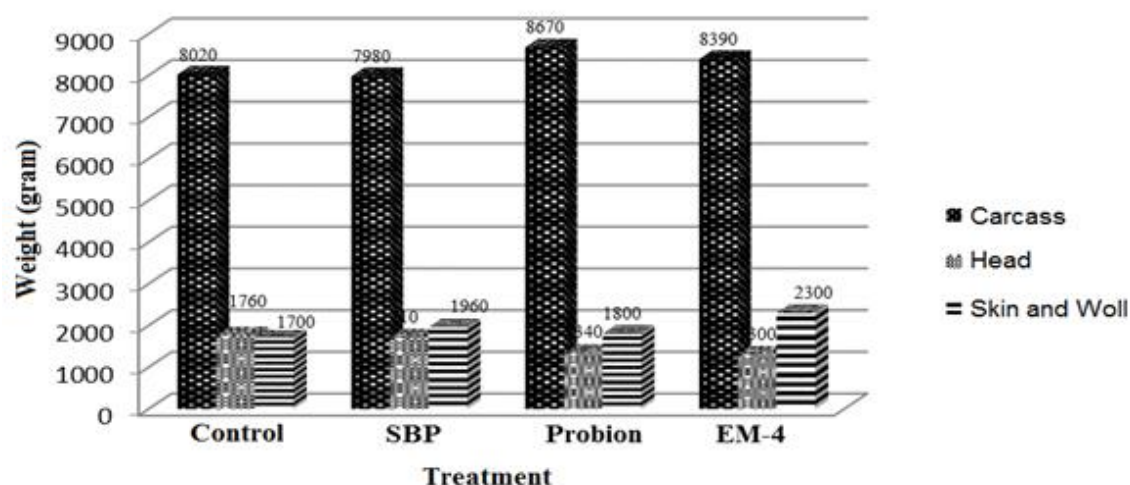
FCR value was calculated by dividing between dry matter feed consumption and the average of BWG per day. In our study, FCR had no significant effect ( $p>0.05$ ) between control and fermented complete feed on FCR of male sheep with the FCR of 10.18; 10.09, 11.89 and EM4 12.06 respectively for control, SBP, Probion and EM4 (Table 1). Kurniawati (2003) defined that inoculum was a group of selected proteolytic, ligninolitic, cellulolytic and lipolytic microbe that were able to decompose complex organic in the feedstuff to be simple organic and easy to be digested. Research conducted by Wulandari *et al.* (2014) FCR of sheep fermented complete feed based on pod cacao was better than non-fermented complete feed with the value of between 7.5-7.7. However, the value of FCR in this study was better with the value of 10.09-12.09. In accordance to Purbowati *et al.* (2009) feed conversion in the tropical sheep was with the range of 7-15. It means that to produce 1 kg of meat required 7-15 g of feed. The



lower of FCR value is the better of feed utilization by animals. Based on BWG and FCR results, it was concluded that complete feed with the basis of sago residues with and without fermentation was able to fulfill both maintenance and production of sheep.

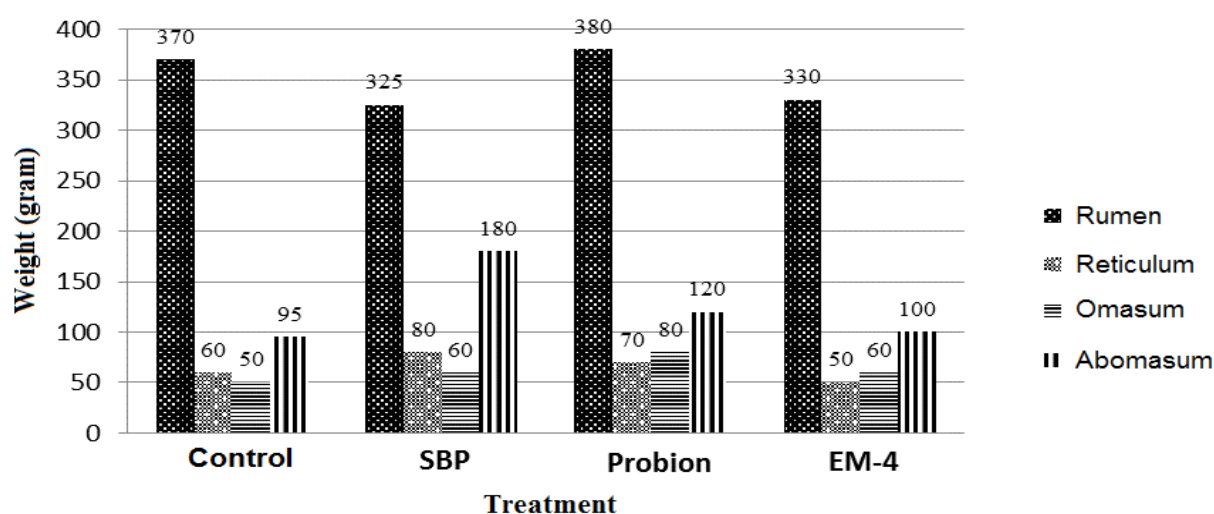
### Carcass and non-carcass

In this study, carcass and non-carcass were not statistically calculated due to limited treatment replication and were only presented in description. Picture 1 presented the weight of carcass, head and feather of sheep.



**Figure 1.** Carcass weight, head, skin, and wool of sheep fed fermented complete feed with the basis of sago residues by using different commercial fermentation products (Note: SBP (Saus Burger Pakan); EM4 (Effective Microorganism-4))

Carcass weight of treatment fermented complete feed and control was 8020 gr (44%), 7980 gr (41%), 8670 gr (43%), and 8390 (43%) respectively for control, SBP, Probion and EM4. Haronto (2000) reported that although administration of probiotic was not significantly effect on BWG but influenced the fat content of carcass. This is due to the change of energy utilization not for fat deposition instead of meat tissue production. This was probably due to the ability of probiotic to digest organic matter in rumen and the most of energy produced was utilized to produce meat and only small amount to be used as fat deposition.



**Figure 2.** Ruminant compartment of sheep fed fermented complete feed with the basis of sago residues by using different commercial fermentation products (Note: SBP (Saus Burger Pakan); EM4 (Effective Microorganism-4))

Carcass weight was strong correlated with feed protein consumption. The higher of feed protein consumption was the higher of animal growth (Boorman, 1980). Feeding of high quality feed based on

requirement would increase BWG and results in high of carcass weight. This is in accordance with Anggorodi (1994) stated that sheep productivity was determined by quantity and quality of feed. Soeparno (1998) informed that feed consumption and digestible nutrient influenced the growth of animal organs and other animal components including ruminant compartments, digestive tracts and internal organs of animals as shown in Figures 2, 3 and 4.

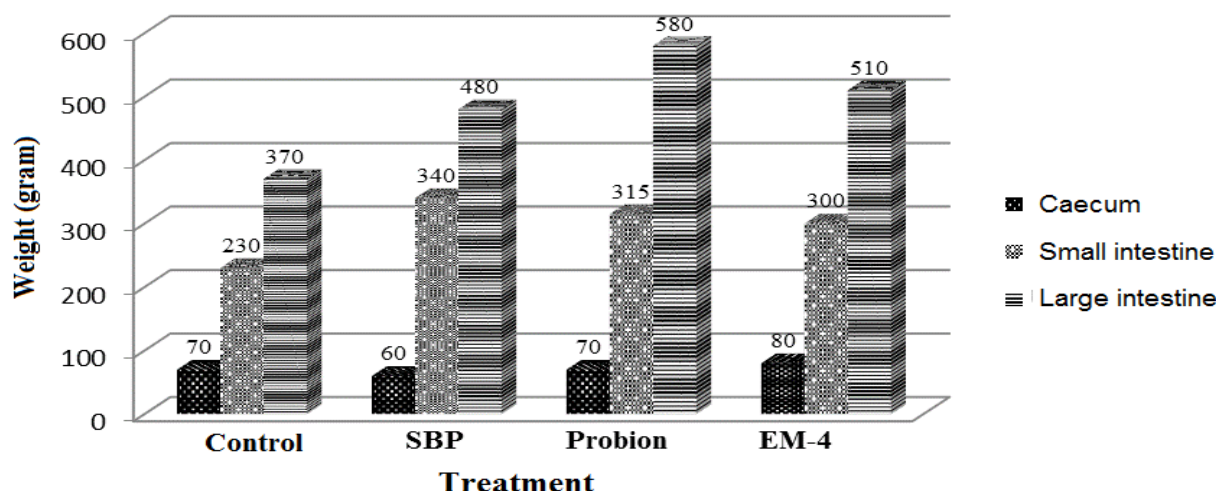


Figure 3. Digestive tracts of sheep fed fermented complete feed with the basis of sago residues by using different commercial fermentation products (Note: SBP (Saus Burger Pakan); EM4 (Effective mikroorganisme-4))

Soeparno (1998) stated that sheep consumed high energy feed having lungs, liver, ruminant compartment and digestive tracts was heavier than that of consumption low energy feed. Nutritional treatments were different effects on internal organ weight such as lungs, kidney, meanwhile external non-carcass such as head and legs were not difference (Soeparno, 1998). It means that sheep with the highest weight had high weight and total edible portion, meanwhile low weight sheep had low weight and total edible portion (Swatland, 1984). Ngadiyono *et al.* (2000) reported that administration of bioplus in the cattle feed increased the percentage of skin, lungs, liver, kidney, tail and fat internal organs (viscera).

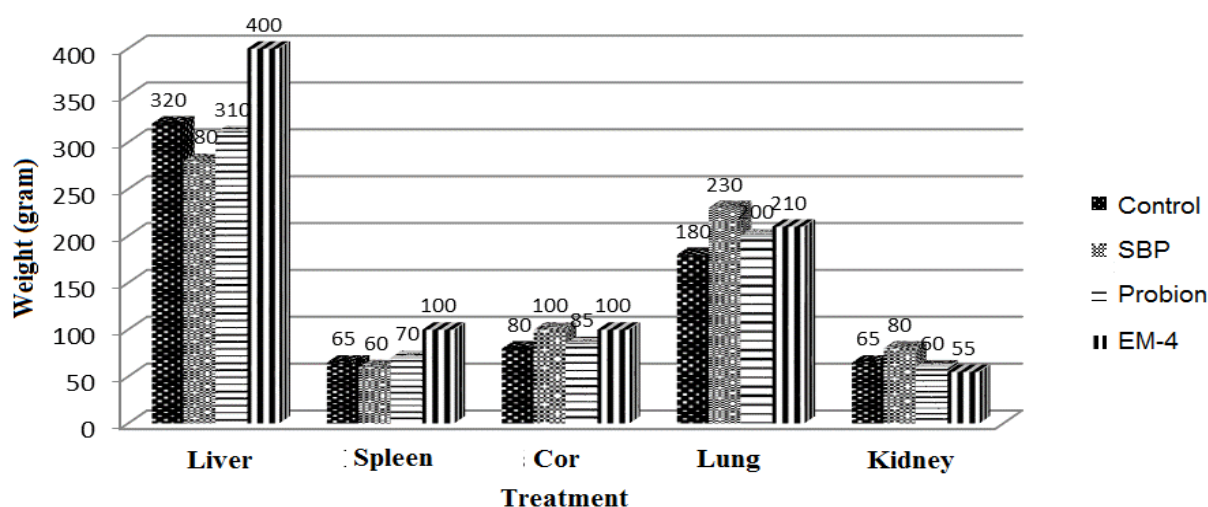


Figure 4. Internal organs of sheep fed fermented complete feed with the basis of sago residues by using different commercial fermentation products (Note: SBP (Saus Burger Pakan); EM4 (Effective Microorganisme-4)).

## Conclusions

The results of this study indicated that administration of different commercial fermentation products into fermented complete feed based on sago residues was not significantly influenced ( $P > 0,05$ ) on sheep

performance. In this study, internal organs were not statistically calculated and presented in describing information.

## Acknowledgements

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## Analysis of Drought Severity and Hydrological Disaster Mitigation Efforts in Krueng Jreue Subwatershed, Great Aceh

<sup>1</sup>\*Helmi, <sup>2</sup>Hairul Basri, <sup>2</sup>Sufardi, <sup>2</sup>Helmi

<sup>1</sup>Agriculture Graduate Programs, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: helmiusi@gmail.com.

### Abstract

Drought is an act of nature most powerful on the availability of the water supply, which necessary for the agricultural benefit and to human need. There are three phases of drought research analysis: (1) Identifying precipitation station at research areas. Monthly precipitation data using rain observation data (2005-2014) from 2 stations in Indrapuri and Jantho; (2) analyzing precipitation to get the drought meteorology index of each station precipitation with the gauge standardized precipitation index (SPI); and (3) interpolation analysis of Inverse Distance Weighted (IDW) method toward drought index value of each station to get the distribution of drought. Analysis using the 3 monthly scale SPI method (SPI-3) showed that the precipitation in 2005-2014 year was not very dry because only in June-July-August (JJA) experienced dry conditions with the drought index average among -0,80 until -1,00 for 10 years. The rest months September-October-November (SON), December-January-February (DJF) and March-April-May (MAM) were at normal condition, with the -0,99-0,99 value range of the SPI. The hydrologic disaster mitigation may be done by structural (optimize the development of the Krueng Jreue dam, rehabilitation and irrigation maintenance. Water storage excess in the rainy season for use in the dry season), and nonstructural (drought prediction, reforestation, planting water saving plan, the use of mulch and organic compound).

**Keywords:** Standardized Precipitation Index, Drought, Distribution, Hydrological Disaster Mitigation, Krueng Jreue Subwatershed

### Introduction

Krueng Jreue Sub Watershed, Krueng Aceh Watershed, cannot be separated from the pressures caused by human activities. The high rate of population growth and land use activities, lead continue to changes in land used. That categorized as critical watershed assigned as priority watersheds based on the Ministry of Forestry regulation No. 328/2009.

The results of the analysis of land cover Citra Spot 5 in 2013, during the 2009 to 2012 period there has been a change in land use in Krueng Jreue Subwatershed which caused a reduction in the primary forest of 1584.81 ha (6.82%) to 1576.51 ha (6.79%) or decreased to 8.30 ha (Bappeda Aceh, 2013). Reduced forest land causes the reduction of water flow in watershed, marked by insufficiency of water. Availability of water present in the Krueng Jreue Subwatershed ranged from 0.24 to 3.22 m sec<sup>-1</sup>. While total water demand for agriculture and households amounted to 0.18 to 6.44 m sec<sup>-1</sup> (Isnin *et al.*, 2012).

Land and water resources relate to the hydrological cycle. Climate change has an effect on changes in the hydrological cycle, including floods and droughts (Nugroho *et al.*, 2013) which are considered as hydrological disaster. In addition to flooding, the impact of other changes in the hydrological cycle due to climate change as well as the criticality of the watershed is drought. The phenomenon of drought impacts on agriculture, forestry, plantation and water resources (Adiningsih, *et al.*, 2014). Drought is a threat that often disrupts the system and the production of food crops (Triatmoko *et al.*, 2012), it also has become a regular problem in some areas, but due to the mishandling, the prevention and the control are running slowly, then the problem becomes prolonged and unresolved (Pratama *et al.*, 2013).

A hydrological disasters cannot be avoided, but with the development of science and technology, supported with accurate data, it can be anticipated to minimize all kinds' of environmental damage. Early warning is a major factor in disaster risk reduction and it is necessary to anticipate a hydrological disaster that will minimize losses, it can be anticipated by taking several measures for disasters and for stakeholders, they are expected to create policy that help society becomes well prepare for disasters (Bokal *et al.*, 2014).

These facts suggest the importance of understanding the characteristics of the region and its response toward the changes hydrological cycle as a result of climate change (Paulson *et al.*, 1985; Van Huijgevoort *et al.*, 2014). Including Krueng Jreue Subwatershed which is an important information for planning, territory management and early anticipation of the negative effects and the risk of hydrological disaster, both in the short and long term.

Based on the above problems, it is necessary to conduct the research aimed at analysing the causes of hydrological disasters parameters that occur in subwatershed based on biophysical and climatological aspects. This study aimed to analyze the severity of the drought by Standardized Precipitation Index (SPI) (Bhuiyan *et al.*, 2006; Hayes *et al.*, 2011), which changes the characteristics of the land has been affecting the classification of the severity of the drought Krueng Jreue Subwatershed. Having this research will be obtained hydrological disaster mitigation efforts in Krueng Jreue Subwatershed, so that the negative impacts and risk of drought damage can be minimized.

## Materials and Methods

Research was conducted in the Krueng Jreue Subwatershed, Krueng Aceh Watershed, Aceh Besar. These 23218.06 ha areas is located at 5°12'-5°28' N and 95°20'- 95°32' E. The study was conducted over three months, in October 2015 - December 2015.

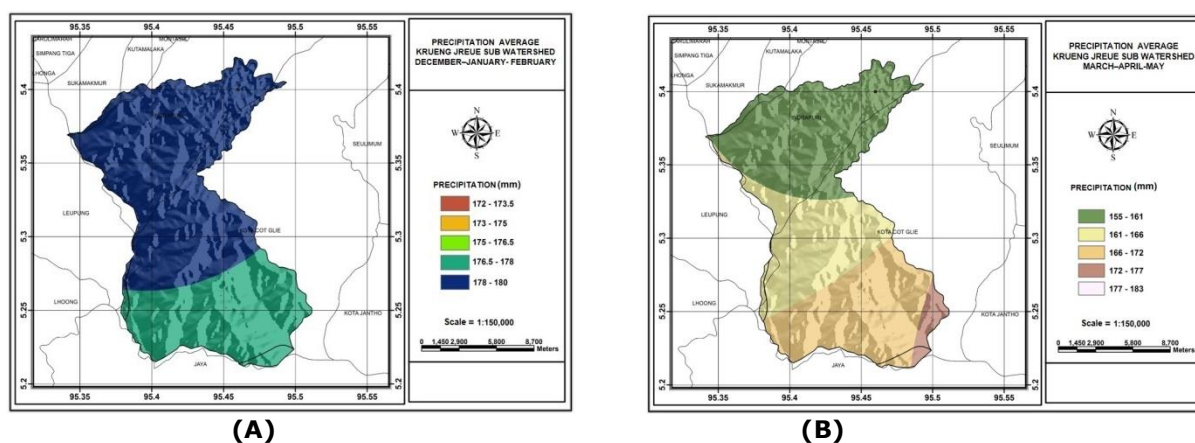
Materials used: administrative maps, observatories station's maps and precipitation map, each scale of 1: 50,000 and precipitation data from 2005 to 2014 period. The equipment are GPS, altimeter and digital cameras.

Descriptive method with field surveys was used in this research. Determination of precipitation in Krueng Jreue Subwatershed done Isohyets method based on precipitation data in stations Indrapuri and Jantho. Drought index values calculated from the rain by the method of Standardized Precipitation Index (SPI). Classification of the severity of a drought based on the SPI index, consisting of five classes: (1) dry ( $\leq -1.50$ ), (2) moderately dry ( $-1.00$  -  $-1.49$ ); (3) normal ( $-0.99$  -  $0.99$ ); (4) moderately wet ( $1.00$  -  $1.49$ ); and (5) wet ( $\geq 1.50$ ) (Ceglar, 2007). The SPI value obtained from precipitation stations in each month starting from January to December in 2005 to 2014. Determination of drought distribution of, the SPI value resulted from interpolated Inverse Distance Weighted (IDW) method.

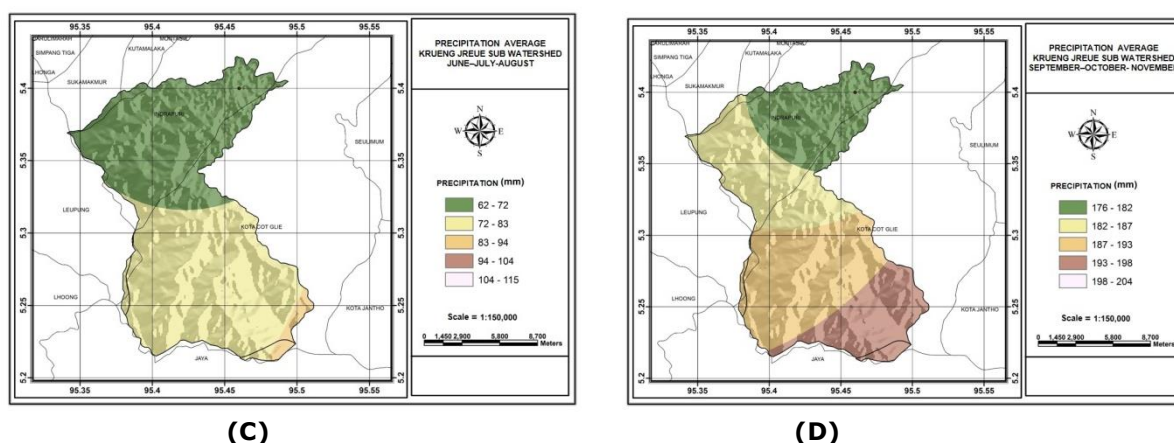
## Results and Discussion

### Spatial Analysis Seasonal Precipitation Territory

Spatial analysis on precipitation was done to identify areas have drought potential based on precipitation volume and to compare the size of precipitation at each precipitation station. The results of spatial analysis on seasonal precipitation in Krueng Jreue Subwatershed starting from January-December can be viewed on a contour map Isohyets in Figure 1.







**Figure 1.** Contour map isohyet in the period of December-January-February (A), March-April- May (B), June-July-August (C), and September-October-November (D)

Figure 1A-D show the northern subwatershed had less precipitation than the southern part, where the region had a minimum precipitation in June-July. The southern subwatershed that dominated by primary and secondary forests had a high precipitation. While the northern part that dominated by settlements and paddy fields have low range of precipitation. Maximum precipitation in the southern subwatershed occurred in November-December. The minimum precipitation on the northern subwatershed was 67 mm per month and occurred in June. Overall, in Krueng Jreue Subwatershed had maximum precipitation in November and minimum precipitation in July.

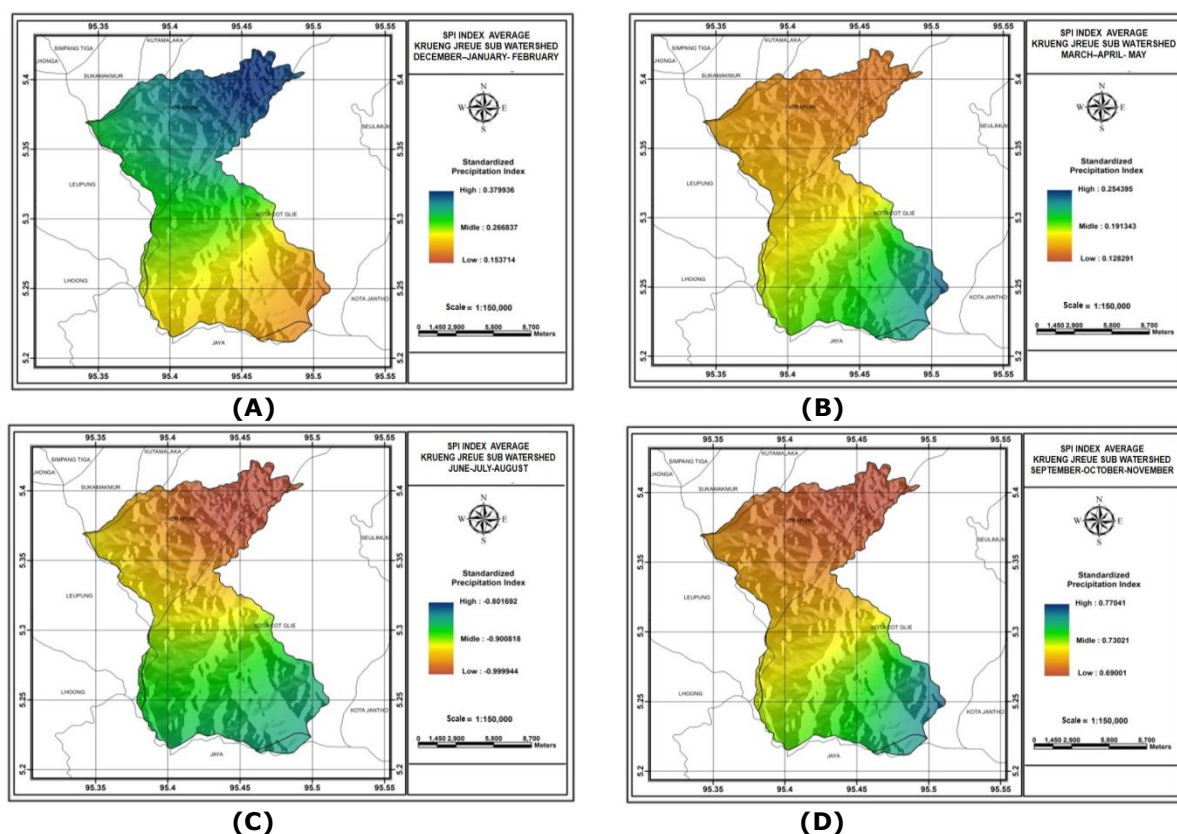
Krueng Jreue Subwatershed has a varied topography with heavy orographic and forest percentage is above 30%. This causes precipitation influenced by monsoon and local winds. Indrapuri precipitation stations located in the northern part of the subwatershed, the rain is more influenced by the local climate. Van Loon and Laaha (2015), shows that the duration of drought and water deficit are governed by a combination of climatic factors and watershed control, but not in the same way. Areas have a maximum precipitation or surplus subwatershed are located in the southern part with the maximum precipitation occurred in the period September-October-November (Fig. 1D), followed by December-January-Feb (Fig. 1A), with a range of precipitation 193-198 mm per month and 176-178 mm per month, respectively. Areas that have minimum precipitation or deficit is situated in the north with the peak of minimum precipitation occurs in the period June-July-August (Fig. 1C) followed by March-April-May (Fig. 1B), with precipitation ranged from 83-94 mm per month and 172-177 mm per month, respectively.

### **Drought Severity Index**

For the years 2005-2014, SPI in Krueng Jreue Subwatershed experienced moderately dry conditions that occurred in July at Indrapuri stations and from June to July in Jantho Station, with values ranged from -1.10 to -1.16. At the station Indrapuri, wet and moderately wet months were November and December, while other months were normal. The wet month in Jantho station was November, while other months were normal. In the same periods, all stations did not experience very dry condition. A moderately dry condition only occurred in the period of June-July-August. The worst drought occurred in July (SPI value was -1.23), where values of index reached the level of moderate drought severity (SPI values ranged from -1.00 to -1.49). Results of previous studies showed that duration and severity of drought have different spatial patterns in each watershed (Agwata, 2014).

### **Distribution of Drought**

Determination of drought distribution, the result of SPI-3 from Indrapuri station and Jantho in December-January-February (DJF); March-April-Mei (MAM); June-July-August (JJA); and September-October-November (SON) interpolated with Inverse Distance Weighted (IDW) interpolation method (Bhuiyan, 2004). Distribution map of drought or drought index contours of each period in Krueng Jreue Subwatershed is shown in Figure 2.



**Figure 2.** Drought index contour map in the period of December-January-September (A), March-April-May (B), June-July-August (C), and September-October-November (D)

Distribution of the drought period of DJF is shown in Figure 2A. Values of drought index ranged from 0.38 to 0.15. The moderate drought spread towards the south while the central and northern subwatersheds tended to be relatively dry. The level of drought for this period was relatively moderate whereas severity of drought is still belonged into the normal category (-0,99 to 0,99).

Average distribution of drought in the period from MAM is shown in Figure 2B. The drought index ranged from 0.25 to 0.13. The moderate drought spread towards the center and north, while the southern region of subwatershed tended to be relatively dry. The level of dryness for this period was moderate. Where the severity of drought was still in the normal category (-0,99 to 0,99), it increased compared to those in December-February.

As illustrated in Figure 2C, the drought index in the period of JJA ranged from -0.80 to -1.00. The mean drought spreads more evenly almost half the area subwatershed towards the north and center, while the southern regions of Krueng Jreue Subwatershed did not tend to dry. The level of dryness for this period was a bit high compared to those of MAM, where the classification of the severity of the drought include the category moderately dry that -1,00 to -1,49.

Distribution of dryness in SON was tended to be uneven, almost the same as in the JJA (Figure 2D). The displacement patterns of drought in this period occurred in the northern regions of subwatershed with lower drought levels (0.77- 0.69). These levels of drought belonged into normal category. Level of dryness in this period was lower than those in the period of JJA. The existed distribution pattern of drought was gradually changing due to the narrow scope of subwatershed. Based on the SPI-3, the driest month were June-July-August, followed by MAM, DJF, and SON, while the wettest month is SON, followed DJF, MAM and JJA. Basically the changing pattern of drought will be obvious when the coverage area is seen by the watershed coverage, where a watershed may include more than one subwatershed. Drought pattern changing is also related to the hydrological cycle and meteorological condition that were affected by the climate of a region subwatershed (Van Loon and Laaha, 2015).

### Hydrological Disaster Mitigation

Structurally, hydrological disaster mitigation was done by optimizing the development Krueng Jreue Dam, rehabilitation and maintenance of irrigation systems. Storage of excess water during the rainy season to be used in the dry season by utilizing Keuliling reservoir adjacent to Krueng Jreue



Subwatershed. The realization of their actions so that the water resources can be managed properly, when heavy precipitation occurs the water can be accommodated and when low precipitation, the water can be allocated to areas lacking of water.

Hydrological disaster mitigation can be done non-structurally by making drought prediction from monitoring and hydrological data processing using telemetry facilities and the aid of computer simulations combined with national and international data bases. Other efforts need to do are erosion control and reforestation with water-efficient plants, decreasing evaporation using mulch and organic matter, forest areas revitalization and socialization to the community in and around the watershed areas.

## Conclusions

SPI method can be applied to analyze the severity of the drought area in Krueng Jreue Subwatershed, Great Aceh. Distributions of drought have different patterns from year to year. These conditions can be used as references to determine the future direction of development on the basis of historical data. Drought assessment based on historical data can be considered to make projections on the distribution of drought in the future.

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## Evaluation of Weevil Productivity and Infestation on Stored Sweet Potatoes in Terengganu, Malaysia

\*Nur Aida Hashim, Nurul Athirah Muhamad Noor, Nurul Adawiyah Zulkifli

Laboratory for Agri-Food Pest and Disease Management, School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

\*Corresponding Author: aida.hashim@umt.edu.my

### Abstract

The productivity and the effects of infestation by the sweet potato weevil, *Cylas formicarius* (Coleoptera: Brentidae), on sweet potato variety Vitato were evaluated under laboratory condition. A mean total of  $361 \pm 135.57$  weevils emerged from two kilograms of infested sweet potatoes throughout 66 days of observation. The sex ratio male to female was approximately 1:1. Two infestation treatments were conducted for a period of eight weeks; 1) low density and 2) high density. Sweet potatoes infested by low and high density of weevils recorded significant reductions in fresh weight compared with the non-infested sweet potatoes (control). The weight of sweet potatoes decreased with time due to water loss but more prominent under weevil infestation. High number of weevils caused high number of punctured holes on the skin surface of infested sweet potatoes compared to control group. After eight weeks of treatments, the damage severity of sweet potato for control group, low density and high density treatments were classified as class 1 (no damage), class 4 (medium-high damage) and class 5 (severe damage), respectively. The level of damage caused by the weevils on sweet potatoes in the storage increased over time. Therefore the weevil infestation decreased the shelf life of sweet potatoes.

**Keywords:** weevil productivity, infestation, *Cylas formicarius*, sweet potato

### Introduction

Sweet potato weevils (SPW), (*Cylas formicarius* L.) poses a serious pest problem in sweet potato crop. The weevil can easily enter the roots through open cracks that appear in the soil when the soil dries up (Stathers *et al.*, 2003).

Major countries that produce sweet potato as their staple food experienced the declining of sweet potato yields. However, the use of sweet potato is increased in the animal feed and industrial starch making. Sweet potato is an important food crop especially in Eastern Africa with an annual production of 11.83 million tons cultivated on an area of 1.84 million ha (FAO, 2011). In Malaysia the total yield of sweet was 58630 tons in 2013 (Agrofood Statistics 2013). The average annual per capita consumption of fresh roots is estimated at 10 kg in Africa, 20 kg in Asia, 5 kg in Latin America, 7 kg in Japan and only 2 kg in the USA (Lebot, 2009).

Among 139 countries, 113 are estimated to have quantity losses, either as weight of edible mass or the volume of food that became discarded due to damage or spoilage. Surface and internal damage by weevil result in average value loss of 12-13% (Affognon *et al.*, 2014).

The SPW infestation causes bitter taste to the sweet potatoes when consume become unmarketable. The feeding activity of SPW is the main cause of quality reduction of sweet potato during cultivation and storage. Therefore the aim of this study is to observe the productivity of SPW from infested sweet potatoes and to determine the damage severity caused by the weevil on stored sweet potatoes.

### Materials and Methods

This study was conducted in the Laboratory for Agri-food Pest and Disease Management, School of Food Science and Technology, Universiti Malaysia Terengganu. Sweet potatoes from variety vitato (orange-flesh) were purchased from the farmer's stalls in Besut, Terengganu. The experiment was conducted under natural condition with temperature ranged between 26 to 30 °C.

For the weevil productivity experiment, six kilograms of damaged sweet potatoes (weevil infested) were used. The sweet potatoes were sorted and cleaned from soils and weevils in the laboratory using a soft brush. Two kilograms of the sweet potatoes were transferred into three separate insect cages measuring 30 cm X 30 cm X 30 cm served as replication. The sweet potatoes were observed daily until the adult

SPWs appeared. The sex ratio for female to male was determined by counting the number of adults that emerged

For the severity of infestation experiment, three insect cages (replicates) with 2 kg of new sweet potatoes were prepared for each high density (40 weevils), low density (20 weevils) and control (no weevil introduced) treatments. Weevils used in this experiment aged between 1-5 days old. The damage on sweet potatoes was observed and a weekly assessment was done for eight weeks. The severity classification in this study followed Agona *et al.* (1999). The classes are; class 1 is no damage, class 2 is light damage, class 3 is medium damage, class 4 is medium-high damage and class 5 is severe damage. Severity photos of the sweet potato before and throughout the experiment were taken for visual data. The appearances of the sweet potatoes after being feed by the weevils were observed and recorded. The percentage of weight loss was calculated by using formula below:

$$\text{Percentage weight loss} = \frac{x - x_i}{x} \times 100$$

Where, x = weight of class 1 of sweet potato

$x_i$  = weight of sweet potato every week

All data were tested for normality distribution test. One-way ANOVA were used to analyse data from different treatments. The T-test was used to determine difference in sex ratio. All statistical analysis was performed by using SPSS version 21 software.

## Results and Discussion

Results showed a mean total of  $361 \pm 135.57$  weevils emerged throughout 66 days of observations (Figure 1). Out of this number,  $163 \pm 65.36$  were males and  $198 \pm 70.21$  were females. The t-test showed that there was no significant difference between male and female emergences, [t (4) = - .372, p > 0.05]. The sex ratio male to female is 1:1.2.

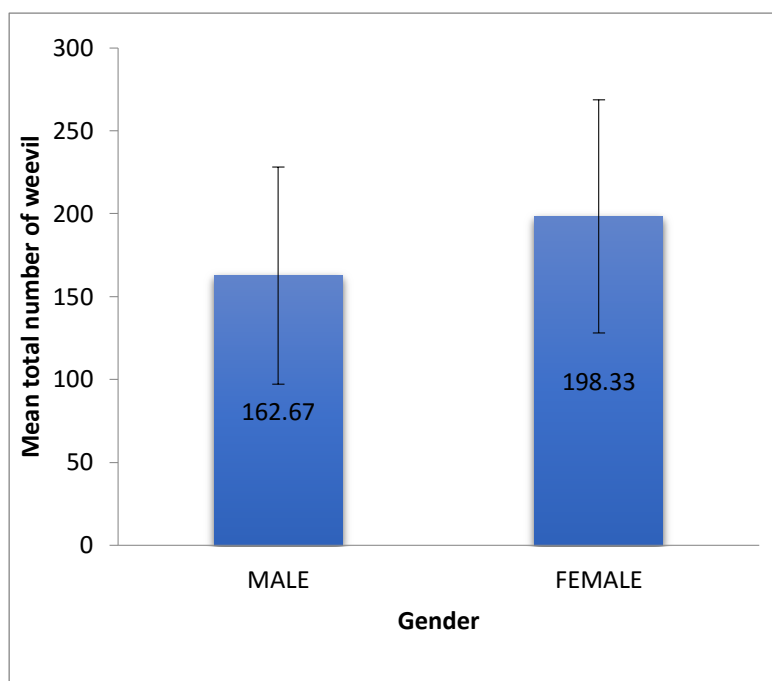
In this study, there was significant difference between weight loss of sweet potatoes [as determined by one way-Anova F (2, 6) = 9.347, (p < 0.05)]. A Tukey post-hoc revealed that high density weevils caused the highest weight loss to the sweet potatoes after eight week exposed to the treatment while control treatment was statistically lowest in weight loss (Figure 2). The total mean weight loss after eight weeks of treatment were  $38.43 \pm 0.63$ g,  $28.81 \pm 3.46$ g and  $22.17 \pm 0.63$ g for high density weevil, low density weevil and control treatments respectively.

After eight weeks exposed to three different treatments, all sweet potatoes had been observed with weight loss except for the control (Figure 3a) where the sweet potatoes were classified in class 1. The characteristic for class 1 indicated there were no damage on the appearance of the sweet potatoes and had not incurred any weight loss. The potatoes in class 1 could be marketable and consumed.

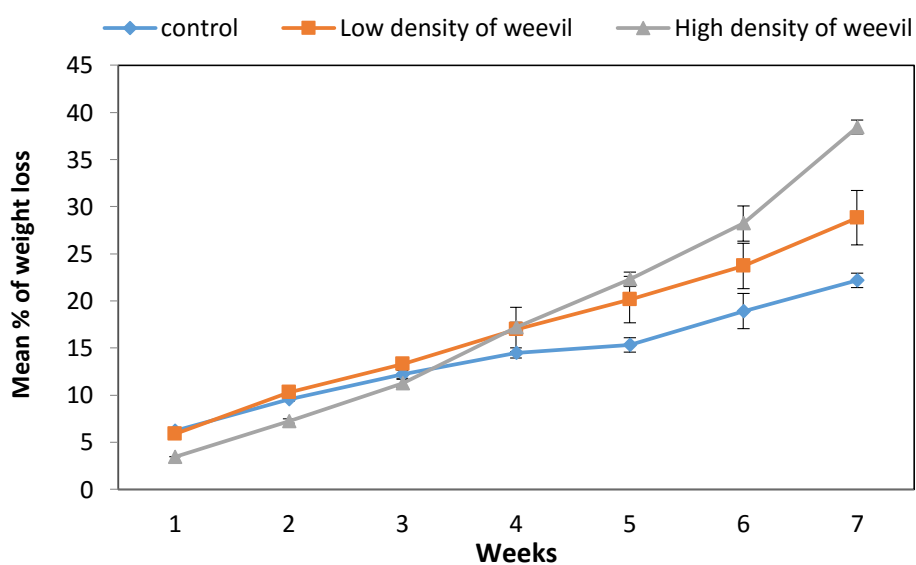
The sweet potatoes infested by low density of weevil showed medium damage and classified in class 4 (Figure 3b). Usually, potatoes in this are class suitable to be used for livestock feeding and in some countries they were used for distilling local gin. All tubers dried up and harden due to weevil feeding activities observed and classified in class 5 after eight weeks exposed to high density of weevil (Figure 3c). The potatoes in this class were considered severely damaged and will be discarded.

Rees *et al.* (2001) reported that there was a significant positive correlation between weight loss and rotting. A similar finding was reported by Agona *et al.* (1999) where the highest weight loss were recorded in class 5 which visually has the most damage. Both works also stated that there were significant differences between damage class categories in term of severity of damage and weight reduction.

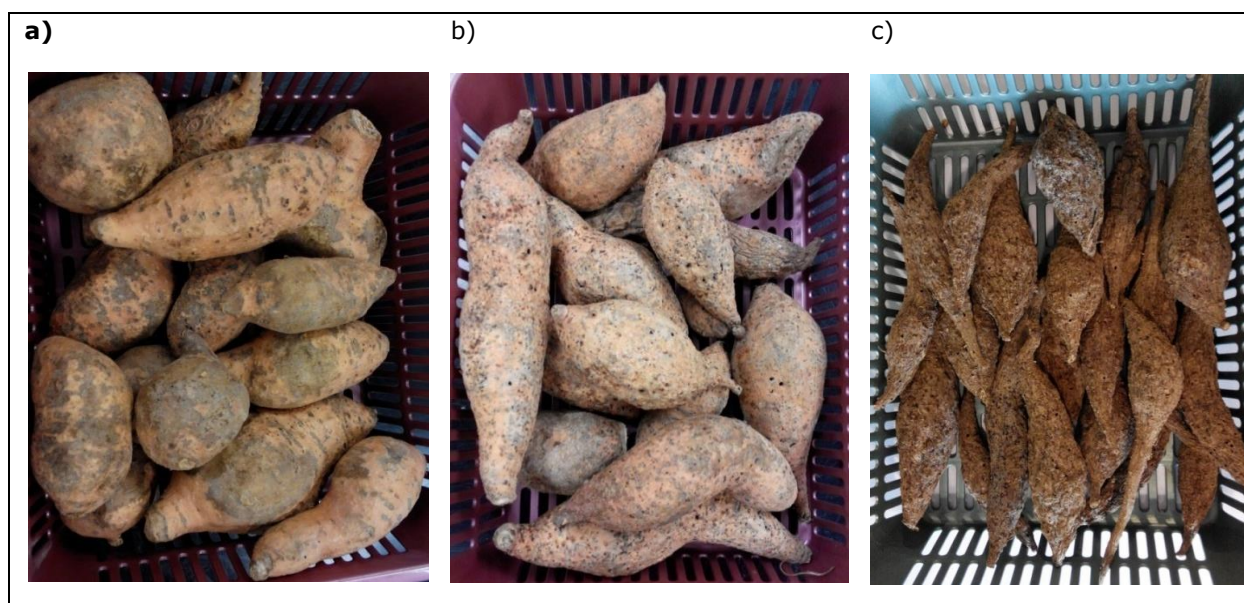
Sweet potato weevil is a major problem to sweet potato production worldwide and the SPW attack sweet potato both in fields and during storage (Mao *et al.*, 2001; Chalfant *et al.*, 1990; Jansson and Raman 1991). The infested sweet potatoes loss its market value due to physical damage during storage and harvesting by 11-63% (Thomlins *et al.*, 2000; Affognon *et al.*, 2014).



**Figure 1.** Mean total number of male and female SPW.



**Figure 2.** Mean percentage of weight loss (%) in sweet potato infested by low and high density of weevil



**Figure 3.** Sweet potatoes in a) control treatment b) low density weevil treatment and c) high density weevil treatment after stored for eight weeks.

### Conclusions

In the present study, more than 300 weevils were successfully emerged from two kilograms of damage sweet potatoes. This study suggests that if the damaged or rotten sweet potatoes are not properly dispose by farmers or retailers, the emerged weevils could continuously infest sweet potatoes in cultivation areas or storage. The weevils will infest the roots and highly causing reduction in quality and shelf life. This finding can give information to farmers on situation of unmanaged rotten sweet potatoes as well as precaution.

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## **Feed Enriched With Fermented Cocoa Pod and Sugar Cane Byproducts Improve Agricultural Business Economy of Beef Cattle Gandapura District, Bireuen, Aceh**

\*Dzarnisa, Didy Rachmadi, Muhammad Fakhurradhi

The Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: dzarnisa@yahoo.com.

### **Abstract**

This study was done to investigate effect of using fermented cocoa pod and sugar cane by products as supplementary feeds in the fattening program of local cattle on the agricultural business economics of farmers in the Gampong Cot Teube, Bireuen, Aceh, Indonesia. Cocoa pad and sugar cane by products collected from local farmers were dried, cut, and grained. Fermentation was carried out using EM4 for 21 days. For feeding treatments, 12 adult local cattle aged 2 years old were randomly assigned into 4 groups with 3 replications each. Group A (control) were cattle fed with traditional feeds (forage and grass); group B were cattle fed with traditional feeds and fermented cocoa pod addition (25%); and group C were cattle fed with traditional feeds supplemented and fermented sugar cane (1.5%); and group D were cattle fed with traditional feeds, fermented sugar cane (1.5%) and fermented cocoa pod (12.5%). Feed treatment was performed for 3 months. The results showed that the average of feed consumption of cattle in the group A, B, C and D were 5.37, 4.53, 0.59, and 0.64, respectively. The feed conversion rates were 7.05 (control), 9.76 (group B), 7.10 (group C) and 7.29 (group D). The feed containing 25% of fermented cocoa pod resulted in the highest feed conversion rate. From the results obtained it can be concluded that feed treatments were effective to increase daily weight gains of local aceh cattle during the fattening program.

**Keywords:** local cattle, sugar cane, byproduct, fermentation, performance

### **Introduction**

The demand for local beef increases every year especially in Aceh where the peoples prefer to consume meats of local cattle in any cultural ceremonies. The increased demand and consumption of the meats of aceh cattle from 6,3334.9 tons in 2002 to 10,721.6 tons in 2006 contradict to the 8.9% decreased in local cattle populations after tsunami disaster attacked the province on December 2004. Around seventeen percent of totally 13,313 local cattle predicted available in Aceh in November 2007 were ready to be slaughtered. This is equal to 350-400 kg live weight. With 5.10% increase in the numbers of cattle slaughtered per year and reduced cattle stocks brought from other provinces will potentially make the cattle into endangered condition.

Efforts in developing animal husbandry agricultural business have been done effectively in Aceh province not only to fulfill this demand but also to bring beneficial for both farmers and agribusiness sectors. Local farmers and businessmen must work hand in hand to create a sustainable, well-developed system of people agricultural farming and animal husbandry.

As pasture lands have been reduced due to their use for non-farming purposes, fattening program for local aceh cattle is among alternatives to address. This is because many farmers usually use commercial concentrates such as rice hull, kernel cake, cocoa pod and sago (metroxylon) by products in their fattening practice. As these materials are also used for producing chicken and duck ransoms, their prices are expensive. Here, producing forages and other feed sources become essential to overcome significant decrease of ruminant grassing land and space.

Agricultural by products available in Aceh such as cocoa pod and sugar cane wastes will be important in providing alternative feeds. Total cocoa pod and sugar cane by products are 75% and 40% of their main products, respectively (Direktorat Jenderal Peternakan, 2015). These will be advantageous to be used as ruminants feed since they contain fibers.



One district in Aceh where farmers have used cocoa pod and sugar cane by products as the feeds to fatten local cattle is Cot Teube Gandapura. This district is located in Bireuen Regency that has 571.958 Ha of lands. The majority of people in this Regency are farmers that plant cocoas and canes and also raise beef cattle for income, saving and farming aids. No wonder large amounts of wastes are produced. More than 2,800 tons were produced in 2010, and the numbers increased more than 3 folds and reached 8.800 tons in 2011. In Gandapura Districts only the products of cocoas waste were around 28 tons in 2012 (Bireuen Dalam Angka, 2013). The wastes, unfortunately, have not been optimally used for cattle raising and breeding due to inadequate skills and knowledge to modified the by products containing high crude fibers into more digestible feed resources.

One technology can be used in producing feeds from agricultural by products are fermentation. This approach increases nutritional values of feed ingredients by increasing feed digestibility, consumption, and rough protein contents as well as by saving high water feed ingredients. It is believed that the use of fermented agricultural wastes in animal feeding may optimally support the production fattening program of local Aceh cattle. A study, therefore, was done to investigate effect of using fermented cocoa pod and sugar cane by products as feeds supplements in the fattening program of local cattle on the agricultural business economics of people in the Gampong Cot Teube, Gandapura District, Bireuen, Aceh.

## Materials and Methods

This research was conducted from October to December 2015 in the Cot Teube Village, Gandapura Subdistrict of Bireuen, Aceh. In this study using completely randomized design 12 adult local aceh cattle aged 2 years old were randomly assigned into 4 feed treatment groups consisting 3 replications each. Group A (control) were cattle fed with traditional feeds (forage and grass); group B were cattle fed with traditional feeds and fermented cocoa pod by product (25%); and group C were cattle fed with traditional feeds supplemented with fermented sugar cane by product (1.5%); and D were cattle fed with traditional feed supplemented with fermented sugar cane (1.5%) and cocoa pod (12.5%) by products.. Feed treatment was performed for 3 months.

Fermented cocoa pod and sugar cane by products were prepared by firstly dried and cut raw materials collected from local farmers. The dried by products were then grained and fermented using EM4 for 21 days. The feed composition was formulated based on cattle nutrition needs as recommended by the National Research Council, 1984 (NRC).

Variables observed were feed consumption/intake, body weight gain, and feed conversion. Feed consumption was measured based on the number of feed consumed by certain period of time. Consumption level (voluntary food intake/VFI) was determined based on feed consumed by cattle under *ad libitum* administration (Parakkasi, 1996). Body weight gain was recorded through weekly repeated measurements. Feed conversion was used as a production indicator since it involved body weight and feed intake/consumption (Rassyaf, 1994). Data collected was analyzed using Analysis of variance and Duncan Multiple Distances test (Steel and Torrie, 1991).

## Results and Discussion

### Feed Consumption

Results of this research showed that fermented sugar cane and cocoa pod wastes increased the cattle body weight. This finding indicated that fermented feed could increase the food digestibility that, in turn, stimulated cattle production during the fattening program. As shown in Table 1, however, feed treatments did not significantly increased feed consumption of the local cattle.

**Table 1.** Average feed consumption of local cattle during fattening (kg/cattle/day)

Period	Feed treatment			
	A	B	C	D
I	3.71	5.15	3.89	2.81
II	5.34	4.60	4.13	5.35
III	7.08	3.84	4.47	6.07
Total	16.13	13.60	12.49	14.33
Average	5.37	4.53	4.16	4.78

**Note:** A (control): cattle fed with traditional feeds; B: cattle fed with traditional feeds and fermented cocoa pod by product (25%); C: cattle fed with traditional feeds and fermented sugar cane by product

(1.5%); D: cattle fed with traditional feeds and by products of sugar cane (1.5%) and cocoa pod (12.5%).

Although multiple range tests did not show significant increase in feed consumption between treatment groups ( $P>0.05$ ), cattle fed with feeds supplemented by fermented cocoa pod by products (B), fermented sugar cane wastes (C), or combination of both (D) consumed less feed compared to cattle consumed traditional feeds only (A, control). This condition might be related to the high lignin bound on the 3 fermented feeds. According to Mochtar and Tedjowahyono (1985), low digestivity of sugar cane was caused by lignin bound with cellulose and hemicellulosa. High lignin content limits the digestive power of ruminants. Baharuddin (2007) added that the cocoa pod wastes given to cattle must be fermented to reduce the number of lignin and to improve its nutrition. However, the minimum feed concentration must be taken into account since it has anti theobromin nutrition. Cocoa shell contains alkaloid theobromin that limits digestibility of this by product as cattle feed.

Ensminger and Olentin (1978) said that feed consumption is also influenced by the feed palatability and physical changes. Chemical characteristics of feed compositions and the size of feed particles in stomach will change rumen buffering capacity that in turn affect fermentation process of the bacteria in the rumen, activity of ruminal digestive enzymes, and metabolism by rumen microba. Chemistry compositions of a ration varied according to the materials used to compose the ration. Tillman *et al* (1991) stated that the amount of feed consumption was influenced by some factors such as palatability, feed materials availability and the environment. On the other hand, feed palatability is influenced by the form, color, and odor of the feed consumed. Since concentrates given to the cattle were in good condition it was not likely feed their odor affected feed palatability.

### **The Increase of Body Weight**

One of the production variables that can be assessed externally is the increase of the cattle body weight as compensation of raising and feed management and control. These three components will be accumulated as the increase of the body weight.

Results of this study indicated that in the process of local cattle fattening the addition of fermented sugar cane and cocoa pod by products, either as single or combination feed supplements, increased body weight gain from the improved processes of nutrition metabolism (Table 2). Daily weight gains of cattle raised by fattening program using feed treatment implemented in this study, which ranged from 0.47-0.77/kg/head/day (Table 3), were substantially higher than that using natural raise (0.2-0.4 kg/head/day). The increase in total and daily body weights resulted, however, were insignificant among treatment groups. Whether this effects related to the short feed treatment period need to be confirmed by further study.

**Table 2.** The local cattle body weight gained during the research (kg/head)

Period	Feed treatment			
	A	B	C	D
I	25	22	32	26
II	41	24	25	34
III	49	24	32	36
Total	115	70	89	96
Average	38.33	23.33	29.66	32

**Note:** A (control): cattle fed with traditional feeds; B: cattle fed with traditional feeds and fermented cocoa pod by product (25%); C: cattle fed with traditional feeds and fermented sugar cane by product (1.5%); D: cattle fed with traditional feeds and by products of sugar cane (1.5%) and cocoa pod (12.5%).

**Table 3.** Average body weight gains of Local cattle during fattening program (kg/head/day)

Period	Feed treatment			
	A	B	C	D

I	0.50	0.44	0.64	0.52
II	0.82	0.48	0.50	0.68
III	0.98	0.48	0.64	0.72
Total	2.30	1.40	1.78	1.92
Average	0.77	0.47	0.59	0.64

**Note:** A (control): cattle fed with traditional feeds; B: cattle fed with traditional feeds and fermented cocoa pod by product (25%); C: cattle fed with traditional feeds and fermented sugar cane by product (1.5%); D: cattle fed with traditional feeds and by products of sugar cane (1.5%) and cocoa pod (12.5%).

Rahardjo (2006) stated that growing process and weight increase of cattle depend on the quality and quantity of feed consumed as well as feed digestibility. Increased digestive level of feed nutrients might be caused by better activity of ruminal bacteria from more optimal ruminal clearing of fermented by products.

### Feed Conversion

Feed conversion is a part of body weight gained and ration consumption during the raise. As it involves both body weight and feed intake, feed conversion can be used as a indicator of production (Rasyaf, 1994). Feed intake is one of indicators for the effectiveness of feed consumption. Lower amount of feed conversion, therefore, reflects a better effectiveness of feed consumption (Anggorodi, 1979). Data on feed conversion of cattle raised using fermented by products feed formulation of this study is presented in Table 4.

**Table 4.** The Average of Feed Conversion of Local Cattle during the Research

Period	Feed treatment			
	A	B	C	D
I	7.42	11.72	6.08	5.59
II	6.51	9.58	8.26	7.87
III	7.22	7.99	6.98	8.43
Total	21.15	29.29	21.32	21.89
Average	7.05	9.76	7.10	7.29

**Note:** A (control): cattle fed with traditional feeds; B: cattle fed with traditional feeds and fermented cocoa pod by product (25%); C: cattle fed with traditional feeds and fermented sugar cane by product (1.5%); D: cattle fed with traditional feeds and by products of sugar cane (1.5%) and cocoa pod (12.5%).

Data in Table 4 showed that cattle given the fermented cocoa pod or sugar cane by products (B, C, and D) had better food conversion than control, but the highest feed conversion was achieved using fermented cocoa pod by products of 25% (B). This could be used as a consideration in formulating fermentation based rations because the lower average feed conversion indicates reduced amount of feed conversion in producing a kg of meat (Kartasudjana and Supriyatna, 2006).

For ruminant cattle, capability to consume feed has strong relationship with their stomach physical capacity and digestive system as a whole (Church, 1975). Giving concentrate feed which contains microbio protein can increase digestive level of feed as a whole.

Bad feed management will disappoint the farmers since the value of biological feed become lower. This will cause the development and growing of the cattle is not optimal. Based on the body weight gained during the treatments of fattening, the combination of two types of treatments could reduce feed consumption on one side, with the same achievement of body weight gained. The more the concentrate digested, the faster the feed can be digested so that it can empty the rumen and cause hungry sensation and make it possible for the cattle to consume more (Soewardi, 1974).

### Conclusion

Based on the study it was proved that the fattening time of local cattle can be shortened by feed treatment. The increase of body weight gain, final body weight gain, and feed consumption effectiveness could be improved through the feed treatments as well. This might potential to increase the income of local farmers and bring beneficial for their economic.

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## Arbuscular Mycorrhizal Fungi Communities at the University Farm of Ie Seuum Station

<sup>1</sup>\*Fikrinda, <sup>2</sup>Syafruddin, <sup>1</sup>Sufardi, <sup>3</sup>Rina Sriwati

<sup>1</sup>Department of Soil Science, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Agrotechnology, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>3</sup>Department of Plant Protection, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: fikrinda@unsyiah.ac.id.

### Abstract

Almost all terrestrial plants are able to form symbiotic association with arbuscular mycorrhizal fungi (AMF). Spore abundance and root colonization of AMF were analyzed in six plant species at the University Farm of Ie Seuum Station. We found four genus AMF namely *Glomus* (8 types), *Acaulospora* (2 types), *Gigaspora* (2 types), and *Scutellospora* (3 types). *Glomus* was the most prevalent of AMF spores found at the University Farm of Ie Seuum Station. The highest spore density and colonization of AMF were found in maize.

**Keywords:** mycorrhiza, glomus, university farm.

### Introduction

Arbuscular mycorrhizal fungi constitute an important component of soil microbial community by forming mutualistic symbioses with more than 80% vascular plant species in many ecosystems throughout the world (Smith and Read, 2008). They provide numerous benefits to their hosts. In exchange of plant assimilated carbon, AMF promote host nutrition and growth (Ortas and Ustuner, 2015; Sanmartín *et al.*, 2014; Smith *et al.*, 2015), provide adaptation to biotic and abiotic factors (Manaut *et al.*, 2015; Wang *et al.*, 2016) and affect formation or maintenance of stable soil aggregates (Leifheit *et al.*, 2015; Mardhiah *et al.*, 2016)

Spore production is highly dependent on environmental conditions (Higo *et al.*, 2011). The environmental parameters such as nutrient availability (Peng *et al.*, 2015), soil type and characteristic (De Beenhouwer *et al.*, 2015), season (Lara-Pérez *et al.*, 2014), and disturbance (Trejo, *et al.* 2016) affect distribution and number of AMF spore. Besides, according to Bencherif *et al.* (2016) AMF root colonization rate and spore species vary according to plant phenology and ecological parameters.

The diversity of AMF has significant ecological consequences because individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors (Abo-Elyousr *et al.*, 2014; Lenoir *et al.*, 2016). As fungal strains may differ in their effects on host plants, detection of AMF diversity in soil is critical for any agro-system. Besides, AMF are well adapted to the environment from which they were isolated (Garg and Pandey, 2015; Liu *et al.*, 2014). Therefore, the potential of native AMF that improves the growth and nutrient uptake of crop plants is a great interest.

Unlike their application, data about AM fungi communities are still limited in Aceh. The University Farm of Ie Seuum Station, an area covered by shrubs and cultivated plants, is a potential place to be explored their biodiversity. Therefore, this study was done to explore the communities of AM fungi and their colonization in six plants at this location.

### Materials and Methods

#### Site study and soil sampling

This study was performed in the University Farm of Ie Seuum Station, Syiah Kuala University. Thirty root and rhizosphere soil samples belonging to six plant species were collected at 0-20cm depth around five randomly selected individuals of each species after removing the litters/biomasses. Rhizosphere soils (approx. 1 kg per plant) attached and adjacent to roots were placed in individual polyethylene bags, labeled and transported to laboratory.

### Assessment of soil properties

The soil samples collected were used for the study of AMF status and chemical properties. From each soil sample, an air dried and sieved subsample was used for analyzing chemical characteristics including pH, organic carbon (C), total nitrogen (N), available phosphor (av. P), exchangeable potassium (exch. K), and cation exchange capacity (CEC). The pH was measured in water (soil:water ratio 1:5) with a digital pH meter. Organic carbon content was determined by the Walkley–Black acid digestion method (Walkley and Black, 1934). Total N was measured by Kjeldahl method. Available P was extracted according Bray 1 method. Cation exchange capacity extracted by 1 N ammonium acetate at pH 7 was measured using atomic absorption spectrophotometry.

### Analysis of AM fungal spores

Spores were obtained from 50 g of each well-mixed soils using wet sieving technique followed by centrifugation in water and sucrose 60% at 2,500 rpm (Brundrett *et al.*, 1996). The total number of spores was counted under a stereo microscope. Thereafter, the healthy and completely in assessorial spores and sporocarps of similar size, color, and hyphal attachment were mounted into slides, using polyvinylalcohol–lactoglycerol (PVLG) following Schenck and Perez (1990).

### Root staining and Assessment of AM fungal colonization

Colonization of various AM fungal structures in the plants observed was visualized by staining and examining under a compound-light microscope (Nikon). Root samples collected were washed in tap water, then cut into small pieces (ca. 1 cm). These root segments were cleared in 10% (w/v) KOH solution for 12 hours, and then washed in tap water several times. As the roots were highly pigmented, roots were bleached in 3% NaOCl solution. Then the roots were stained in a solution of 5% ink (Quink Parker) in household vinegar (5% acetic acid) for 12 hours. Roots were destained in vinegar for 30 mins and stored in tap water acidified with some drops of vinegar (modified after Vierheilig *et al.*, 1998). The stained roots were observed under Nikon compound microscope and counted the colonization percentage. A root segment was considered mycorrhizal (positively colonized) if there were a hypha, hyphal coils, arbuscule, vesicle, auxiliary cells or any combination of these characteristic structures of AMF, within a single field of view of the microscope. The colonization by AMF was calculated and expressed in percent (Brundrett *et al.*, 1996).

### Data analysis

The formula described structures of AM fungi communities include spore density and species richness. These data were analyzed descriptively.

## Results and Discussion

### Soil Characteristics

Arbuscular mycorrhizal fungal communities are influenced by soil properties. Soil chemical parameters viz. pH, C, N, av. P, exc. K and CEC of all the six plant species in the fields are presented in Table 1.

**Table 1.** Soil chemical properties of six plant species in the University Farm of Ie Seuum Station

Plant	pH	Org. C (%)	Total N (%)	Av. P (ppm)	Exch. K (cmol <sup>(+)</sup> kg <sup>-1</sup> )	CEC
<i>Imperata cylindrica</i>	5.91	2.24	0.16	5.43	0.30	19.02
<i>Eleusine indica</i>	6.17	4.87	0.26	14.33	1.01	17.83
<i>Stachytarpheta jamaicensis</i>	5.74	1.39	0.14	19.44	0.41	14.66
<i>Capsicum annum</i>	6.18	1.62	0.20	76.05	1.14	19.02
<i>Zea mays</i> L.	6.23	1.64	0.13	11.54	0.45	11.59
<i>Citrullus lanatus</i>	5.99	2.55	0.18	39.66	0.93	19.02

The range of soil pH did not vary widely in the most of soil samples studied, but other soil properties varied widely irrespective of plant species. Soil pH was slightly acidic (5.91-6.23) in all soil samples studied. Soil C value was low at the rhizosphere of *S. jamaicensis*, *C. annum*, and *Z. mays*; moderate at *I. cylindrica* and *C. lanatus*; and high at *E. indica*. Most rhizosphere of plant species studied has low in total N but moderate at the rhizosphere of *E. indica*. The lowest av. P and exch. K were at the rhizosphere of *I. cylindrica* while the highest was at *C. annum*. Soil CEC was low at the rhizosphere of *S. jamaicensis* and *Z. mays*, but moderate at other plants.

### Spore density of AM Fungi

Plant species can influence communities of AM fungi by hosting different AMF taxon identities and/or richness (Lekberg and Waller, 2016). Table 2 indicated that *Z. mays* had the highest spore density of AMF while *C. annum* had the lowest number. Soil P and K in this studied area influenced AMF

communities. The effect of soil P on AMF communities has been intensively observed (Blanke *et al.*, 2012; De Beenhouwer *et al.*, 2015; Goomaral *et al.*, 2013). This study showed that the lowest number of AM fungi spore density at the rhizosphere of *C. annum* was related to high level of soil P and K.

**Table 2.** Spore density and richness of AM fungi

Plant species	Spore density (50 g soil <sup>-1</sup> )	Species richness
<i>Imperata cylindrica</i>	15.60	10
<i>Eleusine indica</i>	10.60	6
<i>Stachytarpheta jamaicensis</i>	13.80	9
<i>Capsicum annum</i>	10.00	9
<i>Zea mays</i>	28.33	3
<i>Citrullus lanatus</i>	10.80	5

### Species richness

Spore number and types of each genus of AMF varied among plant species (Table 3). *C. imperata* had more species richness than others otherwise *Z. mays* having the highest spore density had the lowest species richness. This result showed that there was no correlation between spore density and species richness of AMF.

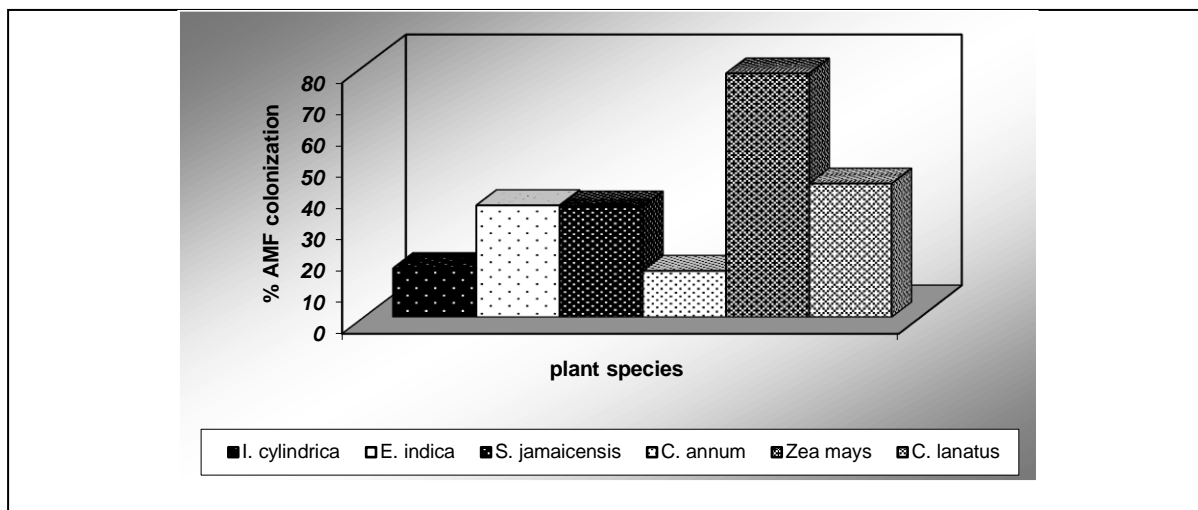
Compared to others, *Glomus* was genus with the highest numbers of species recorded (8), followed by *Scutellospora* (3), *Gigaspora* (2), and *Acaulospora* (2). *Glomus* was the most number of AMF genus found at different areas (Fikrinda *et al.*, 2016; Wei *et al.*, 2015).

**Table 3.** Distribution of AM Fungi

AM Fungi Genus	<i>Imperata cylindrica</i>	<i>Eleusine indica</i>	<i>Stachytarpheta jamaicensis</i>	<i>Capsicum annum</i>	<i>Zea mays</i>	<i>Citrullus lanatus</i>
<b><i>Glomus</i></b>						
<i>Glomus</i> sp.1	2.2	2.8	1.20	0.8	-	2.80
<i>Glomus</i> sp.2	-	-	0.40	-	-	-
<i>Glomus</i> sp.3	0.4	1.2	-	0.8	-	2.00
<i>Glomus</i> sp.4	-	-	-	-	10.33	-
<i>Glomus</i> sp.5	0.2	-	-	0.2	-	-
<i>Glomus</i> sp.6	0.6	2.2	1.00	1.8	-	4.40
<i>Glomus</i> sp.7	0.2	1.6	0.60	0.8	-	1.40
<i>Glomus</i> sp.8	0.6	1.2	0.40	-	-	-
<b><i>Acaulospora</i></b>						
<i>Acaulospora</i> sp.1	-	-	-	-	8.33	-
<i>Acaulospora</i> sp.2	9.0	-	6.80	1.0	-	-
<b><i>Gigaspora</i></b>						
<i>Gigaspora</i> sp.1	1.4	-	-	0.2	-	0.20
<i>Gigaspora</i> sp.2	0.6	-	1.6	1.6	9.67	-
<b><i>Scutellospora</i></b>						
<i>Scutellospora</i> sp.1	-	-	-	2.8	-	-
<i>Scutellospora</i> sp.2	0.4	1.6	1.8	-	-	-
<i>Scutellospora</i> sp.3	-	-	0.4	-	-	-

### AMF colonization

The presence of association by vesicles, arbuscules, hypha, or auxiliary cells in the roots is generally used to determine AMF colonization (Brundrett *et al.*, 1996). AMF roots colonization plays an important role in improving plant establishment and growth (Alguacil *et al.*, 2011). This study indicated that *Z. mays* had higher roots colonization than the others (Fig. 1). Difference in the level of colonization observed among plants may be attributed to abiotic factors, age and phenology, mycorrhizal dependency, and capacity of population of mycorrhizal propagules in the soil to form mycorrhizal association (Smith and Read, 2008)



**Figure 1.** AM fungi colonization of six plant species observed

## Conclusion

*Glomus* was the AMF spore found abundantly at the University Farm of Ie Seuum Station. Our results suggest that *Zea mays* has a good promising as a host plant, presenting high values of sporulation and mycorrhizal colonization.

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## Genomic DNA Extraction of *Lactobacillus* Isolates From Aril Durian Fermentation (*Jruek Drien*)

\*Yulia Sari Ismail, Cut Yulvizar, Novekhana Anelia

Biology Department, Faculty of Mathematics and Natural Science, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author : ysismail@unsyiah.ac.id

### Abstract

*Lactobacillus* isolates, JD-1 and JD-4, were isolated from fermented aril durian (*jruek drien*). This isolates were predicted as probiotic. The aim of this research is to determine the concentration and purity of DNA isolate from JD-1 and JD-4 by using QIAamp DNA Mini Kit (*Qiagen*). The isolates were regenerated in MRSA and TSB medium, then the DNA extraction were performed using QIAamp DNA Mini Kit (*Qiagen*). The isolated DNA measured by nanophotometer and electrophoresis. The result showed that JD-1 has concentration of 40 ng/μL, with the purity ( $A_{260/280}$ ) was 2,000. JD-4 has concentration of 32,5 ng/μL, with relatively low purity ( $A_{260/280}$ ) that was 1,625.

**Key words:** *Jruek drien*, *Lactobacillus*, DNA isolation

### Introduction

The presence of lactic acid bacteria can be identified by the phenotype and genotype. Previous research has succeeded in isolating and identifying four of the nine isolates potentially probiotic lactic acid bacteria from *jruek drien*. Four isolates that has been found were *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, and *Micrococcus lylae* (Ismail et al., 2015).

Phenotype analysis has low in accuracy and unstable because the phenotype of an organism is a result of genotype and varied environment (Widowati et al., 2014). Identifying species of bacteria needs an accurate method that is genotypic analysis (Singleton, 2004). This study was focused on performing genotype analysis using 16S rRNA gene of two isolates of potentially probiotic *Lactobacillus*, that were *Lactobacillus plantarum* and *Lactobacillus acidophilus* (namely as JD-1 and the JD-4).

### Material and Methods

Fermented aril durian (*jruek drien*) were obtained from Aceh Jaya, Aceh Barat, Aceh Barat Daya, Nagan Raya and Aceh Selatan district.

### Isolates Regeneration

JD-1 and JD-4 isolates from stock culture were inoculated on deMann Rogosa Sharpe Agar (MRSA) by streak plate method. The isolates were incubated at 37°C for 24 hours. One of the colonies was transferred into a tube containing 6 mL of Trypticase Soy Broth (TSB) and incubated at 37°C for 48 hours in shaker waterbath.

### Genomic DNA Extraction of Gram Positive Bacteria

The obtained culture were recovered in TSB and transferred into microcentrifuge tube and centrifuged at 13.300 rpm for 10 min. The cell pellet were submitted to genomic DNA isolation using QIAamp DNA mini kit (*Qiagen*). The volume of the pellet was calculated and the buffer ATL added to a total volume of 180 μL. The 20 μL proteinase K was added and mixed by vortexing and incubated at 56°C, then 200 μL buffer AL was added to the sample, homogenized by vortex and incubated at 70°C for 10 min. Furthermore, 200 μL ethanol 96% added to the sample, mixed by vortex for 15s to produce lysate.

The lysate of sample was put into QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim, then the cap was closed and centrifuged at 8000 rpm for 1 min. The QIAamp Mini spin column was placed in a clean 2 mL collection tube, and the tube containing the filtrat was discarded. The 500 μL Buffer AW1 was added, and the cap was closed and centrifuged at 8000 rpm for 1 min. The QIAamp Mini spin column was placed in another clean 2 mL collection tube and the tube containing the filtrat

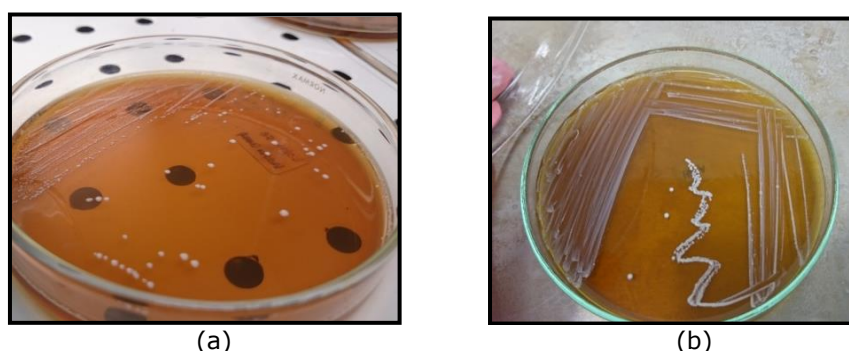
was discarded. Then 500  $\mu$ L buffer AW2 was added, and the cap was closed and centrifuged at 13.300 rpm for 3 min. The QIAamp Mini spin column was placed in a clean 1,5 mL microcentrifuge tube and the tube containing the filtrat was discarded. Carefully, the QIAamp Mini spin column was opened and 200  $\mu$ L buffer AE was added. The DNA isolates were incubated at room temperature for 1 min and then was centrifuged at 8000 rpm for 1 min (Qiagen, 2015).

### **Determination of Quantity and Concentration of DNA**

5  $\mu$ L isolation products were mixed with 1  $\mu$ L SYBR Safe and submitted in 1% agarose gel in 1X TAE to perform electrophoresis. Then agarose gel visualized using GelDoc (*Bio-Rad*). Quantity and concentration of DNA calculated using NanoPhotometer P 330 (*Implen*).

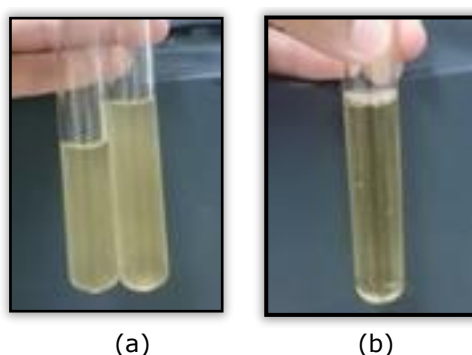
### **Result and Discussion**

Isolates regeneration on media MRSA was done by four quadrants streak method (Fig 1). JD-1 and JD-4 colonies shows good colony growth. The of macroscopic morphology colony from both colonies have similiar charateristics, which are round, convex, smooth, and creamy-colored.



**Figure 1.** Macroscopic growth interpretation on MRSA, (a). JD-1; (b). JD-4

JD-1 and JD-4 showed good turbidity which indicated bacteria grow well (Fig 2). Dwidjoseputro (1994) said that the growth of bacteria in a liquid medium characterized by the formation of sediment, pedicle, turbid, and spread. This phenotypic examination was important to reconfirm the stock culture was the isolated lactic acid bacteria. Furthermore, liquid culture of JD-1 and JD-4 isolates were going to be used for genomic DNA extraction.



**Figure 2.** Macroscopic growth interpretation on TSB. (a). JD-1; (b). JD-4

JD-1 isolate show good result which high purity and concentration of DNA (table 1). On the contrary, JD-4 had relatively low purity. Faatih (2009) stated that pure DNA obtained if the ratio of the absorbance at 260 nm and 280 nm valued in the range of 1,8 to 2. Ratio less than 1,8 indicates that the DNA is contaminated. Contamination could be caused by several factors, one of them was the presence of RNA that need to be added by RNase.

**Table 1.** Quantity and concentration of DNA

No.	Isolate	Purity(A <sub>260</sub> /280)	Concentration(ng/μL)
1	JD-1	2,000	40
2	JD-4	1,625	32,5

However, in this research RNase was not added because the sample derived from bacteria that isolated from *Jruek drien*. Qiagen (2015) stated that RNase additions is necessary to obtain pure DNA. However, RNase generally added when samples come from heart and kidneys. The presence of RNA in the samples may interfere with the enzymatic reaction. But will not inhibit the process of DNA amplification. Another suggestion of different concentration results after calculation of the quantity of DNA by nanophotometer, it was thought to be occurred because a lack of homogenizing duration on DNA isolate using a vortex. Bronner *et al.* (2009) said that vortex is important instrument to homogenize and cell destruction.

### Conclusions

JD-1 and JD-4 were grown well on MRSA and TSB medium. The genomic DNA was extracted from both *Lactobacillus* isolates. JD-1 DNA isolate had high concentration and purity, and JD-4 DNA isolate had high concentration and relatively low purity.

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## The ability of *Leptosphaeria biglobosa* to infect oilseed rape and swede cultivars grown in New Zealand

<sup>1,2\*</sup>Suhaizan Lob, <sup>2</sup>Marlene Jaspers, <sup>2</sup>Hayley Ridgway and <sup>2</sup>Eirian Jones

<sup>1</sup>School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

<sup>2</sup>Ecology Department, AGLS, Lincoln University, PO Box 84, Lincoln University, Canterbury, New Zealand

\*Corresponding Author: [suhaizanlob@umt.edu.my](mailto:suhaizanlob@umt.edu.my)

### Abstract

A survey was carried out in 2011 to determine the cause of Phoma stem canker in New Zealand. Of the 175 isolates obtained, 171 belonged to the species *Leptosphaeria maculans* and four were morphologically identified as *L. biglobosa*, which had not been reported in New Zealand before. Two of these isolates were from oilseed rape and one each from cabbage and kale. Preliminary identification of these isolates was carried out by amplifying genomic DNA using species-specific primers (LmacF, LmacR and LbigF) with all four *L. biglobosa* isolates producing the 444 bp PCR product characteristic of this species. Identification was confirmed by sequencing of the internally transcribed spacer (ITS) of the ribosomal RNA and actin gene regions. The ITS (580-585 bp) and actin (941 bp) sequences of the four isolates were 92-99 and 99% identical, respectively, to the reported sequences of *L. biglobosa* in GenBank, DQ133893.1 and AY748949.1, respectively. The pathogenicity of the *L. biglobosa* isolates was tested on two oilseed rape cultivars (Flash and Excellium), and one swede cultivar (Highlander). Wounded cotyledons (10-day old) were inoculated with 10 µL of conidial suspension ( $10^6$  mL<sup>-1</sup>) or sterile distilled water for controls. Leaf lesions that developed on the inoculated cotyledons were characteristic of *L. biglobosa*, being small and dark in colour with a distinct margin. The pathogen was re-isolated from the symptom developed on the inoculated cotyledon and Koch's postulate was completed. However, the disease did not progress into the stem 65 day-post inoculation. Since *L. biglobosa* has not previously been investigated as a pathogen of brassica crops, this provides an opportunity to examine its significance to crops of oilseed rape and swede in New Zealand.

**Key words:** Cotyledon, conidial suspension, phoma stem canker, *Leptosphaeria maculans*, ITS.

### Introduction

Phoma stem canker disease is known to be an important disease of oilseed rape (OSR; *Brassica napus*) worldwide (West *et al.*, 2001; Howlett, 2004). This disease is caused by *Leptosphaeria maculans* and *L. biglobosa* which both have a worldwide distribution (West *et al.*, 2001). Both species are also pathogens of other crops in the *Brassicaceae* family, generally brassica crops such as *B. napus*, *B. rapa*, *B. juncea* and *B. oleracea* (Fitt *et al.*, 2006a, 2006b). However, there are very limited reports in the literature on the occurrence of dry rot disease of swede and whether both species cause disease. Overseas, research has mainly focused on the OSR as it is their key crop, however in New Zealand, forage brassicas are as, if not more, economically important. Forage brassicas are grown annually as food for stock during winter (Donkers, 2010) while OSR is grown for vegetable oil for human and animal consumption and biodiesel production.

Both pathogens are present on most continents and often co-exist within the same fields, as is the case in Europe (France, Germany and Hungary) and North America (West *et al.*, 2001; Karolewski *et al.*, 2002). However in some countries such as Australia and UK only one species is predominant (West and Fitt, 2005). In China, Russia and Poland, *L. biglobosa* causes widespread disease in the OSR, resulting in large yield losses (West *et al.*, 2000). *Leptosphaeria biglobosa* can be further divided into subclades based on sequences of the internal transcribed spacer (ITS) or fragments of the actin or  $\beta$ -tubulin genes. To date, *L. biglobosa* has six subclades being *L. biglobosa* 'australensis', *L. biglobosa* 'brassicae', *L. biglobosa* 'canadensis', *L. biglobosa* 'erysimii', *L. biglobosa* 'occiaustralensis' and *L. biglobosa* 'thlaspii' (Plummer *et al.*, 1994; Vincenot *et al.*, 2008; Van de Wouw *et al.*, 2008). The different subclades are often associated with specific host plant or geographic origin, for example, *L. biglobosa* 'thlaspii' was isolated from a weed, *Thlaspi arvense* in Central Canada and *L. biglobosa* 'australensis' was isolated from *B. oleracea* or *B. napus* in Australia and the United States (Vincenot *et al.*, 2008).

In addition to OSR, forage brassicas such as swede are grown widely with over 300 000 ha of cropping area in New Zealand (Dumbleton *et al.*, 2012). With both crops being important economically to New Zealand and also significant potential hosts to *L. biglobosa* it is vital to determine if the New Zealand populations vary in their pathogenicity to the commonly grown cultivars. Although *L. biglobosa* is known to infect and cause crop losses in the OSR (Mendes-Pereira *et al.*, 2003) the pathogen has not been recovered from swede and there is no information on its potential pathogenicity. In order to ensure the crop establishment, the choice of resistant cultivar is significant to the future as this is one of the most effective control strategies for this serious disease of brassica crops (Aubertot *et al.*, 2006). Little or no information was available with regards to the susceptibility to *L. biglobosa* of cultivars of either OSR or swede grown in New Zealand.

The pathogenicity of isolates obtained from the different brassica hosts and locations throughout New Zealand were assessed on cultivars of OSR and swede commonly grown in New Zealand to determine their susceptibility to New Zealand isolates of *L. biglobosa*. This will provide information on whether specific isolates are able to infect OSR and other forage brassica crops, and therefore whether infected forage brassica crops can act as an inoculum sources for subsequent OSR crops and *vice versa*.

## Materials and Methods

### Isolates collections

Isolates were recovered from OSR/forage brassica material showing typical *L. biglobosa* disease symptoms, including leaf lesions, stem cankers and dry rot. Small pieces of OSR/forage brassica infected tissue (0.5 cm × 1.0 cm) from leaves, stems and tuber/bulb were surface sterilized and placed in Petri dishes containing potato dextrose agar (PDA; Difco™, New Jersey, USA) amended with streptomycin and penicillin and incubated at 15°C in the dark for 7-10 days. The resulting fungal colonies were examined for characteristics typical *L. biglobosa* which produced abundant and fibrous aerial mycelium, associated with the production of yellow to brown pigmentation in the media (Somda *et al.*, 1996) after 7 days incubation. Likely cultures were subcultured onto PDA and were then identified to species level.

### Molecular identification of *L. biglobosa* isolates

The extraction of the genomic DNA was done using the Puregene® system (Gentra systems, Minneapolis, USA) following the instruction by manufacturer. The DNA concentration in each tube was determined by spectrophotometry and all DNA samples were diluted to 20-25 ng/μL using (SNW) sterile Nano Water for use in PCR.

For species identification, DNA from *L. biglobosa* isolates were amplified using two forward species-specific primers, *LmacF* (5' - CTTGCCCACCAATTGGATCCCCTA - 3') and *LbigF* (5' - ATCAGGGGATTGGTGTCTCAGCAGTTGA - 3'), and a single reverse primer *LmacR* (5' - GCAAAATGTGCTCGCTCCAGG - 3') (Liu *et al.*, 2006). For further species confirmation, the ITS (ITS4:5'-TCCTCCGCTTATTGATATGC-3'; ITS5:5'- GGAAGTAAAAGTCGTAACAAGG-3') and actin gene region (5'- TGGGACGATATGGAIAAIATCTGGCA-3'; 5'-TTAGAAGCACTTNCGGTG-3') of all four *L. biglobosa* isolates were amplified using paired primers for each region and performed as described above. After amplification, the PCR products (7 μL of each) were separated on a 1% agarose gel for 45 min and the stained gels were photographed under UV light using FireReader UVITEX Cambridge. The PCR products were sequenced at the Lincoln University Sequencing Facility. Sequences obtained from this study were aligned with sequences downloaded from the NCBI database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) using MEGA 5.

### Pathogenicity test for *L. biglobosa* isolates on OSR and swede cultivars

Three *L. biglobosa* isolates, two isolates (Lb 237 and Lb 238) obtained from sampling and one isolate (ICMP 10665) from the ICMP culture collection were used. A conidial suspension of each isolate was used as inoculum and prepared freshly on the day of inoculation. The pathogenicity of these three *L. biglobosa* isolates was tested on two different OSR cultivars, Flash and Excellium, and one susceptible swede cultivars, Highlander. Seedlings of each cultivar were grown in a greenhouse and inoculations of the isolates were done on 10-day old seedlings. The eight replicates seedlings set up for each treatment was arranged in a completely randomised design. Inoculated plants were allowed to grow until 65 dpi. The disease symptoms on the inoculated cotyledons were assessed 20 days post-inoculation. Inoculated plants were allowed to grow until 65 dpi to assess stem canker development. Assessment on leaf lesions and stem cankers was carried out, small pieces (0.5 cm × 1.0 cm) of tissue from the inoculated cotyledon and stem region (visible cankers or the region close to where the cotyledon attaches to the

stem) were taken from three randomly selected plants for each of the isolate and control treatments to complete the Koch's postulates.

### Data analysis

To account for the unbalanced design due to not all plants developing leaf lesions, mean leaf lesion diameters were analysed using Generalized Linear Model (GLM) in Minitab 16. Means were separated using Tukey's Honest Significant Difference (HSD) test at  $P \leq 0.05$ . Leaf lesion and stem lesion severity scores were analysed by analysis of variance (ANOVA) in GenStat 14 with means separated using Tukey's HSD test at  $P \leq 0.05$ .

### Results and Discussion

In most cases, *L. biglobosa* was recovered from OSR and kale on the upper stem lesion, which had distinct margins similar to those caused by *L. maculans*. There were four isolates of *L. biglobosa* recovered in this study which had characteristic white fluffy mycelium with a yellow pigment produced in the growth media after 5 days growth. Pycnidia were also produced on *L. biglobosa* colonies but were usually covered with mycelium. Species identity of the representative isolates were confirmed using molecular techniques. Amplification of genomic DNA from four isolates identified as *L. biglobosa* by species-specific PCR yielded a product of approximately 444 bp. Identification was confirmed by sequencing of the internally transcribed spacer (ITS) of the ribosomal RNA and actin gene region. The ITS (580-585 bp) and actin (941 bp) sequences of the four isolates were 92-99 and 99% identical, respectively, to the reported sequences of *L. biglobosa* in GenBank, DQ133893.1 and AY748949.1 respectively. *Leptosphaeria biglobosa* was only recovered from OSR and kale stem lesions, and not from any swede samples. For the four *L. biglobosa* isolates in this study, the sequences of ITS regions and actin gene regions, clustered with the *L. biglobosa* 'brassicae' type specimen. However, the sequences of  $\beta$ -tubulin gene regions showed that only one isolate (Lb 238) was identical to the type specimen while the other three each had the same two polymorphic bases. Previous studies by Vincenot *et al.* (2008) also showed that *L. biglobosa* had some degree of sequence polymorphism, with discovery of six subclades (based on DNA sequence information) which were linked to either host specificity or geographic location. There was no evidence that the three isolates belonged to a new subclade as their sequences were 99.9% similar to Lb 238 and grouped within the 'brassicae' subclade. Actin and ITS were identical to *L. biglobosa* 'brassicae'. Vincenot *et al.* (2008) reported that the different subclades of *L. biglobosa* were different in their pathogenicity on an OSR cultivar. All isolates from this study were equally pathogenic on the tested crops, except for the ICMP isolate, further supporting that all *L. biglobosa* isolates found in this study belong to the brassicae subclade. The colony morphology could characterise the *L. biglobosa* isolate by producing a yellow pigment in the culture media (William & Fitt, 1999). For *L. biglobosa*, the main subclade associated with OSR worldwide, apart from central Canada and Australia, was also reported to be 'brassicae' (Vincenot *et al.*, 2008; West *et al.*, 2001). *Leptosphaeria biglobosa* 'brassicae' has not been isolated in Australia, with *L. biglobosa* 'australensis', 'occiaustralensis' and 'canadensis' being identified as being associated with OSR (Vincenot *et al.*, 2008; Wouw *et al.*, 2008).

No leaf lesions developed on the uninoculated control plants for OSR (cv. Flash and Excellium) and swede (cv. Highlander) at either assessment times. All three isolates tested were able to cause leaf lesions on both OSR cultivars (Flash and Excellium) and swede cultivar Highlander with symptoms characteristic to *L. biglobosa* were observed on all inoculated plants. The mean leaf lesions severity score of two OSR cultivars (Flash and Excellium) and swede cultivar Highlander assessed at 20 dpi are summarized in Table 1. Mean leaf lesion severity score assessed on all tested cultivars were not significantly different ( $P=0.637$ ) from each other with scores ranging from 4.92-5.52. There was a significant effect ( $P<0.001$ ) of isolates on leaf lesion severity score. Plants inoculated with isolate Lb 238 (6.08 score) had the greatest mean severity score and significantly different from isolate ICMP 10665 (3.60 score) but not with isolate Lm 237 (5.83 score). There was no significant interaction ( $P=0.026$ ) between cultivar and isolate on the leaf lesion severity score assessed on the inoculated plants with mean score ranging from 2.80-6.88.

**Table 1** Mean leaf lesion severity score (0-9 scale) on two OSR cultivars (Excellium and Flash) and one swede cultivar (Highlander) inoculated with three *Leptosphaeria biglobosa* isolates assessed at 20 day-post inoculation.

Isolates	Leaf lesion severity score (0-9 scale) <sup>1</sup>			Mean across isolates <sup>2</sup>
	Highlander	Flash	Excellium	
Lb 237	6.88	4.63	6.00	5.83 y
Lb 238	6.88	6.63	4.75	6.08 y
ICMP 10665	2.80	4.00	4.00	3.60 x
Mean <sup>3</sup>	5.52	5.08	4.92	

All three *L. biglobosa* isolates selected for the pathogenicity study showed that they were all pathogenic to susceptible and more tolerant cultivars of OSR, and susceptible cultivar of swede with the disease symptom being observed on all inoculated plants. Of the three isolates, isolate ICMP 10665 produced the least leaf lesion severity score (2.90). This isolate may have lost its virulence after more than 30 years in storage. Leaf lesion symptoms that developed on the inoculated plants were characteristic to what has been reported before for *L. biglobosa*, being small dark lesions with dark margins and pycnidia absent (Vincenot *et al.*, 2008). No stem lesions developed on the inoculated plants which were allowed to grow until 65 dpi. This finding was similar to the Wouw *et al.* (2008) study which found no hyphae of *L. biglobosa* 'canadensis' observed in the inoculated plant stems 10 weeks after inoculation. There are a few possible causes of the reduced efficiency of *L. biglobosa* in colonizing the stem. Of these, the inability to produce the toxin sirodesmin PL (as does *L. maculans*) could be one reason (Elliot *et al.*, 2007). In a greenhouse study conducted by Elliot *et al.* (2011), *L. biglobosa* 'canadensis' was reported to cause stem cankers with low internal infection severity on both *B. juncea* and *B. napus*. In their study, the plants were inoculated by spraying the 10<sup>7</sup> conidia/mL on four-week old seedling. This spraying method provided more infection sites on the seedlings which may lead to the formation of stem cankers. This is the likely reason for no stem lesions being observed in this study, where plants were inoculated with only one conidial droplet at one inoculation point. This suggestion has been supported by Travadon *et al.* (2009) who demonstrated that the disease severity (external necrosis length and necrosis score) increased with increasing number of infection sites on cotyledons. Furthermore, a longer incubation time (approximately 20 weeks after inoculation) was applied in the Elliot *et al.* (2011) study compared to this study which only had until 65 dpi (9.2 weeks). Another study that supported the finding by Elliot *et al.* (2011) was by Thomas *et al.* (2009) who also found that *L. biglobosa* 'canadensis' was capable of causing internal infection on *B. juncea* and *B. napus* although the disease levels were low. However, the inoculation method and incubation period used in both of previous studies were the same and not totally comparable with the present study. Further, the pathogenicity of a different subclade to which *L. biglobosa* 'canadensis' belongs, compared to *L. biglobosa* 'brassicae' used in this experiment, may account for differences in symptom development. In addition, Vincenot *et al.* (2008) also reported that all *L. biglobosa* 'brassicae' isolates used in their study were always ranked as most virulent on all cultivars (Columbus, Darmor, Surpass 400 and Westar) tested in comparison to the other subclades.

## Conclusions

In summary, the survey has confirmed that *L. biglobosa* is present in New Zealand and able to infect OSR and swede. This study has provided valuable new information on the pathogenicity of the New Zealand *L. biglobosa* isolate which can help to build disease control strategies in the future.

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## In Vitro Antimicrobial Activity of Ethanolic Extracts of *Piper nigrum* L.

<sup>1</sup>\*Noni zakiah, <sup>2</sup>Yanuarman yanuarman, <sup>3</sup>Miralena kartika

<sup>1</sup>Department of Pharmacy, Polytechnic of Medicine Of Ministry of Health, Darul Imarah, Aceh Besar 23352, Indonesia;

<sup>2</sup>Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>3</sup>Department of Pharmacy, Polytechnic of Medicine Of Ministry of Health, Darul Imarah, Aceh Besar 23352, Indonesia;

\*Corresponding Author: nonizakiah1981@gmail.com

### Abstract

The infection is a type of disease that most affect to many people. This study was conducted to determine the effect of ethanol extract of white pepper (*Piper nigrum* L.) on the growth of *Staphylococcus aureus* and *Escherichia coli*. This study was an experimental study with Kirby-Bauer method, the disc diffusion method. This study used a Completely Randomized Design (CRD), which is divided into 5 treatments, DMSO 10% (control), white pepper ethanol extract 100%, 75%, 50% and 25%. Each treatment was performed with five repetitions. Based on the ANOVA test, the results showed that the ethanol extract of white pepper (*Piper nigrum* L.) is very influential ( $P = 0.000$ ) against *Staphylococcus aureus* and *Escherichia coli*. Duncan Test results showed that the average diameter zone of inhibition for the ethanol extract of white pepper against *Staphylococcus aureus* at a concentration of 100%, significantly different from the other concentration. As against *Escherichia coli* at a concentration of 100%, significantly different from the other concentration. The results of this study can be concluded that the ethanol extract of white pepper (*Piper nigrum* L.) can inhibit *Staphylococcus aureus* and *Escherichia coli*.

**Key words:** *Piper nigrum* L., diffusion method, *Staphylococcus aureus*, *Escherichia coli*, inhibition zone

### Introduction

Infections are the most types of diseases that affect many people. One of the causes is bacterial infection. Among the pathogenic bacteria that can cause infections are *Staphylococcus aureus* and *Escherichia coli* (Jawetz et al., 2007).

Treatment of infections can be carried out by using antibiotics. However, the termination of antibiotic therapies prior the resolution is a major contributing factor of resistance. Therefore one of the alternatives that can be taken by the public is to utilize active substances contained in medicinal plants which considered safer and have relatively fewer side effects (Sari, 2006). White pepper (*Piper nigrum* L.) can be used in the treatment of abdominal pain, chronic kidney inflammation, malaria with fever, nasal congestion, and headache (Muslihah and Hening, 2000).

A research conducted by Sidarta et al., (2013) showed that the methanol extract of white pepper were able to inhibit the growth of *Streptococcus mutans*. This is because white pepper was thought to contain antibacterial compounds, such as alkaloids, tannins, phenols, coumarins and essential oils (Kumar et al., 2014). Research Singh et al., (2012) have proven that white pepper contains essential oils that can inhibit bacteria and fungi

Therefore, the authors were interested to conduct antibacterial activity assay of white pepper (*Piper nigrum* L.) against *Staphylococcus aureus* and *Escherichia coli*.

### Material and Methods

This study was an experimental through laboratory testing by using a diffusion method to test the antibacterial activity of ethanol extract of white pepper (*Piper nigrum* L.) on the growth of *Staphylococcus aureus* and *Escherichia coli*. Samples used in this study were white peppers (*Piper nigrum* L.) obtained from Lambaro market, Aceh Besar. The design used in this study was Complete Randomized Design (CRD) which divided into 5 groups, namely, DMSO 10% as a control, extracts of

white pepper with a concentration of 100%, 75%, 50% and 25% with each 5 replicates. The making of white pepper ethanol extract with maceration method.

### **Diffusion method assay to *Staphylococcus aureus* and *Escherichia coli***

Each media NA inoculated with the suspensions *S. aureus* and *E. coli* as much as 0.1 mL above the surface of the media. Each media is divided into five regions : the discs containing 10% DMSO as a control, the disc that had been dipped into an extract of white pepper with a concentration of 100%, 75%, 50% and 25%. All petries incubated at a temperature of 37°C for 2x24 hours. the growth of bacteria on each treatment was monitored, afterwards measuring the diameter of inhibition zones. The data were analyzed using ANOVA test dan Duncan test.

## **Results and Discussion**

### **Results of antibacterial tests against *Staphylococcus aureus***

The results of antibacterial activity assay of ethanol extract of white pepper (*Piper nigrum* L.) against of *Staphylococcus aureus* using the diffusion method showed that the ethanol extract of white pepper (*Piper nigrum* L.) could inhibit *Staphylococcus aureus*. It was characterized by the formation inhibition zones around the disc. ANOVA test results showed that the ethanolic extracts of white pepper (*Piper nigrum* L.) was very influential ( $P = 0.000$ ) against *Staphylococcus aureus*. This was due to antibacterial compounds contained in the ethanol extract of white pepper (*Piper nigrum* L.). The results of phytochemical tests, ethanol extract of white pepper (*Piper nigrum* L.) positive for alkaloid, tannin, phenol and essential oil (Sidarta et al., 2013).

Table 2. Further test results of mean diameter of inhibitory zone of white pepper ethanolic extracts (*Piper nigrum* L.) on the growth of *Staphylococcus aureus*

Treatments	Mean Diameter of Inhibition Zone $\pm$ SD (mm)
Control (DMSO 10%)	0,00 <sup>a</sup> $\pm$ 0,00
Extract of 100%	14,09 <sup>d</sup> $\pm$ 1,23
Extract of 75%	11,60 <sup>c</sup> $\pm$ 1,21
Extract of 50%	10,57 <sup>bc</sup> $\pm$ 0,89
Extract of 25%	9,96 <sup>b</sup> $\pm$ 0,41

The different Superscript letters showing the significant differences ( $P < 0,05$ )

Afterwards Duncan further tests were conducted which show the differences between treatments. The results of further assays of inhibitory zones mean diameter of white pepper ethanolic extracts (*Piper nigrum* L.) on the growth of *Staphylococcus aureus* could be seen in Table 2.

Based on Table 2, the widest mean diameter of inhibition zones was at a concentration of 100% of 14.09 mm, significantly different from the concentration of 75% (11.60 mm), 50% (10.57 mm) and 25% (9.96 mm). Meanwhile, at a concentration of 75% (11.60 mm) was not significantly different from the concentration of 50% (10.57 mm) as well as the concentration of 50% (10.57 mm) to 25% (9.96 mm).

### **Results of antibacterial tests against *Escherichia coli***

The results of antibacterial activity assay of ethanol extract of white pepper (*Piper nigrum* L.) against of *Escherichia coli* using the diffusion method showed that the ethanolic extracts of white pepper (*Piper nigrum* L.) can inhibit *Escherichia coli*. The mean diameters of inhibition zones that were obtained for concentrations of 100%, 75%, 50% and 25% were 11,31 mm, 9,92 mm, 9,73 mm dan 8,92 mm respectively. ANOVA test results showed that the ethanolic extracts of white pepper (*Piper nigrum* L.) was very influential ( $P = 0.000$ ) against *Escherichia coli*. Duncan test was conducted which show the differences between treatments (table 3).

Table 3. Further test results of mean diameter of inhibitory zones of white pepper ethanolic extracts (*Piper nigrum* L.) on the growth of *Escherichia coli*

Treatments	Mean Diameter of Inhibition Zone $\pm$ SD (mm)
Control (DMSO 10%)	0,00 <sup>a</sup> $\pm$ 0,00
Extract of 100%	11,31 <sup>c</sup> $\pm$ 1,42
Extract of 75%	9,92 <sup>b</sup> $\pm$ 0,97
Extract of 50%	9,73 <sup>b</sup> $\pm$ 1,49
Extract of 25%	8,92 <sup>b</sup> $\pm$ 0,54

The different Superscript letters showing the significant differences ( $P < 0,05$ )

The Duncan test showed that there were significant differences ( $P < 0.05$ ) at concentrations of 100%, 75% and control while the concentrations of 50% and 25% with no significant differences (table 3). Furthermore, the mean diameter of inhibitory zones of ethanolic extract of white pepper (*Piper nigrum* L.) on the growth of *Escherichia coli* showed that there were significant differences ( $P < 0.05$ ) at 100% concentration and control, but not the case with the concentrations of 75%, 50% and 25% (table 3).

The abilities of ethanolic extracts of white pepper (*Piper nigrum* L.) in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* were different, where the mean diameters of *Staphylococcus aureus* inhibition zones were greater than the mean diameters of inhibitory zones of *Escherichia coli*. It was assumed that occur in bacteria *Staphylococcus aureus* and *Escherichia coli* due to the differences between the structure of cell walls of bacteria that affect the function of the ethanolic extracts of white pepper (*Piper nigrum* L.) as antibacterial compounds. It means that ethanolic extracts of white pepper (*Piper nigrum* L.) can only take effect if the extracts could enter the cell of the bacterias. The structures of the cell walls of Gram-positive bacterias are more simple, single-layered with a low lipid content of bioactive ingredients to facilitate entry into the cell. *Staphylococcus aureus* as gram-positive bacteria have 3 layers namely the plasma membrane, the peptidoglycan layer which is thicker and teichoic acid (Pratiwi, 2008).

According to Jawetz et al., (2007) *Escherichia coli* is Gram-negative bacteria that have cell walls with multiple layered-structures and very complex, containing three polymer layers located outside the peptidoglycan layer namely lipoprotein, outer membrane consists of phospholipid and lipopolysaccharide, outer membrane has phospholipid properties. With the cell wall structures that more complex, posing a stronger barrier against antibacterial compounds contained in the white pepper ethanolic extracts (*Piper nigrum* L.) therefore harder for the compounds to penetrate the bacterial cell membrane, so that *Escherichia coli* is less sensitive to antibacterial compounds contained in the white pepper ethanol extract (*Piper nigrum* L.) compared to *Staphylococcus aureus*.

A research conducted by Ganesh et al., (2014) showed that white pepper was thought to contain antibacterial compounds, such as alkaloids and tannins. The ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall thereby inhibiting the microbial growth (Doss et al., 2009). On the other hand, alkaloid inhibits nucleic acid synthesis, as they inhibit the enzyme dihydrofolate reductase in cell-free assays (Cushnie et al., 2014).

## Conclusion

The ethanol extract of white pepper (*Piper nigrum* L.) is very influential on the growth of *Staphylococcus aureus* and *Escherichia coli*. The above results open the possibility of finding new clinically effective drug and could be useful in understanding the relationship between traditional cures and current medicines. An understanding of these properties would be invaluable in the development of alternative, natural, and safe methods of controlling bacterial infections. The crude extract of *P. nigrum* demonstrated a significant antibacterial activity against the microorganisms investigated and could therefore be added to the potential list of antibacterial agents. This result suggests the need for further studies on this substance to identify, isolate, characterize and elucidate the structure of the active ingredients using some spectroscopic techniques such as nuclear magnetic resonance (NMR), infrared spectrophotometry (IR) and mass spectrometry (MS).

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## **Rapid and Non-Destructive Evaluation by NIRS: Comparison between Partial Least Square and Support Vector Machine Regression Approaches to Predict Total Acidity of Intact Mango**

<sup>1</sup>Rahmaddiansyah, <sup>2</sup>\*Agus Arip Munawar

<sup>1</sup>Department of Agribusiness, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Agricultural Engineering, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia.

\*Corresponding Author: aamunawar@unsyiah.ac.id.

### **Abstract**

To determine acidity of mango or other agricultural products, several methods were already widely used in which most of them are based on solvent extraction followed by other laboratory procedures. However, these methods often require laborious and complicated processing for samples. They are time consuming and destructive. In last few decades, the application of near infrared reflectance spectroscopy (NIRS) as non-destructive technique in food and agricultural product industries is gaining more attentions. The basic idea of this technique is revealing chemical constituent information of biological objects buried in the NIR spectra through a process called calibration modeling. In the present study, two different linear and non-linear regression techniques namely partial least squares regression (PLSR) and support vector machine regression (SVMR) were studied and compared in predicting titratable acidity (TA) of intact mangos. TA prediction models were established based on near infrared diffuse reflectance spectra acquired between 1000 and 2500 nm. The results obtained show that both PLSR and SVMR were able to predict TA satisfactorily with maximum correlation coefficient ( $r$ ) is 0.98. Yet, SVMR is more powerful than PLSR for TA prediction. The coefficient determination of calibration ( $R^2_{cal}$ ) and prediction ( $R^2_{pred}$ ), the root-mean square error of calibration (RMSEC) and prediction (RMSEP), and the residual predictive deviation (RPD) were 0.96, 0.87, 26.94 mg·100g<sup>-1</sup>, 30.13 mg·100g<sup>-1</sup> and 3.80, respectively. The overall results sufficiently demonstrate that NIR spectroscopy coupled with the SVMR regression approach has the optimal results to determine TA of intact mango.

**Key words:** fast, technology, non-destructive, near infrared, spectroscopy.

### **Introduction**

Mango is one of the most popular fruits in tropical horticultures and very popular worldwide due to its high nutritional value, delicious taste and excellent overall flavor. It has very high demand and fetches a good price in the world market. With the increasing demand and consumption of mango, quality control becomes more and more important nowadays. Many national and international authorities are setting such criteria for quality factors to ensure good chain supply of mangoes.

In general, consumers purchase fresh fruits on the basis of quality which is an important subject to those engaged in horticultural industries. Their acceptance depends on highly subjective factors including appearance, touch, smell and taste. Titratable acidity (TA) is one of main quality attributes among others for mango fruit. The TA represents mango total acidities including ascorbic acid which is known as vitamin C. To determine acidity of mango, several methods were already widely used in which most of them are based on solvent extraction followed by other laboratory procedures. However, these methods often require laborious and complicated processing for samples. They are time consuming and destructive, therefore unsuitable for the quality control of fresh agricultural products which requires real time, rapid, on-line and non-destructive measurements.

Nowadays, several rapid and non-destructive methods are available such as ultrasound, microwaves absorption, nuclear magnetic resonance and infrared spectroscopy (Xie et al., 2011). Near infrared spectroscopy (NIRS) is among these methods has been proved to be a fast, simple, chemical free and non-destructive method and widely used for quality control assessment of various agricultural products (Munawar et al., 2013; Agelet et al., 2012; Xiaoying et al., 2012; Jha et al., 2012; Niu et al., 2008).

Recently, in many spectroscopic applications, partial least square regression (PLSR) is often used to make regression models because of its simplicity to use, speed and good performances. However, as

described by Perez-Marin et al. (2007), in many current and potential applications of NIRS measurement, the relationship to be modeled is not always linear. This means that the classical linear regression methods alone are not always the most suitable option and may not provide a complete solution to the regression problem in this work. Therefore, it is necessary that different linear and nonlinear tools should be attempted, and that is the aim of our present study.

The use of support vector machine regression (SVMR) approach as non-linear method for NIR spectroscopic regression purposes may be an option. Therefore, the main objective of this present study is to apply and compare two different regression techniques namely partial least squares regression (PLSR), and supporting vector machine regression (SVMR) used to predict titratable acidity (TA) of intact mangos by establishing calibration models.

## **Materials and Methods**

### ***NIR spectra acquisition***

Diffuse reflectance spectra were acquired and recorded using a benchtop Nicolet-Antaris Method Development Sampling (MDS) system on wavelength range from 1000 to 2500 nm, by co-adding 64 scans per sample. Spectra for each sample were captured 6 times at different points (two in the left and right edge, and four in the center) and the average of the six spectra was stored and used for further analysis.

### ***Reference TA measurement***

The reference titratable acidity (TA) measurement of mango was taken directly after spectra acquisitions. Each sample fruit was sliced at the same marked point of the NIR acquisition and the pulp was taken. TA measurement were carried out by making sample juice from 20 grams of pulp sample and adding maximum 100 ml distilled water. Automatic titration (Titroline 96, Schott) with 0.1 N NaOH to an end point of pH 8.1 was used to obtain TA expressed as  $\text{mg} \cdot 100\text{g}^{-1}$  Fresh Mass (Flores et al., 2009).

### ***Spectra pre-processing***

In order to achieve a reliable, accurate and stable prediction model, NIR spectra of all samples were pre-processed using multiplicative scatter correction (MSC) and standard normal variate (SNV).

### ***Data analysis***

Integrated software, Thermo Integration<sup>®</sup>, was used to develop workflow and run specified tasks of the NIR instrument for spectra acquisition. The Unscrambler X version 10.3 network clients (CAMO Software AS, Oslo) was used for spectra MSC and SNV pre-processing, and building calibration models with PLSR and SVMR algorithms.

## **Results and Discussion**

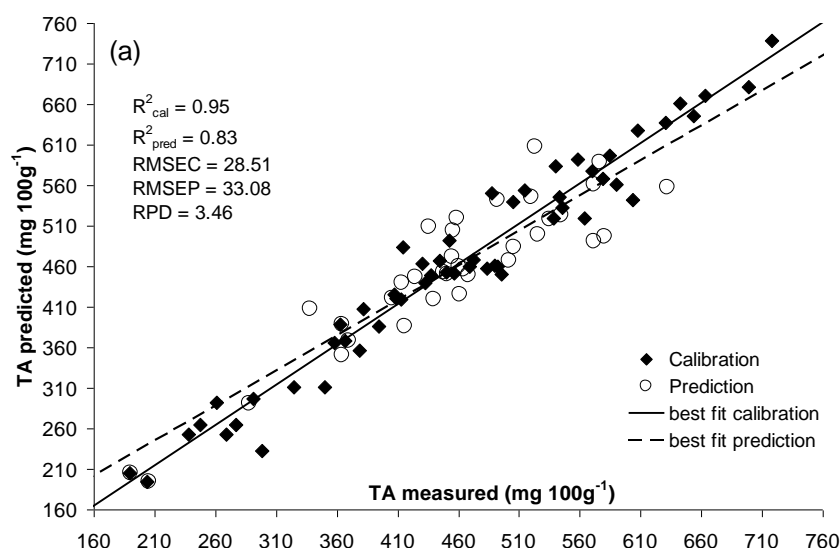
Calibration models were established by plotting reference TA measurement data as y-variable and NIR spectra data as x-variable in the calibration dataset (55 spectra dataset). For TA prediction, calibration models were built based on MSC spectra. The models were then tested by independent samples in the prediction dataset (30 spectra dataset). The performance of the final model was quantified based on calibration and prediction results according to the coefficient determination of calibration ( $R^2_{\text{cal}}$ ) and prediction ( $R^2_{\text{pred}}$ ), the root mean square error of calibration (RMSEC) and prediction (RMSEP), the error difference between RMSEC and RMSEP, and the residual predictive deviation (RPD) indexes obtained by dividing standard deviation of reference data with the RMSEP value. Finally, the numbers of principal components or latent variables required to build the model was also taken into account for optimal model criteria selections.

First of all, the PLSR method was attempted to predict TA. PLSR is one of the most widely used regression methods for NIR calibration. In this present study, during calibration, PLSR was optimized by a multifold cross validation with 10 segments and determined according to the lowest minimum root mean square error cross validation on the respective latent of variable. Based on the best calibration result, the optimum PLSR model was achieved when five latent variables are included for TA prediction as presented in Table 1.

Table 1. Comparison between two regression methods in calibration and prediction

Method	PCs / LVs	Calibration		Prediction		Error difference	RPD
		R <sup>2</sup>	RMSEC	R <sup>2</sup>	RMSEP		
PLSR	5	0.95	28.51	0.83	33.08	4.57	3.46
SVMR	4	0.96	26.94	0.87	30.13	3.19	3.80

For TA calibration, the optimal PLSR model produced the coefficient of determination ( $R^2$ ) and the root mean square error of calibration (RMSEC) are 0.95 and 28.51 mg 100g<sup>-1</sup> respectively. When the model tested by using samples on the prediction dataset, it yields the  $R^2$  prediction and the root mean square error of prediction (RMSEP) are 0.83 and 33.08 mg 100g<sup>-1</sup> respectively. Thus, the error difference between calibration and prediction is 4.57, and the residual predictive deviation (RPD) index is 3.46. This means, PLSR was accurate and robust for TA prediction. Scatter drawn from the PLSR model for predicted TA versus reference measured TA is presented in Figure 1.

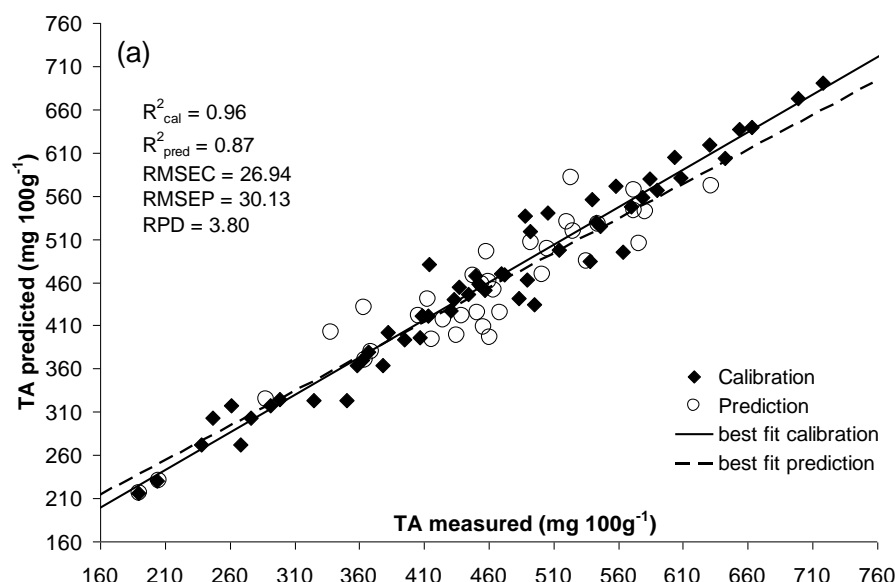


**Figure 1.** Scatter plot of predicted versus measured TA based on PLSR model derived from near infrared spectra data

Moreover, further spectra data analysis was performed to develop TA prediction model using a nonlinear regression approach. It was attempted by support vector machine regression (SVMR) method from which based on grid search optimization, the parameter values for SVMR approach are as follows: constraint parameter ( $C$ ) = 100, regularization parameter ( $\gamma$ ) = 0.01, and loss function ( $\epsilon$ ) = 0.1.

As can be seen in Table 1, SVMR provided superior results compared to PLSR for TA prediction. The use of SVMR improves the  $R^2$  coefficient both in calibration and prediction phases. As a consequence, the RPD index by SVMR for TA is also better (3.80) than by PLSR (3.46). Nevertheless, since the RPD is above 3, we may argue that TA can be predicted well either by SVMR or PLSR. Scatter drawn from the SVMR model for predicted TA versus reference measured TA is presented in Figure 2.





**Figure 2.** Scatter plot of predicted versus measured TA based on SVMR model derived from near infrared spectra data.

### Conclusions

The overall calibration and prediction results sufficiently demonstrate that NIR spectroscopy with the help of two regression approaches (PLSR and SVMR) can be successfully used in determination of TA in mango. The evaluation performance results of PLSR and SVMR with respect to TA prediction accuracy and robustness could be arranged in the following order: SVMR (4 PCs of PCA as input) > PLSR (5 LVs). The obtained results in this present study showed the high potential of NIRS with a nonlinear regression approach for rapid and non-destructive determination of TA in intact mangoes.

### Acknowledgements

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## Antibacterial activity of the extract combinations of *Myrmecodia pendens* and *Zingiber officinale* var. *rubrum*

<sup>1</sup>\*Munira, <sup>2</sup>Muhammad Nasir and <sup>3</sup>Ainun Mardiah

<sup>1</sup> Departement of Pharmacy, Health Polytechnic Aceh, Banda Aceh 23352, Indonesia.

<sup>2</sup> Departement of Biology, Syiah Kuala University, Banda Aceh 23111, Indonesia.

<sup>3</sup> Departement of Dental Nursing, Health Polytechnic Aceh, Banda Aceh 23352, Indonesia.

\*Corresponding author: munira.ac@gmail.com

### Abstract

The study was conducted to determine the antibacterial activity of extract combinations of ant nest (*Myrmecodia pendens* Merr. & L.M. Perry) and red ginger (*Zingiber officinale* var. *Rubrum*) in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus*. This study was an experimental study with completely randomized design consisting of 7 treatments with four replications each. The treatments consisted aquadest (negative control), extracts combinations of ant nest and red ginger extracts with ratios 5:0 ; 4:1; 3:2; 2:3; 1:4; 0:5 (pure red ginger). The microbiological test antibacterial activity was tested using Agar Diffusion. Screening phytochemical extracts ant nest contains saponins, flavonoids, polyphenols, terpenoids and quinones. While the red ginger extract contains alkaloids, saponins, tannins, flavonoids, polyphenols, terpenoids and quinones. ANOVA test results showed that the combinations of ant nest and red ginger extracts were very influential in inhibiting the growth of *E. coli* and *S. aureus* ( $P = 0.000$ ). Duncan test showed that the average diameter of zone of inhibition against *E. coli* showed no significant differences between the combination of ant nest extract and red ginger extract with ratio 5:0 (10.50 mm), 4:1 (12.00 mm) and a 3:2 (13.13 mm), but there are significant differences ( $P < 0.05$ ) with a 1:4 (17.63 mm) and 0:5 (18.38 mm). Duncan test showed that the average diameter of zone inhibition against *S. aureus* showed that no significant difference between the combination of ant nest extract and red ginger extract ratio of 5:0 (14.50 mm); 4:1 (15.73 mm) and a 3:2 (16.68 mm), but there are significant differences ( $P < 0.05$ ) with 2:3 (20.43 mm); 1:4 (24.23 mm) and 0:5 (33.18 mm). The combination of extracts ant nest with red ginger extract did not show any synergy.

**Keywords:** *Myrmecodia pendens*, *Zingiber officinale* var. *Rubrum*, antibacterial, *Escherichia coli* and *Staphylococcus aureus*

### Introduction

Infectious diseases are public health problem that never been completely overcome and remain major causes of death in the world, including Indonesia (Priyanto, 2009). In addition, there occurrence and emerge of antibiotic resistance become current world's attention (Westh et al., 2004). Increasing number of infections and bacterial resistance lead scientists to find new antimicrobial compounds are not only potent but also do not result in resistant cases as well as more affordable (Hertiani et al., 2003). Infectious diseases can be caused by several pathogenic microorganisms such as bacteria, viruses, parasites, and fungi (Jawetz et al., 2005). Among the bacteria that commonly cause severe nosocomial infections are *E. coli* and *S. aureus*.

Various efforts have been made to treat infectious diseases, such as by inhibiting the growth of microbes that cause infections (pathogens). This action generally requires the use of potent commercial antimicrobial compounds (Madigan et al., 2011), that has been challenged by the emerge of antibacterial resistance. Currently, the utilization of traditional medicine in Indonesia is progressing very rapidly as the alternative treatments for currently available modern medicines.

Among some popular medicinal plants are ant nest (*Myrmecodia pendens*) and red ginger (*Zingiber officinale* var. *Rubrum*). Red Ginger is used by the community as headaches reliever, swelling eliminator, menstrual pain killer, anti-emetics, diuretics, stimulants, anti-diarrhea, and anti-dysentery. While ant nest has been used as antibiotics, antiviral and herpes remedy (Cawson and Odell, 2008) as well therapeutic herbs for various diseases such as asthma, cataracts, diabetes, arthritis or rheumatism, migraines, hemorrhoids, periodontitis and cancer (Lok and Tan, 2009). Ant nest has both antibacterial and antifungal activities (Effendi and Hertiani, 2013). Red ginger also has antimicrobial activity (Sari et al., 2013). Flavonoids, tocopherols, polyphenols, glycosides and tannins contained in the ant nest are

bioactive compounds that act directly as antibiotic (Hakim, 2011). Zingerone (Anon, 2016) and gingerol (Miri et al, 2008) found in red ginger are active ingredients showed antibacterial and antifungal activities.

Currently, research have been done using extracts of ant nest or red ginger as antimicrobial. One study on the combination of plant extracts was done by Dwijayanto (2015) showed that the highest antioxidant activity of the extract combination with a ratio ant nest and red ginger extracts of 4: 1. Research on the combination of extracts ant nest and red ginger for antimicrobial purposes has never been done. Therefore it is necessary to do research on testing effect of these extract combinations as antimicrobial considering possible synergistic and antagonistic effects that may mutually reinforce or reduce their benefits.

## **Materials and Methods**

This study used a completely randomized design (CRD), which consisted of 7 treatments and 4 replications each. This research was conducted in June 2016 at the Laboratory of Microbiology of Department of Pharmacy, Ministry of Health Poltekkes Aceh and the Laboratory of Chemistry, Faculty of Mathematics and Natural Science, Syiah Kuala University, Banda Aceh.

### **Bacterial Strains**

*S. aureus* and *E. coli* obtained from the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh.

### **Extraction**

This study uses the ant nest and red ginger obtained from traditional medicine shop in Banda Aceh. Ant nest and red ginger extracts were individually prepared by soaking 100 g of the powder in 750 ml of ethanol 75% in glass jars for 5 days with occasional stirring. The jars should be protected from direct sun light. The marinades resulted were filtered through a funnel glass coated with filter paper. The filtrates were dissolved with 75% ethanol up 1000 ml, and stored for 2 days in closed containers that protected from sun light. A thick extract was obtained by subjecting individual extract to a rotary vacuum evaporator 40-50°C.

### **Media NA Preparation**

Growth media were prepared by pouring 10 g of NA media into an Erlenmeyer flask, added with 500 ml of distilled water and heated until dissolved. After checking the pH, the media were then autoclaved at 121 °C for 15 minutes, cooled down to 45 °C at room temperature and poured into (150x25 mm) petri disks, 40 ml each.

### **Inhibition test**

For agar diffusion method, a NA plate agar was divided into 7 regions (7 treatments) using a marker. Bacterial suspension (*S. aureus* or *E. coli*), 0.1 ml, were inoculated on the surface of ND media and spread using a bent rod. Five millimeter sterile paper disks containing aquadest (negative control), combinations of ant nest and red ginger extracts with ratios of 5:0, 4:1; 3:2; 2:3; 1:4; and 0:5 were applied onto agar plate which 4 replications each. The plate was incubated at 37 °C for 48 hours. Inhibition zones resulted were measured using a ruler.

## **Results and Discussion**

Antibacterial testing showed that the combinations of ant nest and red ginger extracts inhibited the growth of *S. aureus* and *E. coli*. Average diameters of inhibitory zones resulted by combinations of ant nest and red ginger extracts with ratios of 5:0, 4:1, 3:2, 2:3, 1:4 and 0:5 against *S. aureus* were 14.50 mm, 15.73 mm, 16.68 mm, 20.43 mm, 24.23 mm, and 33.18 mm, respectively. The average diameters of inhibition zone resulted by the same extract combinations against *E. coli* were 10.50 mm, 12.00 mm, 13.13 mm, 16.13 mm, 17.63 mm and 18.38 mm, respectively. How about the controls?? Statistical analysis using ANOVA test showed that there were very significant differences in antibacterial potential shown by combinations of ant nest and red ginger extracts in inhibiting the growth of both *S. aureus* ( $P = 0.000$ ) and *E. coli* ( $P = 0.000$ ). These ability might be related to bioactive compounds contained in the extracts. Phytochemical identification indicated that ethanol extract of ant nest contained saponins, flavonoids, polyphenols, terpenoids, and quinines whereas that of red ginger contained alkaloids, saponins, tannins, flavonoids, polyphenols, terpenoids and quinones.

Each chemical might have different mechanism of actions in inhibiting the growth of bacteria. According to Robinson (1995), alkaloids antibacteria work by disrupting components of peptidoglycan in the cell wall of bacteria so that the layer of cell wall structure is not fully formed and then cause cell death. Tannins, on the other hand, inhibit reverse transcriptase and DNA topoisomerase enzymes that important for the synthesis of bacterial cells (Nuria *et al.*, 2009). Tannins also show ability to inactivate microbial cell adhesion, enzyme activity and protein transport in the inner layer of cells (Cowan, 1994). According to Sari and Sari (2011), tannins also have targets in polypeptide cell wall so that the formation of the cell wall becomes less than perfect. This may result in lysis of bacterial cells from osmotic pressure and other physical stress so that bacterial cell will die. The incorporation of iron ions with tannins may relate to their toxicity (Akiyama *et al.*, 2001).

Terpenoids are known to active against bacteria, but their mechanisms of action are still not completely understood. Terpenoids antibacterial activity allegedly involve in membrane breakdown by lipophilic components (Cowan, 1999; Bobbarala, 2012).

In addition, according to Leon *et al.* (2010), phenolic compounds and terpenoids. Quinone in addition to providing a stable source of free radicals also known to form complexes with proteins irreversible nukleofilik amino acid causing protein inactivation. Quinone binding polypeptide and bacterial enzymes. Terpenoids generally occurrence through the bacterial cell membrane destruction because of the nature of terpenoid compounds tend to be lipophilic (Cowan, 1999). Cell membrane damage can occur when the active antibacterial compound reacts with the active side of the membrane or by dissolving the lipid constituents and increase permeability.

The further test by using Duncan test showed that the average diameter of inhibition zone formed against *S. aureus* showed no significant difference between the combination of ant nest extract and red ginger extract ratio of 5:0 ; 4:1 ; and 3:2, but there are significant differences ( $P < 0.05$ ) with extracts of ant nests and red ginger extract ratio of 2:3; 1:4 and 0:5. The combination of extracts ant nest and red ginger extract ratio of 2:3 and 4:1 ratio significantly different from 0:5. Further test results of average diameter zone of inhibition can be seen in Table 1. The results of a further test (Duncan test) the average diameter zone of inhibition against *E. coli* showed that the average diameter of inhibition zone formed showed no significant difference between the combination ant nest extract and red ginger extract a 5:0 with a ratio of 4:1 and the ratio 3:2, but there are significant differences ( $P < 0.05$ ) with extracts of ant nests and red ginger extract ratio of 2:3 ratio 1:4 and comparison 0:5. Further test results average diameter inhibition zone can be seen in Table 2.

Table 1. Extract Combination and average Inhibition Zone Diameter Ants Nest and Red Ginger Extract on Growth of *S. aureus*

No	Treatment	The Average Diameter inhibition Zone (mm) $\pm$ SD	Inhibitor Category
1.	Aquadest (Control)	0,00 <sup>a</sup> $\pm$ 0,00	Weak
2.	Ant nest extract:Red ginger extract (5:0)	14,50 <sup>b</sup> $\pm$ 1,62	Strong
3.	Ant nest extract:Red ginger extract (4:1)	15,73 <sup>bc</sup> $\pm$ 4,69	Strong
4.	Ant nest extract:Red ginger extract (3:2)	16,68 <sup>bc</sup> $\pm$ 2,70	Strong
5.	Ant nest extract:Red ginger extract (2:3)	20,43 <sup>c</sup> $\pm$ 3,89	Very Strong
6.	Ant nest extract:Red ginger extract (1:4)	24,23 <sup>c</sup> $\pm$ 6,36	Very Strong
7.	Ant nest extract:Red ginger extract (0:5)	33,18 <sup>d</sup> $\pm$ 2,60	Very Strong

Superscript different letters indicate significant differences ( $p < 0.05$ )

The results showed that treatment with red ginger extract created inhibition zone are higher. The highest diameter zone of inhibition is a combination of extracts ant nest and red ginger ratio of 0:5 (pure red ginger) and the lowest in the ratio of 5:0 (pure ant nest) either in inhibiting the growth of *S. aureus* and *E. coli*. This could be because the content of antibacterial compounds in red ginger extracts more than the extracts of ant nests. The red ginger extract contains alkaloids, saponins, tannins, flavonoids, polyphenols, terpenoids and quinones, but the ant nest extract containing said compounds except tannins and alkaloids.

Table 2. Average Extract Combination Inhibition Zone Diameter between Ants Nest and Red Ginger Extract on Growth *E. coli*

No	Treatment	The Average Diameter inhibition Zone (mm) ± SD	Inhibitor Category
1.	Aquadest (Control)	0,00 <sup>a</sup> ± 0,00	Weak
2.	Ant nest extract: Red ginger extract (5:0)	10,50 <sup>b</sup> ± 0,57	Strong
3.	Ant nest extract: Red ginger extract (4:1)	12,00 <sup>bc</sup> ± 1,08	Strong
4.	Ant nest extract: Red ginger extract (3:2)	13,13 <sup>bc</sup> ± 3,88	Strong
5.	Ant nest extract: Red ginger extract (2:3)	16,13 <sup>cd</sup> ± 3,56	Strong
6.	Ant nest extract: Red ginger extract (1:4)	17,63 <sup>d</sup> ± 1,43	Strong
7.	Ant nest extract: Red ginger extract (0:5)	18,38 <sup>d</sup> ± 4,49	Strong

Superscript different letters indicate significant differences ( $p < 0.05$ )

The ability of a combination of red ginger extract and ant nest in inhibiting the growth of bacteria there is a difference between *S. aureus* and *E. coli*, where the average diameter of inhibition zone against *S. aureus* is greater than the diameter of inhibition zone *E. coli*. According to Jawetz et al. (2007) stated that the bacterium *E. coli* is a Gram-negative bacteria have a cell wall structure layered and very complex, containing three polymer layers located outside the peptidoglycan layer is lipoprotein, outer membrane consists of phospholipid and liposakarida, outer membrane is phospholipid. This condition can lead to the ability influx of antibacterial substances into the bacterial cell is reduced, so that only a minimal impact on the lives of the bacteria. In addition, much of lipid found in the cell wall of Gram-negative bacteria can also affect timohidroquinon activity can reduce the inhibition produced. According to Pelczar and Chan (2005), Gram-positive bacteria have a thick cell wall structure (15-80 mm) single-layered. Lipid-containing cell walls are low (1-4%), peptidoglycan and teikoat acid. Peptidoglycan is a major component of the bacterial cell wall constituent so that Gram-positive bacteria are more sensitive to antibacterial substances of the Gram-negative bacteria.

## Conclusions

The combination of extracts ant nest and red ginger extract very influential in inhibiting the growth of *E. coli* and *S. aureus* ( $P = 0.000$ ). The average diameter of inhibitory zone with combination ant nest extract and red ginger extract on the growth of *S. aureus* greatest formed in comparison 0:5 (pure red ginger) that is equal to 33.18 mm and significantly different with all treatments. The average diameter of inhibitory zone with combination ant nest extract and red ginger extract on the growth of *E. coli* greatest formed in comparison 0:5 (pure red ginger) that is equal to 18.38 mm and not significantly different in the ratio 1:4 (average a diameter of 17.63 mm) and 2:3 (average diameter of 16,13 mm inhibition zone). The combination of extracts ant nest with red ginger extract did not show any synergy.

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## ***Fusarium* species associated with infected sea turtle eggs in Chagar Hutang, Redang Island**

<sup>1</sup>\*Siti Nordahliawate Mohamed Sidique, <sup>1</sup>Andrew A. Ngadin, <sup>1</sup>Nurul Faziha Ibrahim, <sup>2</sup>Juanita Joseph

<sup>1</sup>Laboratory for Agri-food, Pest and Disease Management, School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia.

<sup>2</sup>Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia.

\*Corresponding Author: dahliasidique@umt.edu.my

### **Abstract**

We noticed that fungi are often found on the exterior of unhatched green turtle eggs (*Chelonia mydas*) at Chagar Hutang, Redang Island. However, no detailed study has been conducted to address the problem and its relationship with frequent hatching failures. Therefore, the project was done to study the morphological characteristics of *Fusarium* species associated with unhatched eggs and to determine the sources of fungus such as soils, debris or plant parts nearby the nesting areas. Samples of infected eggs, soils, plant roots, and debris were collected from several egg chambers. Results showed the greatest number of isolates were *Fusarium*, mostly identified morphologically as *F. solani*. These studies were expected to provide an important information regarding to status of *Fusarium* species associated with unhatched eggs for developing control methods essentially in monitoring the nesting areas and furthermore, to mitigate pathogenic fungi impact.

**Key words:** *Fusarium*, green turtle, hatching failure, Chagar Hutang, Redang island

### **Introduction**

The green turtle (*Chelonia mydas*) is the most abundant sea turtle species in Malaysia, nesting in Sabah, Sarawak, Terengganu, Pahang, Perak, and Johor (Chan, 2006). The International Union for Conservation of Nature (IUCN 2004) listed the green turtle as endangered species. Most current conservation efforts aim to restore depleted populations by protecting nests and increasing the hatch success. Like many conservation programs in the world, it is not possible to protect all sea turtle eggs because of illegal and unsustainable poaching activities, as well as predation by monitor lizards, ants, crabs, and birds (Garcia *et al.*, 2003).

Now, there is yet another threat to this precious creature. Recently, there have been several reports of a fungus known as *Fusarium*, that has infected sea turtle eggs and is responsible for the sea turtles' decline in the Atlantic, Indian and Pacific Oceans from 2005-2012 (Fisher *et al.*, 2012; Sarmiento-Ramírez *et al.*, 2014). The exact similar fungal infection symptom was observed in Pulau Chagar Hutang in 2009 until present. In Malaysia, most nesting areas have been showing fungal infection that may lead to higher mortality rates as reported in Boavista where *Fusarium*, particularly the strain *F. solani*, as an important factor in the decline of sea turtles (Sarmiento-Ramírez *et al.*, 2010). The critical issue is that as climate change and habitat destruction continue, more turtles will deposit eggs in these less suitable nesting areas, increasing the likelihood of being infected by the aggressive fungus such as *Fusarium* species.

Several work has proven that soil-borne plant pathogenic fungi *Fusarium* species has been effecting the nesting areas especially in countries like Ecuador, Columbia, Costa Rica, United States, Australia, Spain and Turkey (Mo *et al.* 1990; Eckert and Eckert 1990; Phillott *et al.*, 2004; OzrGüçlü, 2010; Sarmiento-Ramírez *et al.*, 2014). The *Fusarium solani* was found to be responsible for dead of embryo of the loggerhead sea turtle in Boavista Island, Cape Verde, Spain (Sarmiento-Ramirez *et al.*, 2010). Phillott *et al.* (2004) had stated that the fungus could growth in exterior shell and/or embryonic tissues. Therefore, the work aims to study morphological characteristics of *Fusarium* species associated with unhatched green turtle eggs along the nesting beach and to determine the sources of fungal such as soils, debris or plant parts nearby the nesting areas.

## Materials and Methods

### Sampling site and sample collection

The nesting area is situated at Chagar Hutang beach (5° 48.35' N, 103° 00.50' E), in the northernmost part of Redang Island, which is about 22.5 km from the mainland of Terengganu (Fig. 1). The beach is about 350m long and backed by hills with undisturbed tropical rain forest and accessible only by boat. Chagar Hutang, Redang Island is one of the largest nesting sites for green turtles in Peninsular Malaysia, with 1000 to 2500 nests per year.

Samples were collected after monsoon season in April and June 2016. All unhatched eggs were examined to identify the cause of death. Samples of egg showing signs of fungal invasion, soils, plant roots and organic debris from the egg chambers were collected and kept in paper envelopes for fungal isolation.

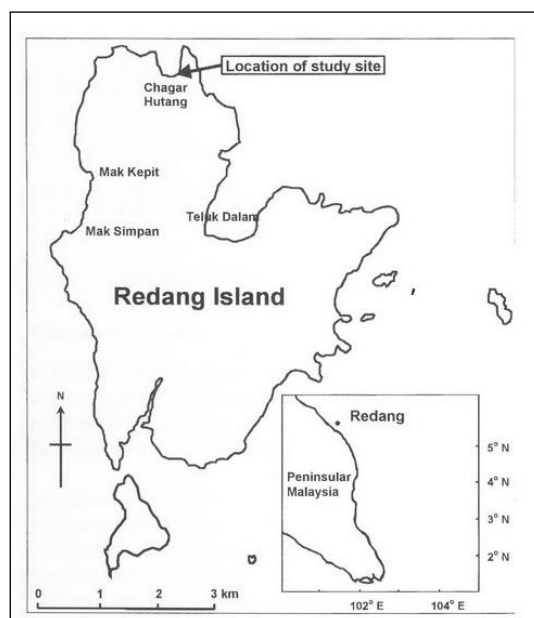


Fig. 1: Sampling of green sea turtle eggs colonised with fungus at Chagar Hutang, Redang Island.

### Fungal Isolation, purification and preservation

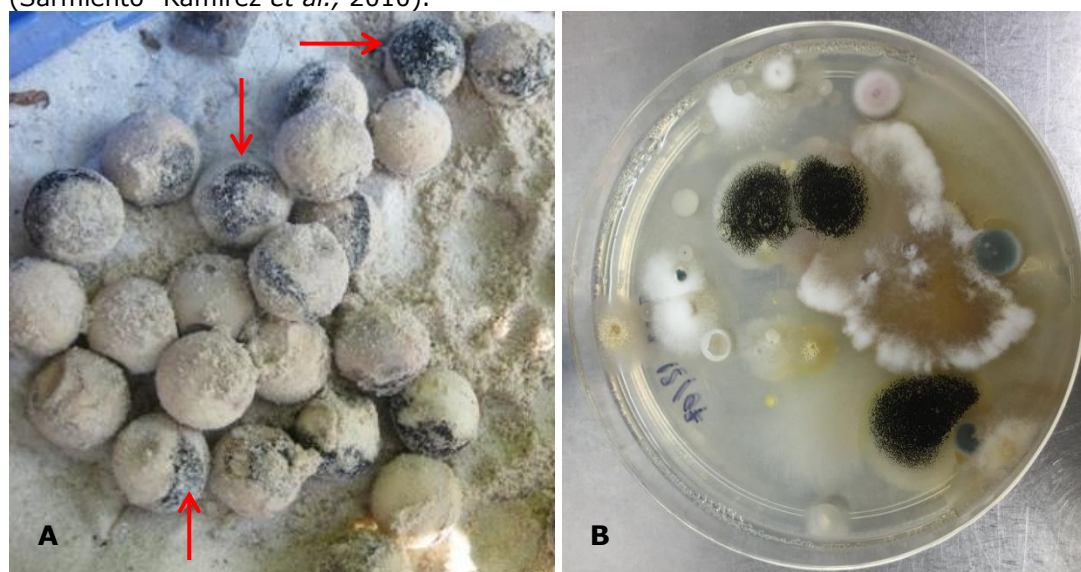
Sections of diseased egg shells, plant roots and organic debris were plated onto rose bengal agar (RBA) and potato dextrose agar (PDA) after surface-sterilized with 0.5% sodium hypochlorite and rinsed in sterile water (Leslie and Summerell, 2006). Soils from each nest were air-dried at room temperature ( $27 \pm 1^\circ\text{C}$ ) for five days, and passed through a 0.5 mm sieve to separate large particles of debris. From this, 10 mg of sieved sand were spread evenly on the surface of the PDA or RBA plates. Plates were incubated for 7 days under standard conditions (12 h under fluorescence and NUV lights, 12 h dark at  $28 \pm 2^\circ\text{C}$ ) (Salleh and Sulaiman 1984). All *Fusarium* species isolated were purified by using a single-colony isolation technique and preserved. The clean cultures of the fungus were used as working cultures. For preservation, the colonized leaves from carnation leaf-piece agar (CLA) (Fisher *et al.*, 1982) were taken out and placed in sterile cryovials for temporary preservation while the conidial suspension were poured into 15% sterile glycerol for permanent preservation stored in a deep-freezer at  $-80^\circ\text{C}$  (Leslie and Summerell, 2006). All *Fusarium* species were deposited to Fungi Culture Collection, Laboratory for Agri-Food, Pest and Disease Management (LAPDiM) Universiti Malaysia Terengganu (webpage: <http://lapdim.umt.edu.my/>).

### Morphological identification of fungal isolates

*Fusarium* cultures were transferred onto full-strength PDA and carnation leaf agar (CLA) plates (Fisher *et al.*, 1982) for observation of macroscopic characteristics (formation of mycelium and pigmentations) and microscopic characteristics (macroconidia and microconidia, phialides and chlamydospores). Morphological characteristics were evaluated and species were identified based on Leslie and Summerell (2006).



Results showed that *Fusarium solani* was the greatest number isolated (95%) and morphologically identified (Fig. 3) and others (5%) were *F. semitectum*, *F. oxysporum*, *Aspergillus* species and *Trichoderma* species. The hyphae of *F. solani* are capable to penetrate the turtle eggs and reducing calcium content of the outer layer (Phillot *et al.*, 2006). Furthermore, *F. solani* secondary metabolite is recognized as pathogenic or toxic to animal (Leslie and Summerell, 2006). In Boavista Island, Cape Verde, Spain, *Fusarium* was found to be responsible for dead of embryo of the loggerhead sea turtle (Sarmiento- Ramirez *et al.*, 2010).



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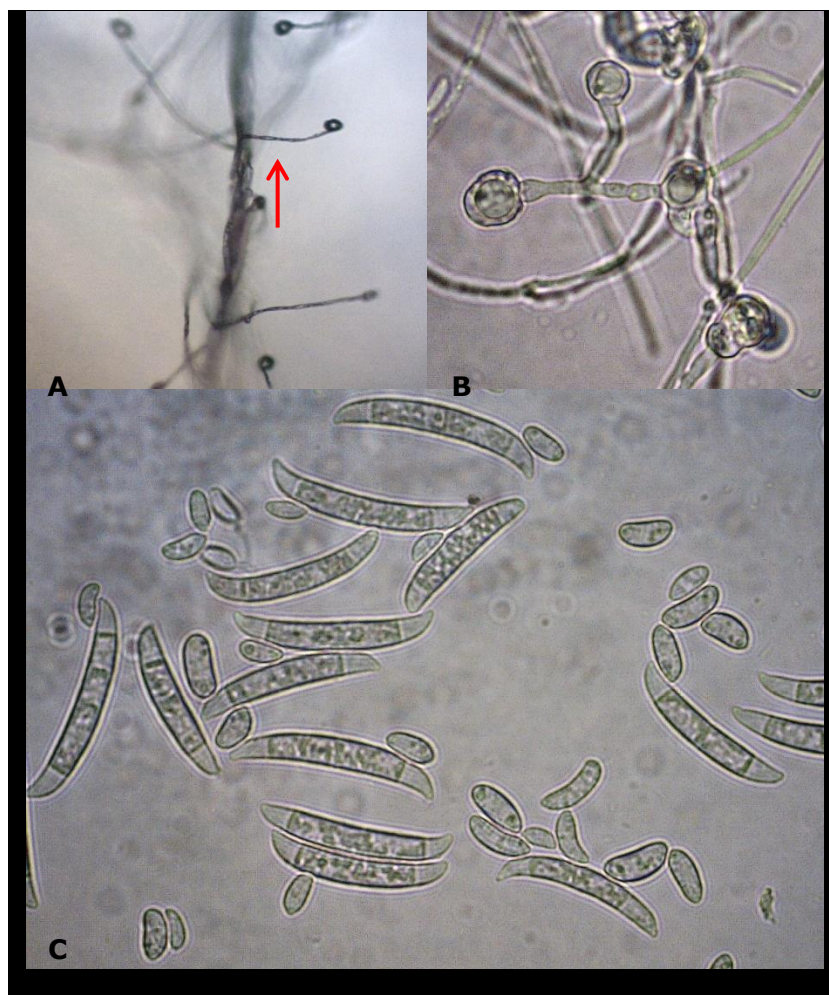


Fig. 3: Morphological characteristics of *F. solani* grown on potato dextrose agar (PDA) with (A) long monophialides (arrow), (B) chlamydospores, (C) macroconidia and microconidia. Scale bar: 20µm. Most of the fungi isolated from debris and soils were similar to fungi that were isolated from plant parts and this result suggested that the inoculum may derive from the vegetation area near to the nesting sectors.

### Conclusions

In Chagar Hutang, Redang Island, there is an urgent need to monitor the nesting areas and to understand the dispersal of *Fusarium* species along the nesting beach. Mortalities in nesting area of sea turtle in Chagar Hutang or other areas may lead to *Fusarium* species. Thus, it is important to understand the morphological characteristic of fungi, how it spread, penetrate and any possible production of mycotoxin. The nesting areas which are near to the vegetation areas may poses high risk of fungal infection and warmer conditions may trigger *Fusarium* colonisation. Finally, this work will contribute as an aid to improve conservation programs as well as to create awareness of the risk of fungal infection to both turtle and human.

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# **THEME : ANIMAL, FISHERIES AND MARINE SCIENCE**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
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## Effect of Salinity on the Growth of Juvenile Giant Trevally (*Caranx ignobilis*)

<sup>1</sup>Firdus, <sup>2</sup>Sayyid Afdhal El Rahimi, <sup>3</sup>Muhammadar A. Abas, <sup>3</sup>Boihaqi, <sup>4</sup>M. Ali S, <sup>5</sup>\*Samadi

<sup>1</sup>Departement of Biology, Faculty of Mathematics and Natural Science, Syiah Kuala University, Banda Aceh, Indonesia;

<sup>2</sup>Departement of Marine Science, Faculty of Marine and Fishery, Syiah Kuala University, Banda Aceh, Indonesia;

<sup>3</sup>Departement of Aquaculture, Faculty of Marine and Fishery, Syiah Kuala University, Banda Aceh, Indonesia;

<sup>4</sup>Departement of Biology Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh, Indonesia;

<sup>5</sup>Departement of Animal Husbandry, Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia.

\*Corresponding Author: samadi177@yahoo.de

### Abstract

Growth and development of fish are highly dependent on the type and character of species and salinity levels. Giant Trevally (*Caranx ignobilis*) is a marine carnivore fish that can be found in estuarine and marine environments. Regional coastal water environments such as estuaries, swamps, and tidal streams can be characterized by the number of abiotic stresses due to changes in temperature, dissolved oxygen and salinity environment, these conditions to affect the growth of fish. Giant Trevally is one type of reef fish that is very potential to be developed because it has some comparative advantages, among others, are able to live in conditions of high density (150 individuals / m<sup>2</sup>), and has a high growth rate. However, further study is needed on the effect of environmental factors to development. This study was done to determine the effect of different salinity on the growth of the Giant Trevally (*Caranx ignobilis*) in the media or pool maintenance. This study was conducted in June-August 2015. The design of the study was completely randomized design (CRD) non factorial with 6 treatments and 3 replications. The treatments were: A = Maintenance juvenile Giant Trevally on the salinity of 15 ppt, B = 18 ppt, C = 21 ppt, D = 24 ppt, E = 27 ppt, F = 30 ppt. The results showed that different salinity levels did not significantly affect the growth of juvenile Giant Trevally. This suggests that the juvenile Giant Trevally has a high tolerance to salinity changes.

**Keywords:** Growth Giant Trevally, *Caranx ignobilis*, salinity

### Introduction

Indonesia estimated sea area of 5.4 million km<sup>2</sup> have the longest coastline in the world at 81.000 km and the islands as many as 17,508. Indonesia has the potential of fish estimated at 6.26 million tons per year and can be managed sustainably with the details as much as 4.4 million tons were caught in the waters of Indonesia and 1.86 million tons can be obtained from the waters of the economic exclusive zones (EEZ) (DKP RI, 2001). From numbers of fish species in the Indonesian EEZ, giant trevally (*Caranx* sp) is one type of fish that is hunted by fishermen. In 2005 the production of giant trevally in Indonesia through the catch reached 47,125 tones (DKP Aceh, 2011). Aceh province is also famous for the production of catch giant trevally. DKP Aceh (2011) reported that in 2011 the catch giant trevally in the province reached 4,280.7 tons. Anonymous (2013) reported that in Aceh productivity of catch giant trevally increased to 6,761 tons. This situation shows that many giant trevally are captured in nature and very little of cultivation. Fishing gears used include fishing, trawl and others.

Giant trevally is one type of reef fish that potential to be developed compared to other types of fish, This is because giant trevally has some comparative advantages such as is able to live in conditions of high density (150 individuals/m<sup>2</sup>), has a high growth rate, is very responsive to the addition of feed, and has efficient feed conversion (Irianto *et al.*, 2002). The fish is popular to consumers due to it is relatively good taste, has no intermuscular bones so that easily consumed (Abdussamad *et al.*, 2008). This fish usually lives in shallow coastal water, coral and rock, and can naturally spawn and unseasonal. However, in some areas of Indonesia giant trevally have been cultivated, such as in Gondol (Bali), Batam and Aceh (Irianto *et al.*, 2002).



In the cultivation of giant trevally feed quality is the decisive factor to be considered for growth and health. The fish needs nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals. The needs vary according to the age and species of fish (Suwiry et al., 2001). Giant trevally in nature consumes 50% of fish, 25% crustaceans, 15% molusca and 10% Polychaeta (Rowling and Raines, 2000). Foods that commonly used in the cultivation of giant trevally are trash fish. One common type of trash fish used in aquaculture of giant trevally was kuniran (*Upeneus moluccensis*). According to Fauzi (2008), the approximate nutritional contents of kuniran trash fish are water (79.12%), protein (70.05%), lipid (6.50%), ash (0.07%) and crude fibers (18.68%).

In addition to the food, environmental conditions also affect the growth of giant trevally. According Mansauda et al. (2013) suitable water quality for maintaining this fish are water temperature 28-30 °C, salinity 24-30 ppt, and brightness 6 to 9.5 m. However, in nature giant trevally was also found in estuarine has salinity lower than that in high seas. It is presumed these fish have a high tolerance to environmental factor "salinity". Based on this evidence, there is a possibility that these fish can be cultured in brackish waters and even freshwater. Since scientific data on this subject have not been found, the study to investigate effects of different salinity levels on the growth of giant trevally was done.

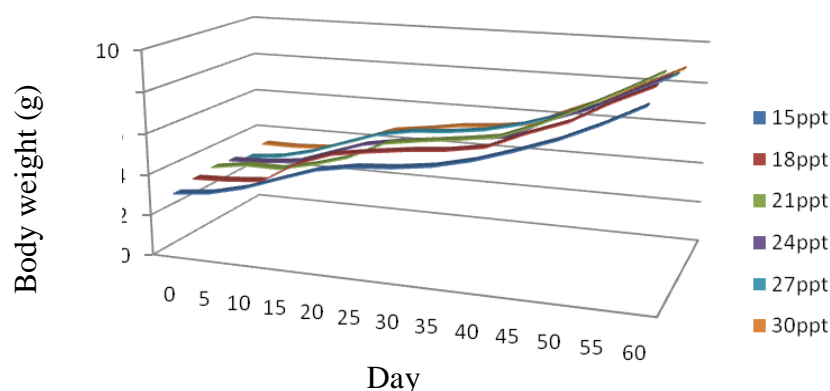
## Materials and Methods

This study was conducted from June until August, 2015 in the Hall of Brackish Water Aquaculture (BBAP) Aceh province. The fish used were juvenile fish. Bishop et al. (2006) said that the juvenile is the phase in which the morphology, physiology and ecology have been similar to the adult stage, but not reproductive. The experiment was performed using a completely randomized design non factorial consisting of 6 treatments and three replications. Before treatment all fish were adapted for 2 weeks. In triplicate, aerator equipped containers (tanks) contained water has salinity of 15 ppt, 18 ppt, 21 ppt, 24 ppt, 27 ppt, or 30 ppt. Treatment was started by subjecting ten heads of juvenile giant trevally to each tank for 60 days. Trash fish feed was given *ad libitum* twice a day, in the morning at 8:30 to 9:30 am and in the afternoon at 5:30 to 6:30 pm. Daily- (specific growth rate, SGR) and absolute (absolute growth rate, AGR) rates were recorded.

## Results and Discussion

### Daily growth

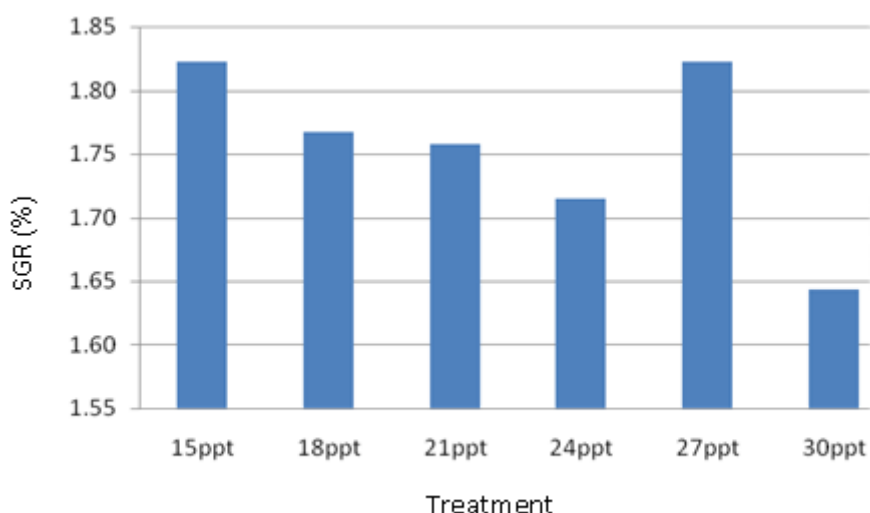
Fish daily growth rate was measured during 60 days of observation. Changes in salinity can affect the activity and growth of fish. Mansauda et al (2013) says that the salinity suitable for the life and growth of giant trevally range from 24-30 ppt. However, results of this study showed that salinity ranged from 15-30 ppt was feasible for the growth of giant trevally.



**Figure 1.** Distribution of weight of juvenile giant trevally reared in water has different levels of salinity for 60-days.

As illustrated in Fig. 1, the juvenile giant trevally continued to experience linear growth at every level of salinity. Daily growth, which is a calculation of weight growth per day, ranged from 1.64% -1.82%.

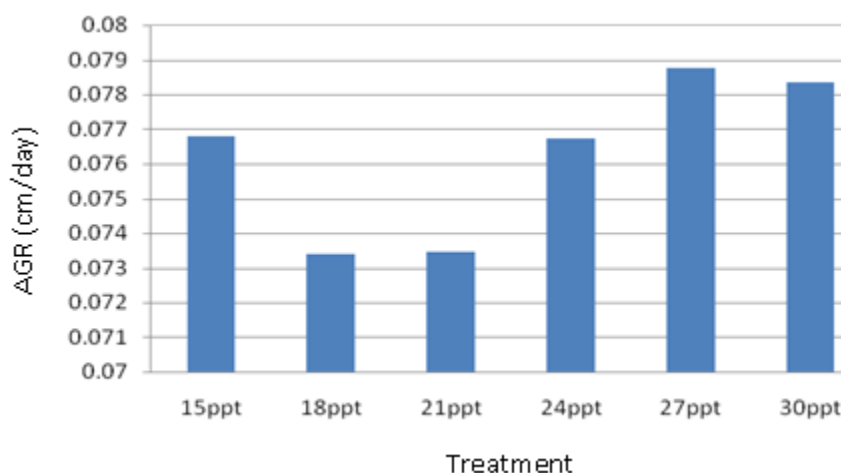
The highest daily growth was found water tank containing the salinity of 27 ppt (Figure 2). The lowest daily growth was found in 30 ppt salinity treatment.



**Figure 2.** Daily specific growth (%) of juvenile giant trevally at 6 levels of salinity

### **Absolute growth rate**

Absolute growth rate (AGR) was calculated from the length data of juvenile Giant Trevally for 60 days of observation. The range of AGR ranged from 0.073 to 0.079 cm/day (Figure 3). The highest value was also found in 27 ppt salinity treatment. The difference value from the smallest to the largest are about 0.006 cm/day.



**Figure 3.** Absolute Growth Rate (cm/day) juvenile Giant Trevally at six levels of salinity treatments

### **Effect of salinity on growth of juvenile giant trevally**

Statistical analysis to examine the effect of salinity on fish weight gain of juvenile giant trevally showed that there was no significant difference in weight gain and length of the fish at given different salinity (Table 1).

**Table 1.** Results test of ANOVA

Parameter	Treatment Salinity (ppt)						Statistic Test
	15	18	21	24	27	30	
SGR	1.83	1.78	1.76	1.72	1.83	1.65	non significant
AGR	0.077	0.073	0.073	0.077	0.079	0.078	non significant

The data indicated that juvenile giant trevally was able to live water has salinity ranged from 15 – 30 ppt. This showed that the fish is euryhaline, an organism is capable of living on a wide range salinity. Euryhaline can also be interpreted as an organism that is able to adapt to a wide range of salinity and can live in fresh, sea and brackish water. Anni *et al.* (2016) also showed no real difference in the survival and growth of juvenile Pompano (*Trachinotus marginatus* Cuvier 1832) maintained in water has different salinity (3-32 ppt) for 28 days. Different salinity also did not affect protein content and growth of bawal bintang (*Trachinotus blochii*) (Retnani and Abdulgani, 2012). Salinity upto 43 ppt did not influenced metabolic rates of inanga fish (*Galaxias maculatus*) (Urbina and Glover, 2015), indicating good tolerance of these fish on salinity stress. Another fish has good tolerance on salinity is milkfish (*Chanos chanos*) Alava (1998).

Mansauda *et al.* (2013) said that the salinity eligible for the life and growth of giant trevally were 24-30 ppt. Nevertheless juvenile giant trevally was able to live and grow normally at lower salinity. Rayes *et al.* (2013) said that decreasing salinity of sea water into fresh water can affect the balance between water and ion concentration in the fish's body, which deals with the process of osmoregulation, a process of adaptation of aquatic animals control water and ion balance between the body and the environment (Fujaya, 2004).

According to Rayes *et al.* (2013) fish lives in high salinity such as bawal bintang performs more active transport to remove excess Na ions from the gills thus requiring higher energy. According Fujaya (2004) and Bone and More (2008) the cells play a role in osmoregulation in the gills of fish, are choride cells located at the base of gills sheets. receptors of these cells are sensitive to salinity levels in the environment. When the fish are euryhaline sea water enters the environment with different salinity then choride cells will send signals to the central nervous system. If the fish enters the environment with higher salinity then the amount of choride cells will increase. If it enters the environment with lower salinity, on the other hand, the amount of choride cells reduced. Moreover, Rayes *et al.* (2013) said that the treatment engineering lower salinity of the sea water salinity will cause a loss of energy because the decreased activity of gill chloride cells in removing ions. This will provide more energy source for fish to grow rather than perform the osmoregulation process at lower salinity water.

## Conclusion

The study concluded that juvenile giant trevally (*Caranx ignobilis*) can live in a wide or broad ranges of salinity (euryhaline). This indicates potential development of the fish in aquaculture units with brackish water conditions or lower salinity.

## Acknowledgements

Our gratitude and appreciation go to the agencies that helped or supported the implementation of research, namely: 1) Institute of Research and Community Service (LPPM) Syiah Kuala University who funded the study, and 2) Center for Aquaculture Brackish Aceh that has given permission to use the premises and research facilities.

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## Biodiversity of Fish in the Krueng Geumpang River After One-Year Mass Kill of Fish in Geumpang, Pidie Regency of Aceh Province

<sup>1</sup>\*Muhammad Nasir, <sup>1</sup>Iqbar, <sup>1</sup>Dalil Sutekad, <sup>1</sup>Najian Haly, <sup>2</sup>Muchlisin ZA, and <sup>3</sup>Munira

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Faculty of Marine and Fisheries Sciences, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>3</sup>Departement of Pharmacy, Health Polytechnic of Aceh, Banda Aceh 23352, Indonesia;

\*Corresponding author: muhd.nasir@fmipa.unsyiah.ac.id

### Abstract

Increased anthropogenic activities that include gold mine extraction by illegal mining in the upstream of Krueng Geumpang River have polluted the river water and threaten aquatic biota. After a massive fish death in 2014, unfortunately there is no study performed to the river that has become the important source of fish consumed by the local community. This study was, therefore, conducted to determine the distribution and diversity of fish after one year the mass kill of fish in Krueng Geumpang River using survey explorative method. Fish samples were obtained by catching fish using fishing nets and fish traps. Total catches obtained nine species of fish, which is dominated by fish Ren (*Tor Soro*) of the family Cyprinidae. Diversity Index ( $H'$ ) ranged 0.00-1.57 (low). The low diversity index showed that the River Krueng Geumpang is still unhealthy or impaired category.

**Keywords:** mass kill of fish, fish biodiversity, poisoning, illegal mining

### Introduction

Anthropogenic activities related to the extraction of gold metal by illegal gold miners have increased in the Krueng Geumpang River upstream region (PETI). Adlim (2016) suggested that the use of mercury for the extraction of gold ore has been widely known and practiced by illegal miners. Wastes water from the gold ore extraction generally still contains high concentrations of mercury and will pollute the river water stream and threaten aquatic biota. Lyons *et al.* (2013) suggested that pollution could disrupt ecological balance of aquatic biota and result in bad effects on the health of humans rely their life on the river.

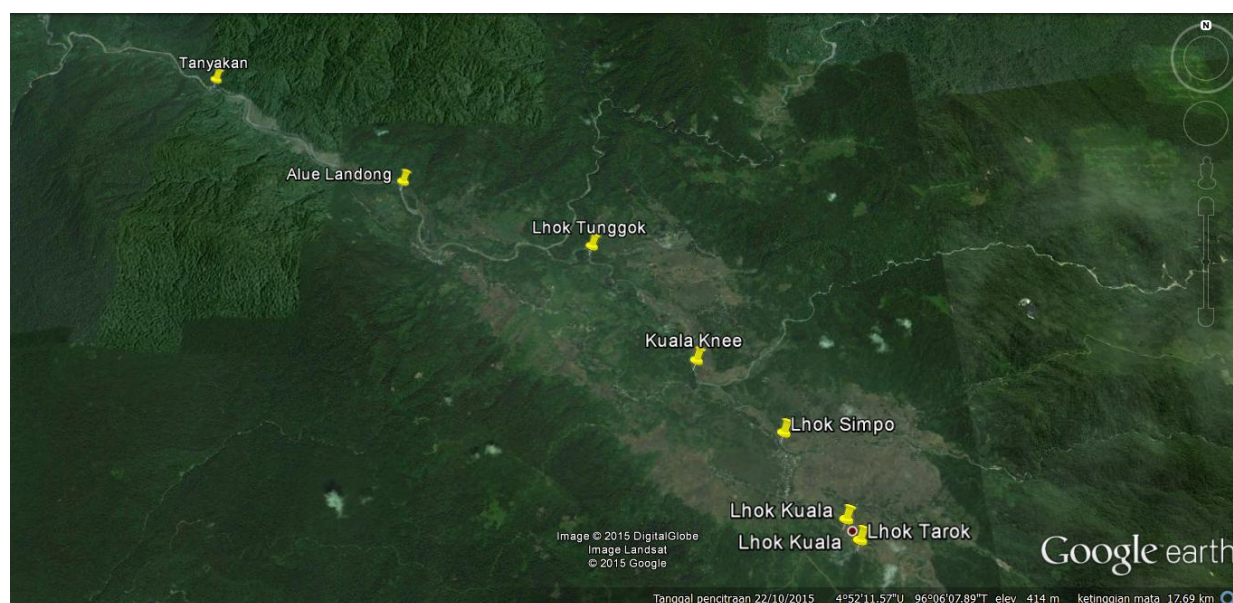
Mine wastes discharged into coastal waters have the potential to be toxic to aquatic biota. The toxicity can occur either directly or indirectly through the food chain (Karrari *et al.*, 2012). This will cause water pollution from mercury (Hg) and cyanide that in turn threaten the potential of Krueng Geumpang Rier as valuable fish resources for the livelihood for fishermen in the Geumpang district.

Pollution of aquatic environments and the mass kill of fish in the Krueng Geumpang river already crowded reported (aceh.tribunnews.com, August 6, 2014). Until this report prepared, however, there is no study performed to investigate the level of contamination in the Krueng Geumpang River and its impacts on the mass death, distribution and diversity of fish in the river. The objective of present studies was to determine the diversity and distribution of fish in the Krueng Geumpang River after one year of the mass kill.

### Materials and Methods

#### Location and sampling

This research was conducted at Krueng Geumpang River, Geumpang Region, Pidie regency in August 2015, approximately one year after the mass death of fish occurred in the study site. Based on characteristics and conditions found along the Krueng Geumpang River, seven sampling locations were determined (Figure 1). Coordinates and characteristics of each location are presented in Table 1.



**Figure 2.** Map showing sampling locations of fish along the Krueng Geumpang River, Aceh, Indonesia

**Table 1.** Locations, GPS coordinates and characteristics of sampling sites

Locations	Coordinates	Characteristics
Lhok Tarok	4°49'40.93"N; 96°8'10.01"E	bordering to the protected forest
Lhok Kuala	4°49'52.66"N; 96°8'3.95"E	around the settlements and paddy fields, additional water from a small river
Lhok Simpo	4°50'43.64"N; 96°7'34.03"E	the settlement and paddy
Kuala Keunee	4°51'29.97"N; 96°6'46.27"E	assembly between Kr. Geumpang with Kr. Keunee
Lhok Tunggok	4°52'51.43"U; 96°5'43.34"E	Many activities taking Quarry
Alue Landong	4°53'43.19"U; 96°3'38.98"E	Bordered by natural forest and Alue Landong River
Tanyakan	4°55'12.66"U; 96°1'18.89"E	River water deeper and larger rivers

### Procedure

Fish were trapped using fishing equipments generally used by local fishermen, namely nets, gill nets, traps (all fishing gears had mesh sizes ranged from 0.5 to 1.5 inches), hooks and line. Fish samplings were done during the day and night over a period of one or two days. Fish caught were placed in a container containing 4% formalin and brought to the Basic Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda for identification following protocol described by Kottelat *et al.* (1993).

### Data analysis

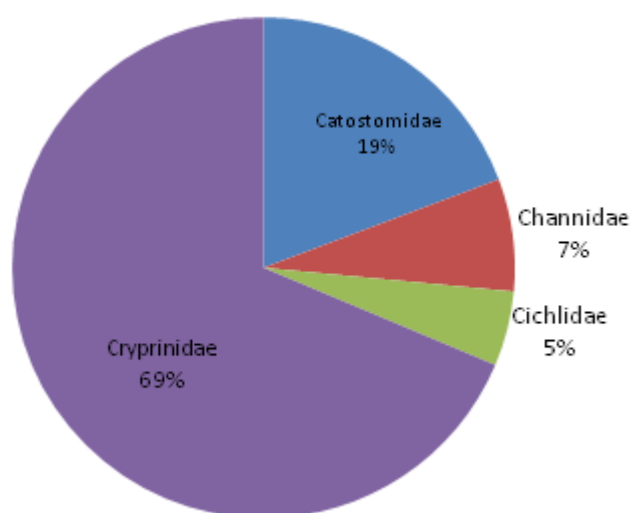
The presence and distribution of fish species in the study area were calculated using the frequency of incidence (FOI) (Muchlisin and Siti-Azizah, 2009; Muchlisin *et al.*, 2015). Diversity index of the fish, a value that can indicate the balance of the habitat, was determined using Shannon-Wiener Index (Brower and Zar, 1977). Dominance index, a calculation to determine the type of fish that dominates in the community, was measured using the Simpson dominance index (Brower & Zar, 1977)

### Results and Discussion

A total of 83 fish were successfully caught in this study. They belonged to 4 families, 6 genera and 9 species. The numbers of fish caught were different according to sampling sites: 14 fish in Lhok Tarok, 13 in Lhok Kuala, 8 fish in Lhok Simpu, 12 fish in Kuala Keunee, 8 fish in Lhok Tunggok, 16 fish in Alue Landong, and 12 fish in Tanjakan (Table 1). Cyprinidae constitutes the prominent fish composition of Krueng Geumpang River (Figure 2). Total largest number of individual found in Alue Landong, and the lowest number of individual found in Lhok Simpu and Lhok Tunggok (Table 2).

**Table 1.** Total genus, species and biological indices of river fish collection Krueng Geumpang

Location	Genus	Species	Number of individuals	Dominance Index	Diversity Index
A. Lhok Tarok	6	6	14	0.24	1.57
B. Lhok Kuala	1	1	13	1.00	0.00
C. Lhok Simpo	3	3	8	0.34	1.08
D. Kuala Keunee	1	1	12	1.00	0.00
E. Lhok Tunggok	3	3	8	0.59	0.74
F. Alue Landong	4	4	16	0.34	1.23
G. Tanyakan	1	1	12	1.00	0.00
Total	6	9	83	0,21	1,75



**Figure 2.** Composition of fish family in the Krueng Geumpang River

Four dominant species found in the Krueng Geumpang river were *Hypentelium nigricans* (FOI = 57.14%), *Oreo chromisniloticus* (FOI = 42.85%), *Rasbora sp.* (FOI = 42.85%), and *Tor soro* (FOI = 42.85%). Each was present in three and four sites (Table 2). Fish diversity index ranged from 0 to 1.75. Dominance index ranged from 0.21 to 1.00. They have economic value because all are consumed by humans.

The low 0-1.7 diversity indexes ( $H'$ ) of fish in the observation sites belonged to the criteria for low and medium. These shows that the water quality of the Krueng Geumpang River has experienced moderate to severe disturbances and fish populations are not yet stable. The small number of species and the number of individual fish one year after the mass death thought that has a close relationship between the distributions of the fish with the quality of the environment. Odum (1971) stated that the species diversity tends to decrease in disturbed ecosystems both physically and chemically because only certain organisms are able to adapt.

Mining wastes received by Geumpang Krueng River flow comes from mining activities available in the upper stream of the river. After the mass death of fish, processing mills of mining located at the borders of the river were closed and moved to the protected forest area. The relocation process was the result of the public protest to shut down mining activities in the border of Krueng Geumpang River. The abandoned processing mill sites can be seen until now. However, the numbers of illegal mining are increasing and become major waste generators that depress environmental conditions.

Fish species that are found few small numbers and in expressions of behavior and morphology are fish that like fast flow water, it indicates that the fish caught that just through the region. In terms of the size generally fish caught in most areas is still relatively small.

Diversity indexes are used to measure how human disorders affecting fish communities in aquatic environments. The site and arrangement of an area is an important factor in the development of effective environmental management strategies. Environmental management is needed to conserve and restore ecosystems impaired. Especially downstream of freshwater river ecosystem of the mine area are growing year by year. Human disturbance factors explain that a vulnerable species will difficult to regeneration so in danger of extinction in the river.

*Tor tambra* and *T. soro* are fish species currently listed as endangered in the IUCN Red List (IUCN 1990). According to Kottelat *et al.* (1993) that genus *Tor* threatened by over-exploitation, waste pollution, and ecological disruption. This is supported by Muchlisin *et al.*, (2015) states that the freshwater fish species are threatened by large overfishing and habitat degradation on a local and global scale.

Muchlisin *et al.*, (2015) suggests fishing Keureling (*Tor tambra*) in the wild should be reduced and may be banned in the future to protect this species. Should the fishermen have to shift their business to cultivation. For this purpose, breed and feed technologies need to be developed. Therefore, information about the biological aspects such as feeding, reproduction and growth are very important to support this program.

**Table 2.** List of species of fish and total individual, economic value and distribution in the river Geumpang Krueng Aceh Province, Indonesia.

Species	Family	Local name	Locations							Total	FOI (%)	Economic Value
			A	B	C	D	E	F	G			
<i>Channa sp.</i>	Channidae	Naleh	-	-	-	-	-	3	-	3	14.28	HC
<i>Channa sp.</i>	Channidae	Ntok	1	-	-	-	-	-	-	1	14.28	HC
<i>Channa striata</i>	Channidae	Bacee	2	-	-	-	-	-	-	2	14.28	HC
<i>Hypentelium nigricans</i>	Catostomidae	Ile	5	-	2	-	1	8	-	16	57.14	HC
<i>Oreochromis niloticus</i> Linnaeus, 1758	Cichlidae	Mujair	1	-	-	-	1	2	-	4	42.85	HC
<i>Puntius brevis</i> Bleeker, 1850	Cyprinidae	Groe	1	-	-	-	-	-	-	1	14.28	HC
<i>Rasbora sp.</i>	Cyprinidae	Depek	4	-	3	6	-	-	-	13	42.85	HC
<i>Tor soro</i> Valenciennes, 1842	Cyprinidae	Ren	-	13	3	12	-	-	-	28	42.85	HC
<i>Tor tambra</i> Valenciennes, 1842*	Cyprinidae	Kerling	-	-	-	-	-	3	12	15	28.57	HC
Number of Individual			14	13	8	12	8	16	12	83		
Number of species			6	1	3	1	3	4	1	9		

Note: \* IUCN red list, A= Lhok Tarok, B= Lhok Kuala, C= Lhok Simpu, D= Kuala Keunee, E= Lhok Tunggok, F= Alue Landong, G=Tanyakan, FOI= Frequency of incidence, HC= Human Consumption

## Conclusion

The diversity of fish is low indicating that the Krueng Geumpang River still not good or impaired category. The degradation and pollution of environmental condition reduced physical habitat, water quality, and sensitive taxa. More small-sized and sensitive species dominated the upstream sections, while tolerant species dominated the middle and downstream sections. Habitat and chemical pollutants have been famous as key factors that structure fish communities, which means that improving habitat value and reducing gold mine wastes of chemical pollutants will encourage the restoration of biological in Krueng Geumpang River. Based on fisherman information that, almost half of the fish species have disappeared in their fish catch area despite being one year after the tragedy.

## Acknowledgements

My special acknowledgement is dedicated to Syiah Kuala University for providing financial support for this project. The other appreciation is dedicated also to Biology Department and DMAS Program who have given permission and support for the implementation of this study and as well as all those who participate and help the implementation of this research.

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## **A Study of Adaptation of Simeuleu Wild Buffalo Behavior for Semen Collection**

<sup>1</sup>Kartini Eriani, <sup>2</sup>Dasrul, <sup>1</sup>Rosnizar, <sup>1</sup>Ria Ceriana , <sup>1</sup>Irma Suryani  
and <sup>3</sup>Syahrudin Said

<sup>1</sup> Department of Biology, Faculty of mathematics and science, Syiah Kuala University

<sup>2</sup> Department of Reproduction, Faculty of Veterinary Medicine, Syiah Kuala University

<sup>3</sup> Laboratory of Animal Cell Culture and Reproduction Biotechnology LIPI

\*Corresponding Author: dasrul.darni@yahoo.com

### **Abstract**

Simeulue buffalo live along the coast of the Simeulue Island. Simeulue buffalo can be used as semen donor so it is necessary to adapt from the wild to the site maintenance. This study used two wild Simeulue buffaloes held in the Saree Central Artificial Insemination (BIB). The study was conducted during five months from March to July 2015. We used standard training methods in Saree BIB. The results showed that Simeulue buffalo needs special handling to get optimal results

**Key words:** simeulue buffalo, artificial insemination, adaptation, sperm collection

### **Introduction**

Buffaloes all over the world can be put in two types; river and swamp buffaloes. River buffalo is kept for its milk, while swamp buffalo for its meat. Simeulue buffalo, one of buffaloes originated from Indonesia, is swamp buffalo and has special characteristics. Buffalo population in the world (Bahri and Talib, 2008; Cruz, 2009) especially in Indonesia (DITJENAK, 2010) tends to decline. To increase buffalo population it can be done by increasing its productivity. Artificial insemination technique is a solution to increase buffaloes' productivity. Artificial insemination (AI) is a technique that can be used to increase animal population and genetic quality (Foote, 2001).

Simeulue buffalo is a local buffalo lives along the coast of Simeulue Island. Its specific trait is a white line above its eyes, looking almost like eyewear, and white color around its neck. Picture 1 shows simeulue buffalo characteristics that distinguish it from other sort buffaloes from outside Simeulue Island. This buffalo lives in the wild along the coast of Simeulue Island. It has a superior genetic quality and has a good potential to meet the demand of buffalo meat in Aceh. Therefore this prime buffalo is expected to be able to be bred and developed in other areas. The breeding needs adaptation process with its new environment. After finding the right handling in breeding this buffalo, artificial insemination can be applied.

Artificial insemination is necessary to increase the efficiency of this genetically superior buffalo. The problem is that most all simeulue buffalo is bred in the wild that makes it very difficult to treat it as semen donor. Therefore efforts should be made to tame and make it ready to fertilize female counterparts.

### **Materials and Methods**

Two male buffaloes were brought from Simeulue Island and they were kept for four months , starting from March until July 2015 in Saree Artificial Insemination Centre (BIB). After taming the wild male buffaloes, the next step was preparing them as semen donor. The buffaloes were fed with grass and concentrate, and once a week was given honey and eggs. Their sexual behavior was observed once a month for five months while treated and used as semen donor. Data was then analyzed descriptively.

### **Results and Discussion**

The buffalo behaviors being observed were its eating, adaptation to the new environment and sexual behavior in order to prepare them as semen donor.



### Eating behavior

Eating begins with sniffing greeneries, snatching food, then lifting, chewing and swallowing (Rasyid, 2008). The results of the observation of the eating behavior and the training of buffalo stud can be seen in Table 1.

**Table 1.** Simeulue buffalo eating behaviors in new environment (Saree BIB)

Buffalo	March	April	May	June
1	Not willing to eat grass	Willing to eat a little	Willing to eat grass	Not willing to eat concentrate
2	Not willing to eat grass	Willing to eat a little	Willing to eat grass and concentrate	Willing to eat grass and concentrate

The buffalo 1 was not willing to eat concentrate at all (Table 1). Buffalo 1 and 2 showed the same eating behavior in March and April. But in May buffalo 2 was already willing to eat concentrate. It shows that Simeulue buffalo needs a long time to adapt to a new environment that is different from its habitat. Their original environment is warm because it is situated in coastal area while Saree is somewhat colder for it is situated in a mountain. The environments are very different so that the buffaloes needed a more serious adapting. Cruz (2010) reported that buffaloes take varying time to adapt to a different environment, depending on individual and species' capability. Although in general buffaloes eat a lot more than cows, and they eat all kinds of leaves, but apparently wild buffaloes show different eating behavior. Their appetite disappears due to environment change. After having a discussion with local people, it seems that the changing of water from salt to sweet water has impact on buffaloes' eating pattern and immunity.



**Figure 1.** Glasses a specific character of Simeulue buffalo

### Environment adaptation behavior and taming

Observation of adaptation behavior can be seen from outer morphology such as hair and body posture. Wild behavior and taming adaptation were observed from approachability and the ability to be herded. The result can be seen in Table 2.

**Table 2.** Environment adapting behavior and taming Simeulue buffalo

Buffalo	March	April	May	June
1	Very wild and aggressive . Trying to rout and made the loud noise. Could not be put nose ring.	Hair loss and looked a bit skinnier. Still wild and aggressive. Trying to rout and made the loud noise. Nose ring could not be mounted	Nose ring was mounted but lost because it was still wild. Body hair started to grow back.	Body weight was back to normal and became a bit tame. Still hard to herd.
2	Very wild and aggressive . Trying to rout and made the loud noise. Nose ring could not be mounted.	Hair loss and looked a bit skinnier. Still wild and aggressive. Trying to rout and made the loud noise. Nose ring	Nose ring was mounted and could be herded. Body hair started to grow back.	Body weight was back to normal. Became tame. Approachable and was able to be herded.

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could not be  
mounted.

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Table 2 showed that adapting behavior of buffalo 1 and 2 in March and April was the same. During the first two months both were still very wild, unapproachable, could not be herded nor unable to mount a nose ring.

In the second month, there was hair loss. In the third month, they became tamer which could be concluded from the fact that a nose ring was well mounted and they could be herded. In the third month, hair started to grow back. It showed that buffaloes started to adapt to their new environment in the third month. Buffalo 1 showed wilder and more aggressive behavior compared to buffalo 2. It was still seen in the fourth month as it was still difficult to herd although already approachable. Handiwirawan et al., (2008) reported that the way buffalo adapt to a new environment is different from the cow because buffalo has only one tenth sweat glands that of the cow, so that heat releasing by way of sweating did not help much. Besides, buffalo has very few hair which gives less protection against the sun. It makes buffalo susceptible to weather changes, whether warm or cold. A sudden weather change can cause pneumonia and sudden death.

In May mud pools were made around Saree BIB so that the buffaloes started to adapt. In a mud pool, their body temperature became colder. It is stated by Ramesh et al., (2002) that the body temperature of buffalo declines faster in a mud pool because its black skin may have a lot of blood vessels that transport and release heat efficiently. Because of its susceptibility to weather changes, buffalo likes to soak in pools or other still water. In June the buffaloes were tame and stable so that efforts to prepare them as semen donor could be done.

#### **Efforts taken to prepare male buffalo as semen donor**

Based on observation on sexual behavior of male buffaloes, it shows that it takes special handling for the buffaloes to copulate and produce semen (Table 3). In this study, male buffaloes were not yet able to get on female buffaloes. Although it seems that the male buffaloes could adapt to the new environment, they were not able to adapt to get on female buffaloes (thus copulate). After being coached several times, they started to show very faint signs of arousal. They finally did get on female buffalo, but there was no copulation. And thus there was no semen production which is the raw material for artificial insemination process. For artificial insemination semen collection should not rely on the presence of female.

Besides coaching to get on female buffalo, the male ones must be given better food to increase their libido. That goes also for the female. Before the male was brought to get on the female, the female must be synchronized with PGF2 (Foote, 2002). A different thing happens to Aceh cow at Saree BIB, the male gets straight on the female when brought closer. They even get on another male cow. The libido of the buffaloes was still low, due to food and probably also to another factor; adaptation to a totally different environment,

from Simeulue to Saree. Agrawal (2003) reports that buffalo reproductive system is quite specific; if they move to a different area, there will be disruption to the reproductive system. In this research, there was a change of environment and living pattern, from the wild to a farm designed to collect semen. Dwiyanto and Handiwirawan (2010) also report that libido of male buffalo declines in the summer and increases in colder seasons. The productivity of buffalo is lower than cow due to its biological characteristics which are the genitalia matures slower than cow.



**Figure 2.** Collecting semen from Simeulue buffalo

**Table 3.** Sex behavior of male buffalo after adapted to get on female buffalo

Animal	Observation of sex behavior per week							
Buffalo	June				July			
	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	week 3	Week 4
1	Refused to approach	Refused to approach	Approached female but not getting on it	Approached female but not getting on it	Approached female but not getting on it	Started showing signs of arousal female (preputium was released)	Got on female but failed to copulate	Got on female but failed to copulate
2	Refused to approach	Refused to approach	Approached female but not getting on it	Approached female but not getting on it	Approached female but not getting on it	No signs of arousal female but started sniffing at female	There were signs of arousal, preputium was seen	Got on female but failed to copulate

Mud pool is also a factor that determines buffalo health and reproduction. According to Saladin (1984), practical management in keeping buffalo as cattle should include a pool, cold water pool or shades. Buffalo has thicker skin compared to cow and fewer sweat glands, which makes it necessary to protect from heat. It needs longer time to adapt and better feeding including the supplement to increase the libido and also semen quality. Handiwirawan et al., (2008) also report that less supportive environment like the excessive sun can obstruct buffalo reproduction and growth. That is why the confinement must resemble its natural habitat. The efforts to collect Simeulue buffalo semen can be seen in picture 2. From this study, it can be recommended that to make Simeulue buffalo as stud semen donor, weather, season and supportive environment should be taken into account. Buffalo sexual behavior is nearly the same as that of the cow, but less intense. Libido is hampered on hot day time, declines during the dry season and gets better in the colder season. Therefore semen collecting for artificial insemination should take those factors into consideration.

## **Conclusions**

Simeuleu buffalo can be trained as genetic resource to increase the population and genetic quality but takes longer time and requires the same environment as in Simeulue Island. It is recommended that to make Simeulue buffalo as stud semen donor, weather, season and supportive environment should be taken into account

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## Wound Healing Effect of the Leaf Extract of *Jatropha curcas* Linn in Mice

<sup>1</sup>\*M. Nur Salim, <sup>2</sup>Darmawi, <sup>1</sup>Ummu Balqis, <sup>3</sup>Cut Dahlia Iskandar, <sup>3</sup>Dian Masyitha

<sup>1</sup>Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Laboratory of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>3</sup>Laboratory of Veterinary Histology, Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [nursalim67@gmail.com](mailto:nursalim67@gmail.com)

### Abstract

The purpose of this study was to determine the **efficacy of methanol extract of *Jatropha curcas* L.** leaves on **wound** healing process of mice skin based on gross observation. Twelve male mice (*Mus musculus*) at the age of 35 days and body weight of 20-40 g. The study was carried using procain cream for local anaesthesia. Artificial wound was created by making 1.5 cm incision that reach subcutaneous skin on the skin in the dorsal region part lateral dexter of mice. The mice were randomly divided into 3 treatment groups. Group A (control) were topically treated with aquadest; Group B were topically treated with **methanol extract of *J. curcas* leaves** and Group C were **given betadine solution**. The treatment was given twice a day until the healing completed. **The gross parameters were existence of blood coagulations, scab formation, wound covering and wound size** used to analyze wound healing process. **The results obtained indicated that methanol extract of *J. curcas* leaves might accelerate the healing process as indicated by decrease in the blood coagulations and wound size but increase in scab formation and wound covering.** In conclusion, methanol extract of *J. curcas* leaves was potential in accelerating wound healing in mice.

**Keywords:** mice skin, **wound** healing, **methanol extract**, *Jatropha curcas*

### Introduction

*Jatropha curcas* L. (*Euphorbiaceae*) is a multiple purposes plant with potential for both biodiesel production and medicinal uses (Abdelgadir and Staden, 2013). This plant is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and Southeast Asia. The genus name 'Jatropha' derives from the Greek word 'jatr'os' (doctor) and 'troph'e' (food), which implies medicinal uses (Sachdeva *et al.*, 2011).

Various parts of *J. curcas* have been used in popular medicine. For example, the latex has been used as wound healing and haemostatic purging, roots are commonly use as diuretic and antileukemic and its leaves can be applied against skin diseases (Soares *et al.*, 2015). In addition, the plant has been used as anti-snake bite, anti-rheumatism, anti-cancer or anti-tumor agents (Uche and Aprioku, 2008). Several studies have confirmed antimicrobial efficacy of *J. curcas* leaves (Igbinosa *et al.*, 2009; Wei *et al.*, 2015). Bioactive potential of *J. curcas* seed oil and its uses in cosmetic production have been reviewed recently (Warra *et al.*, 2012).

Phytochemical analyses have shown that different parts of *J. curcas* plant contain various types and levels of phenolics, flavonoids and saponins. The main constituents are curcin, curcusone B, curcain, campesterol and curcacycline-A (Laxane *et al.*, 2013). Phytochemical screening revealed the presence of saponin, steroids, tannin, glycosides, alkaloids and flavonoids in the extracts (Igbinosa *et al.*, 2009). Latex and leaf extracts showed the highest antioxidant activity. Root and latex extracts inhibit the inducible nitric oxide synthetase in macrophages RAW 264.7 comparable to L-nitro-arginine methyl ester (L-NAME), indicating appreciable anti-inflammatory activities. Cytotoxicity assay results indicated anticancer property of the root extract againsts human colon adenocarcinoma (Oskoueian *et al.*, 2011).

Methanol and hexane extracts of *J. curcas* leaf have been demonstrated to exhibit antimicrobial, antiviral, antioxidant, anticancer, analgesic, anti-inflammatory, and wound healing properties (Wongkrajang *et al.*, 2015). Sesquiterpenoids are responsible for antimicrobial and analgesic effects,

proteins such as curcain are responsible for wound healing (Abdelgadir and Staden, 2013). Considering above information, *J. curcas* leaf was collected locally and its extracts were evaluated for **wound** healing effect using animal models in the present investigation.

## Materials and Methods

### Materials

*Jatropha curcas* Linn leaves were collected around Syiah Kuala University, Darussalam campus, Aceh Province, Indonesia. Male mice (25-30 g) were purchased from Pharmacology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University. Reagents used in this study were methanol, aquadest, alcohol 70%, procain cream, and betadine solution.

### Preparation of plant extract

The leaves (1.0 kg) were sun-dried, pulverized and stored in an air-tight container for further use. About 250g of the pulverized leaf sample was extracted with methanol. The extract was filtered using Whatman No. 1 filter paper and the supernatants were pooled and evaporated by using a vacuum rotary evaporator to obtain dry crude extract. The extracts were re-dissolved in methanol.

### Experimental animals

Male mice were acclimatized for two weeks before the experiment in laboratory animal house at 12 hrs light/dark cycle. The animals were fed a standard animal diet and water *ad libitum* during the experiment. The animal handling protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Syiah Kuala University.

### Surgical procedures

Twelve (12) albino mice were divided into three groups with 4 replications each. All mice were anesthetized locally with procain cream and one wound skin incision was performed 1.5 cm in lenght on the back area (in the middle, on the spine, dorsal region part lateral dexter) of each animal. The animals were handled in accordance with aseptic principles to avoid exogenous bacterial contamination. Then the mice were randomly assigned into three groups. Group A (control or untreated group given aquadest); Group B (treated group with methanol extract of *J. curcas* leaf); and Group C (treated group given betadine solution). The treatment was given twice a day until the healing was completed.

### Macroscopic analysis

The clinical features (gross observation of wounds were monitored regarding to the **existence of blood coagulations**, inflammatory phase, crust, secretion, and necrosis as well as **wound covering and size**. **Data obtained was analyzed using** one-way analysis of variance (ANOVA) followed by Tukey 's post test with *p* values less than 0.05 were regarded as statistically significant.

## Results and Discussion

Methanol extract of *J. curcas* Linn leaf when applied topically did not show any sign and symptoms of skin irritation. Animals treated with topical methanol extract of *J. curcas* Linn leaf showed decreased **blood coagulations**, inflammation period, wound area, **wound covering and wound size** compared to control and those given betadine solution. The application of methanol extract of *J. curcas* leaf to the wound stimulated the healing process. At the 4<sup>th</sup> day the inflammatory exudates were entirely resorbed and a thick crust was formed. At the 5<sup>th</sup> to 6<sup>th</sup> day following the wound was completely closure.

The results showed that blood coagulation, inflammation period, crust presence and **wound size covering** of mice treated with *J. Curcas* extract (group B) reduced significantly in comparison with those of group A (control group) and treated with betadine solution (group C) ( $P < 0,05$ ). Methanol extract of *J. curcas* leaf extract demonstrated hemostatic, inflammation and wound healing activities as illustrated in Table 1.

**Table 1.** Effect **of leaf extract of *J. curcas*** on **Wound** Healingin mice



Group	Blood coagulation (minute)	Inflammation period (day)	Crust presence, (day)	Wound healing (day)
Control (A)	2.12 ± 0.50 <sup>a</sup>	5.66 ± 0.51 <sup>a</sup>	5.33 ± 0.51 <sup>a</sup>	7.33 ± 0.51 <sup>a</sup>
Methanol extract (B)	1.15 ± 0.37 <sup>b</sup>	4.16 ± 0.40 <sup>b</sup>	3.16 ± 0.40 <sup>b</sup>	5.00 ± 0.00 <sup>b</sup>
Betadine solution (C)	1.20 ± 0.37 <sup>b</sup>	4.83 ± 0.40 <sup>c</sup>	4.16 ± 0.40 <sup>c</sup>	5.33 ± 0.51 <sup>b</sup>

Superscripts shown significant (P<0.05)

In this study, the hemostatic effect of methanol extract of *J. curcas* Linn leaf was tested by measuring blood coagulation. Methanol extract of *J. curcas* leaf extract significantly reduced blood coagulation. Wongkrajang *et al.* (2015) reported that **n-hexane extracts of *Jatropha curcas* leaf** possess hemostatic effect by reducing bleeding time, blood coagulation, and platelet aggregation. It was found that *n*-hexane *Jatropha curcas* Linn leaf extract exhibited a significant decrease in bleeding time and blood coagulation. The study suggests the possible mechanism of *n*-hexane extract of *Jatropha curcas* Linn as an enhancer of platelet aggregation due to the activity of phorbol esters or phorbol ester derivatives.

Anti-inflammatory activity of *J. curcas* leave extracts was previously reported by Mujumdar and Misar (2004) who showed good effects of topical application of *J. curcas* root powder in paste form in albino mice suffered from TPA-induced ear inflammation. The methanol extract exhibited systemic and significant anti-inflammatory activity in acute carrageenan-induced rat paw edema. *Jatropha curcas* can be recommended for acute inflammatory disorders and diseases associated with pains (Uche and Aprioku, 2008). Ogunnaik *et al.* (2013) reported that *J. curcas* leaf methanol extracts had anti-inflammatory activity. According to Oskoueian *et al.* (2011) *J. curcas* leaf and latex extracts, contained appreciable amounts of phenolic and saponin compounds. These extracts also showed good antioxidant and anti-inflammatory activities.

Shetty *et al.* (2006) evaluated the wound healing activity of crude bark extract from *J. curcas* in Wistar albino rats. The extract accelerated healing processes by increasing skin breaking strength, granulation tissue breaking strength, wound contraction, dry granulation tissue weight and hydroxyproline levels. Esimone *et al.* (2009) tested the wound healing activity of herbal ointment containing methanolic extract from *J. curcas* leaves incorporated into 10 g of simple ointment base. Ointment was applied topically to the wound at intervals of 3 days until complete wound closure. Blank ointment and gentamicin ointment (1%) served as standard and control respectively. The methanol extract incorporated into an ointment base caused higher rates of wound healing and reduced the epithelialization period in a dose-related manner. Omeni *et al.* (2013) reported that *J. curcas* leaf extracts formulated as ointments have no adverse effect on experimental animals. These findings were in agreement with the belief of users of this plant that the plant is safe. The finding of this study might be extrapolated to humans since the use of leaf extracts in wound healing was safe in rats. Shetty *et al.* (2006) suggested that treatment with fresh homogenized crude extract of *J. curcas* have beneficial influences on the various phases of wound healing such as fibroplasia, collagen synthesis and wound contraction, resulting in faster healing.

## Conclusion

**Methanol extract of *Jatropha curcas*** leaf was potential in accelerating wound healing. Further experiments are needed to evaluate histopathological of wound healing.

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## Supplementation of Aceh Coffee Arabica Extract for Improving Quality of Uterus in Postmenopausal Conditions Using Rats as Animal Models

<sup>1</sup>\*Safrida, <sup>2</sup>Mustafa Sabri

<sup>1</sup>Department of Biology Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Anatomy, Faculty of Veterinary Medicine, University of Syiah Kuala. Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [saf\\_rida@unsyiah.ac.id](mailto:saf_rida@unsyiah.ac.id)

### Abstract

Coffee extract is a natural substance that contains phytoestrogens, having similar estrogen activity. This study was designed to determine the potential of Aceh coffee arabica extract in improving the quality of uterus in postmenopausal conditions, and to compare the natural product of coffee extract with commercially available hormones (ethinylestradiol). Experimental design used was Completely Randomized Design (CRD) consisted of 4 treatments, each consisted of 5 rats. 1) KON = Rat postmenopausal as a negative control, 2) PLS = Rat postmenopausal given distilled water as a placebo, 3) EST = Rat postmenopausal given ethinylestradiol as  $9 \times 10^{-3}$  mg/day/200g BB, 4) KOP = Rat postmenopausal coffee extract 300mg/day/200g BB. The parameters observed were uterus collagen concentrations, uterus water concentrations, and uterus RNA concentrations. Supplementation of Aceh coffee arabica extract in postmenopausal rats could improve the quality of uterus, which was characterized by the increased collagen and RNA concentrations in the uterus in postmenopausal conditions.

**Keywords:** uterus, extract coffee, rats, postmenopausal

### Introduction

Aging changes in the female reproductive system result mainly from changing hormone levels. This is manifested by permanent stop of menstrual periods, a state known as menopause. Hormone therapy with estrogen or progesterone, alone or in combination, may help menopause symptoms such as hot flashes or vaginal dryness and pain with intercourse. Hormone therapy has risks, so it is not suitable for every woman. When the balance of potential benefits and risks is favorable for the individual woman, the use of hormone therapy is appropriate for perimenopausal and postmenopausal women (NAMS, 2012).

One of the natural ingredients found in Aceh and become commodity is Arabica coffee. Today it is known that coffee contains trigonelline as protective auditory neuropathy (Hong *et al.*, 2009). Trigonelline is a novel phytoestrogen in coffee beans that can bind to estrogen receptors that function as endogenous estrogen (Kimberly *et al.*, 2009).

This study is interesting and to be important because of coffee extract supplementation as a natural ingredient good to consume at postmenopausal and it is also an alternative preventive effort to risk disease when entering postmenopausal age. The purposes of this study were to know the potential of coffee extract in improving the quality of uterus in postmenopausal conditions, and to compare natural products from coffee extract with hormone products are already marketed ethinylestradiol

### Materials and Methods

#### **Preparation of aceh coffee arabica extract**

Aceh Coffee Arabica roast done decaffeination, then be heated, and dried in an oven. Furthermore, smoothed with coffee grinding tool so that it becomes powder. The powder is then extracted using maceration method. Furthermore coffee solution filtered using filter paper and purified maserat obtained (evaporation) using Rotary Vacuum evaporator to produce a coffee extract thick. Viscous extract is then dried using freeze drying method (freeze dryer). Strong coffee extract was put in the freezer until

frozen, after it had dried with freeze dryer to a maximum moisture content of 10% in order to obtain a coffee extract in powder.

### Testing aceh arabica coffee extract in mice

Experimental animals used in this study were female rats strain Sprague Dawley in Rat postmenopausal condition, consisted of 4 experiments, each consisted of 5 rats. Rats were placed in individual plastic cages with lids made of wire ram and covered with chaff. Commercial pellet rations and water were provided *ad libitum*. Environment enclosure is made so as not to damp, adequate ventilation and sufficient irradiation with light long 14 hours and 10 hours dark. Postmenopausal rats were adapted to the cage conditions for 1 week before randomly assigned into 4 treatments, namely: KON = postmenopausal rats as a negative control, PLS = postmenopausal rats given distilled water as a placebo, EST = postmenopausal rats given ethinylestradiol  $9 \times 10^{-3}$  mg/day/200g BW, and KOP = postmenopausal rats given coffee extract 300mg/day/200g BW. Treatments were given once a day for 2 months.

At the end of the trial phase diestrus status of all the rats were sacrificed. Before the surgery, the rats first anesthetized with ether. After the rats were sacrificed, the uterus is separated from the soft tissue using small scissors, and then weighed wet weight, then put in a solution of BNF (buffered formalin) 10% for the concentrations of collagen and RNA analysis. Parameter observed is uterus water concentrations, concentrations of collagen uterus, and uterus RNA concentrations in accordance with the method performed by Manalu and Sumaryadi (1998).

### Result and Discussion

Data of water, collagen, and RNA concentrations in the uterus of postmenopausal rats are presented in Table 1. Statistical analysis showed that giving of Aceh Arabica coffee extract affects the concentrations of collagen and RNA, but not water concentration in the uterus of postmenopausal rat.

**Table 1.** Mean water, collagen, and RNA concentrations in the uretus of postmenopausal rats

Treatment	Concentrations of collagen uterus (mg/g sampel)	Uterus water concentrations (%)	Uterus RNA concentrations (mg/g sampel)
KON	37,64 <sup>B</sup>	78,21 <sup>A</sup>	18,63 <sup>B</sup>
PLS	38.43 <sup>B</sup>	79,79 <sup>A</sup>	17,48 <sup>B</sup>
EST	41,16 <sup>AB</sup>	77,38 <sup>A</sup>	21,88 <sup>AB</sup>
KOP	43,57 <sup>A</sup>	77,83 <sup>A</sup>	26,61 <sup>A</sup>

The numbers are followed by different letters in the same column indicate significant differences ( $p < 0.05$ ). 1) KON = Rat postmenopausal as a negative control, 2) PLS = Rat postmenopausal force-distilled water as a placebo, 3) EST = Rat postmenopausal given ethinylestradiol as  $9 \times 10^{-3}$  mg / day / 200g BB, 4) KOP = Rat postmenopausal coffee extract 300mg / day / 200g BB

Concentrations of collagen uterus and concentrations of RNA uterus in postmenopausal rats given Aceh Arabica coffee extract and ethinylestradiol higher ( $P < 0.05$ ), compared with control rat. Coffee extract containing trigonelline compounds similar to estrogen that can function in improving the synthesis activity of rat uterus cells, which is described by RNA concentrations uterus. According to Kimberly *et al.* (2009) trigonelline a new phytoestrogens that can bind to estrogen receptors that function as endogenous estrogen.

Giving of coffee arabica extract can improve of the uterus in postmenopausal conditions. According to coffee bean polyphenols ameliorate postprandial endothelial dysfunction in healthy male adults (Ochiaiet *al.* 2015) Consumption of a dark roast coffee decreases the level of spontaneous DNA strand breaks: a randomized controlled trial. The consumption of the study coffee substantially lowered the level of spontaneous DNA strand breaks (Bakuradze *et al.* 2015). Coffea Arabica leaves extract can prevent photo-damage in skin through inhibiting MMP expression and MAP kinase pathway (Chiang *et al.* 2011). RNA was purified from C. arabica (cv. Catuai Red) flowers, seeds and fruits at 4 successive ripening stages. Degenerate primers were designed on the most conserved regions of the monoterpene synthase gene family, and then used to isolate monoterpene synthase-like sequences from the cDNA libraries. After 5'- and 3'-RACE, the complete transcripts of 4 putative C. arabica monoterpene synthases (CofarTPS) were obtained (Del Terra *et al.* 2013). The roasting of coffee beans generates stable radicals within melanoidins produced by non-enzymatic browning. Roasting coffee beans has further been suggested to increase the antioxidant (AO) capacity of coffee brews (Troup *et al.*

2015). The clinical and experimental findings demonstrate various anticancer properties of caffeine and caffeic acid against both ER(+) and ER(-) breast cancer that may sensitize tumor cells to tamoxifen and reduce breast cancer growth (Rosendahl *et al.* 2015)

## Conclusion

Supplementations of coffea extract in postmenopausal rats could improve the quality of uterus, which was characterized by the increased concentrations of collagen and RNA concentrations of the uterus in postmenopausal conditions.

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## Identification of Cellulase from *Enterobacteriaceae* in the Rumen of Aceh's Cattle Based on Homology 16S rRNA Gene

<sup>1</sup>Wenny Novita Sari, <sup>2\*</sup>Safika, <sup>2</sup>Darmawi, <sup>2</sup>Yudha Fahrimal

<sup>1</sup>Postgraduate student of Doctor of Mathematics and Science, Syiah Kuala University, Banda Aceh 23111, Indonesia

<sup>2</sup>Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh 23111, Indonesia

\*Corresponding Author: [safika@unsyiah.ac.id](mailto:safika@unsyiah.ac.id)

### Abstract

The cattle's rumen is one source of cellulolytic bacteria that ability to degrade cellulose to produce cellulase enzymes that have a high economic value in the industry and feed additive. These bacteria are found in the cattle's rumen especially the cattle feed containing a high crude fiber such as aceh's cattle. This study aims to determine the type of bacteria and the activity of cellulase in the rumen of Aceh's cattle. In this research, isolation of cellulolytic bacteria from rumen fluid samples of aceh's cattle were taken at Slaughterhouse on selective media, that is BHM - CMC agar was incubated anaerobically, then a total DNA isolation to get pure DNA from bacteria produced, DNA amplification and sequencing. The alignment of DNA sequence performed using the BLAST program at the NCBI. The results Homology analysis showed that the isolate closed to *Enterobacteriaceae*, with sequence differences between 89-90%. Furthermore, bacteria that are found will be analyzed and enzyme activity assays.

**Keyword:** cellulolytic bacteria, cellulase, PCR, aceh's cattle, rumen

### Introduction

Aceh cattle is one of the local cattle germ plasma in Indonesia and becomes one of priorities for economic development in Aceh related to its contribution to strengthening food animal security. The cattle that have been raised by the Acehnese by generation are derived from crosses between local cattle (*Bos sondaicus*) and Indian zebu (*Bos indicus*) (Basri, 2006). One of the advantages of Aceh cattle is their capability of degrading feed containing high crude fibers (BPTU-HPT Indrapuri, 2014).

As the degradation of cellulose in the ruminant digestive track is done by microorganisms in the rumen and reticulum, ruminants have high ability of degrading feed contains higher crude fibers such as aceh cattle are supposed to have more cellulolytic bacteria both in numbers and variety. These cellulolytic bacteria produce cellulose enzymes are able to digest crude fibers containing 35-50% cellulose, 20-35% hemicellulose, and 5-30% lignin into simpler carbohydrates that will be fermented further by to produce volatile fatty acids (Yang *et al.*, 2014).

Aerobic cellulolytic bacteria in general belong to Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria (Ulrich *et al.*, 2008). Some studies were performed to investigate fermentation ability of diverse cellulolytic bacteria isolated from the feces and rumen of cattle and swamp buffaloes in Thailand (Chanthakhoun *et al.*, 2012) and India (Chaudhary *et al.*, 2012). Molecular characterization has also been done on cellulolytic bacteria isolated from the feses and rumen of goats, cattle, and deer (Kittelmann *et al.*, 2013; Lin *et al.*, 2013).

Molecular approach to studying the nature and diversity of microorganisms has increased substantially. PCR-sequencing is widely used for identification of microbes and microbial community structure in animals (Favier *et al.*, 2002; Kittelmann *et al.*, 2013), cattle manure (Safika *et al.*, 2013; 2014), land (Dong and Reddy, 2010), and sea water (Gao *et al.*, 2009). Meanwhile, real-time PCR is a sensitive and accurate technique for evaluating the GIT microbiota (St-Pierre and Wright, 2012; Singh *et al.*, 2013).

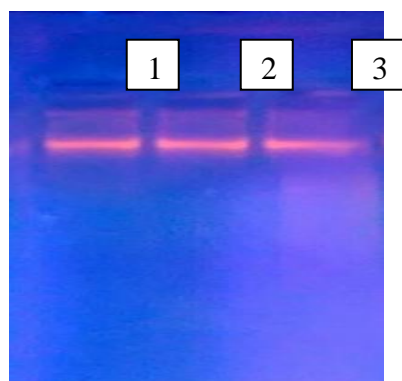
Study on these bacteria in the Aceh's cattle, unfortunately, has been not done before. This paper describe result of our study in determining the type and cellulase activity of celluloytic bacteria isolated from the rumen of Aceh cattle.

### Materials and Methods

Cellulolytic bacteria were isolated from rumen fluid Aceh cattle slaughtered at the Slaughterhouse of Banda Aceh. Bacterial isolation was performed using BHM-CMC agar. Total DNA was extracted, purified and amplified using standard protocols and primers specifically amplify 16S rRNA genes. Nucleotide sequence of the gene was determined using the commercial service of Macrogen Inc., Korea. DNA sequence homology with DNA sequences that have been deposited in the NCBI database was done using web-based BLASTN program.

## Results and Discussion

Cellulolytic bacteria isolated from the rumen of Aceh cattle were designed as isolate FKH\_USK. DNA samples of bacteria isolated by adding some specific compounds with DNA isolation function specific. DNA isolation from cellulolytic bacteria of Aceh's cattle (isolate FKH\_USK) then electrophoresis and visualized for analysis (Figure 1).



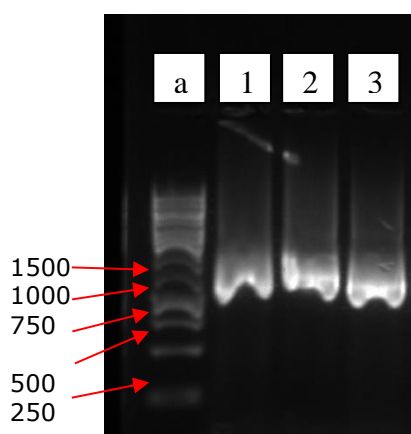
**Figure 1.** Electrophoresis visualized isolate DNA of FKH\_USK.. 1.isolat FKH\_USK\_1 2. Isolate FKH\_USK\_2 3. Isolate FKH\_USK\_3

DNA fragments that have been isolated then electrophoresis to separate the fragments DNA. Figure above shows that the DNA isolation is successful because it shows the whole band formed characterized by the appearance of a single band or ribbon form DNA tailless. The thickness of the band formed from the protein band showing the content or the amount of protein that has the same molecular weight positioned at the same tape. This goes along with the principle of the movement of charge molecules, the charged molecules can move freely under the influence of an electric, the DNA molecule to the charge and the same size will be accumulated in the same or adjacent zone or band (Campbell *et al*, 2002).

### Amplification of the 16S rRNA gene

Amplification process is done by PCR method. This process can be grouped in three successive stages, are template denaturation, annealing primer pair in the DNA strand target and extension (elongation or polymerization), to obtain DNA amplification between  $10^8$ - $10^9$  times (Retnoningrum, 1997). Gen 16S rRNA intended as molecular markers because these molecules are ubiquitous with functions identical in all prokaryotic organisms such as bacteria had similar-producing 16S rRNA gene.

The annealing process at the DNA strands that are already open requires an optimum temperature because the temperature is too high can lead to amplification does not occur, or otherwise the temperature is too low can cause the primer attached to the other side of the genome that is not a side homologous. The result can be amplified many areas are not specifically in the genome. Attachment temperature (annealing) is determined based on the primers used are influenced by the length and composition of the primer (Suryanto, 2003). In this study, using a primer BacF-UNIB ( $\pm$  1500 bp) is based on a sustainable region in the 16S rRNA gene of *E. coli* with the annealing temperature is 50°C.



**Figure 2.** Visualization electrophoresis of DNA amplification product in 1500 bp.a. 1 kb DNA marker, 1.isolat FKH\_USK\_1 2.Isolat FKH\_USK\_2 3.Isolat FKH\_USK\_3

Electrophoresis results showed the tape parallel and separated to the marker 1500 bp (Figure 2). This indicates that the genes are amplified in size  $\pm$  1500 bp corresponding to the length of the primers used. 16S rRNA sequences have three advantages that exist in all bacteria, the function never changes, and its genes are large enough to reach the size of 1500 bp (Janda and Abbott, 2007).

### DNA sequence homology

FKH\_USK isolates DNA sequence similarity to the DNA sequence has been deposited in GenBank, it can be seen through the NCBI using BLASTN program (Altschul *et al.*, 1997).

NCBI Blast:Nucleotide Sequence (1318 letters) <http://blast.ncbi.nlm.nih.gov/Blast.cgi>

**Descriptions**  
Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Enterobacter cloacae strain DSM 30054 16S ribosomal RNA gene, partial sequence	1707	1707	100%	0.0	89%	<a href="#">NR_117679.1</a>
Enterobacter cloacae strain NBRC 13535 16S ribosomal RNA gene, partial sequence	1707	1707	100%	0.0	89%	<a href="#">NR_113615.1</a>
Enterobacter cloacae strain 279-56 16S ribosomal RNA gene, partial sequence	1707	1707	100%	0.0	89%	<a href="#">NR_028912.1</a>
Enterobacter cloacae subsp. dissolvens strain ATCC 23373 16S ribosomal RNA gene, partial sequence	1696	1696	100%	0.0	89%	<a href="#">NR_118011.1</a>
Enterobacter oryzandophyticus strain REICA_082 16S ribosomal RNA gene, partial sequence	1694	1694	99%	0.0	89%	<a href="#">NR_125586.1</a>
Enterobacter cloacae strain ATCC 13047 16S ribosomal RNA gene, complete sequence	1692	1692	100%	0.0	89%	<a href="#">NR_102794.1</a>
Enterobacter cloacae subsp. dissolvens strain LMG 2683 16S ribosomal RNA gene, partial sequence	1692	1692	100%	0.0	89%	<a href="#">NR_044978.1</a>
Enterobacter ludwigii strain EN-119 16S ribosomal RNA gene, complete	1683	1683	100%	0.0	89%	<a href="#">NR_042349.1</a>

**Figure 3.** BLASTN results example of gene sequences FKH\_USK

It is known that 3 isolates FKH\_USK cellulolytic bacteria have sequence similarities of 89-90% of group *Enterobacteriaceae* that found in GenBank. According to Janda and Abbott (2007) if the percentage of homology have close to 100% or above 97% can be confirmed as a species, but on the contrary, if the homology is less than 97% probability of these isolates was a new species or a species not yet to be

confirmed. Therefore, the results of cellulolytic bacterial isolates BLASTN, this possibility is a new species of *Enterobacteriaceae*.

*Enterobacteriaceae* is Gram-negative bacteria, bacil, anaerobic facultative, moving with flagella with their natural habitat in the digestive tract of humans and animals. In general, *Enterobacteriaceae* can ferment glucose and often accompanied by gas production (Brooks *et al*, 2008). *Enterobacter sakazakii* and *Escherichia coli* are known to have the ability to produce the cellulase enzyme (Grim, M. 2008).

## Conclusions

Based on the results of this study concluded that the isolated bacteria cellulolytic possibility is a new species of *Enterobacteriaceae*.

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## Detection of Merozoit Surface Protein-1 (MSP-1) in Erythrocyte Membrane of Mice Infected with *Plasmodium berghei*

<sup>1</sup>\*Rosnizar Rosnizar, <sup>2</sup>Kartini Eriani

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Science, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Biology, Faculty of Mathematics and Natural Science, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: rosnizarjamil@gmail.com

### Abstract

The merozoit surface protein-1 (MSP-1) is a relatively abundant protein which has been found in all *Plasmodium* species. Previous studies had noted its ability to protect mice from challenge against malaria by elicited antibody response. Objective of this study was to detected the availability of several reactive protein bands in sample of mice-infected erythrocyte membrane protein using monoclonal antibody anti-MSP-1 (F4.4) as primary antibody and anti-IgG mouse Horse Radish Peroxide (HRP) conjugated as secondary antibody by western blotting analysis. The level of infected protein density was measured by densitometry analysis. Detection using western blot analysis found several immunoreactive PbMSP-1 protein bands in infected sample that were >225 kDa, ~150 kDa, ~90 kDa, ~60 kDa, ~42 kDa and ~19 kDa. In conclusion, this study revealed that the western blot analysis using monoclonal antibody anti-MSP-1 (F4.4) and anti-IgG mouse HRP conjugated could detect the level of MSP-1 protein clearly and therefore this study believed could give the understanding of the early parasite invasion process to the host cell.

**Key words :** Merozoit surface protein, MSP-1, monoclonal antibody, *Plasmodium* species

### Introduction

The phase of parasite entry into erythrocytes is an important step, and critical to maintain its continuity in the cell (Gaur et al., 2004). The parasite surface proteins and host cell receptors are important elements on attachment and entry process of parasites (Rosa et al., 2006). One protein that is directly involved in the mechanism of entry of *Plasmodium* parasite into erythrocytes is merozoites-surface protein 1 (MSP-1) (Sherman 1998). MSP-1 is an immunodominant antigen that induces the production of antibodies and provide protection to the animals (Wipasa et al., 2002; Egan et al., 2000; Kumar et al., 2000). MSP-1 protein exists as a large precursor molecule (185-210 kDa), found in all malaria species studied (Stowers et al. 2001; Blackman 2000). This protein is considered a protein that acts as a target for the protection and immunity as in several studies in vivo and in vitro (Wickramarachchi et al., 2007; Egan et al., 2000). MSP-1 protein is synthesized in the final stages of development in the intracellular erythrocyte of merozoites differentiation (Conway et al. 2000; Polley et al. 2003). The precursor is processed into small fragments that make up a complex polypeptide (Conway et al. 2000; Polley et al., 2003; Jennings et al. 1998).

To find out its existence in animals or humans infected with *Plasmodium*, several methods of detection of this protein have been reported, including ELISA method, method of Surface Plasmon Resonance (SPR), imunoflouresens method (IFA) and spectroscopic method using protein sequence MSP-1 semi-conserved (Helg et al. 2003).

In this study, some aspects of MSP-1 detection in sample was carried out using multiple detection methods including the used of monoclonal antibodies anti-MSP-1 (F4.4) as the primary antibody and anti-mouse IgG conjugated of horse radish peroxidase (HRP) as the secondary antibody through western blotting analysis. This study also used Enhanced ECL chemiluminescence kit in the process of x-ray film exposure and finally was analyzed by densitometry (Dluzewski & Garcia, 1996; Hasidah et al. 2001). The presence of MSP-1 protein in the sample being studied at the molecular level to detected its involvement in the infection process. In addition, this study was expected to strengthened the information obtained during the assessment of the *P. berghei* entry into erythrocyte so that this information would be useful for detecting *Plasmodium* parasites pathways in infected hosts, apart from its ability as immunodominant antigens.



## Materials and Methods

### Sample of Mice and Plasmodium Parasite

White male mice aged 7-8 weeks of strain Balb/c supplied by vivarium, Faculty of Mathematic and Natural Science. Sample of *Plasmodium berghei* parasites was obtained from cryopreserved stock and then transferred to mice and maintained *in vivo*.

### Protein Analysis

MSP-1 protein detection was conducted through several methods begin with the extraction of erythrocyte membrane proteins, SDS-PAGE, western blotting, x-ray film exposure and densitometry analysis.

### Erythrocyte membrane protein extraction

Approximately 0.8 ml blood sample of mice infected of *P.berghei* and mice without infection (as control) were taken from the vena cava posterior of mice. All mice then terminated using diethyl-eter and the blood was inserted into EDTA tube and then sentrifuged at 650 x g, 5 min, 4 ° C. The pellets were mixed with 5 volumes of phosphate buffer (PBS) (150 Mm NaCl, 5 mM sodium phosphate), pH 8.0 at 4 ° C and then centrifuged. This process was repeated three times and the supernatant containing 'buffy coat' was released (Steck and Kant 1974). Erythrocyte pellet was then transferred into 15 ml centrifuge tube and mixed with phosphate buffer to lysed the erythrocyte (5 mM sodium phosphate, pH 8.0) with a ratio of pellet to PBS solution of 1:20. Results of lysed erythrocyte were centrifuged at 1500 x g, 10 min, and 4 ° C. The resulting supernatant was put in PBS buffer and centrifuged again at 2500 x g, 15 min, 4 ° C for three times (Suetterlin et al. 1991).

The pellets produced were extracted with buffer extraction (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM MSF, 1% Triton X-100) and left in ice for 60 minutes with a little shock every 15 minutes (Johnson et al. 1994). SDS-PAGE sample buffer was added to the extraction of erythrocyte membranes at a ratio of 1: 1 and then boiled at 95°C temperature for 5 minutes. Sample solution was then centrifuged at 1500 x g, 5 minutes, and 4 ° C and aliquot and stored at -30 ° C for the examination of protein concentration using Bradford assay.

### Polyacrylamide- gel electrophoresis (SDS-PAGE) and western blotting analysis

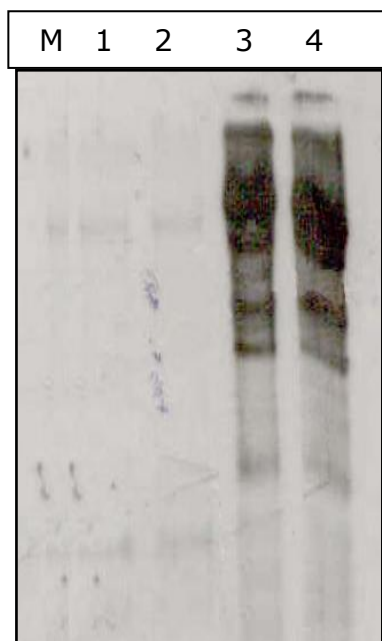
SDS-PAGE electrophoresis was performed according to the method of discontinuous Laemmli (1970). A total of 20 g of protein per sample was put in each well. After the transfered into nitrocellulose membrane, the membrane was processed using mAb F4.4 primary antibody (anti-MSP-1). Recombinant molecular weight marker (10-225 kDa) (Novagen, Germany) was used on one side of the membrane. Reservoir buffer electrophoresis used consisted of 25 mM Tris-base, 192 mM glycine and 0.1% SDS, pH 8.3. Proteins were separated on polyacrylamide gel and transferred into nitrocellulose membrane surface through western blotting method by Towbin et al. (1979). Sponges, Whatman papers, the PVDF membranes and polyacrylamide gel were arranged on cassettes and rolled with a glass rod each time and prepared to get rid of air bubbles. The process of transfer of proteins from polyacrylamide gels to nitrocellulose membranes was performed for three hours with a 40-volt power at 4 ° C. Transfer buffer consisted of 25 mM Tris-base, 192 mM glycine and 20% methanol, pH 8.3.

## Results and Discussion

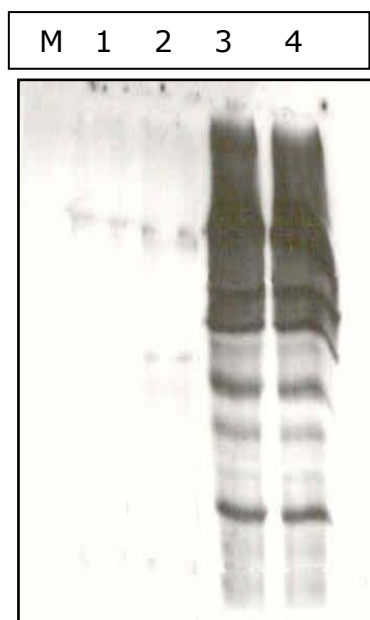
### Detection of MSP-1 Protein using Western Blotting Method

The usage of anti-MSP-1 (MAb F4.4) as primary antibody and anti-mouse IgG horse radish peroxidase conjugated as secondary antibody (Promega Corporation, USA) demonstrated the presence of multiple immunoreactive proteins against MSP-1 with molecular weight >225 kDa, ~150 kDa, ~90 kDa, ~60 kDa, ~42 kDa and ~19 kDa in erythrocyte membrane protein of mice infected with *P. berghei* at parasitaemia level of 10-20% (Figure 1). Meanwhile at group of 30%-40 parasitaemia level, the number of bands detected were approximately seven bands of each size >225 kDa, ~150 kDa, ~90 kDa, ~60 kDa, ~42 kDa, ~33 kDa and ~19 kDa (Figure 2). Analysis of erythrocyte membrane protein in control animals showed that no immunoreactive-MSP-1 protein band could be detected. Based on the size of molecules, almost all bands were Immunoreactive proteins against MSP-

1 (Blakcman 2000; Wickramarachchi 2007), except band with size ~150 kDa protein which was expected as another protein of the surface of merozoite.



**Figure 1.** Western blotting analysis of erythrocyte membrane protein in mice infected with *P. berghei* at 10-20% level of parasitaemia (lanes 3 & 4) and control mice erythrocytes (lanes 1 & 2) (30 g) using a primary antibody anti-MSP-1 (F4.4) and anti-mouse IgG conjugated horse radish peroxidase (HRP) as secondary antibody; M (Marker).



**Figure 2.** Western blotting analysis of erythrocyte membrane protein in mice infected with *P. berghei* at 30-40% level of parasitaemia (lanes 3 & 4) and control mice erythrocytes (lanes 1 & 2) (30 g) using a primary antibody anti-MSP-1 (F4.4) and anti-mouse IgG conjugated horse radish peroxidase (HRP) as secondary antibody; M (Marker).

This study showed that the western blotting method were reliable method that capable of detecting the surface protein of *Plasmodium* merozoites that Immunoreactive to the MSP-1 protein (Wiser 1986; Wiser et al 1997). Results showed that there were significant differences between the samples of erythrocyte membrane protein in infected versus non-infected. Immunoreactive existence against MSP-1 protein in

*P. berghei*-infected samples were detected by the presence of multiple bands of protein with different molecular weight. The fastest antigen band be detected by this antibody F4.4 MSP-1 was a protein that was similar to the polymorphic schizont antigen which had molecular weight of ~225 kDa (Wiser et al. 1997). However, the presence of cross-reacting antibody with another protein band was also detected by the presence of ~150 kDa protein. In terms of molecular weight, this band was not the result of proteolytic fragmentation of protein MSP-1 precursor as previously reported (Leung et al., 2004; Blackman 2000; Wiser et al. 1997; Jennings et al. 1998). This protein may be derived from another merozoite surface protein such as Ring Infected Erythrocyte Surface Antigen (RESA) that had same molecular weight of ~150 kDa (Magowan et al. 1996).

Number of band of Immunoreactive proteins against MSP-1 protein obtained in this study indicated the presence of protein processing mechanism of MSP-1 during infection occurred. The results of some previous studies on *Plasmodium* species including *P. falciparum*, *P. knowlesi* and *P. berghei* showed that MSP-1 protein had been through a process of continuous proteolysis that were early period proteolysis and the second period proteolysis after skizogoni maturation inside infected erythrocytes (Leung et al., 2004; Jennings et al. 1998) and suggested that this processing mediated by an enzyme (Wiser et al. 1997). This initial processing of MSP-1 occurred shortly before the release of merozoites from infected erythrocytes in new erythrocyte (Leung et al., 2004; Jennings et al., 1998) reported involving enzymes protease located in the plasma membrane or in the vacuole of the parasite parasitoforous (Leung et al., 2004; Eipstein et al. 1981). MSP-1 also reported had protease activity, meanwhile Blackman (2000) reported some initial processing to produce protein fragment molecular weight > 225 kDa, 90 kDa, ~ 60 kDa and ~ 42 kDa (Holder and Freeman 1982; Gaur et al. 2004).

The site cutting of 42 kDa fragment was almost equivalent to a cysteine residue of the cysteine-rich C-end domain (Longacre 1995). The absence of intraspecific variation between residue at position P1 showed that the cutting was generated by a chymotrypsin-like protease (Blackman et al. 1993). In gel electrophoresis, MSP-1<sub>42</sub> in the species of *P. falciparum* migrated as fragment of 30-33 kDa molecular weight whereas in *P. chabaudi* as 32 kDa (Blackman 2000). Fragment derived from C end that migrated as 19 kDa species *P. falciparum* (21 kDa on *P. chabaudi*) referred to as MSP-1<sub>19</sub>. Sequence of MSP-1<sub>19</sub> was believed to be a polypeptide that functions like two epidermal growth factor (EGF) (Gaur et al., 2004; Tomley & Soldati; 2001; Olsen et al. 1991) consisting of six residues cysteine and glycine that preserved and secured by disulfide bond on the order of 1-3, 2-4, and 5-6 (Blackman 2000).

## Conclusions

The study has proved the effectiveness of monoclonal antibody F4.4 as primary antibody, in detecting immunoreactive protein bands against MSP-1 with the appearance of several protein with molecular weight i.e >225 kDa, ~90 kDa, ~60 kDa, ~42 kDa, 33 kDa and ~19 kDa respectively.

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## Effect of Hunting Activity on the Level of Blood Calcium, Phosphorus and Magnesium on Local Dogs in Tabek Panjang, West Sumatra, Indonesia

\*Triva Murtina Lubis, Sri Rahmila Indris, Gholib, Azhar

Faculty of Veterinary Medicine, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [triva\\_murtina@unsyiah.ac.id](mailto:triva_murtina@unsyiah.ac.id)

### Abstract

This study aims to determine level of calcium, phosphorus and magnesium in the blood serum of local dogs in Tabek Panjang Village, Baso Subdistrict, West Sumatra, Indonesia. Blood was collected from 15 male local dogs, ranged 2.0-2.5 years old, before and after hunting activity. Serum samples were analyzed using a spectrophotometer in the Balai Veteriner Bukittinggi, West Sumatera. Concentration of blood calcium, phosphorus and magnesium were analyzed using T-test. The results showed that the average levels of blood calcium, phosphorus and magnesium of local dogs before hunting were  $8.27 \pm 1.09$  mg/dl,  $6.35 \pm 1.73$  mg/dl and  $1.93 \pm 0.34$  mg/dl respectively and the average of blood calcium, phosphorus and magnesium after hunting were  $7.94 \pm 1.45$  mg/dl,  $5.78 \pm 0.93$  mg/dl and  $1.80 \pm 0.42$  mg/dl. The result of statistical analysis showed that the average levels of calcium and phosphorus before and after hunting activity were not significantly different ( $P > 0.05$ ), but the average level of magnesium after hunting activity was significantly lower compared to that of magnesium before hunting ( $P < 0.05$ ). Concentration of blood calcium, phosphorus and magnesium tends to decrease after hunting. It can be concluded that hunting activity does not affect the concentration of blood calcium and phosphorus but it influences the level of blood magnesium in local dogs.

**Keywords:** local dogs, calcium, phosphorus, magnesium

### Introduction

Baso is one of subdistricts in Agam district with the majority of the population are Minangkabau ethnic. For Minangkabau society, hunting wild boars is a traditional activity. The activity is conducted in groups and has its own organization. Hunting wild boars is an attempt to secure the area of agriculture and plantation which is done by Minangkabau men accompanied by dogs as the hunters (Arifin, 2012).

In general, dogs used for hunting are local dogs (*Canis familiaris*) which have ability to chase, catch and kill their preys (Budiana, 2008). Hunting dogs should get a good and balanced feed. The main nutritional composition of the feed of dogs is composed of protein, fat, carbohydrates, minerals, and vitamins (Untung, 1999).

Minerals are essential substances required by the animals to maintain the health, growth and reproduction (Georgievskii *et al.*, 1982). Mineral is a component of bone and teeth formation, and it is useful to control the balance of body fluids contained. For example, calcium and phosphorus work together to stimulate the growth (Budiana, 2008).

Calcium and phosphorus are essential minerals that have functions to establish normal bones and teeth in young animals and to maintain the bone system in order to stay healthy in adult animals. Calcium and phosphorus contained in the body by a ratio of 2:1 (Tillman *et al.*, 1984). Although magnesium contained in a small amount compared to calcium and phosphorus, but it is closely related to calcium and phosphorus in the distribution and metabolism. Magnesium has a role in normal nerve conduction, muscle function as well as mineral bone formation (Klasing *et al.*, 2005).

The intake of calcium, phosphorus, and magnesium should be sufficient for dogs while hunting because it is a kind of physical exercise which need a healthy and strong body to catch their preys. According Zorbas *et al.* (1999) resorption of calcium and phosphorus in rats increased when they were doing physical exercises. Rovira *et al.* (2007) stated the levels of calcium and phosphorus plasma decreased significantly after training for 100 seconds in the dogs, whereas according to Lucas *et al.* (2015) the

concentration of calcium and phosphorus of dogs improved significantly after practicing than when resting.

Determination of mineral content can be done through a blood chemistry test. Blood chemistry provide information of the biological conditions of an animal such as physiological status, nutrition, disease condition and stress in animals (Widiyono and Sarmin, 2012). Blood chemistry parameters in physiological status varied and showed an association with age, race and reproductive status of animal (Jain, 1986; Kuhl, 1998; Ahmed *et al.*, 2000; Mercaldo *et al.*, 2003). This study was conducted to obtain information about the concentration of calcium, phosphorus and magnesium in the blood serum of local dogs in Nagari Tabek Panjang and to determine the influence of hunting on those mineral level.

## Materials and Methods

The animals were restrained manually. Blood samples were collected through saphenous veins using a 3 ml syringe with a needle. The serum formed was transferred into a 1.5 ml microtube for laboratory analysis. Tubes for samples, standard solutions and control were prepared. Using a micropipette, 10 ml serum, standard solutions and control were taken then inserted into each test tube. Reagents, 1000 ml, were added into each tube. Reagent used for examinations are *Calcium AS FS*, *Phosphate FS* and *Magnesium XL FS*. The mixture was homogenized and incubated for 5 minutes at room temperature. The result was read using a spectrophotometer.

## Result and Discussion

### Blood calcium levels of local dogs

The data shows the highest calcium level before hunting was 10.60 mg/dl while the lowest level was 6.4 mg/dl. The average calcium levels before hunting were  $8.27 \pm 1.09$  mg/dl. This result was lower than the result from Huntingford *et al.* (2014) who stated that the average level of calcium in Greyhound 24 hours before exercise or at rest was  $10.20 \pm 0.20$  mg/dl. However, the average level of calcium of this study was higher compare to the average serum calcium levels in dogs reported by Pramina *et al.* (2013) namely  $6.90 \pm 0.10$  mg/dl. The difference in calcium levels may be because of differences in the type of dog, the age, the time of blood collection as well as the feed given.

**Table 1.** Mean ( $\pm$  SD) serum calcium, phosphorus, and magnesium levels in local dogs

Time Collection	Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)
Before hunting	$8.27 \pm 1.09$	$6.35 \pm 1.73$	$1.93 \pm 0.34$
After hunting	$7.94 \pm 1.45$	$5.78 \pm 0.93$	$1.80 \pm 0.42$

The highest calcium concentration after hunting in this study was 12.2 mg/dl whereas the lowest concentration was 6.2 mg/dl. The average level of calcium after hunting was  $7.94 \pm 1.45$  mg/dl. The average calcium levels before and after hunting were not significantly different ( $P > 0.05$ ). Huntingford *et al.* (2014) claimed that calcium levels 20 hours after a workout at the Greyhound were  $10.1 \pm 0.2$  mg/dl.

The findings in this study were consistent with results from Huntingford *et al.* (2014) who reported that there was a tendency of decrease in calcium levels 24 hours after the hunt. However, this decline was still within the normal range. According to Nelson *et al.* (2004) normal calcium levels in adult dogs ranged from 9.0 to 11.5 mg/dl. Total serum calcium lower than 7.0 mg/dl indicated the occurrence of tetany. Total serum calcium higher than 16 mg/dl, on the other hand, lead to acute renal failure and cardiac toxicity.

Serum calcium levels of 10 dogs in this study showed a decrease after the hunt. Decreased calcium levels can be caused by increased production of lactic acid by muscles that have a high intensity contraction so that the levels of ionized calcium out of the sarcolemma decline and contribute to muscle fatigue. Muscle fatigue associated with increased tension on the dog bone (Baltzer *et al.*, 2012). Decreased concentration of calcium in dog after exercise may also be associated with changes in albumin concentration and glomerular filtration rate (Rovira *et al.*, 2007). Calcium loss through sweating also can lower serum calcium concentrations 8 hours after exercise (Inoue *et al.*, 2002).

Mineral levels in the serum are also influenced by the absorption of calcium in the body. Calcium can be absorbed if there is in the form of water-soluble and do not settle. When animals are deficient in vitamin

D, calcium absorption can cause stunted in the gut. Oxalic acid contained in the feed can impede the absorption of calcium. Phytic acid also interfere with the absorption of calcium as calcium phosphate to form insoluble and therefore cannot be absorbed (Almatsier, 2003).

#### **Blood phosphorus levels of local dog**

The data showed that the highest and the lowest phosphorus levels before hunting were 8.6 mg/dl and 3.3 mg/dl, respectively, with an average of  $6.35 \pm 1.73$  mg/dl. Phosphorus levels at rest in the Greyhound were  $4.4 \pm 0.5$  mg/dl (Huntingford *et al.*, 2014). Normal phosphorus levels in adult dogs ranged from 3.0 to 6.0 mg/dl (Nelson *et al.*, 2004).

The highest and lowest phosphorus levels after hunting were 7.6 mg/dl and 4.4 mg/dl, respectively. On average phosphorus level after hunting was  $5.78 \pm 0.93$  mg/dl. Huntingford *et al.* (2014) reported that phosphorus level at 20 hours after a workout in Greyhound was  $3.5 \pm 0.5$  mg/dl. Statistical analysis showed that there was no significant correlation ( $P > 0.05$ ) in phosphorus levels of local dogs in Nagari Tabek Panjang before and after hunting.

Ten of the 15 dogs showed decreased phosphorus levels after hunting. These illustrated the need for intracellular negative ions to move due to respiratory alkalosis and increased glucose utilization in skeletal muscle that require more phosphorus intracellular (Huntingford *et al.*, 2014). Phosphorus levels in the blood are regulated by parathormone (PTH) excreted by parathyroid gland and calcitonin hormone secreted by thyroid gland. PTH and calcitonin hormones interact with vitamin D to control the amount of phosphorus absorbed, the amount saved by the kidneys, and the amount released and stored in the bones. PTH lowering phosphorus reabsorption by the kidney (Almatsier, 2003).

#### **Blood magnesium level of local dog**

In this study, the highest and the lowest magnesium levels obtained before hunting were 2.6 mg/dl and 1.9 mg/dl with an average of  $1.93 \pm 0.34$  mg/dl. According to Martin *et al.* (1994), normal serum magnesium levels in adult dogs ranged 1.89-2.51 mg/dl.

The highest magnesium levels after hunting in local dogs is 2.5 mg/dl while the lowest levels of 1.0 mg/dl and the average magnesium level after hunting was  $1.80 \pm 0.42$  mg/dl. Statistically, the results of this study showed significant differences ( $P < 0.05$ ) of magnesium level before and after hunting.

Decreased levels of magnesium according to Dreosti (1995) may be caused by increased secretion of calcitonin so that it decreased serum magnesium. Golf *et al.* (1984) stated that physical activity can improve the movement of magnesium from extracellular fluid to the muscle tissue. Rayssiquier *et al.* (1990) reported that the temporary transfer of magnesium from extracellular fluid to skeletal muscle increase the amount of magnesium contained in skeletal muscle, but reduce its concentration in the blood. Magnesium levels can also decreased due to lipolysis because the body requires fatty acids as an energy muscle.

#### **Conclusions**

Hunting activity did not affect the concentration of blood calcium and phosphorus but decreased the level of blood magnesium in local dogs.

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# **THEME : PHARMACY AND HEALTH SCIENCE**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
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## Drugs Knowledge of School Going Adolescents in Banda Aceh

\*Afriani, Haiyun Nisa

Department of Psychology, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: afriani.ansari@unsyiah.ac.id.

### Abstract

The number of illicit drug users in adolescents has increased alarmingly, especially in Aceh. The high rate of early initiation of drug use has negative impact on adolescents' development such as destructive and irresponsible behavior. Adolescents' involvement in drug use is related to their understanding about drug and its negative consequences. Adolescents who have more information about drug abuse are less likely to be involved as drug users. This study was designed to assess the knowledge regarding drugs and its consequences among adolescents in Banda Aceh. A total of 300 of school going adolescents in Banda Aceh were selected using accidental non random sampling. The variable of drugs knowledge was measured using a multiple choices questionnaire. Descriptive analysis found that more than half of the respondents (62.7 %) correctly answered for nearly all of the questions. It showed that most of adolescents in this study had good understanding about drugs and their harmful effects. Other data showed that majority of adolescents reported the information about drug uses and its effects were obtained from many sources like media, friends, teachers, and parents. Additional analysis found that there was positive and significant relationships between parent adolescent drug related communication and drug knowledge ( $p < 0.05$ ). It could be concluded that most of adolescents had accurate information about drug use and were being aware of its harmful effects.

**Keywords:** drugs knowledge, adolescents, Banda Aceh.

### Introduction

Drug use as an risky behavior is as old as the history of man and appears to be an on the increase throughout the world including Indonesia. More and more of individuals are becoming addicted to drug, hiding from the realities of life or using them for thrills. The cases of substance abuse cuts across gender, age educational background and all strata of society. The use of drug among adolescents has been reported rise and studied extensively in many parts of the world. Specifically in Indonesia, data showed that there was 531 adolescents were reported as drug users in 2010. Further, 605 cases were recorded in 2011, and later saw an increase to 695 in 2012 and 1,121 in 2013 (National Narcotics Agency of Indonesia, 2014). Aceh as one of provinces in Indonesia was also noted whereby it recorded that there were 10,000 cases of drug abuse in 2013 (Kemenkokesra, 2013). Trend shows that drug use continuous to be critical issue especially on adolescents (Serambi Indonesia online, 2014).

Adolescence is a period of considerable emotional stress and strain as caused by tremendous amount of changes in physical, cognitive, and social aspects. Rapid physical growth, encounter larger social network, and the breaking away from family-centered activities may and with increasing frequency do lead to complex emotional and social adjustments. Failure to adjust with this situation may lead adolescents to have emotional or social problems. Adolescents who are emotionally or socially less competent tend to be involved in destructive behavior such as drug use (Papalia, 2009; Rice and Dolgin, 2008). Many factors were believed to affect adolescents' involvement in substance use such as boredom, availability to get substance, family related factors (ineffective or poor parent-adolescent relationship), inadequate knowledge of the negative effect of drug use and peer pressure (Humensky, 2010; Macaulay *et al.*, 2005; Mallick, 2003; Friestad *et al.*, 2003; Goodman and Huang, 2002). Specifically, peer pressure is an important factor when it come to using drugs on adolescents (Bronfenbrenner, 1979). Research confirms that some adolescent drug users are introduced by friends (Rice and Dolgin, 2008). Most of them have lack information about drug and its consequences, thus they easily take and try the substance. It is supported by literature surveyed that knowledge and perception of drug and the harm associated with drug use (Alhyas *et al.*, 2015). Prior studies it was found that non drug users have more knowledge about drug than drug users (Yuen *et al.*, 2003; Kelly *et al.*, 2002). It could be explained that if one knows that something is danger for his or her body, normally will not ingest it, thus the more one

knows about the harmful of misusing substance, the less will be the usage of such drug (Goldstein, 2008; Hunt and Ellis, 2004).

Further, there is a consensus in the literature that family characteristics are associated with drug misuse (Branstetter and Furman, 2013; Macaulay *et al.*, 2005). Previous studies reported that adolescents from families with frequent, warmth and open (bi-directional) communication are less likely to become involved with drugs (Kelly *et al.*, 2012; Mallick, 2012). Other studies showed that effective parent adolescent communication on drug use increased adolescents awareness and understanding about the harmful effect of drug (Williams *et al.*, 2014; Branstetter and Furman, 2013)

As stated earlier, adolescents' understanding and awareness of drug use and patterns of the drugs and associated harm are viewed as protective factors against substance abuse. Therefore, the present study was aimed to describe knowledge on drug and its harmful effect among adolescents in Banda Aceh, Indonesia. In addition, given the central role of parent adolescents communication about drug, this study also examined the relationship between parent adolescent drug related communication and drug knowledge. In particular, drug communication between parent and adolescent including the availability of parents in providing information about drug and discuss it with their children and age at first discussion about drugs.

## Materials and Methods

A total of 300 adolescents aged 12 to 19 years in Banda Aceh were recruited using accidental non random sampling as participants of the study. All respondents were asked to complete a set of questionnaire that had three components: demographic data information (personal and family characteristics, sources of drugs information, drugs knowledge test, and parent-child communication about drugs. Specifically, drug knowledge was measured using a 17 items multiple choice questionnaire that adapted from Chairunnisa (2010) that consisted of questions about definition of drugs or narcotics, types of drugs and their effects; e.g., "Which of the following drugs is usually consumed in group and used for reducing anxiety? a) Cannabis; b) Paracetamol; c) Amoxilin; d) Kodein". Meanwhile, parent child communication questionnaire was developed in this study based on theory of interpersonal communication by Devito (1995) consisting 6 items that is rated on a four-point Likert scale ranging from 1 ("strongly disagree") to 4 ("strongly agree") e.g., "My parents talk to me about the dangers of drugs".

This present study is a cross sectional design quantitative study using structured questionnaire. Descriptive statistics (percentages) were used to present the level of drugs knowledge. Further, correlation analysis was utilized to determine the relationships between contextual factors and parent child communication toward drugs knowledge on adolescents.

## Results and Discussion

The sample included 172 (57.3%) female and 128 (42.7%) male adolescents. The average age of respondents was 15.5±1.5 years. As for the family's characteristics, nearly half of respondents had parents who were in the middle age group (mother=43.4±6.4 years old, father=48.7±6.7 years old) and well educated. In terms of family income, it seemed that most of respondents came from low to middle income families (see Table 1).

**Table 1.** Characteristics of the respondents

No	Variable	N (%)
1	Personal Characteristics	
	Sex (N=300)	
	Female	172 (57.3)
	Male	128 (42.7)
	Age (N=300)	
	12-13	33 (11)
	14-15	113 (37.7)
	16-17	140 (46.7)
	18-19	14 (4.7)
2	Family Characteristics	

Parents' Age		Father (N=283)	Mother (N=282)
30		16 (5.7)	61 (21.6)
40		59 (20.8)	98 (34.8)
45		66 (23.3)	79 (28)
50-54		87 (30.7)	35 (12.4)
55-59		38 (13.4)	8 (2.8)
>60		17 (6)	1 (0.4)
3	Parents' Education	Father (N=278)	Mother (N=282)
	14 (4.7) 11 (3.7)	14 (4.7)	11 (3.7)
	12 (4) 16 (5.3)	12 (4)	16 (5.3)
	95 (31.7) 113 (37.7)	95 (31.7)	113 (37.7)
	17 (5.7) 27 (9)	17 (5.7)	27 (9)
	92 (30.7) 103 (34.7)	92 (30.7)	103 (34.7)
	40 (13.3) 11 (3.7)	40 (13.3)	11 (3.7)
	8 (2.7) 1(3)	8 (2.7)	1 (3)
4	Family Income (N=281)		
	<Rp.1.500.000	44 (15.65)	
	Rp. 1.500.000-Rp. 2.500.000	34 (12.10)	
	Rp. 2.500.000-Rp.3.500.000	46 (16.37)	
	>Rp.3.500.000	157 (55.87)	

### Knowlegde of drugs among adolescents

Knowledge of drugs was overall high among adolescents in this study as presented in Table 2. For 62.75 % of the female and male respondents in various age ranged from 12 to 19 years old, performed well on nearly all of the questions about drugs. It showed that adolescents had accurate information about drugs regarding the definition, classifications (hallusinogen, depressants and stimulants), their usage and effects. Further, they knew the potential physical danger which results from drug use.

**Table 2.** Knowledge of drugs among adolescents (N=300)

Table 2: Knowledge of drugs among adolescents (N=300)						
No	Variable	Knowledge of Drugs among Adolescents (N=300)				Total
		N (%)				
		High	Moderate	Low	Very Low	
1	Sex					
2	Female	111 (37)	35 (11.7)	11 (3.7)	15 (5)	172 (57.3)
3	Male	77 (25.7)	32 (10.7)	9 (3)	10 (3.3)	128 (47.2)
4	Age					
	12-13	13 (4.3)	11 (3.7)	5(1.7)	4 (1.3)	33 (11)
	14-15	63 (21)	29 (9.7)	8 (2.7)	13 (4.3)	113 (37.7)
	16-17	105 (35)	23 (7.7)	6 (2)	6 (2)	140 (46.7)
	18-19	7 (2.3)	4 (1.3)	1 (.3)	2 (.7)	14 (4.7)
	Total	188 (62.7)	67 (22.3)	20 (6.7)	25 (8.3)	300 (100)

### Correlates of drugs knowledge

Pearson correlation analyses were computed to explore what respondents' characteristics and parent child drug communication significantly related with drugs knowledge. As illustrated in Table 3, only two variables, parent child drugs related communication and adolescents' age, had significant relationships with drug knowledge. Specifically, there was a positive relationships between parent child drugs communication and drug knowledge ( $r=0.32$ ,  $p<0.05$ ).

**Tabel 3.** Correlates of drugs knowledge

No	Variable	Drug Knowledge r (p)
1	Parent Child Drug related Communication	0.32** (0.00)
2	Adolescents' Age	0.27** (0.00)

3	Fathers' Age	-0.02 (0.75)
4	Mothers' Age	0.09 (0.15)
5	Family Income	-0.07 (0.27)

\*\* $p < 0.01$

The positive coefficient correlation indicated that adolescents who had frequent and deliberate discussion with their parents about the dangers of drugs tend to have more accurate information about drugs and their effects. The finding is in line with a study by Mallick (2012) that found parent child drug related communication increased adolescents' knowledge on drug. Similarly, other studies (Williams *et al.*, 2012; Branstetter and Furman, 2013) also found that adolescents' understanding about the risks and sanctions against drug use was influenced by parents' knowledge and how they communicate that issue with their children. Parents have an important role in preventing adolescents to be involved in drug use by providing information about drugs using effective communication (King and Vidourek, 2011).

Result of the study supported the notion that parent child drug related communication appears to be important and should be taking place well at first. Further, data analysis found that adolescents' age were significantly and positively associated with drugs knowledge ( $r=0.27$ ,  $p<0.05$ ). It means that older adolescents are more knowledgeable about drugs than younger adolescents. This finding showed there was gain in drug knowledge from early to late adolescence.

### Sources of Drugs Information

As shown in Table 4, adolescents received information about drugs from parents, friends, teachers and media. Media like internet, television, newspaper or magazines was reported by adolescents as the main source of drugs related information compared to the others. Overall, most of adolescents reported using various information drug sources. Result of the study demonstrated that external factors have significant role in affecting adolescents' understanding about drug.

**Table 4.** Sources of drugs information

No	Source	N (%)
1	Parents	25 (8.3)
2	School Friends	10 (3.3)
3	Teman Diluar Sekolah	14 (4.7)
4	School Teachers	42 (14)
5	Media (Internet, television, newspaper, magazine etc.)	82 (27.3)

Given the potential risk and negative consequences of drug misuse, as well as the growing early initiation of drug use among adolescents especially in Aceh, some efforts are greatly needed. Anti-drug media and educational campaigns are effective way as early prevention program to eliminate or lessen drug misuse on adolescents. As stated in literature, cognitive knowledge about drug was significantly related to drug usage (Azwar, 2003; Hunt and Ellis, 2004). It showed that more adolescents know about the harmful affect of drug abuse are less likely to use such substance. In other words, the exposure to cognitive knowledge of drug abuse resulted in decreased drug use.

Limitation of the present study was merely focused on adolescents' level of drug knowledge. The study did not explore how adolescents' cognitive knowledge about drugs are related to their actual use and abuse. However, results of the study give valuable information about how adolescents especially in Banda Aceh have understanding and awareness about drugs. This information could well be the basis in developing effective drug abuse prevention education not only in school but in youth organizations, civic groups and governmental agencies. Knowledge about risk and danger of drug usage play a fundamental role in behavioral intervention program which try to get adolescents to recognize their own vulnerability to negative outcomes of drug misuse. Relationships between drug perception and knowledge on adolescents drug use in Banda Aceh need to be explored further.

### Conclusions

The purpose of the study was to provide information on the adolescents' knowledge concerning drugs. Results of the current study demonstrated that a large proportion of the adolescents have a good general understanding and awareness about drug misuse and its harmful effect. Their awareness of the negative side of substances misuse indicates they have accurate knowledge related to different types of drugs

and its effect. Generally, adolescents received drug related information from various sources including family, friends, school and media (internet, newspaper, magazines). Specifically, parents have an important role in providing information about illicit drug use through bi-directional communication toward adolescents.

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## Antioxidant Activity and Vitamin C of Banana Peel Infused Water Basis on Difference of Infuse Duration and Water Temperature Using DPPH Radical Scavenging and UV-Vis Spectrophotometer Method

Manna Wassalwa, \*Supriatno, Hafnati Rahmatan

Biology Education, Faculty of Education and Teacher Training University of Syiah Kuala, Darussalam, Banda Aceh 23111;

\*Corresponding author E-mail: [supriatno@unsyiah.ac.id](mailto:supriatno@unsyiah.ac.id)

### Abstract

Antioxidant activity and vitamin C of banana peel infused water basis on difference of infuse duration and temperature of water were studied. Antioxidant activity was determined by DPPH (1,1 diphenyl-1-picrylhydrazyl) radical scavenging method. Concentration of vitamin C was measured using UV-Vis spectrophotometer method. ANOVA test shown that the combination of infuse duration and water temperature of infusion gave a significant effect on the antioxidant activity and vitamin C of banana peel infused water ( $p < 0.05$ ). The highest concentration both of antioxidant with value 4.80 ppm and vitamin C value 9.75 ppm were obtained from sample of 37°C of water temperature with 120 minutes of infuse duration. A positive correlation between concentration of each sample with their %inhibition was observed ( $r = 0.99$ ).  $IC_{50}$  value was measured of 3.63 ppm.  $IC_{50}$  value shown that the sample exhibited a strong antioxidant DPPH radical scavenging activity.

**Keywords:** Banana peel infused water, antioxidant activity, vitamin C, DPPH radical scavenging, UV-Vis spectrophotometer.

### Introduction

Bad effects for human health caused by instant drinks such as soft drinks, carbonated beverages, and canned drinks (Popkin *et al.*, 2011) has made infused water as an alternative beverage which is healthy to be consumed daily (Stone, 2014). Infused water is water with added pieces of antioxidant rich ingredients such as fruits, vegetables, or herbs that gives natural taste and benefits for health (Soraya, 2014). One of the infused water is banana peel infused water. Banana peel has been selected because it contains antioxidants and vitamin C (Sulaiman *et al.*, 2011). However, whether there is a link between duration and temperature of the water infusion to yielding antioxidants and vitamin C of banana peel water infused does not known well yet. This study aimed to determine the effects of duration and temperature of the water infusion regarding antioxidant activity and vitamin C in banana peel infused water.

### Materials and Methods

#### Materials

Banana *Musa paradisiacal* var. Raja peel, radical DPPH powder (Sigma Aldrich), ascorbic acid (GCE Laboratory Chemical), methanol (Sigma-Aldrich), filter paper, distilled water, aluminum foil (Total), masks (SENSI), gloves (SENSI) and seal paper (Total). The tool used was spectronic spectrophotometer 20D + (Thermo Scientific), Spekol UV-Vis spectrophotometer (Analytik jena), volumetric flask (Pyrex), Erlenmeyer flask (Pyrex), beaker glass (Pyrex), incubator (Bellstone Hi-Tech International 3755), digital scales (Analytical Balance Radwag AS220 / C / 2), oven (Mido / 4 / ss / f), vial bottle, and micro pipette (Bellstone-1000).

#### Preparation of banana peel infused wáter

Ripe bananas with yellow skin color followed a little green at the tip and the base of the fruit was cleaned from dirt and dust. 5 mg of the inner skin was cut to the size of 2x2 cm and soaked in 50 ml of distilled water with the combination of temperature and length of time in accordance with certain soaking treatment. The water temperature is constant from early immersion to finish. Samples were obtained by filtration (Karlinda *et al.*, 2013).

### DPPH radical scavenging assay

5 ml of the filtrate of banana peel infused water was put in the clean vial bottle that has been coated with aluminum foil, added of 3 ml solution of DPPH 20 ppm, then the mixture was incubated for 30 minutes at 37°C. The absorbance of the solution was measured using a spectrophotometer at a wave length of 517 nm (Nuramanah *et al.*, 2013; Pabesak *et al.*, 2013, with slight modifications). The antioxidant activity stated in percent of inhibition and calculated by the following formula (Bendra, 2012):

$$\% \text{ Inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100\%$$

The level of antioxidant activity by DPPH radical scavenging method expressed by IC<sub>50</sub> values (Table 1).

**Table 1.** Levels of antioxidant activity using DPPH method.

Antioxidant Activity	IC <sub>50</sub> Value
Very strong	<50 ppm
Active	50-100 ppm
Moderate	101-250 ppm
Weak	250-500 ppm
Not active	> 500 ppm

### Vitamin C test

5 ml filtrate sample was diluted into 50 ml flask and homogenized. The absorbance value of the sample was measured using a spectrophotometer at a wave length of 265 nm (Wardani, 2012, with minor modifications). The concentration of vitamin C of the sample was yielded using the linear regression of calibration curve.

## Results and Discussion

### Antioxidant activity test

Results of analysis of variance showed that there is an effect of the combination of time and temperature of infuse water on the ability of antioxidant to dampen free radical activity in the samples with the F-count  $\geq$  F-table (the alternative hypothesis is accepted) at test level  $\alpha$ : 0.05 with a value of 4.06 > 2.19 (Table 2). Due to the significant differences in the results of analysis of variance is obtained it is necessary to further test. Varian coefficient value of 17.38% indicates that a further test to be used is Test Duncan Multiple Range Test (DMRT) (Table 3). The optimum treatment to the concentration of the antioxidant is treatment of S3W4 which was treated at 37°C with an infusion duration of 120 minutes with a value of 4.80 ppm concentration of antioxidants. IC<sub>50</sub> value was found to be as 3.63 ppm (Table 3).

**Table 2.** Analysis of variance regarding to antioxidant concentration of banana peel infused water

Variance	Degree of Freedom	Sum of Square	Mean Square	F count	F Table (0.05)
Temperature	3	34	11.33	34.33*	2.90
Duration	3	8.75	2.92	8.85*	2.90
Interaction	9	12.02	1.34	4.06*	2.19
Error	32	10.49	0.33		
<b>Total</b>	<b>47</b>	<b>65.26</b>			

Note: \* = significantly different at test level of 0.05



**Table 3.** DMRT (*Duncan Multiple Range Test*) regarding to concentration of antioxidant activity

Treatment	Average of Concentration (ppm)	DMRT <sub>(0.05)</sub> = 1.14
S1W1	0.66	A
S1W2	1.40	Ab
S1W3	2.36	Bc
S2W1	2.64	Cd
S1W4	2.93	Cde
S3W1	3.04	Cdef
S3W2	3.38	Cdefg
S4W4	3.38	Cdefg
S2W2	3.49	Cdefgh
S4W3	3.67	Defghi
S2W3	3.89	Efghij
S3W3	4.06	Efghijk
S4W2	4.06	Efghijk
S2W4	4.23	Ghijkl
S4W1	4.46	Ghijklm
S3W4	4.80	Ijklmn

DPPH test is a test method that is based on the reduction of purple free radical DPPH by inhibiting free radical at a wave length of 517 nm maximum (Shekar and Goyal, 2014). DPPH radical compounds that are purple when reacted with sample turns into yellow. The color changes to yellow are caused by the presence of substances that act as antioxidants which is contained in the sample. Sunarni (2005) in Bendra (2012) said that the purple radical compounds when reacted with quencher compound will produce changes in the intensity of the color purple to yellow. The color change indicates that there are compounds that act as free radical scavengers such as vitamin C that capture or reduce DPPH (Khasanah *et al.*, 2014). When radical DPPH antioxidant reacts with hydrogen donors, DPPH accept Hydrogen atom and become DPPH-H. DPPH molecules will donate hydrogen atoms so that radical DPPH turned into non radical diphenyl pikrilhidrazin (Khasanah *et al.*, 2014).

Analysis showed that the antioxidant activity of the sample S3W4 treatment produce the highest concentration of antioxidants which means producing the strongest antioxidant activity when compared to the other treatment that is 4.80 ppm. Infusion process carried out at 37° C with the length of time of 120 minutes. Setyastuti (2004) in Pramita (2008) stated that the high level of antioxidants means the antioxidant activity is great.

Soaking process at 5°C showed a tendency to increased concentrations of antioxidants along with the addition of infusion time, but the increase in value is not significantly different (Table 3). This means that the manufacturing banana peel infused water at 5°C can slow the reaction of antioxidant to dissolve. This is in connection with the statement of Ibrahim *et al.*, (2015) that low temperatures can cause a reaction process running longer. With increasing temperature, diffusion process will increase so that the reaction will also run faster. Soaking process at 45 °C showed that the antioxidant activity goes lower in line with increasing of duration of infusion. Soaking process at high temperatures can increase the rate of oxidation of antioxidants contained in the peel of a banana. In the other hand, soaking at a temperature of 45°C to infuse at long duration can cause damage to the antioxidant compound which is can causes a decrease in the concentration of antioxidants. As mentioned by Husni *et al.* (2014) that antioxidant levels decreased with increasing temperature and long heating duration.

Furthermore, based on the regression of the relationship between the concentration of the sample with the percent of inhibition of antioxidant that is  $\hat{Y} = 14.35X - 2.16$ ,  $IC_{50}$  value can be determined as 3.63 ppm. This shows that the filtrate of banana peel infused water at a concentration of 3.63 ppm was capable to donate electrons and able to reduce 50% of the free radical DPPH. Based on the antioxidant activity classification (JUN *et al.*, 2003) (Table 1) showed that the filtrate of banana peel infused water has antioxidant activity in the range of <50 ppm, which is very strong.

### Vitamin C test

Results of analysis of variance showed that there was an effect of combination of infuse duration and water temperature on the concentration of vitamin C in banana peel infused water. Variance coefficient value of 7% indicate that a further test to be used is the Least Significant Difference Test (Table 4). Table 5 shows that treatment S3W4 (37°C and the infusion time of 120 minutes) is the best treatment to generate the highest concentration of vitamin C in banana peel infused water as value of 9.75 ppm.

**Table 4.** Analysis of Variance regarding to vitamin C of banana peel infuse water

Variance	Degree of Freedom	Sum of Square	Mean Square	F count	F Table (0.05)
Temperature	3	14.82	4.94	15.94*	2.90
Duration	3	1.88	0.63	2.03	2.90
Interaction	9	32.49	3.61	11.65*	2.19
Error	32	9.79	0.31		
<b>Total</b>	<b>47</b>	<b>58.98</b>			

Note: \* = significantly different at test level of 0.05

**Table 5.** Least Significant Difference Test (LSDT) regarding to vitamin C concentration of banana peel infused water

Treatment	Average of Concentration (ppm)	LSDT <sub>(0.05)</sub> = 0.94
S1W4	5.92	A
S4W4	6.75	Ab
S4W3	7.17	Bc
S1W3	7.42	bcd
S3W1	7.75	cde
S4W2	8.25	def
S1W2	8.33	defg
S3W2	8.42	efgh
S2W2	8.42	efgh
S2W1	8.42	efgh
S1W1	8.92	fghi
S4W1	9.00	fghij
S3W3	9.17	fghijk
S2W3	9.17	fghijk
S2W4	9.50	ijkl
S3W4	9.75	ijklm

The results of the regression of ascorbic acid calibration curve obtained *r* value of 0.98. Positive *r* value suggested a perfect, direct linear relationship or correlation (Sudjana, 2005). These results indicate that there was a positive relationship between the concentration of ascorbic acid with absorption. The higher the concentration of ascorbic acid, the absorbance values will also increase. These results consistent with previous research by Karinda *et al.* (2013) that the value increase the concentration of vitamin C, the absorbance values also increase.

Treatment process at a temperature of 5°C was found a slight decrease in the concentration due to the addition of infusion time. The treatment of food storage at 5°C with a storage time 3 days and 7 days

significantly different where the storage of 3 days oxidation process of ascorbic acid which plays a role in the oxidation of vitamin C activity decreased, but on storage of 7 days was found an increase in activity of oxidation ascorbic acid process. This means that the solubility of the reaction of vitamin C by the enzyme is still ongoing but running slow (Safaryani, 2007). Koswara (1992) in Safaryani *et al.* (2007) explained that the stability of vitamin C typically increase with decreasing temperature during storage but storage at low temperatures can cause damage to the tissue because of the tissue covered by a layer of ice.

Treatment at a temperature of 45°C showed results decreased in concentrations of vitamin C along with increased in infusion time. Temperature can accelerate the reaction process, but exposure to high temperatures in a long period of time of infusion may cause damage to the vitamin C compound. Along with Rosida (2013) that vitamin C is easily oxidized with oxygen and this oxidation process can accelerated by high temperatures, but the heat conditions in a relatively long time can damage the structure of vitamin C.

## Conclusions

Based on the research can be concluded that (1) the combination of duration and temperature of the water infusion affect to the activity of antioxidants and vitamin C of banana peel infused water, (2) the treatment S3W4 which is treated at a temperature of 37°C for 120 minutes infusion duration is the best treatment because it showed the highest antioxidant activity that is 4.80 ppm and the highest concentration of vitamin C as 9.75 ppm, and (3) As 3.63 ppm concentration of sample can reduce 50% of DPPH radical compound activity. Subsequently, this research should be furthered investigated in the future.

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## Optimization of Early Warning System Using Climate Data for Malaria Elimination in Aceh Province

<sup>1</sup>\*Rinidar, <sup>2</sup>Zaitun, <sup>1</sup>Hamny, <sup>1</sup>M. Isa

<sup>1</sup>Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam Banda Aceh, 23111 Indonesia

<sup>2</sup>Department of Agrotechnology, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh, Indonesia

\*Corresponding Author: rinidar@unsyiah.ac.id.

### Abstract

The objective of this research was to create a model for detecting the status of malaria outbreak and sustainable malaria prevention management based on climate data. Here climate data (temperature, rainfall and humidity) recorded for seven consecutive years (2006-2012) in Aceh Besar and Aceh Barat, Indonesia were used. Malaria data recorded during the same time interval were obtained from the respective health offices. Data were then analyzed using linear regression. The results showed the occurrence of strongly multiple correlations between temperature and rainfall with the incidence of malaria in Aceh Besar. The  $R^2$  score of 0.707 indicated the representativeness of using temperature and rainfall data to predict the incidence of malaria was large, namely 70.7%. This is contrast to condition in Aceh Barat where weak correlation ( $R^2$  of 0.03) occurred between temperature and rainfall with malarial incidence. Simultaneous F-test and partial test failed to show significant effect of both temperature and rainfall variables on the incidence of malaria in the two regencies.

**Keywords:** mosquito, rainfall, humidity, malaria, temperature

### Introduction

Malaria is still reported to causes high numbers of mortality in the world and put a burden on social and economic aspects (Sach and Malaney, 2002). The incidence of malaria is highly correlated with environmental factors such as the climate, the distance between mosquitoes' breeding place and human residential, and the number of mosquito species in a location (Clive, 2002, Gilles and Peter, 2002). The climate change has resulted in global warming that significantly contribute to change of life-cycle of malarial vector, such as *Anopheles*, sp, and high transmission of the disease (Bruce-Chwat, 1985; Lindblade *et al.*, 1999; Lieshout *et al.*, 2004; Ahmadi, 2007). The increase of rainfall also increases the numbers of potential breeding places that promote growing number of mosquitoes.

The efforts to control malaria should include the identification and collection of comprehensive environmental conditions contribute to the development of potential vectors in a long-term program supported by a model that is able to explain the dynamic malaria transmission in endemic areas (Gilles and Peter, 2002; Yazoumé *et al.*, 2009; WHO, 2009).

Aceh Province is one of malaria endemic regions in Indonesia that has many nested locations of malaria vectors. Aceh Besar and Aceh Barat are two regencies in the province categorized into malarial mesoendemic areas. An initial control program has been implemented based on the model development of vector control in Lamteuba, Aceh Besar (Rinidar, 2010). However, this model was only focused on the behavior of malaria vector in a sub-area of a district without considering the climate data.

In fact, the behavior of malaria vector is very dynamic according to climate changes. The vectors move from an area to other locations following a heterogeneous distribution pattern in a large geographical coverage and variation of malarial cases. Therefore, a model uses climate data and geographical information system (GIS) is needed to support optimization of malaria early warning system. The model developed is expected to provide an estimation of the level of malaria cases based on climate data in Aceh Besar and Aceh Barat.

### Materials and Methods

Climate data collected were daily and monthly temperatures, rainfalls and humidity in Aceh Besar and Aceh Barat in seven consecutive years (2006 – 2012). These data were obtained from the Meteorology

and Geophysics Agency of Aceh Besar and Aceh Barat. Recorded malaria cases in these regencies during the same period were obtained from the Public Health Offices and Community Health Centers of the regencies. The data were then analyzed using double regression analysis (Steel and Torrie, 1991)

## Results and Discussion

### *The profile of Aceh Besar and Aceh Barat*

Aceh Besar is geographically located between 5.2°-5.8° North latitude and 95°-95.8° East longitude. It is surrounded by Malacca Strait and Banda Aceh (North), Aceh Jaya (South), Pidie (East), and Indonesian Ocean (West) with a total of 2,974.12 km<sup>2</sup> (5.2% of the Aceh Province). Jantho is the capital city of the regency that has 23 subdistricts, 68 kemukiman and 601 villages. Topography of the region varies from coastal areas, plains, hills up to the mountains with an altitude between 100-500 meters above sea level (42.64%). Like other areas in Indonesia, climate in Aceh Besar generally divides into dry season that lasts from April to August, and rainy season that lasts from September to February (BPS, 2012)

Aceh Barat geographically located on 04°06'-04°47' North latitude and 95°52'-96°30' East longitude. Administrative boundaries of this regency are North: Aceh Jaya and Pidie, South: Indonesian Ocean and Nagan Raya, East: Aceh Tengah and Nagan Raya, West: Indonesian Ocean. Aceh Barat has a total area of 2,927.95 km<sup>2</sup>. Topographically, the majority (72.6%) of villages in this regency are plain regions. The rest are beaches, valleys and slopes. Average minimum solar radiation is 37 hours and occurs in August and November. Maximum solar exposure (71 hours) occurs in April.

### *Malarial incidence in Aceh Besar and Aceh Barat*

Secondary data of malaria cases in Aceh Besar and Aceh Barat from 2006 to 2012 were collected from primary health care units and local health offices and crossed check with the data collected from the provincial health office of Aceh. The data is presented in Table 1.

### *Climate profiles in Aceh Besar and Aceh Barat*

The climate data recorded included temperature, rainfall and humidity obtained from the Meteorology and Geophysics Agency (BMG) of Blang Bintang as summarized in Table 2.

### *The relationship Between Climate Elements and Malaria Cases in Aceh Besar*

Air temperature in Aceh Besar from January 2006 to December 2012 ranged from 25.97°C to 28.17°C. In the same period average humidity and rainfall fluctuated from 72.35% to 84.77% and 23.36 mm to 273.57 mm, respectively. In November 2006, where rainfall was at the highest and average humidity was high, only 82 malaria cases reported. Malaria incidence increased December. In 2007 the highest rainfall occurred in January but lower malaria cases recorded. The incidence of malaria increased almost 4 fold February when the rainfall was less than 8 mm. This is contrast to the high numbers of malaria attacks reported in December where the average rainfall almost reached 200 mm.

**Table 1.** Average of malaria cases in Aceh Besar and Aceh Barat in the period of 2006-2012

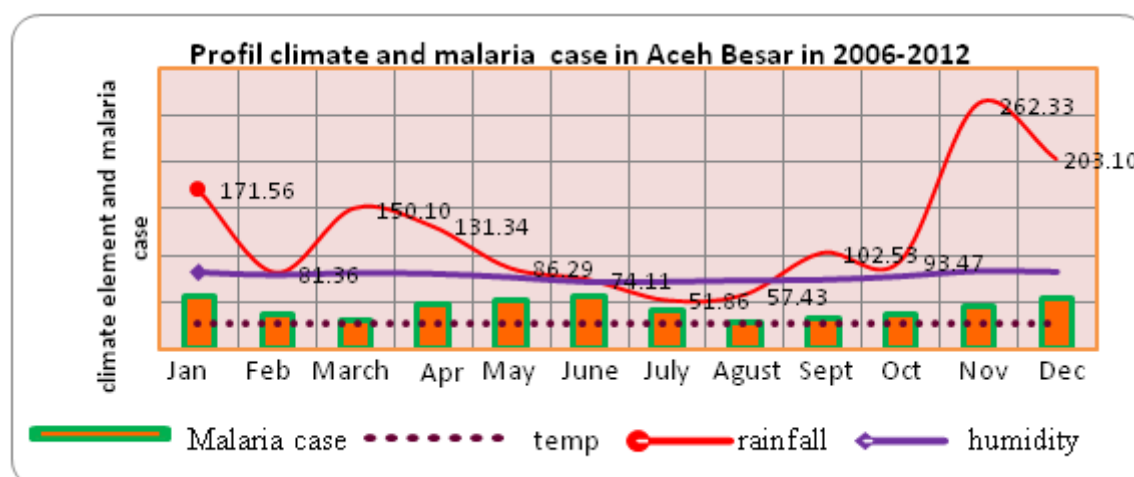
No	Month	Malarial case	
		Aceh Besar	Aceh Barat
1	January	55.29	41.86
2	February	36.86	54.71
3	March	30.86	50.29
4	April	47.29	43.00
5	May	51.57	43.14
6	June	57.14	32.43
7	July	40.00	26.14
8	August	29.00	36.00
9	September	32.71	15.29
10	October	37.00	24.86
11	November	45.71	73.14
12	December	54.14	131.00

Source: Provincial Health Office of Aceh (2013)

**Table 2.** Climate profiles in Aceh Besar and Aceh Barat in the period of 2006-2012

Period	Aceh Besar			Aceh Barat		
	Temperature (° C)	Rainfall (mm)	Humidity (%)	Temperature (° C)	Rainfall (mm)	Humidity (%)
January	26.20	171.56	82.83	26.26	326.50	86.71
February	26.71	81.36	80.44	26.46	211.70	86.29
March	26.61	150.10	82.76	26.41	364.07	87.14
April	27.19	131.34	81.74	26.73	469.56	88.43
May	27.70	86.29	78.09	27.07	226.01	86.29
June	28.01	74.11	72.90	26.50	223.47	85.43
July	27.74	51.86	73.26	26.19	203.56	86.00
August	27.49	57.43	74.69	26.14	313.93	85.14
September	27.27	102.53	75.31	25.66	329.57	86.14
October	26.79	93.47	79.09	26.00	420.06	88.14
November	26.24	262.33	84.73	26.07	476.26	89.14
December	26.20	203.10	83.76	26.01	370.67	89.57

In November 2008, 2009, 2010, and 2012 when the rainfall was at the highest the incidences of malaria were low. In 2011 the highest rainfall occurred in March and malaria attacks reported in this month was also low. Overall description of the linkage between climate element and malaria incidence in Aceh Besar District is shown in Figure 1.

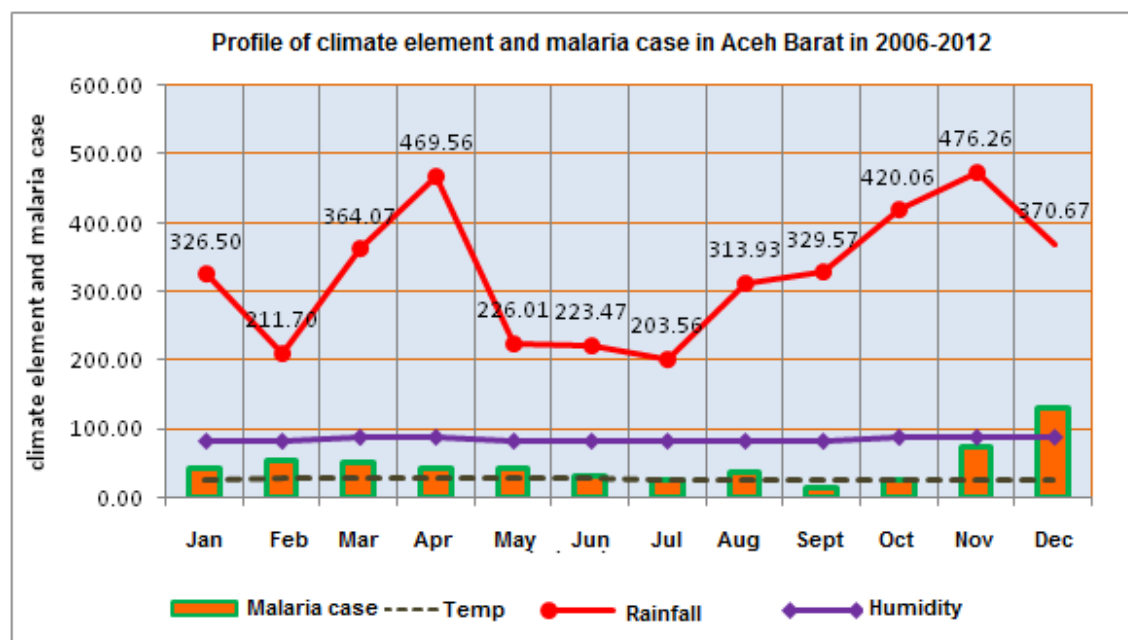


**Figure 1.** Profile of climate element and malaria case in Aceh Besar from 2006 upto 2012.

### **The relationship between climate elements and malaria cases in Aceh Barat**

Climate elements might affect the reproduction of anopheles mosquitoes, the vector of malaria. Climate elements analyzed in this study were temperature, humidity and rainfall. Average air temperature, humidity and rainfall in Aceh Barat from January 2006 to December 2012 ranged from 25.32°C to 27.14°C, 83.71% to 90.85% and 124 to 656.12 mm, respectively. The highest rainfall and humidity occurred in December 2006 looked like contributed to the highest malaria attacks. In 2007, on the contrary, although the highest rainfall occurred in October, the highest numbers of malaria cases occurred in February.

In 2008, 2009, 2010, 2011 and 2012 rainfall reached the peak in April, November, September, August and November, respectively. The highest malaria attacks in these particular years, however, occurred in February, March, May, February, and January. Overall description of the relationship between climate elements and malaria incidence in Aceh Barat is shown in Figure 4.



**Figure 2.** Profile of climate element and malaria case in Aceh Barat from 2006 up to 2012.

### **The relationship between climate elements and malaria cases**

In order to determine relationship between climate elements and malaria cases, a multiple linear regression test was performed. Analysis in Aceh Besar used a lag system based on the facts that scattered data of malaria incidences generally occurred following a month has the highest rainfall. The analysis showed strong double correlation between temperature and rainfall with malaria incidence. A 0.71  $R^2$  indicated large potency of using these climate elements to predict malaria incidence. Simultaneous analysis using F test and partial test at 5% error rate indicated that temperature and rainfall did not influence malaria incidence.

Double correlation analysis showed that both temperature and rainfall weakly related to the incidence of malaria in Aceh Barat. This indicated that the potency of using temperature and rainfall to predict the incidence of malaria is very low. Similarly, result of F test and partial test showed no effect of both temperature and rainfall on the incidence of malaria.

It is interesting that we found that climate elements had no effect on malaria incidence in Aceh Besar and Aceh Barat. A preliminary data showing a strong correlation between temperature and rainfall with malaria incidence ( $R^2 = 0.71$ ) was not statistically strong enough to be used to predict the incidence of malaria. Depinay (2004) and Yazoume *et al.* (2009) suggested that rainfall altogether with temperature and humidity contributed to the abundance of vector populations. Changes in temperature, humidity and rainfall made mosquitoes lay their eggs more frequent so that the number of vector populations increased (Cook, 1996; Zell, 2004; Preston *et al.*, 2006).

These events tended to occur in Aceh Besar district, as in 2007, the highest rainfall occurred in January was accompanied with high malaria case that in the next month. Based on BMG data rainfall in the Aceh Besar varies from 23.36 mm to 273.57 mm. This indicates that in Aceh Besar a rainy day was usually followed by a sunny day, a condition allowing vector to breed optimally. This is due to rainfall, accompanied by the increase in average humidity might provided a convenient breeding place for *Anopheles* mosquitoes in the environment.

The influence of rainfall on malaria incidence varied according to the amount of rain and the demography of the region. The majority of areas in Aceh Barat are plateau zones covering 233 villages (72.59%). The rests are beaches, valleys and slopes. Air temperature in Aceh Barat from January 2006 to December 2012 ranged from 25.32°C to 27.14 °C with an average humidity ranged from 83.17% up to 90.85%. Average length of solar radiation varied from 37 hours in August and November up to 71 hours in April. This climate condition was very convenient for the reproduction process of mosquitoes.



There was a difference in rainfall between Aceh Besar and Aceh Barat. Aceh Barat generally has fairly high rainfall, indicating unequal distribution of rainfall in the region. In Aceh Barat, including all regions in south west coastal line of Aceh, average rainfall was relatively high (>100mm). This causes less breeding places for Anopheles mosquitoes. High rainfall led to an increase in river water flow, thus damage and washed away mosquito breeding places formed along water bodies or river banks. Nevertheless a relatively high humidity due to the heavy rainfall increased breeding activity of Anopheles mosquitoes in the region.

Humidity affected mosquitoes' breeding rate, biting ability and resting time (Harijanto, 2000). At higher humidity mosquitoes became more active and bite more frequent thereby increasing the transmission. The optimum moisture needed for the breeding of mosquitoes is higher than 60% (Chwatt-Bruce, 1985). Humidity in the study area, that was higher than 60%, was quite supportive for the growth of mosquitoes.

Aforementioned description proved that malaria transmission might occur after certain 4-week period of extrinsic and intrinsic incubation time. From the observations of rainfall and supporting malaria morbidity data it could be estimated the emergence of new malaria morbidity (Paijmans, 2007). Therefore, the potential indicator of rainfall for predicting the transmission of malaria in certain regions could be used as one of the considerations in planning malaria control program and anticipating malaria outbreaks (Bates, 1970).

While results of this study tend to be very weak to declare there are significant effects of climate factors on malaria transmission, the literatures mention that global climate change could affect the dynamics of disease transmission, climatic factors do not affect linearly (Focks *et al.*, 1993). This means that the climate is not the main factor of the incidence of malaria. Therefore, it is projected that climate change is very likely to alter the distribution and abundance of this species through space and time. As a consequence, the relationship between climate change and ecological systems are often complex. A complex interaction is often non-linear and difficult to predict before any changes occur (Adger *et al.*, 2009). Our initial hypothesis about climate elements could affect malaria attacks in the district of Aceh Besar and Aceh Barat has not been proven in this study. Hence, the results cannot be used as the next model of early alert system.

This study has raises some questions whose answers require additional data for further research to be able to predict the future incidence of malaria. Therefore, other variables are needed to support the model, for example, by incorporating other environmental elements such as latitude, length of solar radiation and rainfall enriched with a number of rainy days in order to get rainfall index, to be able to determine any fluctuations in rainfall.

Another factor must be considered is that malaria cases should be made malaria morbidity rate fluctuations by calculating the monthly parasite incidence (Mopi) by dividing the number of infected patients with total population multiplied by a constant of 1000. Not only the amount of rainfall but also the frequency and precipitation pattern will likely change. This change will interact with changes in temperature and affect to evaporation and relative humidity. In addition to the above-mentioned variables, secondary data used, both climate fluctuation and malaria cases, should be supported by a primary data collected from field studies so that data obtained to be more valid.

## Conclusions

The climate elements had no effect on malaria incidence in Aceh Besar and Aceh Barat. The model for early warning system was not statistically strong enough to be used to predict the relationship of climate and incidence of malaria.

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## **Syneresis and Acidity Evaluations On Probiotics Milk Added By Different Levels Of Lactic Acid Bacteria and Carrot (*Daucus carrota* L) puree**

\*Yurliasni, Yusdar Zakaria, Zuraida Hanum and Raudhatul Jannah

Department of Animal Husbandry, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: yurliasni62@yahoo.co.id

### **Abstract**

A research about evaluations of syneresis and acidity on probiotics milk with the addition of carrot and lactic acid bacteria (LAB) at different levels has been conducted. Starters used were *Lactobacillus acidophilus* and *Lactobacillus casei* known as probiotics. The objectives of this study were to determine the effect of using *Lactobacillus acidophilus* and *Lactobacillus casei* along with the addition of carrot puree on acidity and syneresis of probiotic milk. This research was held at the Science and Milk Processing Technology Laboratory of Department of Animal Husbandry, Agriculture Faculty, Syiah Kuala University. Completely randomized designed (CRD) with factorial pattern used with two factors, A was level of the starter combination (*Lactobacillus acidophilus* and *Lactobacillus casei*) namely as  $a_1$ (2.5%) and  $a_2$  (5%), B was level of carrot puree consisted of three different levels  $b_1$ (5%),  $b_2$ (10%),  $b_3$ (15%) with 4 repetitions each. The parameters measured were acidity (pH) and the levels of syneresis. The results showed that milk fermented with the addition of probiotics and carrot puree at different levels very significantly resulted ( $p < 0.01$ ) effect in decreasing the pH, and significantly ( $p < 0.05$ ) effect on levels of syneresis. In conclusion, the best treatments given were combinations of either 2,5 or 5% starters and 5% carrot puree on pH value and syneresis level consecutively 3.46, 45,05% and 3.61 44.31% .

**Keywords:** probiotic milk, starter, syneresis, carrot puree, LAB

### **Introduction**

The role of fermented milk in human nutrition has been known very well, particularly those fermented using probiotics. Probiotics are defined as selected viable microorganisms used as dietary supplement having potential for improving health of human following ingestion. Several benefits of consumption of probiotic fermented milk products are alleviation of lactose intolerance, protection of the body against gastrointestinal infection, anticarcinogenic effect, lowering of serum cholesterol, alleviation of constipation (Panesar *et al.*, 2009). Besides to make it is more useful and better preference, probiotic product needs to have a variety of flavors.

Generally, fermented milk products sold in the market are products have fruit flavors. With the aims of making fermented milk products to be more variable it is needed to find new flavors for the products such as by adding vegetables into a fermented milk in the form of carrot puree. The addition of carrots in manufacturing probiotics milk may be able to increase their nutritional values, taste and consumers' acceptance. It is widely known that carrot has good nutritional values because it contains protein, vitamins, minerals, fiber and high antioxidants. However, good probiotic milk products have to meet criteria of SNI standards such as low syneresis values and appropriate acidity.

Syneresis and acidity are two of several factors that affect the quality of fermented milk products. It is highly expected that a fermented product has a low syneresis and appropriate acidity at the end of fermentation process. In relation to these factors it is necessary to evaluate syneresis and acidity levels of fermented milk that supplemented with carrot puree and probiotics at different levels.

### **Materials and Methods** **Experimental design**

This experiment used a completely randomized factorial design consisted of 2 factors: two levels of probiotic (2.5% and 5%) and three concentrations of carrot puree (5, 10 and 15%), with four replications each.

### Preparation of carrot puree

Carrots (*Daucus carota* L) bought from public markets in Banda Aceh were peeled, washed, and cut into cubes. The carrots were blended and filtered to produce a smooth texture extract. The extract was pasteurized at 90-95°C for 5 minutes to produce a puree.

### Preparation of fermented milk

Fresh milk samples obtained from mall lives stock farms located at the ring road around the campus of Syiah Kuala University farm were pasteurized at 90-95 °C for 10 minutes, and then cooled at 30 °C. Pasteurized milk, 100 ml, were poured into 24 sterile bottles. A combination of probiotics *Lactobacillus acidophilus* and *Lactobacillus casei* (2.5% or 5%) and carrot puree (5, 10 and 15%) were added into the milk. The culture starters of probiotic lactic acid bacteria (LAB), *Lactobacillus acidophilus* and *Lactobacillus casei*, were purchased from the Food Science Microbiology Laboratories, Bogor Agricultural Institute. The mixtures were then, incubated at 37°C for 13 hours.

### Measurement of the pH and syneresis

The pH of probiotic milk was measured using a calibrated pH-meter (AZ 86502) at room temperature. Fermented milk, 10 grams, were poured into the syneresis tube, closed tightly and stored in a refrigerator (5 °C) for 1 hour. Samples were centrifuged at 3,000 rpm for 10 minutes. Syneresis value (%) was quantitated by dividing the weight of supernatant (liquid whey) obtained with total weight of sample.

### Data analysis

Data obtained were analyzed by using two-way analysis of variance (ANOVA). The differences among treatments were examined by Duncan multiple range test (Steel and Torrie 1995).

### Results and Discussion

As shown in Table 1, the addition of carrot puree and probiotics in the fermented milk affected the pH values of probiotics milk. This is indicated by the differences in pH values between treatments. The pH values obtained, however, were lower than that of normal yogurt that ranges from 4.5 to 4.6.

It was clearly seen that at the level of 2.5% probiotics and at all levels of carrot puree led to an increase in the pH value. Fleet (1990) reported that LAB, especially *L. acidophilus* and *L. casei*, excrete organic acids which lead to a lowering in the pH.

Results of analysis of variance showed that the addition of probiotics and carrot puree had very significant effects ( $P < 0.01$ ) on the pH of fermented milk and there was interactions between the two factors. Duncan's multiple range tests suggested that the addition of the two levels of probiotics and 5% carrot puree decreased pH of probiotic milk. The addition of same levels of probiotic and 10% carrot puree, however, tended to increase pH values. Furthermore, there was no difference in the pH between probiotics milk added with 15% carrot puree and 2.5% probiotics with those added 10% carrot puree and 2.5% probiotics. On the other hand, the addition of 5% probiotic and 15% carrot puree decreased pH value of milk equal to those resulted by the addition of 2.5% probiotics and 15% carrot puree.

**Table 1.** The pH of probiotics milk added with different levels of probiotics and carrot puree

Probiotics	Carrot Puree			average
	b1	b2	b3	
a1 (2.5%)	3.46±0.03 <sup>c</sup>	3.54±0.01 <sup>b</sup>	3.58±0.05 <sup>b</sup>	3.53 <sup>a</sup>
a2 (5%)	3.61±0.02 <sup>b</sup>	3.71±0.03 <sup>a</sup>	3.57±0.02 <sup>b</sup>	3.63 <sup>b</sup>
avarage (%)	3.54 <sup>b</sup>	3.62 <sup>a</sup>	3.58 <sup>b</sup>	3.58

Note: means with different superscripts at the same column differ significantly ( $p < 0.01$ ); a<sub>1</sub>= Probiotics 2.5%; a<sub>2</sub>= Probiotics 5%; b<sub>1</sub>= carrot puree 5%; b<sub>2</sub>= carrot puree 10%; b<sub>3</sub>= carrot puree 15%. Probiotics (*Lactobacillus acidophilus* and *Lactobacillus casei*)

Carrot puree levels in the fermented milk were able to determine the activity of lactic acid bacteria and bacterial activity can lower the pH. It was clearly seen in this study that the average of pH of probiotics milk ranged from 3.46-3.71. This was consistent with the statement of James (2005) that pH of standard yogurt ranged from 3.5-4.5. In addition, Kumalasari *et al.* (2012) stated that decreased milk pH is caused by the use of lactose as a source of energy and carbon by bacteria to produce lactic acid. The accumulation of this acid will lead to decreased pH.

Data in Table 1 shows that the addition of 2.5% of probiotics at every level of carrot puree had limited ability to convert lactose and other sugars into lactic acid, but at the level of 5% of probiotic the ability to transform lactose into lactic acid is greater that in turn lowered the pH. The activity of lactic acid bacteria (*L. acidophilus* and *L. casei*) was also influenced by the nutrients contained in carrot puree. Lactic acid bacteria will develop well if supply of nutrients is adequate (Murpi, 2008).

Table 2 explains that the addition of probiotics and carrot puree affected the syneresis values of probiotic milk. It is obvious that increased levels of carrot puree would increase the syneresis value. Based on analysis of variance, the data obtained showed that the addition of carrot puree significantly ( $p < 0.05$ ) effected syneresis values. The addition of carrot puree up to 15% can increase the value of both the concentration of probiotics 2.5% and 5%. The higher the addition of carrot puree the higher the number of syneresis. Having tested by Duncan's Multiple Range, there is a difference between the addition of 15% with the addition of 5 and 10% carrot puree. The increase of syneresis value can be influenced by carrot puree and probiotik level. The higher level of carrot puree, the greater the amount of free water content available so that increasing of syneresis value, and finally lower the quality of probiotic milk.

**Tabel 2.** Average syneresis values of probiotics milk added with probiotics and carrot puree

Bacteria ( <i>L a</i> + <i>Lc</i> )	Carrot puree			Avarage
	b1 (5%)	b2 (10%)	b3 (15%)	
a1 (2.5%)	45.05± 3.41	44.46 ± 4.21	49.94 ± 1.93	46.48
a2 (5.0%)	44.31± 4.77	46.86 ± 4.11	52.18 ± 2.23	47.78
avarage (%)	44.68 <sup>b</sup>	45.66 <sup>b</sup>	51.06 <sup>a</sup>	47.13

Note: means with different superscript at the same column differ significantly ( $p < 0.01$ ). a<sub>1</sub>= Probiotics 2.5%; a<sub>2</sub>= Probiotics 5%; b<sub>1</sub>= carrot puree 5%; b<sub>2</sub>= carrot puree 10%, b<sub>3</sub>= carrot puree 15%. Probiotics (*L. acidophilus* and *L. casei*)

Based on the result it can be said that the addition of 15% carrot puree at two levels of probiotics caused low quality of probiotic milk because from the consistency. Furthermore, Wulandari and Setiadi (2010) stated that the occurrence of syneresis is due to reduced ability of protein network to bind water.

Manab (2008) also explains that one cause of syneresis was changes in solubility of casein and shrinkage of casein particles. In another study Kalab (2002) found that physical disturbance contributed to the syneresis as stirring intensity was too high, the hydrogen bonds between protein and water molecules weakened in acidic environment, the state of the pores between casein molecules unwound and can be passed by water molecules which initially bound to protein. In addition, Savitri *et al.* (2008) describes that the acidity (pH), and water holding capacity can also affect syneresis of fermented milk. syneresis also can be affected by the protein content of raw materials and additives.

## Conclusions

The best probiotic milk obtained from this study was that added with probiotics at levels 2.5 and 5% and at 5% usage of carrot puree because it has a pH that meet the standar of SNI, 1992, and the lowest syneresis value.

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## Fast and Simultaneous Detection of Honey Adulteration and Soluble Solids Content using Near Infrared Reflectance Spectroscopy

<sup>1</sup>Agus Arip Munawar\*, <sup>1</sup>Hendri Syah, <sup>1</sup>Yusmanizar

<sup>1</sup>Department of Agricultural Engineering, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia.

\*Corresponding Author: aamunawar@unsyiah.ac.id.

### Abstract

Honey is one of the most important foodstuffs that can be used as a medicine. Study was performed to detect adulteration in honey and predict soluble solids content rapidly and simultaneously by applying infrared spectroscopic technology in wavelength ranged from 1000 to 2500 nm. Diffuse reflectance spectra data of honey were acquired using near infrared reflectance spectroscopy (NIRS) instrument with co-added 32 scans per acquisition while soluble solids content (SSC) were obtained using digital refractometer. Honey adulteration was detected by subjecting spectral data onto principal component analysis (PCA). On the other hand, SSC was predicted by establishing prediction models using partial least square regression (PLSR) with k-fold cross validation method. The results showed that NIRS combined with PCA could distinguished all honey adulteration accurately with total explained variance of two PCs is 98%. Moreover, SSC could be also predicted simultaneously and satisfactorily with correlation coefficient of calibration ( $r_{cal}$ ) and prediction ( $r_{pred}$ ), and the residual predictive deviation (RPD) were 0.96, 0.91, and 2.41, respectively. The overall results sufficiently demonstrate that NIRS could be used to detect adulteration and predict SSC in honey.

**Key words:** rapid, infrared, detection, spectroscopy.

### Introduction

In the last few decades, since authenticity is also considered as a relevant food quality criterion, fast and single methods to detect adulterations and to ensure the quality and the geographical origin of different commodities are widely required by consumers, producers, and regulatory bureau. One of the related food products needed to ensure its authentication is honey in which can be used as a traditional medicine.

Honey is defined as a natural sweet substance produced by *Apis mellifera* bees from the nectar and other secretions of plants. It is a valuable food product because its high soluble solids (mainly glucose and fructose) composition provides immediate energy to organism. It includes other beneficial substances such as proteins, amino acids, organic acids, aromatic compounds, vitamins, enzymes, minerals, trace elements and polyphenols (Escriche et al., 2011).

However, since there is a great demand for high quality honey and consumers also intend to pay high prices for this product, valuable honeys have become a target of adulteration with cheap sweet foreign components worldwide. Adulterated honeys are often labeled as natural and priced the same as pure honey, which is both fraudulent and unfair to consumers (Batista et al., 2011; Chen et al., 2011). In general, the adulteration of honey does not pose a health risk. However, it does influence market growth negatively by damaging consumer confidence (Chen et al., 2011). Thus, identification and authentication of unadulterated honey is important to control and maintain honey chain market.

Near infrared reflectance spectroscopy (NIRS) has become one of the most promising and non-destructive methods of analysis in many field areas including in agriculture due to its advantage: simple sample preparation, fast, and environmental friendly since no chemicals are used. More importantly, it has the potential ability to determine multiple quality parameters simultaneously (Munawar et al., 2016). Numerous studies have been carried out to investigate and apply NIRS in quality assessment of foods and agricultural products (Chen et al., 2011; Cozzolino et al., 2011; Vesela et al., 2007; Jaiswal et al., 2012; Liu et al., 2008; Bobelyn et al., 2010; Penchaiya et al., 2009; Munawar et al., 2013). Therefore, the main aim of this present study is to apply NIRS as a fast method to detect adulteration in honey and predict soluble solids content (SSC) of honey simultaneously.

## **Materials and Methods**

### ***Honey samples***

Pure honey samples were obtained from Trienggadeng, Pidie Jaya, Aceh Province and stored in room temperature (25 °C) for approximately one month until near infrared (NIR) spectra acquisition. Samples were placed in 50 ml bottles for a total of 100 bottles. One day before NIR scanning, 22 samples were added with white sugar and commercial syrup for adulteration purpose.

### ***NIR spectra acquisition***

Near infrared diffuse reflectance spectra of all honey samples were acquired using a Fourier transform near infrared (FT-NIR) instrument. Sample measurement with integrating sphere was chosen as a basic measurement in this study. Background spectra correction was performed every hour. Diffuse reflectance spectra in wavelength range of 1000 – 2500 nm with the increment of 2 nm resolution were acquired 32 times and averaged.

### ***SSC measurement***

Soluble solids content of pure honey samples were performed using a hand-held digital refractometer (Atago Inc.) directly after spectra acquisition. It performed in triplicate and averaged.

### ***Spectra pre-processing***

To achieve a robust and accurate prediction model, spectra pre-processing was performed prior to prediction models development. Multiplicative scatter correction (MSC) was used as a spectra pre-processing algorithm.

### ***Data analysis***

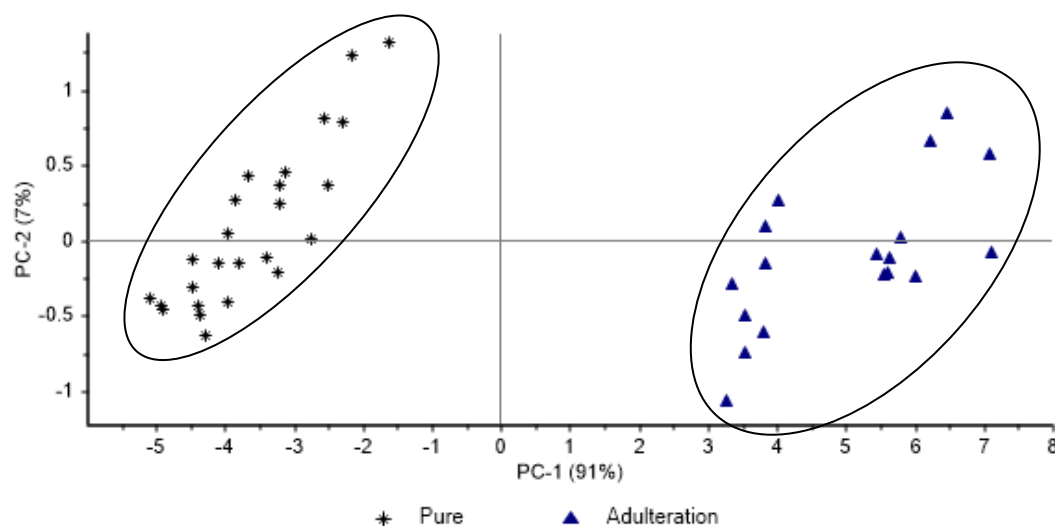
NIR spectra data were analyzed and projected onto principal component analysis (PCA) to differentiate between pure and adulterated honey. On the other hand, calibration was performed to develop models used for SSC prediction. Partial least square regression (PLSR) with k-fold cross validation ( $k = 10$ ) was used as a calibration method. Calibration models were established between reference measurement data as y-variable and NIR spectra data as x-variable. The models were validated and tested using a 10 fold cross validation method. The performance of prediction models was quantified based on calibration and cross validation results according to the correlation coefficient of calibration ( $r_{cal}$ ) and validation ( $r_{val}$ ), the residual predictive deviation (RPD), and the number of principal components or latent variables (LVs) required to establish the model.

### **Results and Discussion**

The NIR spectra indicate the presence of organic materials as derived from the bands that result from the interaction of molecular bonds of O-H, C-H, C-O and N-H with the incident radiation. These bonds are subject to vibrational energy changes in which two vibration patterns exist in these bonds including stretch vibration and bend vibration (Cen and He, 2007). The presence of water absorbance bands was observed at around 1460 nm and 1930 nm because of O-H tone combination and its first overtone. Moreover, the absorption bands in the range of 2200 - 2300 nm are suggested to be related to C-H-O structures such as glucose, fructose, vitamin A and C; whilst absorption bands at around 1400, 1800 and 2100 nm are associated with organic acids.

The PCA result as shown in Figure 1, provide an obvious differentiation between pure and adulterated honey based on electro-optics properties derived from near infrared spectra data. Total cumulative explained variance from two principal components (PCs) was 98% with 100% classification accuracy.

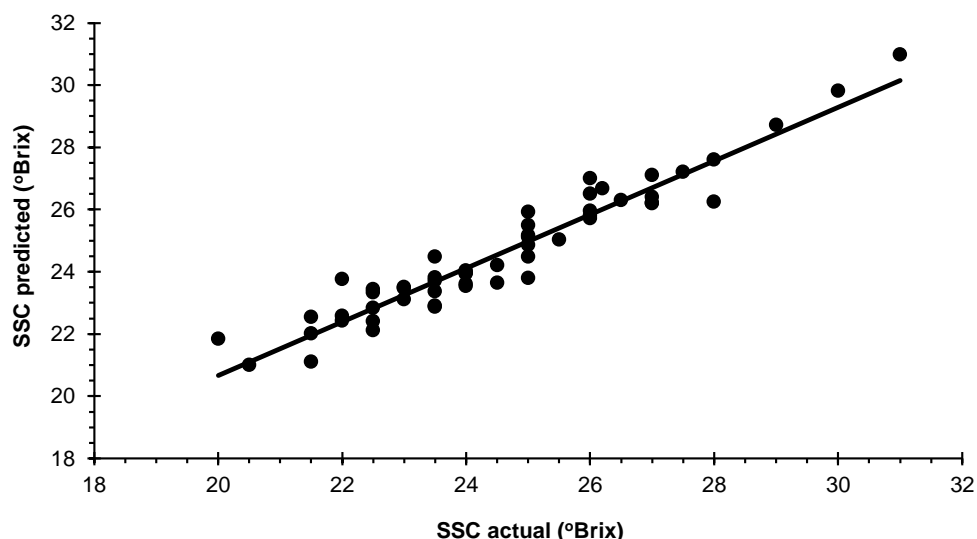




**Figure 1.** PCA result derived from NIR spectra data to detect honey adulteration.

Moreover, further spectra data analysis was performed to predict soluble solids content (SSC) since SSC was formed from C-H-O. Partial least square regression (PLSR) was used as a calibration method and validated using k-fold cross validation method. Figure 2 shows calibration result from which the correlation coefficient for calibration ( $r_{cal}$ ) is 0.96 with the number of latent variables (LVs) is 5 and residual predictive deviation (RPD) index is 2.41 which is categorized as a good prediction model performance.

This result is also in agreement with Nicolai et al. (2007) where the RPD between 1.5 – 1.9 means that coarse quantitative prediction are possible, but still need some improvement in calibration. A value between 2 and 2.5 indicates that prediction model is sufficient. Meanwhile, an RPD value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy respectively (Cozzolino et al., 2011; Munawar et al., 2016).



**Figure 2.** PLSR calibration result to predict SSC in honey derived from near infrared spectra data.

### Conclusions

The present study investigated the feasibility of NIRS method as a fast and non-destructive method in detecting honey adulteration and predicting SSC in honey. Obtained result shows that NIRS combined with PCA and PLSR were able to detect adulteration and predict SSC content in honey precisely.

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## The Correlation Between The Level of Knowledge, Educational Degree and Family Support to The Drug Compliance in Leprosy Patients in North Aceh District

<sup>1</sup>\*Fitria, <sup>2</sup>Vera Dewi Mulia

<sup>1</sup>Dermatology and Venereology Department, Faculty of Medicine Syiah Kuala University/dr. Zainoel Abidin Hospital, Banda Aceh;

<sup>2</sup>Pathology Anatomy Department, Faculty of Medicine Syiah Kuala University/dr. Zainoel Abidin Hospital, Banda Aceh;

\*Corresponding Author: [fitria.spkk@gmail.com](mailto:fitria.spkk@gmail.com)

### Abstract

Leprosy is the chronic infection disease that affect skin and peripheral nerve system. It causes handicap and affect the patient's quality of life. There are many steps that have been done in order to increase the drug compliance. This compliance is influenced by many factors such as level of knowledge, educational degree and family support. The aims of this study want to observe the correlation between level of knowledge, educational degree and family support to the drug compliance in leprosy patient. This study was performed using cross-sectional analytic method with structural questionnaire. The sample is the leprosy patients in North Aceh district. In total of 78 leprosy patients, there were 61 patients (78,2%) who consumed the medication regularly. Seventeen Patients (21.8%) were not consumed them regularly. Statistical analytic shown that the level of knowledge ( $p=0,843$ ) and educational degree ( $p=1,000$ ) were not related with drug compliance, but the family support ( $p=0,001$ ) gave the opposite result. It report that family support correlated with drug compliance in leprosy patient.

**Keyword:** Leprosy, level of knowledge, educational degree, family support, drug compliance.

### Introduction

Leprosy causes complication in eyes and nerve damage that can lead to handicap, paralytic and deformity (Lookwood, 2010; James *et al.*, 2011). Leprosy is contagious therefore it rise the phobia in society. The phobia creates the negative perspective and isolation toward the leprosy patients.

North Aceh district is one of the highest load of leprosy in Aceh province. The high level of leprosy load is measured from (a) NCDR >5 per 100.000 inhabitants or (b) Total newly identified cases is bigger than 30 cases per 3 years subsequently. According to Department of Health in Aceh province, the number of NCDR in 2013 reached 9 per 100.000 inhabitants. In addition, the total of newly identified cases in 2011, 2012 and 2013 subsequently were 48 cases, 51 cases, and 45 cases (Kemenkes RI, 2012, Dogra *et al.*, 2013).

The treatment of leprosy is aimed to cure the patient, to avoid the handicap and to demolish the contagion effect. If the patients do not consume the medication regularly, the leprosy strain will be immune and causes the permanent symptom or even a new worse symptom. One of the most frequent obstacles in Leprosy treatment is the less compliance to consume the medication regularly. The discrimination from the society generates shame, fright, worries and unconfidence state of the leprosy patients. Misunderstanding and lack of education become the main reason why the society discriminates the Leprosy patient (Dinkes Sulsel, 2003).

Rao, 2008 assumes that the failure of Multi Drugs Treatment (MDT) can be categorized into 3 main reasons; Personal factor, health condition problem and health service problem. Personal factor, which consists of negative perspective and psychosocial, is the most frequent reason of all. Another study from Wiyarni *et al.*, 2013 also suggested that the drug compliance is highly related to patient's handicap. Therefore, both the family support and drug compliance are pivotal to avoid the handicap.

Hutabarat, 2008 categorized the drug compliance into two factors based on its source, internal and external factor. In addition, several factors are also involved, such as gender, education, the role of health workers and leprosy manifestation. Other factors such as age, knowledge, occupation, attitude, family role, handicap, duration of drug consumption and drug's side effects, are not affected the drug compliance. Another study from Masykur, 2010 mentioned that both the perception toward Leprosy and the family support influenced the drug compliance in Jangka region in Bireun district.

Based on those literature, we assume that it is important to perform a research that investigates the correlation between the level of knowledge, educational degree and the family support to the drug compliance in the leprosy patients in North Aceh district.

## Materials and methods

This study was performed by using cross-sectional analytic with the interview using structural questionnaire. The samples were the leprosy patients in North Aceh district. The respondent was asked to sign the informed consent and answered all questions in the questionnaire. Research data were analyzed by using non-parameter test chi-square with SPSS

## Results and discussion

There were 78 respondents that participated in this study. The characteristic of each respondent can be seen in table 1. Based on the research, the majority of respondents are in the productive age (25-44 year old/43,6%). There was no difference between male and female. Most of the patients are uneducated (38,5%). Only a small number with the higher education background(1,3%). Occupationally, Some of the respondents work as farmer and labor (46,1%) and jobless (44,9%). The most common type of Leprosy in this study is MB (79,5%).

**Table 1.** Basic characterisation of the respondents

Characterisation	Frequency (n)	Percentage(%)
<b>Age</b>		
< 14years old	6	7,7
15-24year old	12	15,4
25-44 year old	34	43,6
45-64 year old	23	29,5
≥ 65 year old	3	3,8
<b>Gender</b>		
Male	43	55,1
Female	35	44,9
<b>Education</b>		
Uneducated	30	38,5
Elementary school	18	23,1
Junior high school	15	19,2
Senior high school	14	17,9
Higher education/ University	1	1,3
<b>Occupation</b>		
Jobless	35	44,9
Self employee Private sector	6	7,7
Farmer/Labor	36	46,1
Public sector/Police	1	1,3
<b>Leprosy type</b>		
PB	16	20,5
MB	62	79,5

The distribution of respondents based on their level of knowledge can be seen in table 2. The highest distribution is the group of respondents with adequate knowledge (39 respondents / 50,0%). The

spreading of the respondents based on their education can be seen in table 3. Respondents with the low educational degree are the highest group (50,0%) and the respondents with higher education background are at the lowest point (1,3%).

**Table2.** The distribution of respondents based on their level of knowledge

Quality of life	Frequency (n)	Percentage (%)
Good	33	42,3
Adequate	39	50,0
Less adequate	6	7,7
<b>Total</b>	<b>78</b>	<b>100</b>

**Table 3.** The distribution of respondents based on their education

Quality of life	Frequency (n)	Percentage(%)
Higher education	1	1,3
High school	14	17,9
Low Education	63	80,8
<b>Total</b>	<b>78</b>	<b>100</b>

The distribution of respondents based on their family support can be seen in table 4. Most of the patients gain adequate family support (89,7%) and no patient have an unsupportive family. The distribution of the respondents based on their drug compliance can be seen in the table 5. Most of the patients consumpt the medication regularly (78,2%).

**Table4.** The distribution of respondents based on their family support

Quality of life	Frequency (n)	Percentage (%)
Supportive family	70	89,7
Less supportive family	8	10,3
Not supportive family	0	0
<b>Total</b>	<b>78</b>	<b>100</b>

**Table 5.** The distribution of the respondents based on their obedience of medication consumption

Quality of life	Frequency(n)	Percentage (%)
obedience	61	78,2
less obedience	17	21,8
<b>Total</b>	<b>78</b>	<b>100</b>

Data analysis was performed in order to correlate the level of knowledge, educational degree and family support to the drug compliance in Leprosy patient. The result can be seen in table 6. Those all results indicate that the level of knowledge and educational degree have no correlation with the drug compliance in leprosy patient. In contrary, family support has a significant effect on the drug compliance.

**Table 6.** Correlation between level of knowledge, educational degree and family support to the drug compliance in Leprosy patient

			Drug compliance		
	compliance		Less compliance		P value
Level of knowledge	n	%	n	%	0,843
Good/Enough	57	79,2	15	20,8	
Less knowledgeable	4	66,7	2	33,3	
Educational degree					1,000
Higher education	12	80,0	3	20,0	
Low education	49	77,8	14	22,2	
Family support					0,001
Supportive	59	84,3	11	15,7	
Less supportive	2	25,0	6	75,0	

This study concludes that the level of knowledge and educational degree does not have the significant effect on drug compliance in leprosy patient. This result is similar to the research that has been performed by Afifah in Rembang district, Central Java. In her report, Afifah mentioned that the level of knowledge and the educational degree has no significant effect on drug compliance in leprosy patient (Afifah, 2014). Another study by Selum and Wahyuni in Pamekasan district, East Java, also shown the same tendency (Selum and Wahyuni, 2012).

In the opposite, a study by Chalise in Nepal reported that 86% of fail-treated leprosy patient were caused by lack of knowledge. Most of those patients assumed that the leprosy lesion is an indication of the healing process, which is incorrect (Chalise, 2005). A research by Hutabarat in Asahan district in North Sumatera, reported that the level of knowledge and educational degree were significantly involved in drug compliance ( $p=0,019$ ). Yet, the respondents of this research were dominated by higher educational degree's patient. Nevertheless, the role of public health workers is pivotal to educate the patient, the families and the societies. The increase of knowledge will help to promote the drug compliance which then leads to elimination of leprosy disease in the future (Hutabarat, 2008).

Our study reported that the patients with a lack of the family support were 4,7 times less compliance compared to those who have family support. This result supports a study from Griffiths and Ready in Mozambique. Griffiths and Ready stated that the failure of leprosy medication commonly happens because of the lack of support in the beginning of the treatment. The early phase of Leprosy treatment is the toughest part of the treatment due to the leprosy handicap and neighborhood's perspective. Therefore, the family support is critical to minimize those stressors (Griffiths and Ready, 2001).

Moreover, a study in Blora district, Central Java, by Khotimah, briefly reported that the patient with the lack of family support was six-time riskier to decline the medication. Family support in term of attention, help and other supports are pivotal to assist the patient to stay in line to the medication steps. In addition, Afifah stated that the role of patient's family is critical not only to control the patient to consume the medication regularly but also to accompany the patient to take the routine medication in the nearest public health service (Khotimah, 2014).

## Conclusion

The level of knowledge and educational degree was not related to drug compliance, but the family support has the correlation with drug compliance in Leprosy patients.

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## Factors Affecting Alterations of Gut Microbiota in Pregnancy

<sup>1\*</sup>Marisa, <sup>2</sup>Juwita

<sup>1</sup> Department of Nutrition, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Biochemistry, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: marisa@unsyiah.ac.id.

### Abstract

Pregnancy is a physiological condition in human's life cycle. There are an alterations in anatomy, physiology, and metabolism of nutrients in a pregnant women, which aims to provide an adequate substrate for the growing fetus and prepare mother for lactation. Beside metabolic changes, there is an alteration in gut microbiota. It has been known that gut microbiota plays an important role in various diseases and metabolic changes, including pregnancy. Metabolic alteration and several changes in inflammation marker in pregnancy which is similar with alteration in metabolic syndrome, is related with the alteration of gut microbiota in line with older gestational age. This metabolic changes is a state of physiological condition, but in the other hand has a potential to become pathological circumstances. Gut microbiota in human body is ten folds higher than human cells, and influenced by various factors like age, genetic, use of antibiotics, and diet as a major factor. However, a few current research on gut microbiota in pregnancy revealed that gut microbiota alteration in pregnancy related with others factor. The aims of this review is to summarize the recent scientific information about the factors influenced the gut microbiota alteration in pregnancy, in relation to the metabolic changes.

**Key words:** diet, gut microbiota, pregnancy, metabolic alteration.

### Introduction

The human body is a habitat for the growth of various microorganisms, one of them called the microbiota (Gilliland et al., 2012). Approximately there are  $10^{14}$  microbiota in human body, which is ten folds higher than the number human cell.  $10^7$  of them residing in human gut, and have an important role for human health, through the production of short chain fatty acids and produces the energy, the production of vitamin K, and ion absorption in the intestine (Conlon and Bird, 2014). In addition, the gut microbiota can also control the proliferation and differentiation of epithelial cells, stimulate the body's defense system, as well as protect the body against pathogen bacteria (Mikami et al., 2012).

Pregnancy is a physiological state and associated with the gut microbiota (Cani, 2009). Change of metabolism in pregnancy already strongly associated with intestinal microbiota composition, with a mechanism that is not fully understood yet. The "good" composition of gut microbiota guarantees the continuity of the normal pregnancy and avoid disturbance of metabolism in pregnancy, such as excessive weight gain during pregnancy, even gestational diabetes mellitus (Koren et al., 2012; Santacruz et al., 2010). The presence of gut microbiota in pregnancy is also worthy to note, because it can affects the gut microbiota composition of the newborn. The mechanism of microbiota transfer from maternal to her newborn can be explained through direct transfer via placenta during pregnancy, as well as contamination at the time of delivery (Mikami et al., 2012, 2009; Rigon et al., 2012; Thum et al., 2012).

### Gut Microbiota, Human Metabolism, and Pregnancy

The gut microbiota is dominated by five large phylum such as Firmicutes, Actinobacteria, Verrumicrobia and Proteobacteria (Gilliland et al., 2012). 98% of the gut microbiota is dominated by the phylum Firmicutes and Bacteroidetes. Ratio of Firmicutes and Bacteroidetes associated with the magnitude of the impact to the metabolic problem (Cheng et al., 2013). However, other types of bacteria still plays an important role, such as Bifidobacterium, one genus of Actinobacteria, account for only about 10% in a normal adult. Bifidobacterium plays a crucial role to maintain the integrity of the intestinal mucosa (Turroni et al., 2008).



The difficulty of doing research on humans, causing there is no definition of "normal" gut microbiota definition, or eubiosis (ILSE Europe, 2013). Generally, gut microbiota is affected by age, genetic factors (Clemente et al., 2012; Kovatcheva-datchary et al., 2013; Turnbaugh et al., 2009), methods of delivery (Makino et al., 2013), chronic inflammation in digestive tract (Prideaux et al., 2013), antibiotic consumption in a given period (Lozupone et al., 2012; Robinson and Young, 2010), and diet (Karen P Scott et al., 2013). Diet is a major factor that affects the intestinal microbiota composition. This can be seen from the alterations of the gut microbiota from baby to adult. Breastfeed infant has a higher number of Bifidobacterium than formula feed infant (Azad et al., 2014; Turroni et al., 2012). In adults, consumption of high-fat diet cause dysbiosis or a change in the composition and function of gut microbiota, reversing the ratio of Firmicutes against Bacteroidetes and correlated with the incidence of obesity and other metabolic diseases. Meanwhile, the consumption of high fiber diet returns the Firmicutes to Bacteroidetes, and increase the number of Bifidobacterium (De Filippo et al., 2010; Hildebrandt et al., 2009; Kabeerdoss et al., 2012; Wu et al., 2012).

High fat diet consumption tendency and an imbalance between energy intake and energy expenditure cause an obesity state. High fat diet, consistently, either in animals or humans, cause changes in the composition of the gut microbiota. This condition characterized by a ratio changes of Firmicutes to Bacteroidetes, which show an increasing number of Firmicutes (gram-negative bacteria) (Brinkworth et al., 2009; Hildebrandt et al., 2009; Y Lee, 2013). In addition, at the level of genus, there is a decrease in the number of Bifidobacterium and Lactobacillus which play role in gut mucosa defense mechanism. Also there is a decrease in the number *Eubacterium rectale*, butyric acid producing bacteria, involved in producing antiinflammatory mediators in digestive tract (Cani et al., 2012).

Gut microbiota dysbiosis in obesity due to the consumption of a high fat diet happened by several mechanisms. High fat diet damage a tight junction protein, called Zonula Occludens-IE-1 (ZO-1) and Occludin, involved in the defense mechanism of the gut. In addition, increase the expression of the endocannabinoid system and its receptors, thus causing an increase in intestinal permeability (Pachikian et al., 2010). The consumption of a high fat diet reduces the function of intestinal alkaline phosphatase (IAP) enzyme, which plays an important role in the detoxification of lipopolysaccharida (LPS) from the bacterial cell wall (Lallès, 2010; Malo et al., 2010). This conditions simultaneously induced metabolic endotoxemia and low grade systemic inflammation, and also decreased insulin sensitivity in liver, muscle, and adipose tissue, which ended with insulin resistance (Cani et al., 2012; Chassaing and Gewirtz, 2014)

Pregnancy causes numerous alterations in anatomy, physiology and metabolic. For example, alteration in digestive tract are include slowing gastric emptying time and increased intestinal transit time (Tan and Tan, 2013). Moreover, pregnancy also changes the macronutrient metabolism such as carbohydrate, protein, and fat, and also influenced by hormonal changes during pregnancy (Hadden and McLaughlin, 2009). Progesterone and estrogen increase during pregnancy and placenta is a major source of this hormone. In addition, the placenta also produces a number of peptide such as human chorionic gonadotropin (hCG) and human placental lactogen (HPL) (Abeysekera et al., 2016). The hormone works as an antagonist to hormone insulin, causing insulin resistance and thus decrease maternal insulin sensitivity. This led to increased lipolysis, hyperlipidemia, hipertriglyceridemia, emphasis the use of glucose by maternal cells and increase the amount of amino acids that are transferred to the fetus through the placenta (Johnson, 2010). Changes in the form and function of fat cells, include cell hypertrophy and increased insulin receptor, causing an increase in fat storage capacity on two last trimester of pregnancy (Hadden and McLaughlin, 2009). These changes resemble the state of the metabolic syndrome, however, aims to provide energy for the growth of the fetus and prepare mother to face period of lactation (Bazer et al., 2014; McIntyre et al., 2010).

### **Gut Microbiota Alterations in Pregnancy**

In addition to an anatomical and metabolic changes, there is also an alteration in gut microbiota. There are several research focussing on gut microbiota in pregnancy. Latest research, to our knowledge, revealed that there was no changes of gut microbiota composition over pregnancy. Gut microbiota composition also relatively stable in vagina, saliva, and tooth/gum (DiGiulio et al., 2015). In contrast to that, some previous studies showed that there was an alteration of gut microbiota during pregnancy. A research on 91 pregnant women, conclude that there are several changes in gut microbiota composition in pregnancy. The composition of gut microbiota in third trimester of pregnancy is differ from its composition in first trimester. Gut microbiota composition in first trimester is similar to other "normal" adult, but in the third trimester there is a decline in diversity and its composition is similar to gut microbiota in metabolic syndrome state., this state called dysbiosis. This two form is basically has some similarities: decreasing diversity, an increasing number of Proteobacteria, and a wide interindividual

variation (Collado et al., 2008). In contrast to the metabolic syndrome state, gut microbiota in third trimester of pregnancy also have an increasing number of Actinobacteria, and no significant difference in Bacteroides and Firmicutes ratio (Jost et al., 2014; Koren et al., 2012).

Other study also revealed that there is a changes in gut microbiota composition affected by term of pregnancy. This prospective study involved 18 overweight pregnant women and 20 normal-weight pregnant women (weight classification based on pre-pregnancy weight), until the third trimester of pregnancy. Gut microbiota composition affected by pre-pregnancy wieght, Bacteroides and Staphylococcus were higher in overweight group. This study also suggested that gut microbiota (Bacteroides) affects weight-gaining process during pregnancy. Bacteroides and Staphylococcus are related to low grade inflammation process and high rate of fat storage (Collado et al., 2008).

Pre-pregnancy weight and weight gain also influenced gut microbiota composition in pregnancy. Staphylococcus, *Escherichia coli*, Enterobacteriaceae were commonly found in overweight group than in normal-weight group. This study also revealed that some microbiota related to biochemistry markers. High number of Staphylococcus were related to high level of plasma colesterol, while high number of Bifidobacterium related to increased folic acid, ferritin, and transferrin levels, which is important for pregnancy state (Santacruz et al., 2010). The number of Bifidobacterium, some of "good" bacteria in the gut, were higher in normal-weight pre-pregnancy state and in normal weight gain over pregnancy group. This fact was caused by a stabilization effect on mucosal integrity and antiinflammation effect of Bifidobacterium in gut environment, prevented systemic low grade inflammation as mention above. (Collado et al., 2008; Santacruz et al., 2010)

The major factor affecting the gut microbiota composition is diet (Conlon and Bird, 2014; Karen P. Scott et al., 2013). One study revealed that alterations of gut microbiota in pregnancy is not related to diet, but is caused by hormonal and immunological changes. (Koren et al., 2012). In other studies, diet (especially selective dietary components) showed as one of factors which caused gut microbiota dysbiosis in pregnancy (Mokkala et al., 2016; Santacruz et al., 2010). Increased number of total gut microbiota inversely correlated with reduced total energy, animal protein, total cholesterol and poly unsaturated fatty acids intake (Santacruz et al., 2010). Increasing diversity of gut microbiota were inversely correlated with the serum zonulin concentration, a marker for intestinal permeability. Low zonulin concentration is related to higher fiber and PUFA intake (Mokkala et al., 2016). In agreement to that, another animals study revealed that oligosaccharides suplementaion to the high fat/ fructose diet improved gut microbiota dysbiosis, increased Bifidobacterium and *Clostridium sp.* count, and ameliorated inflammation and metabolic profile (Paul et al., 2016).

Gut microbiota have changed in early onset of pregnancy dependently upon maternal peri-conceptional and pregnancy diet. In this study, two group of mice were fed by either a control diet (17% of fat, 29% of protein, and 54% of carbohydrate) or a high fat diet (45% fat, 20% protein, and 35% carbohydrate) for 6 weeks before mating. This study revealed that there was a significant increasing number of Akkermansia and Bifidobacterium in high fat diet. This maternal high fat diet was also related to increasing rate of maternal metabolism of fatty acids, cysteine, methionine, ketone, and vitamins. This study also proposed that this metabolic alterations linked to gut microbiota alterations, but the mechanism is still not well understood (Gohir et al., 2015).

## Conclusions

Alterations of gut microbiota in pregnancy cause metabolic changes which important to the growth of fetus and lactation periode. Gut microbiota alterations influenced by some factors such as hormonal and immunological changes, gestational age, and also maternal diet prior to and during pregnancy. However, those factors still need further research especially in human.

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## Another Way to Trace Microbes in Human Tissue Section

<sup>1</sup>\*Wilda Mahdani

<sup>1</sup>Department of Microbiology, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [wildamahdani@unsyiah.ac.id](mailto:wildamahdani@unsyiah.ac.id)

### Abstract

Infections can affect all parts of the human body. Culture method is the best way to identify the etiologic agents of infectious diseases, as well its antimicrobial sensitivity testing. Unfortunately, some of the agents causing infections are non-cultivable, while some grow slowly or fastidious. Microorganisms such as *Loboa lobi*, *Rhinosporidium seeberi*, *Pneumocystis jiroveci*, *Treponema pallidum* and *Mycobacterium leprae* still can not be grown in microbiology culture media. Identification of the infectious agents by microscopic visualization remains a major diagnostic tool, beside the development of diagnostic techniques in immunohistochemistry and molecular science. Histopathology technique is very useful for diagnosis when cultures are not feasible. This method also allows us to assess tissue reactions such as infiltration of inflammatory cells or the appearance of typical inclusion bodies. The successful identification of infectious agents requires proper characterization of the inflammatory response, knowledge of associated pathogens, and good management of patient specimens. Clinical microbiologists, pathologists and clinicians need to communicate effectively, so that the correct diagnosis of infectious disease cases can be made efficiently. This mini review discussed about histopathology technique as an excellent microbiology diagnostic tool. Especially whenever culture method is impossible to be done.

**Keywords:** Tissue infection, Histopathology techniques, Microscopic visualization

### Introduction

A number of techniques have been developed to reveal the infectious microorganisms in human body by culture and staining histology specimens (Brewer & Weed, 1976). Culturing harmful microorganisms such as *Histoplasma capsulatum* should be performed under biosafety level 3 (BSL-3) conditions (Frickmann *et al.*, 2015). These microorganisms can cause invasive infections even in the immunocompetent person. Transmissions to laboratory workers had been reported (Sewell, 1995; in Frickmann *et al.*, 2015).

Histopathological evaluation of the tissue excision specimens has a very important role in infectious diseases management (Wilson & Winn, 2008), especially for the diagnosis of infectious etiology that is difficult or can not be cultured. This technique is particularly important for the evaluation of chronic lesions which contained only a small amount of viable microorganisms. There are so many clinical features of lesions that caused by microbes. Precise identification of the causative microorganisms and tissue reactions will lead clinician on an appropriate therapy (Satyanarayana & Kalghatgi, 2011).

### Specimen Management

Well managed specimens can provide important diagnostic informations, otherwise can be missed (Brewer & Weed, 1976). Samples selection and collection are very important. We should decide to take only the representative area or the extensive one. For example, caseous granulomas are the clue for *Mycobacterium tuberculosis* infection.

Before collection it is necessary to perform decontamination procedures to eliminate colonizations. Tissue damage should be prevented such as by avoid any compression. Tissue cutting can be done using sterile scissors or scalpel (Gupta *et al.*, 2009). Once the specimens have been collected, preserved tissue samples by passing it through a fixative. Tissue sample were then treated with a solvent to dissolve fatty materials and cleared. Cleared tissue sample is infiltrated with wax, which replaces the

solvent. Samples then might be sliced for microscopic slides (H. Lee Moffitt Cancer Center and Research Institute, 2014).



**Figure 1.** Specimen sampling from a chronic wound. Precise selection and collection determine the best diagnostic result.  
(Source: private archive).

## **Tissue Processing**

### **Fixation**

Fixation aims to preserve tissue specimens from damage due to autolysis, keeping the original structure. Fixative volume should be 15 to 20 times higher than the specimen's. Fixation time depends on the volume of specimens. Fixation speed is approximately 1mm/ hour at room temperature (IRIC, 2016). Fixation and sectioning still have been not changed much despite many advances in immunology and molecular techniques. Before 1970 mercuric chloride was the fixative frequently used in the laboratory, it has perfect fixation quality. However, these compound is harmful to the environment and laboratory staff, so its use was banned and replaced by others (Suvarna *et al.*, 2013). Formalin has been a choice for decades. Nowadays, the term formalin-fixed and paraffin embedded (FFPE) sample is very familiar (Kap, *et al.*, 2011). Fixation is a key step in diagnostic pathology. Commercial formalin gas available in a 37% concentrated solution. It is then stabilized with ethanol. A 10% dilution of concentrated formalin (in water or buffer with a final concentration approximately 4% formaldehyde) named 10% formalin (Kiernan, 2008). The main reasons for formaldehyde popularity are: low cost, simple preparation procedures, old traditions and international use (Zanini *et al.*, 2015).

### **Paraffin Embedding**

Paraffin embedded tissue cassette can be stored for decades, reach 30 years (Frickmann *et al.*, 2015). Tissue hardening technique allows the thin slicing (4-5 $\mu$ m). Formalin 10% fixed tissue placed in a histology cassette. Paraffin infiltration procedure consists of three steps; dehydration, clearing and wax infiltration. Dehydration done by using three alcohol baths with an increased concentration. First alcohol 70% bath, second alcohol 85% bath, and third alcohol 90% bath, three minutes each. This prevents tissue distortion and hardening. Water was removed with three alcohol absolute baths. Three toluene baths allow it to replace the alcohol trapped in the tissue and dissolved with the wax. Hot wax bath (44°C - 60°C) will allow wax infiltration (IRIC, 2016).



**Figure 2.** Paraffin embedding preparation  
(Source: private archive).

### **Slicing**

The surface of paraffin embedded tissue is needed to be trimmed prior to sectioning. Tissue sections are 4  $\mu\text{m}$  thick most of the time. Wax ribbons are transferred onto a warm water bath (43°C - 45°C) and spread on a microscope glass slide before drying at least for one hour at 45°C. The glass slides need to be processed with the Aminopropyltriethoxysilane (AES) reagent so preparations can be fixed properly and not detached when staining procedures consist of lots washing steps. AES solution of 2% (245 ml acetone, 5 ml Aminopropyltriethoxysilane), distilled water in two staining jar were prepared. Slides are laid out in racks, put in the AES solution for 2.5 minutes. Slides then rinsed by dipping in the first and second distilled water. Slides are dried with a tissue paper, laid in a dryer box slide. Slides are put in an incubator at a temperature of 37°C for 24 hours (Pohan, 2001).



**Figure 3.** Microtome slicing  
(Source: private archive).

### **Staining**

It is very important to do deparaffinization before staining. This is the opposite of dehydration procedure. The goal is to eliminate wax from tissue sections, by using two baths of toluene (100% -95%), three alcohol baths, first alcohol 100% bath, followed by alcohol 95% and alcohol 80% bath, 3 minutes each. Most routine staining is Hematoxylin-eosin (HE). This staining technique gives an excellent tissue reactions overview. While, there are a lot of special staining techniques. Twort modification for tissue Gram staining, Periodic Acid Schiff (PAS) and Grocott Gomori Methenamine Silver (GMS) to find fungi, Ziehl Neelsen for *Mycobacterium*, modified Wade Fite for *Mycobacterium leprae* and *Nocardia*, Warthin Starry to find Spirochetes and donovan bodies, and many more (Pohan, 2001). Some of these staining can be combined in order to obtain a better contrast, such as GMS-HE and PAS-McManus. Cover slipping or mounting allows slides to be stored in a longer period. Special staining techniques selections are done based on clinical suspicion.

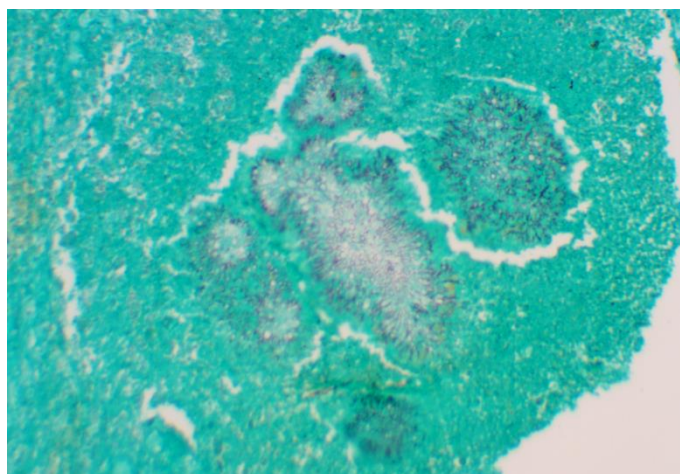




**Figure 4.** GMS staining, one of special staining techniques to detect fungal infections.  
(Source: private archive).

### Microscopic Visualization

Microscopic examination of tissue sections reveals whether microbes are present only in the dead tissue or have invaded the viable tissue. True pathogenicity is actually represented by invasion (Church *et al.*, 2006). Inflammation is the hallmark of most infectious diseases (Gupta *et al.*, 2009). It may also be associated with non-infectious disorders that need to be treated with corticosteroids. The inappropriate use of corticosteroids may exacerbate infection. Therefore, it is critical for the microbiologist to be able to differentiate between inflammatory conditions either caused by infectious or non-infectious etiologies. Usually one type of pathogen elicits one particular type of response (Gupta *et al.*, 2009). Polymorphs infiltration predominates in bacterial infections, whereas in viral infections it is replaced by lymphocytic infiltration (Woods *et al.*, 1996, Chandler, 1997; in Gupta *et al.*, 2009).



**Figure 4.** Microscopic visualization of infectious agent in tissue section. Fungal structures seen in a GMS stained specimen. Magnification 400x.  
(Source: private archive).

### Conclusions

Microbiology diagnostic aims to find and identify the etiologic agents of infectious diseases. Culture method with identification and antimicrobial susceptibility testing is a diagnostic gold standard. However, this procedure is not always possible. Certain microorganisms are nonculturable, some need longer time to grow in culture media. Histopathological evaluation is the major diagnostic tool for these kinds of specimens. Selecting and collecting high quality specimen as well as fixation, embedding, microtomy, staining and coverslipping will determine the final outcome (Gonzales *et al.*, 2010). Microscopic evaluation can provide rapid diagnosis, since it allows direct assessment of pathogen's specific morphology (Gupta *et al.*, 2009). Important information might be missed if careful microscopic visualization of the tissue sample is not carried out. Excellent histology image collection also have a positive influence on education and research (Gonzales *et al.*, 2010).

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## Antimicrobial Susceptibility Pattern of Gram Negative Bacteria from Urine Samples in the Primary Hospital Care of Banda Aceh, Indonesia

<sup>1</sup>\*Masra Lena Siregar, <sup>2</sup>Hijra Novia Suardi

<sup>1</sup> Department of Internal Medicine, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

<sup>2</sup> Department of Pharmacology, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [masra\\_lenadr@yahoo.com](mailto:masra_lenadr@yahoo.com)

### Abstract

The most common etiology of urinary tract infection (UTI) are Gram negative bacteria. Currently, antibiotic resistance against this bacterial have increased sharply, and resistance patterns may differ based on place and time so that every hospital should make a map of germs and sensitivity patterns of its own on a regular basis to guide antibiotic therapy empirically in the hospital. The purpose of this study was to determine of the antimicrobial susceptibility pattern of Gram negative bacteria against antibiotics collected from urine samples. A descriptive study of retrospectively collected data from January to December 2014 at Zainoel Abidin Centre Hospital, Banda Aceh. Sampling technique used total sampling was done by filling in the form of this study. Out of the total 170 urine samples collected in this study, the most common have been the Gram negative which accounts for 93 (54.7%), respectively: *Klebsiella pneumonia* 40 (43.01%) followed *Pseudomonas aeruginosa* 21 (22.58%) and *Escherichia coli* 32 (34.4%). The susceptibility pattern of *Klebsiella pneumonia* showed that 92.5% of the bacterial were sensitive to meropenem, 70% to fosmicin and 57.5% to amikacin. In case of *E. coli*, 90.6% bacterial were sensitive to meropenem, 78.1% to fosmicin and 62.5% to amikacin though *Pseudomonas aeruginosa* that 71.4% bacterial were sensitive to meropenem and 42.9% to levofloxacin. The pathogenic microorganisms are most resistant against penicillin, cephalosporins and quinolone. In conclusion, the majority antimicrobial of the Gram negative organisms are sensitive to carbapenem while the highest drug resistance against penicillin, cephalosporins and quinolone.

**Key words** : Antimicrobial, Gram negative bacteria, Susceptibility pattern

### Introduction

Infectious diseases are still the serious problem and get ranked number one in Indonesia, as in other developing countries. Urinary tract infection (UTI) is the second common infection after upper respiratory tract infections in the community (Smith, 2004). Urinary tract infection are also the most common nosocomially acquired infection, accounting for 40–60% of infections in the hospital setting (Naber, 2004).

Since antimicrobial was discovered in the 1940s, the drug has been widely used to reduce the number of death caused by infectious disease (Inglish, 2003). However, the use of antimicrobial is not necessarily based on culture causative agents to the antimicrobial especially in countries with the level of supervision is not strict. The situation of antibiotics use in hospital, generally prescribing with a new generation that can be prescribed by any physician, all antibiotics can be used without restriction and should not be used without limitation (RSCM, 2007). This situation tends to increase of use antimicrobials is not rational, that ultimately resulted ineffective treatment, increase funding for patients and most importantly to cause resistance to the microba (Soewondo, 2002).

Among all the problems of bacterial resistance, Gram negative pathogen is the very alerting because these pathogens become resistant to almost all medications that commonly use for treatment. Infection of Gram negative are the most serious occurred associated with health care and the most common pathogens that cause this condition is *Enterobacter*, *Pseudomonas aeruginosa* and *Acinetobacter*. Treatment against Gram negative resistance is currently a challenge in many hospitals (CDC, 2013).

A group of bacteria that most commonly cause urinary tract infection (UTI) is a Gram negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella spp*, and *Enterobacter spp*. The data obtained from laboratory and clinical microbiology, University of Indonesia shows the most common bacteria were *Escherichia coli* (19%) and *Klebsiella pneumoniae* (13%) (Samirah *et al.*, 2006).

Antibiotic resistance against bacterial groups have increased sharply, and resistance patterns may differ based on place and time so as every hospitals should make a map of germs and sensitivity patterns of its own on a regular basis to guide antibiotic therapy empirically in the hospital. Thereby, the aim of the present study was to determine antimicrobial susceptibility pattern of Gram negative bacteria against antibiotics from urine specimen of outpatient and inpatient at Zainoel Abidin Centre Hospital, Banda Aceh.

## Materials and Methods

A descriptive study of observational with cross-sectional design which take on secondary data from Clinical Laboratory Installation at Zainoel Abidin Centre Hospital, Banda Aceh that is a reception room of various specimens for culture and antibiotic resistance were obtained from various inpatient. Study population consisted of the result data from culture examination of urine samples and antibiotic sensitivity testing that was done using total sampling indoor patients both female and male from January to December 2014. Samples were recording from culture examination of urine specimen that has a complete medical record. The medical records were obtained recording data of characteristic patient (name, age, sex), specimen source, causative organism, and type of antibiotic sensitivity and resistance.

## Results and Discussion

A total of 170 urine samples from inpatients at Zainoel Abidin Hospital, Banda Aceh, 96 samples (56.5%) are Gram negative, 14 samples (8.2%) are Gram positive and the others samples for 53 (31.2%) are fungi (Table 1). Table 1 shows the majority specimens that Gram negative bacteria compared to other infectious agent are *Klebsiella pneumonia* (23.5%), *Escherichia coli* (18.8%), *Pseudomonas aeruginosa* (12.4%) and *Acinetobacter baumannii* (1.8%), respectively. This study in accordance with the study of other researcher in Pekanbaru (Endriani R *et al.*, 2010) shows that Gram negative bacteria is most common causative agent infectious, respectively *Escherichia coli* (28%), *Klebsiella sp* (26%) and *Pseudomonas* (18%). In another recent study in Surabaya (Sutandhio *et al.*, 2015) reported the most common organism are *Escherichia coli* (59.6%) followed by *Klebsiella pneumonia* (18.1%) and *Enterobacter spp* (10%).

One of study abroad in Iran shows similar results. The most common causative organism in urine specimen is Gram negative bacteria although in consecutive was different that *Escherichia coli* (59.5%) followed by *Klebsiella spp* (11.65%), *Enterobacter* (9.8%) and *Pseudomonas* (7.2%) (Amin *et al.*, 2009). This correlates was reported other study (Shaifali *et al.*, 2012) in which 33.1% are *Escherichia coli* and 7.9% are *Klebsiella pneumonia*. Hence, based on data shows among the bacteria causing UTI are Gram negative, this condition due to Gram positive is the common nosocomial infections in the before use of antibiotic in 1940, but after antibiotics became widely used, the causative agent of Gram positive is rare (Di Piro, 1997).

**Table 1.** Pattern of Bacteria

Causative Organism	Total number of cases	Frequency(%)
Gram negative		
1. <i>Pseudomonas aeruginosa</i>	21	12.4
2. <i>Klebsiella pneumonia</i>	40	23.5
3. <i>Escherichia coli</i>	32	18.8
4. <i>Acinetobacter baumannii</i>	3	1.8
Total	96	56.5
Gram Positive		
1. <i>Staphylococcus aureus</i>	-	-
2. <i>Streptococcus sp</i>	14	8.2
Total	14	8.2
Fungi	53	31.2
Others	7	4.1
Total	170	100

On performing antimicrobial susceptibility testing of the *Klebsiella pneumonia* shows out of total 40 specimen, meropenem is found to be the most sensitive against *Klebsiella pneumonia* (92.5%) followed by fosmicin (70%) and amikacin (57.5%). On the other hand, the highest resistance against commonly ampicillin-sulbactam (92.5%) and ciprofloxacin (87.5%). Study in India (Manikandan and Amsath, 2013), amikacin and imipenem were found to be 86.1% effective against strains of *Klebsiella pneumonia* followed by gentamicin and tobramycin (80%), ofloxacin (79.2%) and ciprofloxacin (76.4%). However, this situation is quite different with the antimicrobial sensitivity of quinolone (ciprofloxacin). In the past was reported that the sensitivity of organism Gram negative like *Klebsiella* which most commonly used as antibiotic were quinolone, as far as the overuse of these antibiotic results antibiotic resistance. Resistance to antibiotics of quinolone has become an increasing problem in some European countries. (Schaeffer & Schaeffer, 2007)

**Table 2.** Antibiotic Sensitivity and Resistant Pattern of *Klebsiella pneumonia*

Antibiotic	Sensitive No (%)	Resistant No (%)
Penicillin		
• Ampicillin Sulbactam	3 (7.5%)	37 (92.5%)
• Amoksi-Clavulanat	7 (17.5%)	33 (82.5%)
Cephalosporins		
• Ceftriaxone	6 (15%)	34 (85%)
• Ceftazidime	11 (27.5%)	29 (72.5%)
Aminoglycosides		
• Amikasin	23 (57.5%)	17 (42.5%)
• Gentamisin	8 (20%)	32 (80%)
Quinolone		
• Ciprofloxacin	5 (12.5%)	35 (87.5%)
• Levofloxacin	13 (32.5%)	27 (67.5%)
Fosmicin	28 (70%)	12 (30%)
Meropenem	37 (92.5%)	3 (7.5%)

The antimicrobial susceptibility pattern of *Escherichia coli* shown in table 3 that 32 specimens are sensitive to meropenem (90.6%), 78.1% to fosmicin and 62.5% to amikasin whereas this organism have high level of resistance to penicillins, cephalosporin, gentamicin, and quinolone. This study show similarities with the study in India that *Escherichia coli* were resistance to many antibiotics especially penicillin, cephalosporin and quinolones. Overall, the isolates seems sensitive to amikacin, piperacilin-tazobactam, nitrofurantoin and imipenem that members of the carbapanem class of antibiotics (Niranjan & Malini, 2013).

**Table 3.** Antibiotic Sensitivity and Resistant Pattern of *Escherichia coli*

Antibiotic	Sensitive No (%)	Resistant No (%)
Penicillin		
• Ampicillin-Sulbactam	2 (6.3%)	30 (93.7%)
• Amoksi-Clavulanat	8 (25%)	24 (75%)
Cephalosporins		
• Ceftriaxone	5 (15.6%)	27 (84.4%)
• Ceftazidime	10 (31.3%)	22 (68.7%)
Aminoglycosides		
• Amikasin	20 (62.5%)	12 (37.5%)
• Gentamisin	9 (28.1%)	23 (71.9%)
Quinolone		
• Ciprofloxacin	4 (12.5%)	28 (87.5%)
• Levofloxacin	16 (50%)	16 (50%)
Fosmicin	25 (78.1%)	7 (21.9%)
Meropenem	29 (90.6%)	3 (9.4%)

Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* as shown in table 4 from 21 specimens show high level susceptible to meropenem whereas resistance of other classes of antibiotics that have high levels of resistance rates (>60%) especially in ceftazidime (90.5%), penicillin, aminoglycosides and ciprofloxacin. This finding agree with other recent Indian reports which have indicated that high level of resistance of *Pseudomonas aeruginosa* to antibiotic monotherapy like penicillin, cephalosporins and fluoroquinolones meanwhile the use in combination (piperacillin-tazobactam, cefotaxime-sulbactam, cefoperazone-sulbactam) demonstrated significantly higher

antibacterial activity, even though the highly sensitive remains shown to the carbapenem group (Javiya VA *et al.*, 2008).

**Table 4.** Antibiotic Sensitivity and Resistant Pattern of *Pseudomonas aeruginosa*

Antibiotic	Sensitive No (%)	Resistant No (%)
Penicillin		
• Ampicillin Sulbactam	4 (19%)	17 (81%)
• Amoksi-Clavulanat	8 (38%)	13 (62%)
Cephalosporins		
• Ceftriaxone	6 (28.6%)	15 (71.4%)
• Ceftazidime	2 (9.5%)	19 (90.5%)
Aminoglycosides		
• Amikasin	8 (38%)	13 (62%)
• Gentamisin	6 (28.6%)	15 (71.4%)
Quinolone		
• Ciprofloxacin	6 (28.6%)	15 (71.4%)
• Levofloxacin	9 (42.9%)	12 (57.1%)
Fosmicin	5 (23.8%)	16 (76.2%)
Meropenem	15 (71.4%)	6 (28.6%)

Gram negative bacteria are more easily transferred the genes of disease resistance to antibacterials. These results are the pattern of resistance to antibiotics, so the pattern of resistance is more dynamic than Gram positive bacteria. It will very difficult to treat these bacteria especially while bacteria have formed a formation that will inhibit the penetration of antibiotics and even which neutralize antibiotics at the place, particularly in the urinary tract (Sjarrurachman *et al.*, 2004).

Generally, among of three types of Gram negative bacteria are resistance against to class of  $\beta$ -lactam *antibiotics* such as penicillin and cephalosporin, especially the early generation so these drugs not recommended its use to overcome the Gram-negative bacteria. The most important mechanism in causing bacterial resistance against these groups is the formation of enzyme *beta-laktamase* by Gram negative bacteria, these enzymes attack on the amide of the  $\beta$ -lactam *ring release the inactive antibiotic* (CDC, 2013).

Apart of resistance to penicillin and cephalosporin, this study also show reduced of the sensitivity in quinolone class. This is due to high use of quinolone in community result in rapid resistance. Aminoglycosides is one of choice the treatment for Gram negative bacteria, but clinicians rarely use of this drugs because of the risk of side effect and increased resistance to this antibiotic (CDC, 2013).

Currently, the class of carbapenem have been used as the "last-line" treatment for infections that producing of extended spectrum  $\beta$ -lactamase (ESBL). In other condition, widely used of this class become expanding rapidly pose a threat to the resistance (CDC, 2013). However, used of these drugs should be determined to avoid prescribing irresponsible that have result *emergence of carbapenem resistance* (Mayers DL *et al.*, 2009).

Hence, based on the data of present study will assist in the selection of antibiotics empirically to patients with UTI especially those caused by Gram negative bacteria in the primary hospital care areas in Aceh, although should surveillance study on antibiotic resistance to a variety of organisms still being done periodically and become the policy of the use of antibiotics in the hospitals.

## Conclusions

According to finding, the most antimicrobial susceptibility pattern are meropenem (*carbapenem class*) whereas the highest drug resistance against penicillin, cephalosporins and quinolone.

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## Riboflavin Deficiency: What Do We Really Know?

<sup>1</sup>\*Juwita, <sup>2</sup>Marisa

<sup>1</sup>Department of Biochemistry, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Department of Nutrition, Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [juwita.attaya@gmail.com](mailto:juwita.attaya@gmail.com)

### Abstract

Riboflavin is a vitamin B namely vitamin B<sub>2</sub> is a watersoluble vitamin and stable to the high temperatures condition. These vitamin has a chemical structure of an isoalloxazine ring, bound to ribitol side chains. Source of riboflavin can be derived from vegetable or animal such as milk, eggs, and dark green vegetables. The active form of riboflavin is FAD (Flavin Adenine Dinucleotide) and FMN (Flavin Mononucleotide), serves as a prosthetic group in a variety of enzymes, in particular as a catalyst for oxidation-reduction reactions. Riboflavin through its active form which is FAD and FMN, have an important role in energy production in the form of ATP and the metabolism of carbohydrates, fats, protein, amino acid synthesis, as well as the activation of other vitamins. Deficiency of this vitamin can cause a variety disorders. This review aims to discuss the riboflavin deficiency and its effects, in particular against tampering hyperhomocysteinemia (a risk factor for cardiovascular disease), migraine, anemia, and cataracts.

**Key Words:** Riboflavin, FAD, FMN, deficiency, hyperhomocysteinemia

### Introduction

Riboflavin (aka vitamin B<sub>2</sub>) is a watersoluble vitamin and has the chemical name 7,8-dimethyl-10-ribityl-isoalloxazine (Powers, 2003). The chemical structure is an isoalloxazine ring bound to a ribitol side chain. The form of riboflavin which most common is FAD (flavin adenine dinucleotide), and then FMN (flavin mononucleotide) (Belinda, 2014). These forms of flavin (FAD and FMN) can bind covalently or non-covalently with the enzyme. Riboflavin is relatively stable to heat, but can be quickly degraded through exposure of light (Powers, 2003). The chemical structure of riboflavin and its flavin (FMN and FAD) can be seen in figure 1 below.

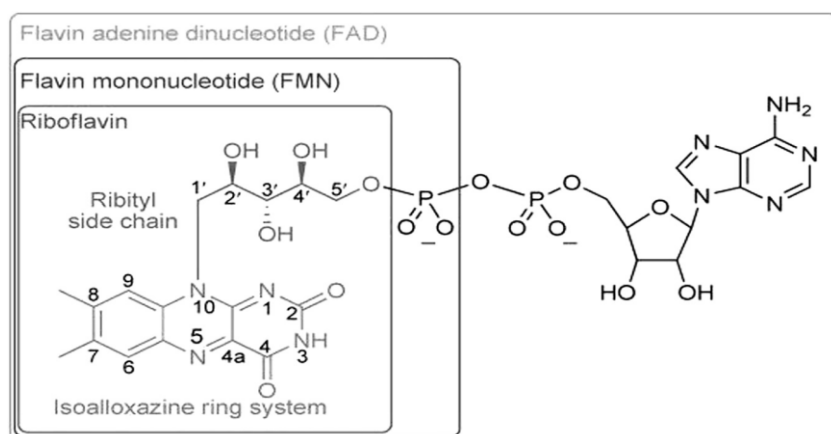


Figure 1. Chemical Structure of Riboflavin, FMN, and FAD (Mazzotta *et al.*, 2014).

### Source of Riboflavin

Riboflavin can be obtained from various sources of food, such as milk and its dairy products, cereals, meat, fish, fruits, and vegetables (especially dark green vegetables that contain high concentrations of riboflavin). Breakfast with cereal and milk is very good to maintain adequate intake of riboflavin (Powers, 2003). Mediterranean Diets, which are characterized by high consumption of fruits, vegetables, and



cereals, but low consumption of simplex carbohydrates, olive oil, and red wine, are associated with increased levels of vitamins (including vitamin B<sub>2</sub>) and minerals in the body (Kennedy, 2015).

### Role and Mechanism of Riboflavin

Riboflavin plays an important role in the body's metabolism. It is a precursor to the formation of FMN and FAD molecules, the biologically active forms of riboflavin. These flavins act as coenzymes which are important for the activity of enzymes involved in energy metabolism (Agostoni *et al.*, 2013; Dai and Koh, 2015). Metabolic reactions that produce energy include oxidation-reduction reaction, electron transfer chain, amino acid oxidation, fatty acid oxidation, and the citric acid cycle (Agostoni *et al.*, 2013; Anshoori and Saedisomeolia, 2014; Kennedy, 2015).

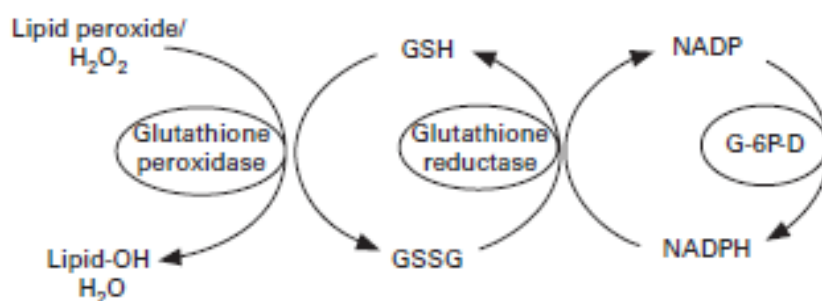
Riboflavin can work together with other B vitamins such as B<sub>6</sub>, B<sub>9</sub>, and B<sub>12</sub> (Belinda, 2014). Coenzyme FMN and FAD have a role in synthesis and conversion of other vitamins such as niacin, folate, and vitamin B<sub>6</sub>. These compounds also have a role in protein synthesis of hemoglobin, synthesis of nitric oxide, oxidation of xanthine, P450 enzymes, and fatty acid metabolism (Ma *et al.*, 2008; Belinda, 2014). Some enzymes which are requiring flavin can be seen in Table 1 below.

**Table 1.** Enzymes with Flavin as Coenzyme

Enzyme	Coenzyme	Function
Dihydrolipoyl dehydrogenase	FAD	Energy metabolism
Fatty acyl-CoA dehydrogenase	FAD	Fatty acid oxidation
Succinate dehydrogenase	FAD	Krebs cycle
NADH dehydrogenase	FMN	Respiratory chain
Xanthine dehydrogenase	FAD	Purine catabolism
Glutathione reductase	FAD	Reduction GSSG to GSH
Methylene-tetrahydrofolate reductase	FAD	5-Methyl-ethyl- tetrahydrofolate
Pyridoxine phosphate oxidase	FMN	Vitamin B6 metabolism
Monoamine oxidase	FAD	Neurotransmitters metabolism

Source: Thorne Research (2008)

Riboflavin also have antioxidant properties. Riboflavin may increase endogenous antioxidant through its role as a coenzyme in glutathione redox cycle. Glutathione reductase enzymes needs FAD as coenzyme and NADPH to convert the oxidized glutathione (GSSG) to the reduced glutathione (GSH). Hydrogen ion from NADPH, transport by FAD to GSSG, so that GSSG could be converted to GSH. GSH is an endogenous antioxidant which can inactivate free radicals such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). GSH role as an antioxidant is mediated by the glutathione peroxidase enzyme (GPx). GPx transport hydrogen ion from GSH to H<sub>2</sub>O<sub>2</sub>, in order to convert H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O (Anshoori and Saedisomeolia, 2014). Therefore collaboration of glutathione reductase, FAD, NADPH, GSH, and glutathione peroxidase can reduce free radicals level in the body, as seen in Figure 2 below.

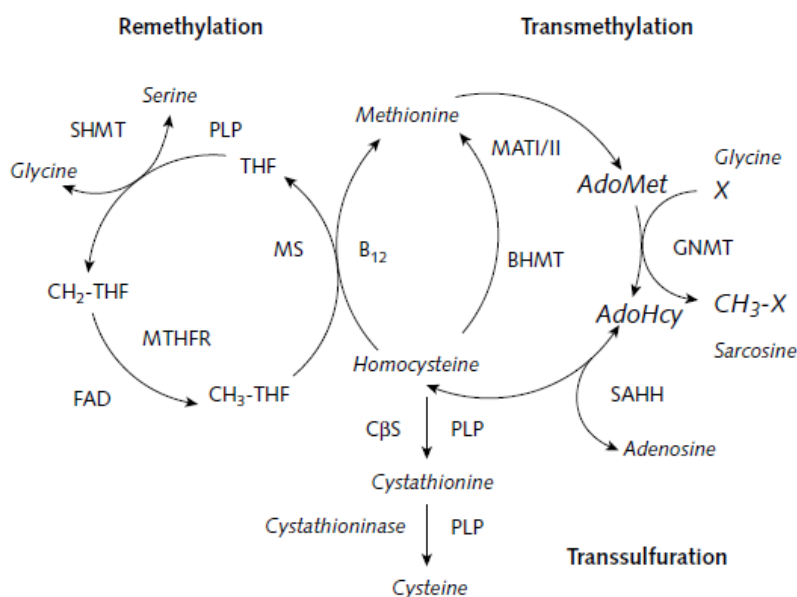


**Figure 2.** Collaboration of Glutathione Reductase, FAD, NADPH, GSH, and Glutathione Peroxidase to Inactivate Free Radicals (Anshoori and Saedisomeolia, 2014).

Coenzyme flavin has a role in the cycle of folate and methionine. In the folate cycle, flavin acts as a coenzyme for the methyltetrahydrofolate reductase (MTHFR), while in the methionine cycle flavin works together with methionine synthase reductase (MTRR) (Kennedy, 2015). Both MTHFR and MTRR enzymes play an important role in homocysteine metabolism (García-Minguillán *et al.*, 2014). In the folate cycle,



along with FAD, MTHFR enzyme catalyzes the conversion of 5,10-dimethyltetrahydrofolate (CH<sub>2</sub>-THF) to 5-methyltetrahydrofolate (CH<sub>3</sub>-THF) (Shane, 2008; Kennedy, 2015). This reaction provides a methyl group, which is important in remethylation process of homocysteine to form methionine (García-Minguillán *et al.*, 2014; Kennedy, 2015), as can be seen in Figure 3 below.



**Figure 3.** FAD Role in Folate and Methionine Cycle (Shane, 2008).

### Riboflavin Intake

Riboflavin intake is very important for all ages, especially infants and children, to help the body produces energy (Agostoni *et al.*, 2013). Recommended Daily Intake (RDI) for adult men and women is 1,3 and 1,1 mg/day, respectively (Institute of Medicine, 1998).

**Table 2.** Riboflavin recommended daily intake (RDI)

Criteria	Age (Year)	Intake Recommendation
Children	1-3	0,5 mg/day
	4-8	0,6 mg/ day
Boys	9-13	0,9 mg/ day
	14-18	1,3 mg/ day
Girls	9-13	0,9 mg/ day
	14-18	1,0 mg/ day
Men	19-70	1,3 mg/ day
Women	19-70	1,1 mg/ day
Elderly Men	>70	1,3 mg/ day
Elderly Women	>70	1,1 mg/ day
Pregnancy	14-50	1,4 mg/ day
Lactation	14-50	1,6 mg/ day

Source: Institute of Medicine (1998)

### Riboflavin Deficiency

Deficiency can result from inadequate intake or due to malabsorption (Kennedy, 2015). In Cambodia, Whitfield *et al* (2015) found the riboflavin and thiamin deficiencies in women of childbearing age in both rural and urban areas (Whitfield *et al.*, 2015). Although riboflavin plays an important role in metabolism, and riboflavin deficiency may occur in many countries, but this deficiency is not lethal because its efficient used in the body (Murray *et al.*, 2009).

Clinical signs and symptoms appear after inadequate intake of riboflavin about 3-8 months (Thorne Research, 2008). Riboflavin deficiency will affect vision, and cause symptoms such as weaknesses, pain,

itching or burning eyes, or cheilosis. Moreover, it can cause cancer, nerve degeneration, peripheral neuropathy, dermatitis, increased risk of cardiovascular disease, and anemia (Belinda, 2014; Kennedy, 2015). If deficiency affects the brain, there will be symptoms of personality changes, fatigue, and brain dysfunction (Kennedy, 2015). Yazdanpanah *et al.* (2008) found that low intake of riboflavin is associated with high risk of fractures in postmenopausal women. Some disorders caused by riboflavin deficiency include hyperhomocysteinemia, cataracts, anemia, and migraine.

### **Hyperhomocysteinemia**

Amino acid homocysteine may be toxic if the levels are excessive in the body (Kennedy, 2015). Level of homocysteine is influenced by genetic and nutrition factors. High level of homocysteine in circulation is associated with an increased risk of cardiovascular disease (Ganji and Kafai, 2004; McNulty *et al.*, 2006). High level of homocysteine is also associated with lower level of riboflavin in individuals with polymorphisms of MTHFR enzyme has reduced enzyme activity (García-Minguillán *et al.*, 2014). Riboflavin supplementation to individuals with MTHFR polymorphisms results in lower homocysteine levels significantly. One of efforts to lower homocysteine level is riboflavin and folic acid fortification in food intake, so the risk of heart disease and stroke will decline (McNulty *et al.*, 2006). Ganji and Kafai (2004) found that intake of milk, yoghurt, cereals, and vegetables (all of which rich in riboflavin and folic acid) can significantly reduce the level of homocysteine in circulation.

### **Cataracts**

Cataract is one of the eye-lens disorders that mainly caused by oxidative stress process (Chiu and Taylor, 2007). Riboflavin has antioxidant effect. FAD as an active form of riboflavin, works as a coenzyme for glutathione reductase. This enzyme is FAD-dependent, which catalyzes the conversion of GSSG into GSH (Anshoori and Saedisomeolia, 2014). GSH is an important endogenous antioxidant, and works to prevent damage to lens of the eye due to ROS. To perform its function as an antioxidant, GSH must be converted from GSSG continuously, and this process requires FAD (Mazzotta *et al.*, 2014).

Riboflavin deficiency is associated with the incidence of cataracts due to inadequate coenzyme FAD required for the activity of the glutathione reductase enzyme (Mazzotta *et al.*, 2014). The low level of this vitamin is a risk factor for the vulnerability of the eyes from oxidative stress that may lead to the formation of cataracts (Bhat *et al.*, 1993). High intake of riboflavin or riboflavin supplementation can reduce the risk of cataracts and cope with or recover the early stages of a cataract (Bhat *et al.*, 1993; Chiu and Taylor, 2007).

### **Anemia**

Anemia can be defined as a reduction of hemoglobin (Hb) concentration in red blood cells (Northrop-Clewes and Thurnham, 2013), resulting in the inadequate oxygen supply to the cells of the body. Riboflavin has an important role in the formation of red blood cells (erythropoiesis). Given high-dose riboflavin to the anemic patients has proved to overcome anemia quickly (Belinda, 2014). A study has shown improvements or positive effects of riboflavin and retinol supplementation to pregnant women have anemia and iron deficiency (Ma *et al.*, 2008). Young women in the United Kingdom who are deficient in riboflavin showed the improvement or increased both hemoglobin status and significant riboflavin levels due to riboflavin supplementation. Increased riboflavin level may lead to an increase in the number of red blood cells in circulation and thus the concentration of hemoglobin (Powers *et al.*, 2011).

### **Migraine**

Migraine is a chronic disorder that is often found, could be due to dysfunction of energy metabolism in the mitochondria (Schoenen *et al.*, 1997). Metabolism in mitochondria involves various important molecules, such as FAD and FMN in terms of electron transfer in oxidation-reduction reactions. Riboflavin as a precursor of FAD and FMN are needed for these types of reactions. Administration of riboflavin as a prophylactic dose of 400 mg/day can reduce the severity and duration of migraine attacks (Schoenen *et al.*, 1997). Riboflavin may increase the activity of complex I and II electron transfer in mitochondria in terms of energy production. Research by Nambiar *et al.* (2011) also found that the administration of riboflavin 100 mg/day as prophylaxis can reduce the frequency, duration, and severity of migraine attacks. Thus, riboflavin can be used as a prophylactic for patients suffered from migraine.

### **Conclusion**

Riboflavin (aka vitamin B2) has an important role in the metabolism, through FMN and FAD as it active form. Both of this flavin are essential for the enzymes activity in metabolism such as energy produces metabolism, glutation cycle, and folate-methionine cycle. The daily intake of riboflavin is low, but it is efficiently used for the metabolism reactions. Riboflavin deficiency may occur due to inadequate intake

or malabsorption. There will be variety of clinical manifestation and disorders associated with the deficiency. Therefore, high intake or supplementation of riboflavin is suggested to avoid deleterious impacts of riboflavin deficiency.

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# **THEME : SOCIAL SCIENCE**

**AAC Dayan Daood, Darussalam-Banda Aceh, Indonesia  
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## **Integration of Oil Palm and Cattle to Empower Farmers' Economic in East Aceh, Indonesia**

<sup>1</sup>\*Saifuddin Yunus, <sup>2</sup>Suadi Zainal, <sup>3</sup>Suryadi and <sup>4</sup>Fadli Jalil

<sup>1</sup> Department of Sociology Faculty of Social and Political Sciences Universitas Malikussaleh, Lhokseumawe Aceh 24352, Indonesia;

<sup>2</sup> Department of Sociology Faculty of Social and Political Sciences Universitas Malikussaleh Lhokseumawe Aceh 24352, Indonesia;

<sup>3</sup> Department of Agro-Business Faculty of Agriculture Universitas Malikussaleh Lhokseumawe Aceh 24352, Indonesia;

<sup>4</sup> Department of Agro-Business Faculty of Agriculture Universitas Malikussaleh Lhokseumawe Aceh 24352, Indonesia

\*Corresponding Author: saifuddin\_my@yahoo.com

### **Abstract**

This study examines the integration of oil palm commodities and cattle to empower farmers' economic in East Aceh, Aceh Province, Indonesia. Even though, this paper only focus on empowerment process with aim to discover an integration process of oil palm and cattle made by oil palm farmers. This study used a qualitative approach to analyze data. The results showed that the process of integration of oil palm and cattle are practiced by oil palm farmers without adequate knowledge. In addition, the integration also has done between cattle breeders and oil palm farmers. However local authorities (Aceh Province and East Aceh) have not given the attention for this program to empower the farmers with education and training skill to integrate oil palm and cattle. Whereas such as this program is commonly practiced in Indonesia.

**Key words:** Integration, Oil palm, Cattle and Empowerment.

### **Introduction**

The economic empowerment of Aceh people in post-conflict period, especially the rural communities of former combatants and conflict victims have been done through agricultural assistance, both plantations and farms revived the local economy was collapsed by the conflict. However, its implementation is generally still done separately between plantations and farms. Whereas the integration of plantations and farms might increase the income of farmer communities (Handaka, et al, 2009; Wake, 2010) and the Government of Indonesia has encouraged the integration of plantations and farms to achieve the targets of meat self-sufficiency quickly, especially beef. Thus, Indonesia's dependence on higher imported beef will be decreased. According to the General Director of Foreign Trade, Bachrul Chairi (2014) that self-sufficiency of beef can be accelerated by the concept of integrated farms. The plantations are most likely to be integrated with livestock are oil palm and rubber plantations.

Realizing that, District Government of East Aceh has integrated the cocoa plantations and goats since 2014, to empower economic of rural people. Then in 2015 East Aceh Government planned the same program in order to ensure the integration of oil palm and cattle. Where people who have gained the favor of oil palm in the previous year will also be given assistance of cattle, with an expectation is the economy of society should arise rapidly. This program will be implemented by optimizing the cooperation and synergy among Local Government Units (SKPD) in the Government of East Aceh, especially between the Department of Plantations and Agriculture with the Department of Stock Husbandry, and also the cooperation between the government unit (SKPD) with privates which associated with economic empowerment. The Integration model is the success key of community empowerment process (Miftah and Syarbaini, 2014).

But this program will certainly stack the assistances to the same community, hence this program potentially arise jealousy and social inequality caused by the distribution of government aid is uneven. The increase of revenue only felt by conflict victims and former combatants who have received government's assistance during planting oil palm.

This situation will certainly hamper integration of communities as a whole to realize sustainable positive peace in Aceh. Indeed, this study will examine the process of establishing a model of cattle – oil palm integration to improve farmers' economic.

### **Research Method**

This study used a qualitative approach. The Informants are oil palm farmers who have cattle or vice versa, cattle breeders who do not have oil palm plantation but relinquished his cow in the oil palm plantations of others with cooperation system, the Department of plantation and livestock services of East Aceh Government. Data collected through non-participant observation, in-depth interviews, and documentation. The data analysis was conducted in three stages: data reduction, data display and conclusion (Neuman, 1997).

### **Results and Discussion**

The area of oil palm assistance to former combatants Free Aceh Movement (GAM) and the victims of the conflict in East Aceh is around 3,726 hectares, spread in 23 districts with involvement of 3,408 farmers, and only one district that did not receive assistance for the development of oil palm is Simpang Jernih (Saifuddin, et.al, 2015). However, not all of the oil palm farmers raise cattle in those areas of oil palm plantations. But there are some cattle breeders releasing their cattle in others' oil palm plantations with permission of the landowner, either cattle grazed with raising system or illegal grazing during the day. Most cattle are raised by the community are their own or other's cattle with shared profit management (mawah). Generally cattle are village cattle or pea cattle. While cattle of Aceh Government assistance and East Aceh Government assistance are Bali cattle given to the people who propose it in groups, it's given to each group in different amount, such as Farmers in Seneubok in Nurussalam got 5 bali cattle for ten members of the group. While the Farmers in Alue Dong-Dong got 10 bali cattle for 5 members of the group.

The differences of integration system, freely grazing and kept grazing are seems to strongly influence by the traditional knowledge of farming communities on its raising and fattening. They have not got enlightenment of knowledge and modern technology to integrate cattle with oil palm. They only use the grass or weeds in oil palm plantation areas which palm estimated could not be reached by cattle that interfere the growth of oil palm. Even though, the number of cattle farmers increase every year, in 2012 there were 38 groups, and 2013 were 49 groups. Even in 2014 increased significantly, reaching 100 groups with production reaching 361.232 kg of beef. But the cattle population has decreased, in 2012 as many as 70.874 cattle, in 2013 are 45.030 cattle, and in 2014 are 51.428 cattle (BPS Aceh Timur, 2015).

Nevertheless, the fact according to Aceh Agricultural Instructor, Nani Yulizar (2016) East Aceh District has great potential to develop beef in Aceh province. East Aceh is now listed as the second area that has great potential in developing beef after Aceh Besar District, by comparison East Aceh now has 51,428 heads of cattle and Aceh Besar has 56,000 heads of cattle. However, the problems faced in developing cattle is a system of cultivation still use semi-intensive with simple technology, so the feeding does not meet the nutrition of cattle. Even so, farmers believe that raising cattle in oil palm plantation could increase their revenue. Hanani and Jamilah (2011) stated that integrated cattle-oil palm program is a policy in developing cattle in Aceh to increase people's income. Technology Innovation for integration system of crop - livestock in farming was proven to increase the efficiency of farming (Kusnadi, 2008).

The following are some resumes of interviews with several informants who worked as farmers and ranchers: Oil palm plantations chosen as location for raising cattle based on no other location for grazing cattle. They did not know the benefits of cattle-oil palm integrated system in detail. The cattle grazed in oil palm plantation are privately owned, while government aid is only one cattle in the location managed by Mr. Dahlan and Saifullah. Total cattle assistance from the government to the village of Alue Siwah Serdang in 2015 were 5 cattle for 10 people that distributed by the Department of East Aceh farms. However, we graze it separately with shared one for two people. The cattle assistance are varieties of Bali. The number of cattle in this location are 40 heads with land area is seven hectares (interview with Saifuddin, Kadesh Alue Siwah Serdang, June 3, 2016).

The next, other informants also have in common, their cattle grazed in oil palm plantation is aid of the Animal Husbandry Department of East Aceh in fiscal year 2009/2010, but they received cattle more than the previous informants, there are 10 heads of cattle for five members in group. So each member

gets two heads. The aid cattle is female Bali cattle. One Informant added, his cattle developed from two heads to 12 heads for six years. Some of cattle were sold for the purpose of the economy (Interview with Sutimin, Chairman of the Cultivation of Farmers, Rural Alue Dondong 2, District Rantau Panjang Perlak, July 16, 2016).

According to him, six heads of cattle are grazed in 3 hectares of oil palm. The cattle released from its cage in the afternoon, at about 3 pm using kept grazing systems (Peurabe Leumo) and then return to be stabled at 05 pm. This purposes to maintain weeds growing in sustainability. By this way, the need of grass for cattle might be met every day, because the cattle will not roam everywhere, so that it given times to weeds to grow up. If it runs out at one location, moved to another, and the location continued to alternate on the next turn. Thus, when it comes to the turn, weed has grown back and ready for the cattle. In their opinions, stabling cattle back at 5 pm should still be given food and drink at night to ensure insufficient nutrients and accelerate the growth and fattening. According to their experience, farmers believe that feeding and drinking cattle at night better and faster for growth and fattening.

While Abdurrahman said that the cattle grazed in his palm plantation is his own, not an assistance of Aceh Government. He grazes cattle in a number of 30 heads, but some are other's. Only 13 heads are belonging to him, and 17 heads are his friend's. All their cattle grazed freely in 13 hectares of oil palm. Although the owners of cattle are two people, they keep cattle grazing to gather in one and same area of oil palm. Only at night, the cattle were placed in three hectares of oil palm which has been fenced with no cages, except during the rainy season made tents to shelter cattle at night. Meanwhile, to fatten cattle usually five to six months before being sold, cattle caged to feed by adding concentrate on feed. This thing was done alone or cooperated with other people who want to do with mawah system (shared profits) with profits shared equally, 50: 50 (Interview with Abdurrahman, Seuneubok Village, District Nurussalam, July 18, 2016).

Nevertheless, the integration process of oil palm with cattle conducted by Abdurrahman is also a natural process, which means that he do not has specific knowledge about the integration of oil palm with cattle. However, he has been doing this process since 2010. Previously, he had oil palm area with only 3 heads of cattle that kept by utilizing the weeds around oil palm. While rice planting season paused for moments, his cattle released to the rice fields. After he has oil palm plantation, he initiate to take advantage of the oil palm plantation to raise cattle in it. Since then, the number of cattle grows because the presence of oil palm land, it is very helpful in raising cattle (Interview with Abdurrahman, Seuneubok Village, District Nurussalam, July 18, 2016).

The explanations above illustrate that the integration of oil palm - cattle carried out by the farming community occurs naturally, without modern knowledge and technology. Oil palm land was chosen as cattle grazed on the basis of necessity, because of less land freely for grazing cattle, so that the utilization of palm tree such fronds and leaves to feed is not treated yet with modern technology. The cattle just eat weeds that grows wildly in the area of oil palm, and palm leaves on the tree. Moreover farmers prefer to look for grass or weeds in other areas to feed their cattle at night or planted grass in specific land areas for being cut to feed cattle daily.

These integration models differ from the integration models were conducted by the companies of oil palm that categorized into three models, namely intensive, extensive and as transportation, as stated by the Director General of Animal Husbandry and Animal Health Ministry of Agriculture, Iwantoro (2014) the company's goals of doing cattle-palm integration program are; (1) to obtain organic fertilizer, cattle raising is done intensively in cages. For example, the Oil Palm Research Center (PPKS) in Bukit Sentang; (2) The cattle enabled to eat weeds / grass growing in the oil palm plantation and given additional feed amplifier / concentrate and water. The integration cattle-palm performed extensively. The example is in PT. Sulung Ranch, Kota Waringin Barat, Central Kalimantan; and (3) Integration with the aim of utilizing the labor force of cattle to transport the oil palm for lack of labor in the oil palm plantation. It's conducted by PT Agrical in North Bengkulu.

In addition, the integration model also takes place through the cooperation of farmers with cattle raisers. The farmers do not have cattle and ranchers do not have oil palm, but they cooperate mutually beneficial to both parties. This type of integrations can be advantageous for both sides, because the cattle raisers do not have to think place to graze their cattle, while for oil palm farmers do not have to clean the weeds in the oil palm plantations (Interview with Jufri Alias Jabrik, Alue Siwah Serdang, Nurussalam sub-district, East Aceh district, June 3, 2016).

However, the integration of cattle with oil palm in East Aceh has not been conducted by the government in particular. Government department, such agriculture and estate, and livestock department work separately and less of coordination. The result is livestock aid cannot be integrated with the assistance of oil palm through the estate agency. Unless cattle aid given to farmers by chance. It was confirmed by farmers' statement in Alue Siwah, that they got five female cattle for ten people from government. Most of them keep it in the area of oil palm, but others do not so (Interview with Mr. Basaruddin, staff of the Ministry of Forestry and Plantation East Aceh, June 4, 2016)

## Conclusions

Cattle-oil palm integrated system in East Aceh conducted by farmers based on their own initiative, without a policy of government. This occurred naturally without knowledge and modern technologies. So that integration models do not have mutual advantage between cattle and oil palm, they perceive oil palm plantation is useful for grazing cattle, while dung of cattle is still useless for oil palm fertilizer. Only few of them have mutual advantage, but without technology treatment. Thus integration models are different from many concepts applied by companies outside East Aceh. Farmers need enlightenment from government or academicians to gain mutual advantage of cattle-oil palm integration model.

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## **The Effects Of Sukuk (Islamic Bonds) In The Economy**

Derry Fahrian<sup>1</sup>, Chenny Seftarita<sup>2</sup>

<sup>1</sup>Development Economics Department, Economics and Business Faculty, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Development Economics Department, Economics and Business Faculty, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: derryfahrian@outlook.com;

### **Abstract**

This study aimed to analyze the influence of sukuk towards Gross Domestic Product (GDP) in the short term and the long term. The data used in this research is sukuk and Gross Domestic Product (GDP) quarterly data from 2009 until 2015 in the form of time series, processed and analyzed by the method of estimation of Autoregressive Distributed Lag (ARDL). The results of this study indicate that factors influencing the Gross Domestic Product (GDP) in the short term and the long term is the Gross Domestic Product (GDP) and the Sukuk. In the short term Gross Domestic Product (GDP) influencing the Gross Domestic Product (GDP) itself positively and significantly, while Sukuk influencing the Gross Domestic Product (GDP) negatively. In the long term both of Sukuk and Gross Domestic Product (GDP) influence the Gross Domestic Product (GDP) positively and significantly. The study conclude that the increase in funding sources could increase the Gross Domestic Product (GDP) in order to increase economic growth. Thus it is critical to increase the sources of financing to stimulate economic growth in Indonesia.

**Key words:** Sukuk, GDP, ARDL.

### **Introduction**

Sharia finance is growing significantly from time to time, the development is not only in areas dominated by moslem population, it has now recovered the world scale. One of sharia financial instrument which is currently growing rapidly is sharia bond known as sukuk. Sukuk is an innovative solution for those who need financing source and investment. According to IFIS data quoted by the economist during the decade 2002-2010, sukuk issuance grew at an average 35% per year. Moreover, based on the results of the study that Thomson Reuters examined, world total sukuk issuance will reach \$ 250 billion by 2020. (Dian Handayani).

Global sharia financial which is developing rapidly represent a great opportunity for the government to link global investors with domestic economic sector by providing financial instruments needed by financial institutions. According to Diaw (2011), sukuk provide many advantages with regard to government spending on infrastructure. Sukuk improves the relationship between markets and financial institutions due to sukuk must be based on tangible assets, and sukuk will strengthen the relationship between the real sector and the financial sector. Similarly with sukuk holders, the sukuk holders prefer to have a predictable and stable cash flow for long-term periods of time.

Sukuk was first published in Indonesia in 2002 that issued by PT. Indosat worth 175 billion rupiah, the success of PT.Indosat in issued sukuk lead other companies to be sukuk issuance. Until the end of 2015, total of corporation sukuk outstanding reached 8.444,4 billion rupiah. The development of global sukuk and corporate sukuk is growing rapidly in Indonesia has been attracted the government, so that in 2009 the government has been began issued sukuk for global sharia financial institutions called Sukuk Negara Indonesia (SNI). The issuance of global sukuk got a good response from investors. The issuance of global sukuk SNI 25 series issued in 2015, 41% investors are from Middle East country, 21% are from US, 12% are from Asia, and 10% are from Indonesia. Indonesia government not only focus on the global sukuk, the government also issued Government Bonds (Sovereign Sukuk) which is known as Sukuk. Sovereign sukuk grows rapidly as well as corporate sukuk. In the early, the value of outstanding sovereign sukuk is only worth 10.256 billion rupiah and in the last quarter of 2015 it has reached 201.017 billion rupiah. This record indicates the investors are interested to invest over sharia obligation in Indonesia that increase constantly from time to time. In 2016, the contributions of outstanding sukuk is 15% of total government financial source. This amount is relatively small compared with government

bond, but the progress showed by sukuk that constantly increasing each period makes sukuk has the potential to increase its contribution in government financing in the future.

The increase of sources of financing, especially infrastructure financing are able to accelerate economic development and economic growth of a region. As Calderon (2011) stated that economic growth is positively and significantly correlated with the stock and infrastructure quality in an area. To increase sources of financing, especially infrastructure financing, sukuk is an alternative. This is because sukuk required the underlying assets in the form of tangible assets. So when the investors invest on sukuk, they has strengthened not only the real sector but also the financial sector in the economy. The real sector which is growing well will encourage the growth of Gross Domestic Product (GDP) of a country.

## **Literature Review**

Islamic capital markets have witnessed the issuance of shari'a compliant financial instrument known as sukuk. Sukuk basically same like bonds, sukuk have a maturity date and holders. However, sukuk are asset-based rather than asset-backed securities, with the underlying asset being necessarily shari'a-compliant in both nature and use. The eligibility of sukuk rests on identifying an existing or a well-defined asset, service, or project. In May 2003, the Accounting and Auditing Organization for Islamic Financial Institutions (AAOIFI) officially defined sukuk as certificates of equal value representing undivided shares in ownership of tangible assets, usufructs, and it identified at least fourteen possible sukuk structures. The AAOIFI distinguishes sukuk from stocks, bonds, and from the conventional process of securitization as well, emphasizing that sukuk are not debt certificate with a financial claim to cash flow.

Although sukuk were first issued in the 1980s, nearly all growth has come within the past decade. According to Moody's (2007,2008), the global outstanding volume of sukuk exceeded \$90 billion in 2007. Data from the Islamic Finance Information Services (IFIS) indicate that corporate sukuk quickly gained a dominant market share on the sukuk market relative to sovereign sukuk. Indonesia, sukuk were first issued by PT.Indosat in October 2002. The bonds have suffered double oversubridger, so that PT.Indosat increase the amount of sukuk in the amount of Rp175 billion. Sukuk continuous to grow from year to year until the end of 2015, outstanding corporate sukuk reached Rp9 billion. As the development sukuk is growing constantly, the government finally issued sukuk in 2009 and in the last quarter of 2015 the amount of outstanding sukuk reached Rp201.017 billion. Sukuk which is growing countinously from time to time make sukuk has the potential to become an alternative source of financing in the future.

Bonds is related to the financial market, based on research conducted by Lucas (1998) and Favara (2003) estimated the positive role of financial markets in economic growth. This result indicates that the development of financial markets in one of the factor encouraging the economic. Hering and Chtusripitak (2000), Braun and Briones (2005) and Fink, Haiss, Kirchmer, and Moser (2006) examined the relationship between bond market and economic growth. The result shows that there is a positive relationship between the bond market and economic growth. Pataraa, Yoonbai, Chong (2013) examined the effect of bond markets on economic growth. The results conclude that (i) capital market development is positively significant assosicated with economic growth, (ii) the contribution of the bank loan portofolio reduced in line with the development of bond market, (iii) government bonds positively correlated with economic growth. Based on the studies that had carried out, the study conclude that bond market is needed to encourage the economic growth of an area.

## **Research Method**

To examine the effect of Sukuk on Gross Domestic Product (GDP), this research estimated the variables with ARDL (Auto Regressive Distributed Lag) model. ARDL model contains the dynamic effects of specific models by including lag value of variable for transform data into the model (Laurenceson and Chai, 2003). ARDL model generally expressed in the following equation (Bekhet and Matar, 2013) :

$$\ln Y = \beta_0 + \sum_{i=1}^k \beta_1 \ln Y_{t-i} + \sum_{i=0}^k \beta_2 \ln X_{1t-i} + \sum_{i=0}^k \beta_3 \ln X_{2t-i} + \sum_{i=0}^k \beta_4 \ln X_{3t-i} + \theta_1 \ln Y_{t-1} + \theta_2 \ln X_{1t-1} + \theta_3 \ln X_{2t-1} + \theta_4 \ln X_{3t-1} + \varepsilon_t$$

This study aimed to investigate the effect of Sukuk on Gross Domestic Product (GDP) in Indonesia. The general equation of ARDL model must be transform becomes :

$$GDP = \beta_0 + \sum_{i=1}^k \beta_1 GDP_{t-i} + \sum_{i=0}^k \beta_2 SBSN_{t-i} + \theta_1 GDP_{t-1} + \theta_2 SBSN_{t-1}$$

The data used in this study is quarterly data from 2009 to 2015 that collected from website of Bank Indonesia and The Asia Regional Integration Center.

## Results and Discussion

To determining the best equation that will be used on ARDL model, Unit Root-Test is necessary to find out whether the variables are stationer at level  $I(0)$  or at first difference  $I(1)$ . Software used in this stationary test is Eviews 9.

**Table 1.** Stationary Test

No	Variabel	PP		
		At level	1 <sup>st</sup>	2 <sup>nd</sup>
1	GDP	-4,4947 (0,0070)	-	-
2	SBSN	-2,7679 (0,2200)	16,6339 (0,0000)	-

Based on stationary test on table 1, it can be seen that the ordo of each variable are different. The Gross Domestic Product (GDP) variable is stationer at level  $I(0)$ , while the *Sukuk* variable is stationer at first difference  $I(1)$ .

To determine the best model will be used on ARDL estimation model, determining the optimal lag of the equation is need to be done. According to Azuma (2014) the amount of lag selected is the equation with the smallest value of Akaike Information Criterion (AIC) or Auto Correlation Function (ACF).

**Table 2.** Optimal Lag Test

Lag	AIC	SC
1	58,34815	58,59009
2	58,14527*	58,48656*
3	58,33389	58,77566

Based on the result on table 2, it can be seen that the amount of optimal lag for the Gross Domestic Product (GDP) and *Sukuk* variables is the second lag. Based on optimal lag test, ARDL equation that will be used in this study is ARDL with two lag.

**Table 3.** Auto Regressive Distributed Lag (ARDL) Estimation

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	5.69E+13	1.83E+13	3.113814	0.0060
D(GDP(-1))	0.369131	0.201591	1.831088	0.0837
D(GDP(-2))	0.160662	0.069824	2.300969	0.0336
D(SBSN(-1))	-0.084568	0.036347	-2.326666	0.0319
D(SBSN(-2))	-0.048804	0.033523	-1.455831	0.1627
GDP(-1)	0.972690	0.010619	91.60014	0.0000
SBSN(-1)	0.103081	0.040342	2.555177	0.0199

The estimation results are shown in table 3 describes that the Gross Domestic Product (GDP) variable is affected by Gross Domestic Product (GDP) in the first lag and second lag, while *Sukuk* affects Gross Domestic Product (GDP) in the first lag. This is based on the value of the estimated coefficients, 0.369131 for the first lag of Gross Domestic Product (GDP) in the short term with 90% confidence level, 0.160662 for the second lag of Gross Domestic Product (GDP) in the short term with 95% confidence level and -0.084568 for the first lag of *Sukuk* in the short term with 95% confidence level. This results suggest that changes of Gross Domestic Product (GDP) in this period will affect the Gross Domestic Product (GDP) itself in the next quarter and the next two quarters. While changes of *Sukuk* will affect Gross Domestic Product (GDP) in the next quarter. In the long term, both of the variables affect Gross Domestic Product (GDP) positively and significantly with estimated coefficients 0.972690 for Gross Domestic Product (GDP) and 0.103081 for SBSN. To find out whether Gross Domestic Product (GDP)

and *Sukuk* variable will be in equilibrium state in the long term, it is necessary to estimate short term equation with ECT (Error Correction Term). ECT is used to determine the level of speed adjustment in the short-term to long-term equilibrium. When ECT estimated coefficient is negative and significant, indicates that the variable will be in equilibrium state in the long-term. The value of estimated coefficient of ECT showed the speed of adjustment level in correcting the imbalance of the variables to be back to the balance point (equilibrium) (Bekhet and Matar in Fahmi, 2015).

Based on the optimal lag, short-term estimation results are obtained as follows:

**Table 4.** Short-Term Estimation

GDP =	3,15E+15	-1,521149	$GDP_{t-i}$	+12,96395	$SBSN_{t-i}$	+3,114273	$\varepsilon_{t-i}$
t-ratio	3,125160	-0,113870		2,282576		1,579111	
p-value	0,0056	0,9105		0,0341		0,1308	

Based on the results of short-term estimation on table 4, obtained that the value of the ECT variable is positive and not significant with estimated coefficient 3.114273 and p-value (0.1308 > 0.05). This results indicate that the variable Gross Domestic Product (GDP) will not adjusted short-term balance towards long-term. To find out whether *Sukuk* variable jointly with Gross Domestic product (GDP) variable correlated with Gross Domestic Product (GDP) in the short-term, this study use wald-test.

**Table 5.** Short-Term Wald Test

Variabel	Wald-Test
GDP	10,89446 (0,0278)**

Based on the Wald-test contained on table 5, it can be seen that p-value shown significant result (0.0278 < 0.05), which means that the variable *Sukuk* has significant effect on Gross Domestic Product (GDP) in the Short-Term. Based on ARDL estimation result on table 3, in the short-term changes of Gross Domestic Product (GDP) variable in a period will affect Gross Domestic Product (GDP) itself on the next quarter and the next two quarter with estimate coefficient 0.369131 and 0.160662 with 90% and 95% confidence level. While change of *Sukuk* in a period will affect Gross Domestic Product (GDP) on the next quarter negatively and significant with estimate coefficient -0.084568 and 95% confidence level. This result indicates that increase *Sukuk* in a quarter will reduce Gross Domestic Product (GDP) in the next quarter. This mechanism allegedly associated with the decision of investors to invest in *sukuk*, when investors shift their investment to *sukuk*, the investment that increase Gross Domestic Product (GDP) in the short-time would be switched on *sukuk* that will reduce Gross Domestic Product (GDP) in the short-time. This is due to mainly *sukuk*, especially mudharaba based *sukuk* are based on project that take time to become productive.

**Table 6.** Long-Term Estimation

GDP =	-6,72E+12	+ 1,020009	$GDP_{t-i}$	-0,064733	$SBSN_{t-i}$
t-ratio	-0,216548	-0,113870		-0,877820	
p-value	0,8304	0,0000		0,3887	

**Table 7.** Long-Term Wald-Test

Variable	Wald-Test
GDP	134920,1 (0,000)*

Based on Wald-test in the long-term that are contained on table 7 show that the p-value is significant (0.0000 < 0.05), it means there is a long-term relationship between the *Ssukuk* variable and Gross Domestic Product (GDP) variable. Based on table 3, estimate coefficient for *sukuk* and Gross Domestic Product (GDP) is 0.103082 and 0.97269 with 95% confidence level. Based on estimate coefficient obtained in ARDL estimation shows that increasing 1 trillion rupiah in *sukuk* will increase Gross Domestic Product (GDP) 103 billion rupiah. Based on the result of these estimation, this study conclude that the

contribution of *sukuk* to Gross Domestic Product (GDP) is relative small. This result allegedly caused by the lack of *sukuk* issuance by government and corporation.

## Conclusions

The results of thi study indicate that; (i) Gross Domestic Product (GDP) is affected by Gross Domestic Product (GDP) itself and *sukuk* in the short-term and long-term; (ii) in the short-term, changes of Gross Domestic Product (GDP) in a period will affect Gross Domestic Product (GDP) itself in the next quarter and the next two quarter. The changes of 1 trillion Gross Domestic Product (GDP) in a period will increase Gross Domestic Product (GDP) in the next quarter amounted to 369.131 million rupiah and the next two quarter amounted to 160.662 million rupiah; (iii) in the short-term, changes of SBSN in a period will affect Gross Domestic Product (GDP) in the next quarter. The changes of 1 trillion rupiah *sukuk* will decrease Gross Domestic Product (GDP) in the next quarter amounted to 84.568 million rupiah; (iv) in the long-term, Gross Domestic Product (GDP) is affected by the Gross Domestic Product (GDP) and *sukuk*. The increase of 1 trillion rupiah *sukuk* will increase Gross Domestic Product (GDP) amounted to 103.081 million rupiah.

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## **The Role Of Knowledge Management On The Performance Of Coffee Company In Southeast Asian Countries: An Initial Meta-Analytic Review**

<sup>1</sup>Hendra Syahputra, <sup>2</sup>Edwar M Nur

<sup>1</sup>Ph.D Student of Management Science, Syiah Kuala University, Banda Aceh, Indonesia

<sup>2</sup>Universitas Abulyatama, Banda Aceh, Indonesia;

\*Corresponding Author: hsyahputra@gmail.com

### **Abstract**

The objective of this paper is to review some researches on knowledge management, business environment as well as competitive strategies applied in small and middle companies that will provide best practices for the improvement of performance of coffee processing company in ASEAN. This study consists of systematic reviews of 17 empirical scientific papers in regard to knowledge management, business environment, and competitive strategies that influence the performance of coffee processing company in ASEAN. Given the importance of the existence of coffee processing company in Indonesia, the application of knowledge management has to be prioritized by the concerned companies in order to accelerate their competitive advantages. This paper provides a better understanding on how to develop good knowledge management application, industry environment as well as strengthen the competitive strategies specifically in coffee processing company by learning from other sectors. The result of this study is believed to give worth understanding about actual situation in this field of research. It proposed some notions that will stimulate more intensive researches in this field in the future. However, there are no systematic literatures in academic journal that cover all the topics discussed in this paper.

**Key words:** Knowledge Management, Business Environment, Competitive Strategies Strategy, Coffee Industry, SMEs

### **Introduction**

Coffee ranks as the first most traded commodity in the world, outperform oil, with total sales of \$9 billion during 1999 and 2006. As a commodity coffee create new jobs for about 25 million people, filling about 400 billion cups each year with about 40 percent of the world's population is believed to consume coffee regularly (Kaplinsky, 2005). About 25 million families who derive their main income from this commodity are settled in developing countries (Potts, 2008). Coffee productions and its trading have an important role in most of families' life, even though the price had ever been drop in 1989 following the collapsed of International Coffee Agreement (ICA). The decline in price at that time is known as the biggest-ever coffee crisis in this world.

ASEAN is a major producer of coffee in the world and coffee products of a wide variety. Thus, coffee is one of leading export commodities that have a significant contribution for the national economy of ASEAN countries. Besides as a strategic commodity trading, coffee also has an important role as the source of foreign exchange, employment provider and the source of farmers' income or other economic actors whom are involved both on-farm and off-farm. Coffee processing companies also provide more jobs for million families and a decent income for them. Those companies are able to employ people from production, processing to marketing of the commodity.

The two largest coffee producer countries in this region are Vietnam and Indonesia where both are ranked as second and third in world biggest countries that produce coffee. With per-capita coffee consumption on the rise in this region, there is obvious room for further growth, but there is also an obvious need for investment. The capital required to take ASEAN's coffee industry to the next level presents appealing prospects for investors, while the countries' burgeoning coffee culture also brings opportunities for foreign exporters. At present, the principal destinations for ASEAN coffee are the US, Japan, China, Western Europe, and also for the fast-rising demand in the ASEAN region itself.

The coffee productions in ASEAN countries are mostly produced by smallholders and/or small and medium size enterprises (SMEs). Therefore the SMEs in coffee production play a crucial role in the ASEAN countries economy in terms of economic growth and providing employment. Thus, it is very important for those SMEs in this industry to continue competitive and sustain in today dynamic economy. The ongoing issues that limit production in this industry include limited knowledge of best practices, widespread use of low quality and uncertified planting materials and an abundance of older, less productive trees. These factors make the coffee production in ASEAN particularly prone to random changes and/or severe weather patterns.

Another key issue in the long-term survival of coffee industry in ASEAN is sustainability. To survive this important industry, especially in ASEAN, it is important to adopt a Knowledge Management (hereinafter abbreviated as KM) system since it is meaningful and strategic for that such organization. The core of knowledge-based view has to be understood to appreciate the value of knowledge for the company. The objects in this study are business units in coffee industry in ASEAN countries, a potential plantation commodity that is exported to the world market. This export commodity needs a well-managed knowledge commodity to compete in a tight competitive industry environment.

To explore existing literatures, this paper defines KM as a process and structure that is available in coffee industry in ASEAN to support a wide range knowledge of different processes, such as transfer, storage and creation. Actually, companies have to find ways to manage these aspects in a balanced manner, which presents particular challenges for SMEs because they usually lack of resources needed to produce full utilization over the stock of knowledge that they possess. Knowledge has become the most important strategic factor in the operations of cooperation (Spender, 1996) as it relates to the ability of the company to achieve competitive advantage (Teece, 2000). However, this does not mean that the appropriate approach to address issues of KM is not significant for the success of coffee industry unit in ASEAN. It may be just emphasized that an appropriate treatment for knowledge is a very important factor that determines whether a business will survive or not.

Since 2001, McAdam and Reid, said that KM is created and developed in large organizations to be applied in other types of community-based businesses, such as SMEs. But the question is, whether KM received full attention from actors in coffee industry sector?. Moved from that question, this paper would like how KM system takes place in a different business unit. Therefore, the business unit in coffee industry might adopt best practices from other business units. The purpose of this paper is to examine the problems and learn what had happened with KM in the coffee business units in ASEAN in the last ten years. This will help highlighting the current understanding of this topic and identify the existing gaps.

The objective of this paper is to review researches on KM in coffee processing company in Aceh Province to identify the gaps in the field and asses the future research activities and clarify other research areas which are no need to be studied in the future. According to the objective, some research questions are formulated as follows: Which KM topics have been researched and which topic have not been researched yet? What is the main finding in the research? What method is used in the research? How the research deal with particular challenge faced by SMEs related to KM?. In summary, this paper is arranged as follows: in section two, the literatures related to the research objectives are discussed, the third section describes the methods used to address the research problems, the next section presents the results of research, and at the end, presents the conclusions and implications of the study.

## **Literature Review**

### *Knowledge Management within Organization*

Knowledge is information combined with experience, context, interpretation, and reflection which is ready to apply to decisions and actions (Davenport et al., 1999) and KM is often defined as 'the management of organizational knowledge to create and retain greater value from the core business competencies and generate competitive advantage' (Klasson, 1999; Chong & Choi, 2005). In addition, KM is affected by the management of cultural, behavioral, operational, technological and organizational factors (Wong, 2008). Therefore, in a holistic sense, KM can be defined as the optimal management of a complex mixture of 'knowledge based system, artificial intelligence, software engineering, business process improvement, human resources management and organizational behavior concepts' (Liebowitz, 2000).

KM plays an important role as a dynamic dialectical element of various activities which are developed and experienced by organization. KM also extends significant contributions as the capitals for all of organization's members. The objective is to build organizational capacity through knowledge sharing interactions among organization's members in order to create the same deals. Thus finally it will creates knowledge conversion from tacit to explicit, vice versa, fundamentally and sustainably (Nonaka &

Takeuchi, 1995). KM in an organization also plays an important role in facing the challenges of complexity and the business environment turbulence in this sector. Business people, business entrepreneurs and even business units from small to large scale have to understand KM. This becomes one of the important issues that should be considered in order to keep the competition on track and to sustain good industry. A Competitive advantage is essentially derived from the value of a knowledge that is created for their customer's outweigh the costs they spent for creating that value (Porter, 1990). Furthermore, KM is the essence of an innovation and competition. One indicator of the occurrence of excellence strategy process in an organization is the knowledge and innovation those are generated in harmony by not rule out a thriving business environment. Moreover, to strengthen trade and investment of the coffee industry in this region, the knowledge management system in this industry must be technically and practically feasible.

The concept of KM as a focus of knowledge has been developed and became the focus of many organizations since 1995. Many organizations grapple with information overload. KM is becoming increasingly inseparable from the business function for many organizations as they realize that the competitiveness hinges is the effective management of intellectual resources (Grover and Davenport, 2001). If knowledge is decided to be the most important resource for the company, then obviously the need to secure existing resources should be the primary responsibility. Most of the delay in dealing with security management of knowledge is due to the misconception of securing knowledge which is considered the same as securing data and information. If the knowledge is a power and a source of competitive advantage (Salisbury, 2003), it is necessary to put special attention on it to ensure the sustainability and the repository of knowledge in the company to secure its core assets. Managing data, information and knowledge within the organization and use it to gain a competitive advantage in an organization has evolved in the field of KM. KM basically consists of processes and tools to capture and share data as well as using the knowledge of individuals within an organization effectively. The last decade has witnessed an explosion of information generated in the company because of the increased use of technology. The utilization of information and knowledge which is stored in the data warehouse is one of the methods to achieve the advanced industrial performance (Matusik and Hill, 1998). Companies that develop and utilize knowledge resources achieve greater success than companies that depend more on intangible resources (Autio et al., 2000).

In some other literatures reviewed the by authors, a variety of different approaches of KM are continue to grow, as research conducted by Wong and Aspinwall, 2004; Egbu et al., 2005; Hutchinson and Quintas, 2008. Nevertheless, it is true as asserted by Beesley and Cooper (2008) that a uniform definition of KM building is poor. Studies of KM has tended to focus on the processes and structures within the organization, such as transfer of knowledge from tacit to explicit, culture and organizational learning, and technologies for the storage and sharing of knowledge in order to improve productivity and sales, reduce costs, or improve innovation and quality (Kluge et al., 2001; Quintas, 2002; O'Dell, 2003; Edvardsson, 2011; Jashapara, 2011). As KM plays an important role in SMEs, the concern to establish and sustain good knowledge management practices in SMEs in order to ensure their competitiveness in the new business environment have to be raised (Hanzidc, 2006).

#### *Business and Industry Environment*

The business environment is strongly associated with a sustainable innovation. It takes serious concern for an organization's future, anticipating market changes, technology, competition and the presence of other products and services. Search results of some scientific reference studies on strategy and organizational performance tend to be expressed in the form of internal resource base of KM as a competitive advantage, which includes valuable, rare, difficult to imitate and hard to replace resources. Based on that view, some experts assess that KM is the most strategic resource that is owned by the company (Nonaka & Takeuchi, 1995; Tuomi 1999; Probst et al. 2000). Moreover, Sharkie (2003) state that the ability to manage KM is a management function that enables organizations to explore and develop sources of competitive advantage compared to its competitors, as well as creating a uniqueness that contributed to its successful in the future.

Ghani et al. (2010) studied the critical internal and external factors of business environment in Malaysia and found that external factors which become opportunities for companies are supported and encouraged by the government, and the threats are represented by bureaucratic procedures that companies must face to obtain various approvals and certifications. This bureaucratic procedures often being complicated processes that has to be faced by most of the companies. This led to the delay on the development of coffee processing unit in this region. Company has to create a more attractive and favourable business environment that will attract export-oriented investor (Jegathesan, 1996).



According to Jegathesan, this argument calls for governments to play the character of provider and initiator of a safe and favourable regulatory environment that provide suitable returns on investment.

The influence of industry environment is considered most significant for the stage of the life cycle of the industry on firm performance but not significant for other industry structural measures such as industry concentration, entry barriers and product differentiation (Robinson, 1998). Some researchers argues that industry structure is not too significant impact on the company performance. This is because company does not exist in a static environment. The industry environment will differ from one marke to the next (Sundqvist et al., 2000). Different industry environment will require different decision about strategic choice and implementation (Craven, 2000).

#### *Competitiveness and Competitive Strategy*

ASEAN countries have to realistically asses its competitive advantages in light of reduced government intervention and increasing integration into the global trade market. They future competitiveness will depend on an understanding of these new trade dynamics and on preparing themselves with well-functioning institutions that is transparent to increase competition. Increasing competition means that coffee producer countries in ASEAN need to seek more new markets and market channels thus likely to be very competitive in its core business: the coffee production and delivery. In a more industrialize commodity processes pursued by most coffee industry today, once an acceptable baseline quality standard is met, consistency and price are the key factors for competitiveness.

According to Porter (1998) competitive strategy is the search for a favorable competitive position in an industry, the fundamental arena in which competition occurs. The sustainability of this positional advantage requires that the business sets up barriers that make imitation difficult, because these barriers to imitation are continually eroding, the firm must continue to invest to sustain or improve the advantage. A firm's choice of competitive strategy will be dictated by its ability to create and sustain competitive advantage. Competitive advantage is the condition which enables a company to operate in a more efficient or otherwise higher quality manner than the companies it competes with, and which results in benefits accruing to that company (Bryson 1995).

Kergley (1990) carried studies in which they compared a set of companies which carried out strategic planning with those which did not using on sales value, sales and earnings per share, and net incomes as parameters. They concluded that companies which practiced strategic planning succeeded in a big way and posted better results than better performers than those that did not plan. David (2003) cited in Lawrence, (2011) argues that this is not to say that all companies that used strategic planning are necessarily successful. In case of small and medium companies, especially in coffee industry, they need to constantly improve their efficiency, reduce production cost and enhance their product reputation by investing in research and development, acquiring new technology, improving management practices, and effectively marketing their products to increase their competitiveness.

#### **Research Method**

This research adopted meta-analytic review since this method is proved to be the most concerned to the quantitative approaches the most suitable method in reviewing the similar researches. The meta-analysis also serves as a useful starting-point for further primary research into the relationship between business/industry environment and competitive strategy/competitiveness and the performance of organization because insights derived from existing studies are of pivotal importance for the development for future research guidelines.

According to Glass (1981), meta-analysis is a secondary analysis or reanalysis to a number of data to answer the research question using better statistical techniques or to answer new questions with new data. Borg (1983) stated that meta-analysis is a newest approach that developed to help researchers find the consistency and inconsistency of their cross studies.

The advantage of meta-analysis over the more traditional literature review is obviously that it contributes to summarizing relationships and indicators, comparing the effect of the use of different methods, and tracing factors that are responsible for differing results across studies. However, there are a number of (practical) difficulties and limitations as well.

We decided to use KM implementation in SMEs and coffee industry as the keywords. To answer the research questions, first, we established the inclusion and exclusion criteria. Inclusion criterions are: publications within 2000-2012 periods, empirical research paper, peer-reviewed, KM focus in general, English, and ASEAN countries. The papers which are published before 2001, the papers which are related to cooperation and regional cluster, grey literatures like reports and non-academic researches, another

language besides English, and non-ASEAN countries represent exclusion criteria. As an additional, an excel data which consists of key aspects that related to research is produced. In the given case, among others: authors' name, publication year, research objectives, theoretical perspective/framework, method, and main findings.

The second is the search for appropriate articles. We used the keywords both "knowledge management and "KM" which is combined with SMEs and coffee industry and results 121 findings. The third is work out with abstract and, if relevance, will go further to the next section of the article to ensure that those articles are truly covers the scope that had been determined. The fourth, insert relevant data that related to research objectives into excel sheet, then working on the data entry and discuss the contents. In case of there are parts that might be passes to be read, the papers will be re-working through questions. This approach can diminish the likelihood that the analysis and the conclusion drawn might be inconsistent. Fifth, the final excel sheet is summarized that allows in categorizing the findings with the theme.

## Results and Discussion

With regard to the likelihood of analysis result in the research model, this will deliver an alternative condition as follow:

### a) *Knowledge Management and its effect on the competitive strategy and the company competitiveness.*

Ghani et al. (2010) studied the critical internal and external factors of business environment in Malaysia and found that external factors which become opportunities for companies are supported and encouraged by the government, and the threats are represented by bureaucratic procedures that companies must face to obtain various approvals and certifications. This bureaucratic procedures often being a complicated process which has to be faced by most companies in developing countries like ASEAN. This led to the delay on the development of coffee processing unit in this region.

### b) *Business environment and its effect on the performance of coffee industry in ASEAN*

Company has to create a more attractive and favourable business environment that will attract export-oriented investor (Jegathesan, 1996). According to Jegathesan, this argument calls for governments to play the characterter of provider and initiator of a safe and favourable regulatory environment that provide suitable returns on investment.

In addition, the influence of industry environment is considered most significant for the stage of the life cycle of the industry on firm performance but not significant for other industry structural measures such as industry concentration, entry barriers and product differentiation (Robinson, 1998). Some researchers argues that industry structure is not too significant impact on the company performance. This is because company does not exist in a static environment. The industry environment will differ from one marke to the next (Sundqvist *et al.*, 2000). Different industry environment will require different decision about strategic choice and implementation (Craven, 2000).

### c) *Competitive strategy on the performance of coffee industry in ASEAN*

In case of coffee processing company as one of the small and middle enterprises form, knowledge management and industry environment do not play important roles in for their performance, because it rarely implemented in such those companies level.

However, according to the alternative condition above, the variables reflects that the SME is actually able to apply new knowledge within their firm.

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## Dividend Policy in Developed and Developing Countries: A literature Review

Husaini<sup>1\*</sup>, Said Musnadi<sup>2</sup>, Faisal<sup>3</sup>

<sup>1</sup>Department Economics and Business Faculty, Malikussaleh University, Lhokseumawe, Aceh, Indonesia

<sup>2</sup>Department of Economics and Business Faculty, Syiah Kuala University, Darussalam, Aceh, Indonesia

<sup>3</sup>Department of Economics and Business Faculty, Syiah Kuala University, Darussalam, Aceh, Indonesia

\*Corresponding Author: [husaini@fe-unimal.org](mailto:husaini@fe-unimal.org)

### Abstract

This article aims to know the application of dividend policy in developing and developed countries. After reviewed 29 articles that located in ASIA and Europe and published by Elsevier and J-Stor publisher found the system of Common law is more dominant used in developed countries where the protection of the investors in stock market tends to be higher. Meanwhile, the system of Civil law tends to be applied by developing countries where the protection of the investors in the share market is relative low. Based the research results in some developed countries found that the application of the outcomes model related to dividend policy, although there exist different outcomes related to tax on dividends and profitability of the firms. While in developing countries, dividend policy is relatively lower that depends on the management of firms, and indicated the application of substitution model related to dividend policy.

Keywords: *Developed and Developing Countries, Outcome and Substitution Model.*

### Introduction

Nowadays, the dividend is still become a topic of discussion related to firms because research results are still contradictive or debatable. Those conditions caused by the various attitudes of investors and managers on dividend policy. The policy of firms related to dividend payment interacts to the potential conflicts between the owners of firms and non-owner shareholders. The conflict is called "The first agency problem" (Villalonga and Amit, (2006).

Besides, the conflict also occurs between the large owners and the small owners that called as "Second agency problem". The large ownership concentration could reduce the First agency problem yet will improve the Second agency problem, (Becht et al, (2003). The first agency problem seriously occurs in the Common Law countries. This problem analyzed and found the existence of a conflict of interest between the owners and managers and between the owners and creditors. (Becht et al, (2003), while the second agency problem occurs dominantly in Civil Law countries where the ownership concentration is higher (Shleifer and Vishny (1997). Second agency problem focused on taking over the majority shareholders to the minority shareholders, Fauver and Fuerst, (2006).

There are some previous research conducted about the conflicts among shareholders in the dividend policy of firms that have the ownership concentration (Rozeff (1982), Renneboog and Szilagyi (2006), Renneboog and Trojanowski (2007). But the results still cause a "serious ambiguity" because dividends are paid to make contrary predictions toward two agency problems. The high dividend payment will cause retained gains, capital gains and the welfare of unchanged shareholders which proves that dividend policy is still Puzzle, Laporta, et al (2000).

In the other hand, when a dividend divided, the investors will incur high tax. Meanwhile, the firms could reduce the tax by holding and reinvesting as the compensation toward gains. Although dividend could be coherent because of the changing of investment policy yet the other problems appear if the firms fail in investing because of liquidation. In this case, the shareholders desire the manager to used the fund from debt so that the risk of investor will be diminished, Modigliani and Franco (1982). Those views explain that dividend payout is higher in the countries that apply a good investor protection (common law) that also occurs in order to be a country with developed capital market. This view would mean that the company is poor in the Capital Market of Developed Countries and should reveal the extreme sensitivity of dividend payout for growth and investment opportunities because they can rely on the external fundraising

Meantime, in developing countries that apply the system of Civil law, if the firms rely on a higher debt, the firms should pay a higher interest. Most of the Civil law countries, especially France that has poor law protection of shareholders and creditors and cause a smaller debt and a smaller equity market, Laporta, et al (2000). Based on those phenomena, this research aims to know the evidence of tendency on the dividend payout in developing and developed countries. Both types of these countries do not only have a different economic growth but also apply different legal system toward the investor protection.

## Literature Review

### *Agency Problem and Legal Regime*

Conflict of interest between the people of firms such as managers and shareholders controllers, and the external investor such as minority shareholders used as the central analysis of modern firms, Berle and Means (1932), Jensen and Meckling (1976). The internal controller of firms' assets could use the assets in various purposes to prejudice the external investors. Simply, the external people can divert the assets of firms to themselves through indirect stealing, reducing external investors by selling stock to the internal people, excessive salary, selling asset for self interest or the other firms interest that are controlled by them with a favorable price or through transfer pricing with the other entities under their controls (Shleifer dan Vishny (1997). Besides that, the external people can use the firm assets to pursue the investment strategy that produces personal gains, such as growth or diversification without making gains to the external investors, Baumol (1959), Jensen (1986).

One of the main solutions of the agency problem is law. Law of firms and others provide the particular strength to the investors (include shareholders) to protect their investments toward taking over by the internal people. In fact, the existence of Law protection might explain why being the minority shareholders is the more feasible strategy of investment giving money to the strangers as a debt. La Porta et al. (1998), the level of Law protection of the external investor is extremely different in ever country. Law protection consists of Law protection consists of the content of legislation and the quality of enforcement. Some countries, especially the rich common law countries such as USA and the UK that provide the effective protection of minority shareholders so that the direct taking over of firm assets by a person is rarely occurs.

La Porta et al. (1998) particularly shows that the Common law countries have the best law protection to minority shareholders, while civil law countries such as France that has the weakest protection. The quality of investor protection can be recognized as a proxy to achieve the lower agency cost. This condition is a critical issue in corporate finance. For example, the company ownership is more concentrated in countries with the lower protection of stakeholder (La Porta et al. (1998), La Porta, Lopez-de-Silanes, and Shleifer (1999). Appraisal and the extent capital markets are greater in the countries with the better protection of the investors (La Porta et al. (1997), Demirguc Kunt and Maksimovic (1998). Finally, there is some evidence and perform that a good protection of investors contributesto the efficiency of human resource allocation and general economic growth (Levine and Zervos (1998), Rajan and Zingales (1998).

### *Dividend as the Outcome Model*

Dividend is the result of law protection to the shareholders based on the *outcome model*, the dividend is the result of an effective law protection toward shareholders. Under the effective system, minority shareholders use their legal powers to force companies to give cash. This condition will hinder the managers as people who use too high free cash flow and profit of the firm for their own benefit, Laporta et al (2000).

In addition, a good protection for investors makes the transfer of assets is legally riskier and more costly for the manager so that it can increase the appeal of the dividend that makes the rights of minority shareholders more protected. If there is any good protection for minority investors, the dividend relationships with investment opportunities is inverse which means that the higher funds invested then the less dividend given, and the less money invested, the higher dividend granted to investors. However, if law protection to minority investors is weak, the investment will be a bit and the dividend payout to investors is also not too high, La Porta et al (2000).

### *Dividend as the Substitution Model*

In this approach, dividend used as a substitute to Law protection of shareholders. This view depends on the needs of firms in the capital market in obtaining external funds. To get the external funds, the firms should establish reputation and the mediation of taking over the right of shareholders. One of the way of establishing reputation is paying out dividend. In order to make this mechanism work, the firms do not need "cash in" to establish reputation by stopping dividend and taking over the entire

shareholders. The firms will never require cash flow, if there is the uncertainty about the future cash flow, the capital market always provide the space, Bulow and Rogoff (1989).

In the countries of Law protection of minority shareholders, reputation is the most valuable and reliable. In result, the need of dividend in creating reputation is frequently happen in those countries. The countries of high Law protection of shareholders come about in return. The needs of reputation mechanism is weak and the lack need to pay out dividend. These views indicate that Dividend payout Ratio should be higher in the countries with poor law protection of shareholders that the countries with high law protection of shareholders. Moreover, the better prospect firms also have strong incentives to establish reputation because of having larger potential necessary to the external funds. As the result, The better growth prospect firms select a higher dividend payout ratio than the firms with poor growth prospects. However, the good prospect growth of firms also has a better use of funds then the poor growth prospects firms, La Porta et al (2000).

#### *Tax Issues*

The economists assess the effect of tax at dividend assessment, Poterba and Summers (1985). The traditional view states that heavy taxes on dividends, both corporate and personal are the strong deterrent for pay out dividends and retain profits. There are two important purposes in this view. One of them, as stated by Miller and Scholes (1978) tat investors, have the access to a variety of dividend strategies in avoiding taxes that allow them to effectively escape from the dividend tax. The purpose is not so closely related to what is actually done by investors (Feenberg (1981). Another purpose, called the new view of dividends and taxes as stated by Raja (1977) and Auerbach (1979) that the money should be paid out as dividends sooner later. Therefore, earlier paying out the dividend does not impose a greater tax on shareholders than the delay. According to this theory, the tax does not preclude the payment of dividends. Harris et al. (1997) support this new view.

#### **Research Method**

This article applies literature review based on 29 articles published in an international journal powered by Elsevier and J-Stor publisher. The sample in study is Developing and Develop countries especially in Europe and Asia. This article focuses on the application of dividend policy at the countries that related common law and civil law systems. Based on the results of several researches are reviewed and written on this paper.

#### **Result and Discussion**

##### *Dividend in Developed Countries*

In developed countries, there are different research result related to a good law protection to minority and develop firms reputation related to the tax of firms and investors. It is alike with a research by Amihud and Murgia (1997), Germany with the system of non-tax toward dividend do not face the loss because of law tax toward most of the large class of investors. However, it has found that the share price reaction to the news of dividends in Germany is similar to that found in the US. This suggests another reason beyond the tax. In addition, a research by Brockman and Unlu (2009), at 53 countries during 1990-2006 indicated that paying out dividend was significantly lower in low creditor right countries. The creditor has a right to play an important role in determining the dividend policy to the whole sample of observation. The weaker rights of creditors in a country the more likely the company will restrict the payment of dividends as a substitute for a binding mechanism.

Then Misra and Ratti (2013) find out that the dividend tax reduction eliminates multiple taxes on domestic income. The effective tax reduction on domestic investment will make foreign securities investments to be reduced. Domestic investors that the dividend is paid under a system of "dividend imputation" receives the credit to the tax paid at the firm level and reduces the effective tax. The equity investments between countries increase if the tax credit rises to taxes paid abroad.

##### *Dividend in Developing Countries*

Dividend payouts in developing countries are relatively low in line with the financial ability of firms either on the level of company profitability or the ability to get fund from the external part. It is alike with a research conducted by Indra and Tandelilin (2014) that claim payout dividend in Indonesia move down by the time. The recommendation from this research should be asked to pay the dividend. Based on the research results, the firms should be obligated to pay the dividend when the firms reach the stages. Serious actions should be carried out to the firms that do not pay dividends.

In a research carried out by Sawicki (2003) indicates that there is some evidence claims that the dividend acts as a substitute for the other firm's management which is the mechanisms before the crisis.

However, there is a strong positive relationship between the government and the emersion of post-crisis dividend. The relationship is an addition to the effect of the legal regime (legal regime) which states that the protection of shareholders at the firm level is important to force the firm to devote cash in "Outcome model" dividend.

In addition, research conducted by Pandey (2013) finds that the probability of a dividend increase, decrease and loss are high with the increase in income, decline and loss. This causes volatility in dividend payments. KLSE firms seem reluctant to eliminate dividends except when they suffer losses. The study found no evidence supporting the usual conditions, but the less stable policy is pursued by dividends of KLSE firm. Then, a research by by Setiawan and Phua (2008) summarized that the firms in Indonesia tend to pay compensation toward the weak of corporate governance by paying the dividend which is a negative effect of corporate governance toward paying out the dividend. Research confirms that there are applications of "substitution model" rather than the "outcome model" in Indonesia.

## **Conclusion**

The system of Common law is more dominant used in developed countries where the protection of the investors in stock market tends to be higher. It indicates that dividend as a outcome model. Even though, in some countries got the different result such as Netherland and Germany. In Netherlands, dividend policy is relatively low dividend policy in line with the development of firms' profitability, but the shareholders, institutional investor and manager strongly enforce and pay higher. While, In Germany claimed that the dividend policy is less affected even if the government does not impose the tax on dividend. The weaker rights of creditors in a country, the more likely the company will restrict the payment of dividends as a substitute for a binding mechanism, Laporta et al (2000).

In Civil law countries where the lack protection of government to the minority investors found that the applications of dividend policy are still different. Generally, it could be claimed that the dividend policy in developing countries is still relatively poor and weaker because of lack of government protection. The applications of dividend policy strongly related to the management of firms. The better management of the firms, the higher dividend payout to the investors. In the other part, pay out dividend also useful as the compensation of the weak corporate governance that indicates the application of Substitution Model.

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## **The Family Firm's Performance: A Literature Review**

Iswadi<sup>1\*</sup>, Said Musnadi<sup>2</sup>, Faisal<sup>3</sup>

<sup>1</sup> Department of Accounting, Faculty of Economics, University of Malikussaleh, Lhokseumawe, Aceh, Indonesia

<sup>2</sup> Department of Management, Faculty of Economics, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

<sup>3</sup> Department of Management, Faculty of Economics, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [iswadi292@yahoo.com](mailto:iswadi292@yahoo.com)

### **Abstract**

The purpose of this article was to provide the understanding of the performance of family firms, explain the relationship of family ownership and family involvement in management and the performance of firms, and to show the differences in the performance of family firms and non-family firms. The analysis carried out based on the results of the review of 29 articles published in the international journals. The selected article is an article that proves the role of family ownership and family involvement in management as a monitoring of firms in order to increase the performance of firms. The results of the literature review found that family ownership and family involvement in management improved the performance of firms. Small scale firms, middle scale firms, and young company positively contributed to the performance of firms. The performance of family firms was better than the performance of non-family firms.

**Keywords:** Family Firms, Performance of Firms, Family Ownership, Family Involvement in Management.

### **Introduction**

Financial research on the ownership of family firms is the crucial component in the capital market even in developed countries. González et al (2012) claim that the economy of countries in the world are dominated by family firms, especially in Europe, Asia, and Latin America. Culasso et al (2015), La Porta et al (1999) conducted a research about the ownership of firms in 27 countries and performs that more than 50 % of firms used as the sample were controlled by families. Faccio and Lang (2002) prove that family firms listed in France, Italy, and Germany more than 60% of firms in France are managed by founder family, Sraer dan Thesmar (2007).

The ownership structure of firms at a country is decided by the system of law. La Porta, et al (1997) argue that the countries that follow the system of Civil law with low protection toward the shareholders cause the higher ownership concentration and the consequence proportion of family ownership become higher. In return, the common law countries with protection for large shareholders tend to occur high dispersion of the ownership structure. Al-Saidi and Al-Shammari (2015) add the characteristic of firms in developed countries such as America and England are distinguished based on the ownership of dispersion stock, while the characteristic of firms in Europe, Asia and other developing countries are distinguished based on the concentrated ownership. The opinions above indicate that the ownership of firms in a country that included family firms are decided based on the law of the country.

Family control of company determines the performance of the company. Marcelo et al (2014) said that the studies on the performance of the family firms are growing very rapidly. The family is a common class and important for investors around the world which provides unique additional value to the firm thereby increasing the financial performance of firm and stock market. The approaches are used to examine the theory that the role of the family in influencing the performance of companies. Some of the theory are agency theory (Villalonga and Amit, 2006), (González et al, 2012), (Al-Saidi and Al-Shammari, 2015), agency theory and the theory of institutions, (Jiang and Peng, 2011), agency theory and asymmetry of information, (Martínez-Ferrero et al, 2016), agency theory and stewardship (Basco, 2013), agency theory and efficient monitoring hypothesis, (Alipour, 2012).

Based on the perspective of agency theory, mixing the ownership with management has some advantages that cause the appropriateness between stakeholder and manager, and some studies indicate that family firms have better performance than non-family firms even included the minority of

stakeholders, (González et al, 2012). The family involvement in management attracts the researcher in family firms yet performs a different performance implication, (Kim dan Gao; 2013). International evidence about the family ownership is various, (González et al, 2012). The researches about family firms examine the agency theory in developed countries and produce different results. Basco (2013) for example, examines the agency theory about the effect of family firms toward firms' performance and donot fully support the agency theory. Similarly with the research conducted by Villalonga and Amit (2006) that less support the agency theory on the family firms. Otherwise, Martínez-Ferrero et al (2016) conducted a research about family firms of 1.275 nonfinance companies listed in 20 countries during 2002-2010 was successful supporting the agency theory.

The contradiction result of research about the agency theory on family firms also occurs in developing countries. Jiang and Peng (2011) examines the agency theory and institution at 744 family firms listed in 8 countries in Asia in 1996 (Hong Kong, Indonesia, Malaysia, Filipina, Singapura, Korea Selatan, Taiwan, and Thailand) found that there were not any concrete evidences which claime that family firms are good, bad or irrelevant in influencing performance of company. In the other word, the examination of agency theory at family firms is unproven to all the country in Asia. Otherwise, a research by Alipour (2012) in Iran supports the agency theory and the efficient monitoring hypothesis. González et al (2012) also conducted a research about the family firms in Columbia that supports the agency theory.

The dimensions used in examining the involvement of family firms are the ownership of family stock, involvement of management, and control mechanism, (Villalonga and Amit; 2006), the ownership of family members stock, the family involvement on head manager, and the involvement of family members on board director, (Chu; 2011), family monitoring, family ownership, and family management, (Audretsch et al; 2013), the ownership of family members stock at least 20% and the involvement of family members in top management of firm, Vieira (2014), the involvement of family members in management, the ownership, and control, (González et al; 2012).

This article discusses the relationship between the ownership of family, the involvement of family in management and firm performance. This article also discusses the performance of family firms comparing with non-family firms, and the implication of size and the age of firms toward performance. This article is important to provide the description whether family ownership and family involvement in the management becomes a monitoring tool to improve the firm performance and whether these two variables can be the mean of monitoring in all size and age of firm?

## **Research Method**

This article applies literature review based on 29 articles published in an international journal. The selected articles for review should perform the results of family ownership and the involvement of family in management that contributes positively and significantly on the performance of firms. The article should also prove the relationship between family firms and the involvement of family firms in the management of the firm to illustrate the role of family ownership and the involvement of family in management as the monitoring of firms. Most of the articles obtained from Emerald, Elsevier, and Springer.

This article focuses on the relationship between family ownership, family involvement in management and firm performance, the influence of size and age of family firms toward performance, and the differences between family firms and non-family firms. The results of various researches are reviewed and described in an article.

## **Results and Discussion**

### *The Definition of Family Firms*

The researchers define family firms in various criterias. Generally, the researchers deal with the definition of family firms in which the ownership of firm stock owned by the family, and the family members involve in firm management. Villalonga and Amit (2006) define family firms as the firms that involve the founder or family members as the employee, director, or the owner of equity at least 5 % either individually or in a group. Anderson and Reeb (2003) define family firm in which the founder either the generation or son or daughter in law act out as the employee, director or the owner of a block either individually or in a group. Audretsch et al (2013) define a family firm as family monitoring, family ownership, and family management. González et al (2012) define a family firm as the involvement of family in management, ownership, and control. Martínez-Ferrero et al (2016) define a family firm as the family ownership more than 10% of voting either individually or in a family. Culasso et al (2015) define a family firm as participation that is controlled by the owner of family capital and the existence of family member minimally a family member in Board director.

Martin-Reyna and Duran-Encalada (2012) define family business as the firms those are controlled either directly or indirectly by the family members based on the criterias; the existence of founders or their generation in management in which the vote more than 20 %, of the stockholders is CEO or the key member in board, the member of board at least has relationship one another, and the first stockholder and related group have more than 50 % vote in the firm. Vieira (2014) claims that family firms as the firms which are controlled by founder family or family members around 20% or more, and involve in the top management of the firms. Jiang and Peng (2011) define the family firm as the firm that owned by a family or family members as the greatest owner. Achmad et al (2009), family firm is the ownership of family more than 20 % of the vote either individually or in a group, and the family members are the biggest block controller in the firm.

The ownership of the family firms are not only measured through direct ownership but also through indirect ownership. Indirect ownership is measured through the pyramid ownership .González et al (2012), the pyramid ownership, Audretsch et al (2013). Pyramid ownership is the ownership of the stock majority of the firm at the turn also owns majority stock in the other companies, González et al (2012). Through the ownership, the family may carry out monitoring toward the firm. Monitoring by the ownership of stock also could be carried out by the involvement of family members in board supervision and management, Audretsch et al (2013) dan Surifah (2013).

#### *Family Ownership and Firm Performance*

Muttakin et al (2014) conducted a research about family firms, family generation, and firm performance at 141 non-financial firms in Bangladesh during 2005-2009 with the observation sample 705 proven that family ownership influenced positively toward firms performance. While family/personal becomes the great stockholder, they will follow their family concerns, then the other stockholders. Family/personal could be better in monitoring toward family firms and could reduce the agency cost. (Al-Saidi dan Al-Shammari, 2015). Arouri et al (2014) claimed a positive influence of family ownership toward bank performance that indicated that family wealthy were positively related to the prosperity of family ownership, family members were motivated to improve theirs wealthy through the improvement of family performance.

Martínez-Ferrero (2016) proved that family ownership relates to a greater controlling and monitoring toward the managerial decision in order to avoid information asymmetry and to prevent the risk of decretion. Martin-Reyna and Duran-Encalada (2012) proved that family ownership influenced positively toward firm performance.

#### *The involvement of Family in Management and Firms Performance*

Villalonga and Amit (2006) documented that family management improved the value of firms if the founders act out as the CEO of family firms and decreases the value of firms if the founders act out as a chairman or CEO. Chu (2011) proved the relationship between family ownership and firm performance is awaken if the family members are not involved in management. Basco (2013) indicate that the involvement of family in family management influences positively toward decision making through family orientation strategy. Poutziouris et al (2015) proved that the involvement of family in management increases firms performances. Jiang and Peng (2011) stated that the existence of family CEO relates positively to the firm's performances. In Indonesia and Taiwan, González et al (2012) proved that most of the family firms have better performance than nonfamily firms if the founders are involved in the operations of the firms. Chu (2011) stated that the relationship between family ownership and firm performance is awakened while the family members do not involved in firms managements.

#### *Size, Age and Performance of Family Firms*

Culasso et al (2015) stated that family firms with a small market capitalization index are better than non-family firms toward all economy indicators and financial ratio. González et al (2012) proved that the involvement of family positively toward firms performance at a small and young firms, especially while the founder is getting responsibility. Vieira (2014) claimed that the age of firms influenced negatively on the market to book ratio.

Hereinafter, González et al (2012) found that the influences of famly firms are diluted and changed to be negative toward firms performance at the larger family firms or firms managed by heirs. Culasso et al (2015) stated that a middle-level company, family existence are the relevant variables to achieve a better performance than the same level non-family firms performance. If the firms carry out a great expansion, the existence of family is the irrelevant variable in influencing profit and financial Leverage (Loan Ratio).

### *Performances of Family Firms and Non-family Firms*

Halili et al (2014) proved that family firms with concentrated ownership structure have better performances than non-family firms. Marcelo et al (2014) documented family firms in Portugal and Spain mostly have better performances than nonfamily firms. Pindado et al (2014) found the empirical evidence on family firms that has better performances than non-family firms. Vieira (2014) found that family firms have better performances than nonfamily performances that were examined by using the market to book ratio. Culasso et al (2015) said that family firms have better performances than nonfamily firms that were analyzed by using ROA, ROI, and ROS. Muttakin et al (2014) proved that family firms have better performances than nonfamily firms. Singapurwoko (2013) indicated that non-family company had better performances than family firms.

Family firms have better performances than non-family firm because the family ownership causes a stronger discipline and encourage the non-family manager to carry out beneficial efforts, (Martinez-Ferrero et al, 2016). Family monitoring describes the behavior of family in protecting family assets in order to get a better performance (Audretsch et al, 2013).

### **Conclusion**

General family firms are founded in all country either in developed or developing countries. Family or family member involves in the firm ownership and management. The family ownership of equity positively influenced on the firm performance and the involvement family in the management of firms positively contributed on the firm performance. These results illustrate that family ownership and family involvement in management can be used as a mechanism of monitoring enterprise. These results are not consistent with research by Alipour (2012) which proves the ownership of the family or individual negatively influenced on the performance of the enterprise, and Kim and Gao (2013) which proved that involvement of family in management had no direct impact on the enterprise performance.

Generally, the performance of family firm is better than nonfamily firm that caused by the conformity of management and motivation with the owner in order to protect the firm asset to achieve the welfare of family members. Small scale, middle scale and young family firms positively influenced on the performance of firms. This results are consistence with the research conducted by Achmad et al (2009) which proves that the performance of non-family firms were better than the performance of family firms. Based on the results of the review provides the evidence that the owner-agent conflict does not occur in a family firm because of the suitability of interest both of them. The next study hopefully could be focused on the conflict of majority owner with a minority owner of the family firms. The family owners are suspected of committing the expropriation of minority shareholders.

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## **Critical Theory And Accounting Research: A Critical Review**

Irsyadillah\*

Department of Economics Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh, Indonesia

\*Corresponding author: [irsyadillah@unsyiah.ac.id](mailto:irsyadillah@unsyiah.ac.id)

### **Abstract**

This paper is concerned with providing a literature review regarding the use of critical theory in accounting research. In Indonesian context, critical theory has not commonly used to study accounting issues. The fact that both accounting practice and accounting education continue to be undermined by a single prevailing worldview coming from Anglo-American capitalism promoting maximizing shareholder value. The paper argued that given the underlying context of accounting, critical theory is so important to be adopted in order to be able to challenge the prevailing dominant paradigm and at the same time providing alternative perspective. The alternative could be even developed according to indigenous cultural and ethical values. More importantly, critical theory is about providing liberation and emancipation including in the area of accounting.

**Key words:** critical theory, accounting research, liberation, emancipation,

### **Introduction**

It cannot be denied that inequalities still exist in societies including in area of accounting. For example, accounting education has continuously become a contested arena, whereby many different parties attempt to promote their interests and viewpoints. Accounting education has become a terrain of socialisation of ideological values coming from Anglo-American capitalism, in which the main objective of business is introduced to promote maximisation shareholder value (MSV) discourse. However, 'many accounting academics seem to accept this, unquestioningly, as a natural and self-evident ideology. Nonetheless, the most crucial fact is that accounting students are greatly indoctrinated with that only ideological perspective. They are not provided with alternative ways of exercising critical abilities that can be used to challenge the power asymmetries underpin the dominant perspective existing within accounting education that they encounter (Fergusson et al., 2011). This means accounting students are trained to accept and not to speculate the status quo. Consequently, accounting education produce accountants who will only judge a favourable business performance solely based on profit maximisation.

Regarding accounting practice, Cooper (1995) has argued that 'accounting, with its roots in the economic, made it a perfect tool for use by the new right in presenting certain understandings of the world and in closing off alternative understandings' (p.117). Therefore, it is reasonable to take an analytic position that engages with accounting as a technology that has been occupied by capitalist ideology. Indeed, the birth of double-entry bookkeeping has been considered as a significant milestone in the development of capitalism. In this regards, the main concern of critical theory is to problematize the worldview underpinning accounting. Therefore, the purpose of this paper is to discuss the importance of critical theory in accounting research. In particular, this paper attempts to discuss critical theory and its connection to accounting research in Indonesian context. The following section addresses critical theory by elaborating how was it emerged? And how it is understood in literature? This is then followed by the discussion on how critical theory has informed accounting research and the justification for using critical theory in accounting research. The penultimate section draws the conclusion.

### **Literature Review**

The enlightenment era in Europe started at the end of the eighteenth century when the societies began to believe that religions and traditions as a foundation of social life should be replaced with priority of science and reason (Kincheloe & McLaren, 2011). At that point, critical theory started to emerge and it is usually referred to Institute of Social Research at the University of Frankfurt where the early ideas or the first generation of critical theories were developed (Kincheloe & McLaren, 2011; Laughlin, 1995). Marxist Carl Grunberg, an Austrian Professor of political economy, was the director of the Institute of Social Research when it was established in 1923, the period between World War I and World War II

(Held, 1980). Actually, the main reason for establishing the Institute was to protect the elimination of left-wing ideas as the left-wing leaders including Karl Liebknecht and Rosa Luxemburg were murdered in 1919 (Held, 2003). In its beginning, Kant's, Hegel's, Weber's and Marx's philosophies and social thoughts, based at the Frankfurt school, became the basis of critical theory (Kincheloe & McLaren, 2011).

However, according to Hoque (2006) it was due to Horkheimer, from 1923 critical theory began to grow. It was when Max Horkheimer led the Institute of Social Research in 1930, critical theory was officially formed. At that time the figures, such as Theodor Adorno and Herbert Marcuse were also based in Frankfurt School. They were known as the first generation of Frankfurt school of critical theorist (Held, 1980). Nonetheless, after a decade of establishment of Frankfurt school, those critical theorists had to leave Germany because the Jewish membership was in danger as the Nazis took control the country (Kincheloe & McLaren, 2011). They went to America and settled themselves in California where they were shocked by American culture. However, they were challenged to respond on the empirical practices of American social science researchers. In 1953, Horkheimer and Adorno returned back to Germany and again built the Institute of Social Research, whilst Herbert Marcuse kept living in the United States, trying to gain the recipients for his work in social theory (Kincheloe and McLaren, 2011). Critical theory is then continually developed, so that, figure, such as Jurgen Habermas and Axel Honneth and Seyla Benhabib appeared to be the most influential and well known critical theorist (Kincheloe & McLaren, 2011).

Critical theory is built on philosophy of Marxism trying to enlighten people's behavior of receiving the world as normal, which is actually full of inequalities and systemic exploitation of minority by majority (Kincheloe & McLaren, 1997; Hoque, 2006). According to Kamla (2005) critical theory stresses on the significance of questioning and challenging. Nowlan (2001, p.2) explains that 'seeming obviousness, naturalness, immediacy, and simplicity of the world around us, and in particular, of what we are able to perceive through our senses and understand through the application of our powers of reason'. For Alvesson and Willmott (1996, p.13) 'the intent of critical theory is to challenge the legitimacy and counter the development of oppressive institutions and practices'. Likewise, Laughlin (1987, p.482) 'critical theory is a vehicle through which understanding about reality can be achieved and transformation of concrete institutions occurs'. In the same fashion, Chua (2004, p.259) explained that 'a critical theory, then, is a reflective theory which gives agents a kind of knowledge inherently productive of enlightenment and emancipation'.

It seems difficult to answer question what exactly critical theory is due to (a) there are many critical theories, not just one; (b) the critical tradition is always changing and evolving; and (c) critical theory attempts to avoid too much specificity, as there is room for disagreement among critical theorists (Kincheloe & McLaren, 2011). However, according to those critical theorists mentioned in the previous paragraph, the role of intellectual within critical theory is to provide social imaginary by using powers of reasons. The legitimacy of status quo should be challenged in order to create radical change, enlightenment and emancipation, and to free people from oppression, domination and exploitation. Nonetheless, there is no guarantee of change to society and its institutions. It is through exploiting repression, unfairness, asymmetrical power relations, and by opposing and challenging the status quo, critical theory creates possibility for dynamic transformation and development of marginalized people. Actually, the distinctive characteristic of critical theory is its inability to create transformation, although the transformation is flexible and not static, because the transformation process should be aligned with the social circumstances, perspective, and the nature of the problem (Kincheloe and McLaren, 2011; Kamla et al., 2012).

However, instead of being a specific theory, critical theory is considered as a broad framework used for an intellectual movement through which social justice and social change can be pursued and emancipated (Kincheloe & McLaren, 1997). The theory tries to elaborate the present generative issues and identify the real themes with the purpose to modify, emancipate and empower it. In this context, critical point of view is employed in order to obtain practical goals for social changes, emancipations and empowerments (Hoque, 2006). It proposes a variety of perspectives drawing from multi disciplines and cultures to offer a focus on study that eliminates obstacles generated by established academic disciplines (Falconer, 2004). Therefore, it is contended that a critical social theory project needs a group, supra-disciplinary synthesis of philosophy, the science and politics that aims for radical social-political changes and transformation (Kellner, 1989). In this sense, critical theory is absolutely vital to be employed in educational, business and accounting studies, art, philosophy, or literature (Burrell and Morgan, 1979).



It is clear that critical theory is a methodological tool that can be employed to discover and understand the reality and to mediate ideas and reality. Moreover, it is a way to scrutinize the current practice and institution by questioning the claim of authorities (Falconer, 2004; Houqe, 2006). This means that the status quo of domination of particular ideology in certain circumstances or the global world order could be challenged and criticized by employing critical theory. It has ability to criticize ideological frames that used to make sense of the world (Gallhofer & Haslam, 2003; Kamla, 2012). In general, critical theorist have '(a) advocated research that empirically investigate expressions of dominating systems of thoughts in particular communicative situations rather than explain outcomes; (b) refrained from directive statements regarding what people should do (revolt, liberate) but while emphasizing the problematization of dominating belief and values; (c) recognized pluralistic qualities, while still insisting that there are strong asymmetries between various interests and perspective; (d) treated ideology as dominating without seeing them as a simple instrument or in the interest of elite group (Gallhofer & Haslam, 2003). However, it is important to note that in order to achieve its goals, gaining positive transformation, emancipation and enlightenment, critical theory must not only stress on negatives and failures, but it also needs to evaluate all situations with their strengths and weaknesses.

### **Research Method**

This paper is based on reviewing the literature including books and journal articles. This process was conducted through searching the relevant literature with the help of google scholar. There were many books and journal articles discussing critical theory in its own and its connection with accounting issues. For the purpose of this paper, the two sources of literature were extensively used in order to form a complete picture of critical theory and its relation to accounting. However, there were no relevant sources specifically discussing accounting issues with critical theory as the theoretical framework in Indonesian context. Therefore, this paper is greatly beneficial for engaging accounting issues and critical theory in Indonesian circumstances.

### **Results and Discussion**

As explained in previous section that critical theory is able to empower, enlighten and emancipate societies from their particular oppressive circumstances (Dillard, 1991). This is why critical theory is important to be employed in accounting research because the status quo of accounting is problematic. The current accounting features are unable to solve the social problem caused by business activities and, more importantly, it should not be expected to unravel social problem by itself (Boyce, 2000). Therefore, critical theory has become more influential in accounting research in the last two decades (Gallhofer and Haslam, 2003; Hoque, 2006). For example, Baker and Bettner (1997) stated that it is absolutely essential that accounting research adopts a critical perspective, because, in so doing, there will be potential for accounting emancipation attempting to distance accounting practice and disclosure from being repressive (see also Gallhofer and Haslam, 2003). Regarding change and emancipation through critical theory, Laughlin (1995) explained that everything is basically required for change because there is nothing satisfactory that should be maintained, but that something may need for change while others need to be continued. In addition, Gallhofer and Haslam (2003) elucidated what does it mean by emancipatory vision of accounting.

'A vision of accounting as an emancipatory force is consistent with seeing accounting as a communicative social practice that functions as a system of informing that renders transparent and enlightens with the effect of social betterment. It is a vision in which a progressive community comes to control accounting rather than be controlled by it, a reflection of a proper accountability' (p.7).

Notwithstanding, accounting has been generally accepted to be mostly in the form of number, double entry and budget, so it just looks something technical (Morgan, 1988). Conventional accounting function, bound within limited requirements of the entity and boundaries, has been in huge critics (Tinker, 1985). In fact, accounting affects broad stakeholders, people's attitudes, ways of thinking, and behaviour. Therefore, accounting should be viewed comprehensively because it not only relates to financial matters but also social issues and problems. Gallhofer and Haslam (2003, p.6) contend that:

'Accounting has been theorised critically, interpretively, and contextually as a social practice that is problematically constituted and has problematic consequences.'

Moreover, Laughlin and Lowe (1990, p.7) deemed that accounting is:

'a formal system with structural and behavioural characteristic whose terms are expressed in fundamentally financial form, whose meaning is derived from the organisation of which it is an integral part.'

This means accounting is not restricted within economic focus concerning on making shareholders wealth. Thus, it is argued that these accounting views are not narrow but comprehensive. It is important to note that this rises in critical accounting thoughts.

All in all, discussing critical theory is all about resistance to capitalism, this is also a particular aim of using critical theory in accounting. If it is not rejected that the domination of capitalist power is manifestation of capitalist countries, so accounting, which is viewed no more than just a technical and context free activity, is a technology of capitalism determined by capitalistic power and advocated by professional ideology (Braverman, 1974; Lehman and Tinker 1987). In many ways the global world today strengthening capitalism with its free market ideology is basically the roots of critical research (Kincheloe and McLaren, 2011). Therefore, critical accounting research is empowered in order to oppose this assumption which is based on capitalist system. Inimically, the capitalist system also constructs accounting to be around the privileged in the social order. As highlighted by Gray et al. (1996, p.63):

'...the very way in which society is ordered, the distribution of wealth, the power of corporations, the language of economics and business and so on, are so fundamentally flawed that nothing less than radical structural change has any hope of emancipating human and non-human life. The social, economic and political systems are seen as being fundamentally inimical'.

It can be concluded that the fundamental purpose of using critical theory in accounting research is to achieve liberation/emancipation, meaning the intention is to promote changes of conventional accounting to a new accounting (see Boyce, 2000; Gallhofer and Haslam, 2003; Gray, 2002; O'Dwyer, 2002). Catchpole et al. (2004) elucidated that the thing that distinguish critical theory in any context of social analysis is to reveal the alienation, domination and exploitation of human practices, cultures and relations. In particular, critical theory is concerned with power operated to dominate and build the consciousness in which accounting is a political technology of capitalism that promotes shareholder primacy. Collison et al (2011) stated that the shareholder superiority of Anglo-American capitalism is indeed the real character of Anglo-American accounting tradition that focuses decision important information for investors (Collison et al., 2011). Therefore, accounting is basically pushed in order to preserve and enhance the capitalist's control over the means of production (Dillar, 1991). However, critical theorists have noted that power topic is extremely complicated and ambiguous required comprehensive and detailed study and analysis (Kincheloe and McLaren, 2011).

Accounting education is an area that is significantly relevant for using critical theory. It is expected that by using critical theory, the status quo in accounting education will be challenged for liberation and emancipation and thereby produce alternative thoughts and outcomes. More importantly, accounting education is in the process of reform concerning on the perceived insufficiency in relation to its narrow boundaries, functionalist dominance of the discipline (Johns, 1996; Nelson, 1995). Moreover, one of the present concerns in accounting education is the tendency to inculcate students with a particular worldviews, Anglo-American capitalism promoting Maximizing Shareholder Value (Collison et al., 2011; Ferguson et al., 2011). Thus, through critical theory, there will be potentiality to challenge and oppose the dominant and ruling power imposing particular ideology in accounting education. However, it is argued that the hegemony position is still unclear showing by significant unevenness and ambiguity. Additionally, Boyce (2000) contend that although this position is ideologically, materially and culturally supported and even backed up by the state power and multilateral institutions, it still remains contingent.

This particular critical accounting research needs to be considered in Indonesia because the Indonesian context in relation to social, politic, economy, geography and history is particularly significant. As generally known that accounting profession and accounting system in Indonesia was originally built by Western colonialism. The advent of independence did not necessarily mean the control was withdrawn with formal decolonization. In fact, this is being continually shaped by current ruling and dominant power under the auspice neocolonialism and globalization. Hence, the present Indonesian accounting education, that should actually be reflected local values and context, is extremely in line with Western ideology. Therefore, it is argued that one of critical theory, postcolonial theory is a useful and accurate way of developing the critical approach to examine the status quo of accounting education in Indonesia. The present Indonesian accounting education is required to be challenged because it will probably provide opportunity for indigenous people to promote local values (constitutional and religious values).

Thus, inequality and marginalization of local values in accounting education will be halted. More importantly, this will bring chance for local accounting students to understand and experience local values and perspectives in the context of accounting. Boyce (2004) reported that in order to make accounting education more relevant to its socio-historical context and, in particular relevant to the lived experience of student, teaching and learning should be conducted 'outside the circle'.

## Conclusion

This paper has elaborated the importance of critical theory in accounting research. The discussion starts by reviewing the critical theory which emerged to be the basis of criticizing, challenging and opposing the status quo of the world that is full of social, economic and political marginalization and inequality. The need for disrupting the status quo according to critical debate is in order to make radical change, enlightenment and emancipation, and to free people from systemic exploitation and oppression. One area of accounting research is accounting education. This paper in general supports the contention that globally and, in particular, Indonesian accounting education is subjugated by certain dominant power imposing particular worldviews, Anglo-American capitalism, promoting Maximizing Shareholder Value (MSV) (see Collison et al., 2011; Ferguson et al., 2011). It is argued that those representations are the consequence of colonization, neocolonization and globalization. That directly or indirectly facilitates former Western colonizers to expand imperialistic and capitalistic view which is not only to the countries colonized in the past, but also throughout the world touching even non-colonized world. Therefore, this paper proposes the argumentation that critical theory of the stages of global expansion can provide insightful lens in renewing our understanding of the ideological domination in the accounting education that, in effect, shapes the accounting profession and the practice of accounting in ex-colonial country, particularly Indonesia.

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## **Determinants of Budgeting Consistency in Local Government– A Case of Sabang Local Government Indonesia**

Heru Fahlevi<sup>1</sup>, Islahuddin<sup>2</sup>, Didi Wahyudi<sup>3</sup>

<sup>1,2</sup> Economics and Business Faculty, Syiah Kuala University

<sup>3</sup> Post-graduate program, Syiah Kuala University

\*Corresponding author: [hfhahlevi@unsyiah.ac.id](mailto:hfhahlevi@unsyiah.ac.id)

### **Abstract**

This study aimed to examine the influence of human resource quality, budget politics and budget transparency for consistency of budgeting process with the leadership style as a moderating variable. The population of this study were all local government work units in (*satuan kerja*) kota Sabang – Aceh. The respondents were 163 people that consist of 34 budgeting information users and 129 head of divisions and subdivisions. The data used was primary data which was collected through questionnaires. The data analysis was performed using a regression model of interaction or moderate regression analysis (MRA). The results showed that the quality of human resources, budget politics, budget transparency and leadership style has positive influence on the consistency of the budgeting process either simultaneously or partially. The leadership style partially strengthen the link between the quality of human resources, budget politics and transparency with the consistency of the budgeting preparation process.

**Keywords:** Government budgeting, budget politics, transparency, style leadership and budgeting process.

### **Introduction**

Implementation of the Indonesian Government Regulation No. 8 of 2008 regarding stages, Procedures for Preparation, Control and Evaluation of the Implementation Plan for Regional Development is expected to ensure democratic, transparent, accountable, efficient and effective regional planning in governmental budgeting. In this context, consistency of proposed budget and approved can be one of the determinants of the performance of local governments. The program proposed by local government working task unit (*Satuan Kerja Pemerintah Daerah*) in the proposed budget, which have been designed and proposed based on the vision and mission of the local governance, might be substantially replaced by the legislative who try to align their political interest to the proposed budget.

A synchronization between the budget document proposed by SKPD and document KUA-PPAS (*Kebijakan Umum Anggaran (KUA) -Prioritas Plafon Anggaran Sementara (PPAS)*), revised by the executives is one of budgeting step in all Indonesian local government. However, the synchronization is not easy as there is political interest negotiated between the parties. Infact, it is often found that program proposed by SKPD are shifted from one activity to another, which in turn lead to a significant difference between the budget document and the document KUA PPAS. As a result, major revision is required and thus lengthen the budgeting process.

Since 2014 Sabang local government experienced delays in its local budget revision. The delay have an impact on the lack of uptake of the budget realization for each program and activities that have been budgeted for each Work Unit (SKPD) resulting from budgeting programs and activities that are no longer in line with the planning document that has been agreed between the executive and the legislatures. The capacity of human resources that affect the synchronization of the budget document by document KUA-PPAS. This matching is performed by Amiruddin (2009), Iskandar (2013), who found the result that the quality of human resources affect the synchronization of the budget document by document KUA-PPAS.

Political budgeting has been applied from the era of the former government, the new order, for example, as disclosed by Thontowi (2007) that the regions often are not supporters of the ruling party would not be touched development, so if an area wants to forward the leaders of the area should be incorporated in the ruling political party to preserve the power of certain groups. According to (Loina 2005, p.7)

transparency is a principle that guarantees access or the freedom for everyone to obtain information about government administration, information on policies, the process of making and implementation and the results achieved, while the meaning of information is information about every aspect of government policy that are accessible to the public.

To motivate subordinates perform their responsibilities transactional leaders rely heavily on a system of rewards and punishment to his subordinates (Krishnan and Srinivas, 1998, p.4). Instead Burns (1978) states that the model of transformational leadership is essentially emphasizes a leader needs to motivate subordinates to perform their responsibilities more than they expect. A transformational leader must be able to define, communicate and articulate the vision of the organization, and subordinates must admit (Krishnan and Srinivas, 1998, p.4), so that the model of transformational leadership style will provide a strong influence on the consistency of the budget process because subordinates are motivated to achieve accordance with the planning objectives set.

This study aimed to examine the influence of human resource quality, budget politics and budget transparency for consistency of budgeting process with the leadership style as a moderating variable. Furthermore, on the literature review section at the concept of consistency of the budget process, the notion of the quality of human resources, budgeting politics, transparency and leadership style are elaborated. Following that, research methods is presented in section four including population, analytical methods, and the operationalization of variables. While in the discussion will be explained on the hypothesis testing results and discussion of the results penelitian. In the next section will explain the conclusions, limitations and suggestions about the study.

## **Literature Review**

### *Consistency concept Budgetary Process*

Consistency is fit or match between programs and activities in KUA-PPAS, with programs and activities in APBK Qanun (Arniati, 2010). It aims to implement the cornerstone of the regulation on budgeting mechanism that has been set in a number of laws and regulations.

### *Quality Human Resources.*

According to Schultz (1961), founder of the theory or concept of basic human capital (human capital concept), considers that human capital is a form that is reflected in the form of knowledge, ideas (ideas), creativity, skills and productivity of labor. The quality of human resources according to Rucky (2003, p.57) is the level of knowledge, ability, and willingness can be shown by human resources. The rate was compared to the level required from time to time by the organization that has human resources stretcher.

### *Politics Budgeting*

Political according to Hague et.al (1998) is the political activities regarding how groups reach a decision that is collective and tying in with the attempts to reconcile the differences between the member-member. Based on the explanation of the concept of political and budgeting it is the politics of budgeting is how to achieve the goal of a collective nature and binding through power, decision making, public policy, allocation and distribution of the translation process planning activities into the financial plan (Arniati et al., 2010 ).

### *Transparency*

Transparency can be defined as the implementation of tasks and activities that guarantee access or the freedom for everyone to obtain information about government administration. Information about the policy-making process and implementation and the results achieved can be accessed or obtained by people with a good and open (Mardiasmo, 2002). Sumarsono (2003) defines transparency as the openness of government policy making local fiscal policy, so that it can be seen and monitored by the legislature and the public. Transparency of financial pengelolaan area will eventually create horizontal accountability between local governments and their communities, so as to create local government that is clean, effective, efficient, accountable and responsive to the aspirations and interests of the community.

### *Leadership style*

Burns (1978) states that the model of transformational leadership is essentially emphasizes a leader needs to motivate subordinates to perform their responsibilities more than they expect. A transformational leader must be able to define, communicate and articulate the vision of the organization, and subordinates must accept and recognize the credibility of its leaders. Bass and Avolio

(1994) states that the transformational leader is the charismatic leader of character and has a strategic role in bringing members and the organization achieve its objectives.

Following are the hypotheses that tested in this study:

Ha(1): The Influence of Quality of Human Resources, Political Budgeting, transparency and consistency of leadership style for the Budgetary Process

Ha(2): The Influence of Quality of Human Resources for the Budgetary Process Consistency

Ha(3): The influence of Budget politics for the Budgetary Process Consistency

Ha(4): The Influence of transparency for the Budgetary Process Consistency

Ha(5): The Influence of Leadership Style for the Budgetary Process Consistency

Ha(6): The Leadership Style strengthen the Relationship between the Quality of Human Resources and the Budgetary Process Consistency

Ha(7): The Leadership Style strengthen the Relationship between Political Budgeting and the Budgetary Process Consistency

Ha(8): The Leadership Style strengthen the Relationship between the Consistency Transparency and Budgetary Process Consistency

### Research Method

The population in this study are all SKPD which includes offices, departments, and agencies that exist in the City of Sabang which consists of 34 SKPD. The respondents were 163 people that consist of 34 budgeting information users and 129 head of divisions and subdivisions. The data used was primary data which was collected through questionnaires. The data analysis was performed using a regression model of interaction or moderate regression analysis (MRA). The regression equation of interaction or Moderate Regression Analysis (MRA) is as follows:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_1 X_4 + \beta_6 X_2 X_4 + \beta_7 X_3 X_4 + \epsilon$$

Y = Budgetary Process Consistency

X<sub>1</sub> = Quality human resources

X<sub>2</sub> = Politics budgeting

X<sub>3</sub> = Transperancy

X<sub>4</sub> = Leadership Style

$\alpha$  = Contants

$\beta_{1,2,3,4}$  = The regression coefficient

$\epsilon$  = error term

In this study, researchers used the independent variables and the dependent variable. The independent variables consist of the quality of human resources (X<sub>1</sub>), political budgeting (X<sub>2</sub>), transparency (X<sub>3</sub>), and the style of leadership as a moderating (X<sub>4</sub>) while the dependent variable is the consistency of the budget process (Y).

To test the effect of independent variables X<sub>1</sub> is the quality of human resources, X<sub>2</sub> is political budgeting, X<sub>3</sub> yaitu transparency, and X<sub>4</sub> is the style of leadership both simultaneously and partially on the dependent variable (Y) is the consistency of the budget process, to test the quality of the data that is test validity , reliability test, test the classical assumption of normality test, heterokedatisitas and multicollinearity test.

### Result and discussions

Results of testing the hypothesis

Results of testing the hypothesis by using SPSS program can be seen in Table .1

**Tabel. 1**  
**Hyphotesis Test Result**

Model	Standardized Coefficient $\beta$	Std. Error
(Constant)	1.138	14.124
Quality human resources	0.712	4.607
Budget politic	0.827	2.901
Transparency	0.661	3.859

Leadership style	0.487	3.322
Quality human resources*leadership style	0.419	1.086
Budget Politic* leadership style	0.790	0.696
Transparency* leadership style	0.509	0.904

Based on Table 1, can be obtained by multiple linear regression equation as follows:

$$Y = 1.138 + 0.712X_1 + 0.827X_2 + 0.661X_3 + 0.487X_4 + 0.419 X_1.X_4 + 0.790 X_2.X_4 + 0.509 X_3.X_4 + \epsilon$$

Correlation coefficient (R) 0,483

Determination coefficient (R<sup>2</sup>) 0,711

The results of the research hypothesis testing constant value of 1.138, then from the regression equation in the table 1 can be seen the following results:

- 1.The correlation coefficient (R) of 0.843 indicates the degree of relationship (correlation) between the independent variable on the dependent variable amounted to 84.3.
- 2.The coefficient of determination (R<sup>2</sup>) of 0.711, indicating that variations (changes) that occur in the consistency of the budget process variable (Y) by 71.1% due to the changes that occur together on the variable quality of human resources, political budgeting, transparency and leadership style.

The test results together indicate the regression coefficient ( $\beta$ ) of each variable is 0.712 to the variable quality of human resources ( $\beta_1$ ), 0.827 to budget politic ( $\beta_2$ ), 0.661 for variable transparency ( $\beta_3$ ), and 0.487 for the variable force leadership ( $\beta_4$ ). Determining hypotheses mentioned if at least one  $\beta_i > 0$  (i = 1,2,3,4):  $H_a$  accepted, meaning that the quality of human resources, budgeting politics, transparency and leadership style together positive effect on the consistency of the process of drafting budget.

The test results showed the regression coefficient ( $\beta_1$ ) to the variable quality of human resources is equal to 0.712 This means that any increase in the variable quality of human resources at one point, the consistency of the budget process would increase by 0.712. Determining hypotheses mentioned if  $\beta_1 > 0$ :  $H_{a2}$  accepted, meaning that the quality of human resources positively affects the consistency of the budgeting process.

The test results showed the regression coefficient ( $\beta_2$ ) for political variables budgeting amounted to 0.827 This means that any increase in political variables budgeting at one point, the consistency of the budget process would increase by 0.827. Determining hypotheses mentioned if  $\beta_2 > 0$ :  $H_{a3}$  accepted, meaning budget politics has a positive effect on the consistency of the budget process.

The test results showed the regression coefficient ( $\beta_3$ ) for variable transparency amounted to 0.661 this means that any increase in the variable transparency of the figures, the consistency of the budget process will be increased by 0,661. Determining hypotheses mentioned if  $\beta_3 > 0$ :  $H_{a4}$  accepted, meaning transparency has a positive influence on the consistency of the budgeting process.

The test results showed the regression coefficient ( $\beta_4$ ) for leadership style variable is equal to 0.487 this means that any increase in the leadership style variable at one point, the consistency of the budget process will be increased by 0.487. Determining hypotheses mentioned if  $\beta_4 > 0$ :  $H_{a5}$  accepted, meaning that leadership style has positive influence on the consistency of the budget process.

The test results showed the regression coefficient ( $\beta_5$ ) for leadership style variable is equal to 0.419, this means that any increase in the leadership style variable at one point it weaken the relationship between the quality of human resources with the consistency of the budget process would increase by 0.419 units. Determining hypotheses mentioned if  $\beta_5 < \beta_1$ :  $H_{a6}$  is rejected, meaning that the leadership style weaken the relationship between the quality of human resources with the consistency of the budget preparation process.

The test results showed the regression coefficient ( $\beta_6$ ) for leadership style variable is equal to 0.790, this means that any increase in the leadership style variable at one point it will weaken the relationship between politics budgeting with budgeting process consistency of 0.790 units. Determining hypotheses mentioned if  $\beta_6 < \beta_2$ :  $H_{a7}$  is rejected, meaning that the style of leadership to weaken the relationship between budget politics with the consistency of the budget preparation process.



The test results showed the regression coefficient ( $\beta_7$ ) for leadership style variable is equal to 0.509, this means that any increase in the leadership style variable at one point it will weaken the relationship between transparency with the consistency of the budget process at 0.509 units. Determining hypotheses mentioned if  $\beta_7 < \beta_3$ :  $H_{a8}$  is rejected, meaning that the style of leadership to weaken the relationship between transparency with the consistency of the budget preparation process.

The test results together indicate that there are significant human resources, budgeting politics, transparency and leadership style on the success in maintaining the consistency of the budget process in the Environment Government of Sabang. This means that the consistency of the budget process in the Environment Government of Sabang can be achieved if SKPK of Sabang has the human resources quality, political budgeting balanced, transparency in the process of planning and budgeting and leadership style of leadership that is transformational.

Moreover, to improve the quality of human resources in every SKPK, Sabang City Government always include employee to attend training and courses on planning and budgeting. So with the qualified human resources are expected in the budgeting process will always be consistent. It can be seen during the deliberation process KUA PPAS legislative party was instrumental in changing the programs and activities that have been discussed in Musrenbang ranging from district level up to Musrenbang city. Likewise, during the discussion on the draft APBK, the legislature also was instrumental in approving or rejecting programs and activities that have been listed in the planning document that PPAS KUA document if it is not consistent with the objectives of the programs and activities that have been planned.

The application of transparency in the budgeting process can be run with either the City Government Sabang will be consistent in the budgeting process, this is a form of accountability from the Government of Sabang in implementing programs and activities in accordance with the objectives that have been planned so that programs and activities can be carried out effectively and efficient. Accountability becomes a logical consequence of the relationship between the agent and the principal. In terms of agency theory, it can be stated that the surveillance conducted by the legislature on the budget implemented by the executive which is a mechanism to reduce the asymmetry of information or reduce uncertainty.

The charismatic leader will generate pride and confidence for subordinates by giving that inspires motivation in achieving organizational goals, and provide intellectual stimulation by generating new ideas, provide solutions to the problems faced. Thus, consistency in the budgeting process will remain intact. SKPK head as users of the budget in Sabang City Government Environmental implementing transformational leadership style is not too much, this was due to a limited budget for educational activities and leadership training for Echelon II, III and IV. Most of the activity budget allocated for capital expenditures and other spending on goods and services that are consumable and social assistance expenditure and grants.

Leadership styles in Sabang City Government Environmental less supportive of improving the quality of human resources due to the limited budget that comes to training and courses. The budget allocation largely allocated to activities that are allocated to the physical and social activities. The results of this study do not agree with the research conducted by Nurmandi (2006), which states that leadership is a significant factor in conducting a series of innovations in the field of governance, especially innovations in the process of planning and budgeting in order to plan programs and activities conceived just repeat the year previous.

The head SKPK as the budget and staff have not been able to sustain the argument about the objectives to be achieved in programs and activities that have been planned. Besides less able to sustain the argument about the program and activities, some head SKPK also received the intervention of the legislature in determining the programs and activities that will be proposed in the document planning and budgeting, and no part of the head SKPK who filed the programs and activities without the knowledge of the Budget Team Municipal Government Sabang personal interests and certain groups.

In every organization both private and public transparency as just a slogan to gain public support, but behind all of that transparency is actually just an accessory (support) program as a support for the public interest itself. Due to the fact none of the leaders who are ready to openly discuss the conditions of his government (except for matters relating to the safety of the state, the rights of private and confidential positions).

## **Conclusions**

The conclusions of this study is the quality of human resources, political budgeting, transparency and leadership style affect the consistency of the budget process, the quality of human resources affect the consistency of the budget process, political budgeting affect the consistency of the budget process, transparency affect the consistency of the process budgeting, leadership style affect the consistency of the budget process, the leadership style strengthen the relationship between the quality of human resources with the consistency of the budget process, the leadership style strengthen the relationship between politics budgeting with the consistency of the budget process, the leadership style strengthen the relationship between the transparency of the consistency of the process of drafting budget. Further limitation of the study is the conclusion drawn based only on data collected through questionnaires, leading to problems if the respondent's answer is different from the actual state. While suggestions for research on the consistency is not only limited to the suitability of the programs and activities as well as the number of programs and activities but to how the results of the program's goals and activities that have been determined to be useful, so it is necessary to study more in-depth about the consistency of the budget process by adding another variable such as process planning, commitment SKPD, and supervision inherent supposed to influence the consistency of the budget process. It can also complement the study with implications for the consistency of the budget process.

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## The Contribution of Risk Management to Profit and Cost Efficiency in Rural Shariah Banks (BPRS)

\*Anggraeni

STIE Perbanas Surabaya

\*Corresponding Author: [angqi@perbanas.ac.id](mailto:angqi@perbanas.ac.id)

### Abstract

The development of Shariah banking in Indonesia has been so tremendous that the banks should try their efforts to improve their operation. Risk management is vital to be implemented in order that the operation can minimize any risk. This study examines the impact of risk management practice on the profit and cost efficiency in the rural Islamic banks. The data were collected from 33 BPRS but only 29 could be analyzed using the current software. This study also tried to implement better software for financial report. Initially, the initial report shows that, on average, the efficiency is 64% in which the minimum is 30% and maximum is 100%. In fact, only five banks could achieve the efficiency that is from 29 banks during the investigation. From city of origin, it was found that the banks in Pasuruan and those which are in Probolinggo are the most efficient.

**Keywords:** risk management, cost efficiency, profit efficiency, Islamic Rural Bank.

### Introduction

The Sharia Rural Banks (BPR-Sharia) is one of the financial institutions of Islamic banking in which their operations are obliged to follow the principles of sharia or Islamic *muamalah*. SRBs were established under the Law No. 7 of 1992 on Banking and Government Regulation (PP) No. 72 of 1992 concerning the banks based on the Principles of Shari'ah. In Article 1 (point 4) of Law No. 10 of 1998 on the Amendment of Law No. 7 of 1992 on Banking, it is stated that the SRB is a bank conducting business based on sharia principles and they do not provide services in payment traffic.

Since 2013, the growth and development of sharia banking institutions in Indonesia has been so fast. For example, the asset growth of Islamic banking not only shows endurance in the midst of the global financial crisis but it also shows their achievement of a good performance. The banking intermediation of these banks still continue to perform well with FDR 103%. These data indicate that the Islamic banking intermediation function can drive the economy. The budget grew relatively high 32.2% (while nationally only 23.2% in Q3 2013). The growth in assets is 31.8% (while nationally 18.2 in Q3 2013).

Until October 2015, the number of SRB in Indonesia was 163 units with 435 service offices. BPRS average growth over the last 6 years (January 2008- June 2015) had reached by 38% . The average growth in financing extended for 6 years even could reach by 31.52% a year. In addition, the Raising funds in the form of deposits SRB was Rp 2.09 trillion, while savings of Rp 558 billion. The portfolio is dominated *murabaha* financing disbursements (80%) and 12% of revenue sharing (*mudharabah*), the rest *Ijara multi* services (6%) and *qardh* 2 %).

According to the World Bank survey (2010), only 49 percent of Indonesia's population has access to formal financial institutions. Thus, the number of people who do not have a good savings in the bank or in the non-bank financial institutions is still relatively high, 52%. The presence of Islamic banks in such rapid growth is expected to be closer to the people to formal financial institutions, like an Islamic banking.

As presented in Figure 1, it indicates that the performance of the IRB is declining. Almost all indicators show a negative sign indicating there is a difficult situation faced by IRB. The CAR is 20% far above the minimum 8 percent. This also indicates the capital strength of the bank. ROE is also downward indicating the profitability problem. Non-performance finance (NPF) also increases, indicating the problem in economic situation. The NPF of IRB in that condition can be predicted to still continue to increase due to the present economic situation. The cost efficiency is also increasing due to expensive fund in the

market. The condition above attracts the researchers and therefore, this study is conducted to find the constitution of risk management on such banks' efficiency. It is salient to conduct a study related to risk management to see the contribution to the banks efficiency so that they can improve their operations to have their competitiveness and grow much more efficient.

In order to have a precise scope of the research, this study attempts to raise the problems as the following:

1. What is the general level of cost efficiency of the Islamic rural bank?
2. To what degree does the risk management contribute to the cost and profit efficiency of islamic rural banks?

### **Literature Review**

Mghaieth and Mehdi (2014) studied the Islamic bank's scores of efficiency and their determinants were from 16 countries before, during, and after the 2008 financial crisis. The predictors for bank efficiency used are total assets, capital adequacy, profitability, credit risk and operational costs. They used the model both cost efficiency and profit efficiency. They did a random effects model for the three periods and found that a high total asset and high operation cost showed the most efficient in terms of cost. With regard to the profit efficiency model, they apply fixed-effects. The result shows the level of cost efficiency of 82.13% and an average score of profit efficiency of 82.47%. A study by Mongid and Notodihardjo (2009) found that rural banks charged higher interest due to scale inefficiency.

Cyree and Spurlin (2012) examine the impact of competition in rural markets after the presence of large banks in the bank rural areas where they are the genuine small markets. When Competing against major banks, BPR operates at a lower level of efficiency gain but higher, increasing the level of interest income and fee from the loan. The efficiency of much lower profits and benefits that are higher in rural areas suggests that the large banks have market forces in the rural market and that they can extract rent to get the benefits that are higher than the average efficiency. Therefore, of small banks in rural market should not be scared of other competitors.

On the contrary, the customers rely on loans from rural areas, of small banks that suffered negative impact on a higher level. The more competitors are usually seen as a threat for the BPR in rural Market. Rural small bank managers need not fear because BPR can get higher profits when they are more efficient and have a competitive advantage against competitors in rural market. This research implies that BPR should not consider big banks as their competitors so that their managers cannot be inflicted on a higher interest rate and fees for credit.

Beck Demirgüç-Kunt and Merrouche (2013) discussed the implications of Sharia-compliant products of Islamic banking for agency problems using traditional theory of financial intermediation. They found the equity-like nature of Islamic banking and they suggest that Islamic banks' business model might not be different from conventional banks' business model. Their empirical estimations show little significant differences between Islamic and conventional banks. From cross-bank comparison of conventional and Islamic banks, they suggest opposing the effects of Sharia-compliant business model. Tahir and Haron (2010) studied the cost and profit efficiency of the Islamic banking from around the globe for 2003-2008. They found that the average cost and profit efficiency are 43.6 percent and 41.1 percent. This result implies that Islamic banks are relatively better in controlling cost than generating profits.

From time perspectives, the efficiency scores suggest that the trend for both the cost and profit efficiency of Islamic banking is improving. Due to better environment condition, Islamic banks from Europe are more cost and profit efficient than the other groups of Islamic banks. In contrast, Islamic banks from the Far East and Central Asia show lower cost efficiency and African Islamic banks worst in their profit efficiency. To provide the way forward of the research, the reseacrhrs present the road map of the research, especially, on its relationship with previous research and especially to the first year result. The first year research focuses on the risk management practice especially on how islamic rural banking manage their risk taking (lending/financing), liquidity risk, and operational risk. This study produced two manuscripts for scopus indexed journal and one has been published and the second is under blind review process.

### **Research Metode**

This study applies parametric methodology known as the Stochastic Frontier Analysis (SFA). This methodology is regarded as superior as it can produce efficiency more accurately and consistently to economic theory. In order to estimate the cost efficiency of banks, this study employs the so called two-stage SFA model. It also employs three approaches; (SFA assuming the efficiency follow

normal-exponential distribution, SFA assuming efficiency follow normal- half normal distribution and SFA Panel Time Invariant that efficiency distribution follow truncated distribution) and then compare the empirical results to select the best one.

The result implies a common frontier is based on the belief that efficiency differences across banks are mainly attributable to managerial decisions within banks. Managerial decision is defined as risk management practice. Banking technology can be defined as the set of specific methods that banking firms employ to combine financial and physical inputs to generate a certain amount of banking services, such as loan and service for their customers. These methods are diversification, risk pooling, financial information collection and evaluation, risk management are assumed to have impact on bank cost and profit efficiency.

Previous studies showed that the bank-specific variables should be taken into account because these variables are evidenced to have very important role in variability differences in the banking cost and profit. To measure the cost and profit efficiency of French banks we employ the stochastic frontier approach (SFA), as developed by Aigner et al. (1977). The SFA specifies a particular form for the cost and profit function. The model assumes that these errors consist of inefficiencies, which follow an asymmetric distribution and random errors that follow a symmetric distribution. The reason for this particular structure of the composite error term is that, by definition, inefficiencies cannot be negative. Both the inefficiencies and random errors. This study is classified as quantitative study and using secondary data collected from IRBs. The input in this study is total deposits, workers and capital or fixed asset. These data were collected via survey and interviews with IRBs sample. Worker is total personnel expense and fixed asset include premises. The outputs are total loan and other productive assets.

The next step is modelling the determinant of profit and cost efficiencies of the banking firms. The efficiency score using the SFA model is then used for further analysis. To examine the determinants of bank cost efficiency, we use a Tobit regression model which regresses the efficiency scores obtained from the SFA models on a number of bank specific variables especially related to risk management practices of the individual Islamic rural bank and bank specific variable such as size and capital. The environmental variables such as economic growth and inflation are all excluded although they are a standard for bank efficiency study determinant because we assume all Islamic rural bank are exposed to the same conditions. In this study, the researchers adopt the intermediation approach in defining the outputs and inputs (price) of banking services. The researchers chose this approach as it is simpler and previous empirical research is relatively abundant. In this study we use the SFA methodology. To estimate the efficiency score, the stochastic requires inputs and output specification. We include input prices; interest, labour and other price for physical capital. Referring to Hasan and Marton (2003), Mongid, Tahir and Haron (2012) and use the ratio of total other expenses to total fixed assets as the best available proxy measure for the average cost of non financial inputs to banks. Table 1 presents the variable and the definitions.

**Table 1.** The Total Expenses, Total Output, Prices

No	Variable	Definition	Measurement Mean
1	Ltc	Log Total cost	Total IRB operating expenses
2	lq1	Log Total loans	Total loan disbursed
3	lq2	Log Other earning assets	Total Placement in other bank
4	lp1	Log Price of funds	Total profit sharing to total deposits
5	lp2	Log Price of labour	Total salary to total assets
6	lp3	Log Price of other expenses	Other expense to total fixed asset

The total banking costs are both of interest expense and operating costs. The Stochastic Frontier Approach (SFA) is used to analyze the efficiencies of IRB samples. This methodology is well documented in the literature. In this study we refer and follows the methodology based on Coelli (1996) and Coelli, Rao, O'Donnell and Battese (2005). SFA has been widely used by a considerable number of studies for evaluating banking efficiency. The SFA starts with a standard cost or profit function and estimates the minimum cost or maximum profit frontier for the entire sample from balance sheet data. According to Greene (2002) the stochastic frontier model may be written

$$y_{it} = f(\mathbf{x}_{it}, \mathbf{z}_i) + v_{it} \pm u_{it} = \beta' \mathbf{x}_{it} + \mu' \mathbf{z}_i + v_{it} \pm u_{it}, \quad (1)$$

where the sign of the last term depends on whether the frontier describes costs (positive) or

production (negative). The second component,  $u_{it}$  represents technical or cost inefficiency, and must be positive. The approach aims to estimate not only the cost and profit efficiency scores of IRB but also to identify the determinants that affect these scores. Therefore, we adopt the Battese and Coelli (1995) approach, where  $u_{it}$ , the technical inefficiency effect, is assumed to be a function of a set of bank specific variables. In this regard, cost efficiency gives a measure of how close a bank's cost is to what a best-practice bank's cost would be for producing the same bundle of output under the same conditions. Profit efficiency indicates how well a bank is predicted to perform in terms of profit relative to other banks in the same period for producing the same set of outputs.

Evaluating bank efficiency is a complex process that involves assessing interaction between the environment where banks operate, internal bank condition and external activities. Currently, primary method of evaluating internal performance of banking firm is by analyzing accounting data. Financial ratios usually provide a broader understanding of the bank's financial condition since they are constructed from accounting data contained on the bank's balance sheet and financial statement. However, economic efficiency measured using SFA is currently getting popularity as it is immune from creative accounting. Figure 2 shows the model of the factors in this research.

The study uses risk management aspects that influence the cost and profit efficiency of Islamic rural banking firms. This study is to find a link between bank-specific factors and the macroeconomic environment meaning that the finding can be useful for academic knowledge and policy assessment. The framework basically replicates the work previously done by Louzis, Vouldis, Vasilios and Metaxas (2012) and Mongid, Tahir and Haron (2012).

To examine the determinant of Islamic rural bank cost and profit efficiency (EFFI), the researchers use simple linear regression model which is a linear relationship between response variable,  $y$  and the predictor variable,  $x_i, i = 1, 2, \dots, n$ . The model is

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n + \varepsilon \quad (2)$$

where  $\beta_0, \beta_1, \dots, \beta_n$  are regression coefficients and  $\varepsilon$  is the error due to variability in the observed responses. In our study, the model can be formulated as:

$$\text{EFFI}_i = \alpha + \beta_1 \text{SIZE}_{it} + \beta_2 \text{PERSTEX}_{it} + \beta_3 \text{CAR}_{it} + \beta_4 \text{LLRGL}_{it} + \beta_5 \text{LIQMGM}_i + \beta_6 \text{CREDIMGM}_i + \beta_7 \text{OPRMGM}_i + \varepsilon$$

To assess the ability of the model to explain cost efficiency (CIR), we use linear regression testing technique such as t-tests and F-test. F-test is used to test the capability of the model to explain the variability of the CIR. To assess the capacity of the individual variable, we use t-test.

Variables will be employed in this study are derived from theoretical as well as previous empirical studies. There are two types of variables in this study. The first is data derived from individual bank balance sheet and income statement. These data are to measure the individual bank characteristics.

**Table 2** Variable and Sources of Data

Number	Variable	Observation	Sources of Data	Measurement
1	EFFI	SFA Cost and Profit Efficiency	Bank Level	Percentage
2	OPERATIONMGM	Operational management	Bank Level	Percentage
3	LIQMGM	Liquidity Management	Bank Level	Percentage
4	LASSET	Logarithm of Asset Size	Bank Level	Logarithm
5	LLRGL	Loan Loss Provision to Total Loan	Bank Level	Percentage
6	PERSTEX	Personnel Expense to Total Expenses	Bank Level	Percentage
7	CAR	Capital Adequacy Ratio	Bank Level	Percentage
8	CREDITMGM	Credit management	Bank Level	Percentage

In this study we use time series and cross sectional model mostly known as panel data. Panel data models combine a cross-section observations with a time series dimension. The cross-section nature of the panel explain the variability in the for bank-specific factors and how these vary across banks in the samples. However, if as our samples are not fully balanced, we will only apply simple linear regression for simplicity.

### **Result and discussions**

Total samples are 93 banks of the east Java member of ASBISINDO. For 2013, there are 31 banks. As our study will apply panel data, total sample for every year should be similar to make it balance panel. The interesting point of the data collection process is that not all regency own Islamic rural bank (BPRS). It means even though Islam is the main religion the east Java, the awareness to establish BPRS is not rigorous. This may as result of a very time consuming process to establish BPRS. Another interesting point is BPRS flourish in the region that assumed as religious city. Pasuruan, for example, is known as the most business active city where Moslem is the dominant player. Compared to Surabaya, economic capacity of Surabupaten folds of Pasuruan but the number of BPRS in Pasuruan exceeds Surabaya. The interesting point is Batu city where BPRS is also very flourished due to regional government initiative to push the establishment of BPRS.

As an Islamic bank should not apply usury in their business, the musyarakah financing is regarded as the most Islamic bank contracts compare to other such mudarabah. Second best city is Gresik as the amount of musyarakah financing also dominant. BPRS from Batu and Malang are mostly applied Musyarakah. Unfortunately, BPRS from Kediri is reluctant to use this type as it is more risky. The problem is BPRS from Magetan that only doing interbank placement but not financing. The result may as a result of the problem of BPRS especially on capital constraint. In terms of customer fund, Sumenep is the biggest BPRS. It is not strange but the local government initiatives to establish the bank. It makes all government servants eager to serve in this BPRS.

The researchers conducted an initial investigation on how BPRS operate by looking at its efficiency score. Using SFA software we conduct the initial assessment how the cost efficiency of samples. We estimate 30 BPRS as the rest contains 0 information on assets that cannot do done by our current software. However, for the final report we are going to use more sophisticated software. The estimation is using data set for 2013 (See Table 3).

**Tabel 3:** Efficiency Score

No	DMU	Efficiency	BPRS
1	<b>f2</b>	77,56	BPRS Amanah Sejahtera
2	<b>f3</b>	35,55	BPRS Mandiri Mitra Sukses
3	<b>f4</b>	39,35	BPRS Asri Madani
4	<b>f5</b>	31,18	BPRS LANTABUR TEBUIRENG
5	<b>f6</b>	80,08	Artha Pamenang
6	<b>f7</b>	41,71	Rahma Syariah
7	<b>f8</b>	100,00	Tanmiya Artha
8	<b>f9</b>	33,32	Bumi Rinjani Batu
9	<b>f10</b>	100,00	Bumi Rinjani
10	<b>f11</b>	100,00	Mitra Harmoni
11	<b>f12</b>	44,08	Madinah
12	<b>f13</b>	30,39	BPR Syariah Magetan
13	<b>f14</b>	44,87	Bhakti Haji
14	<b>f15</b>	64,30	Bumi Rinjani kepanjen
15	<b>f16</b>	31,72	BPRS Kota Mojokerto
16	<b>f17</b>	49,05	Sarana Prima Mandiri
17	<b>f18</b>	100,00	Al Hidayah
18	<b>f19</b>	67,71	Daya Artha
19	<b>f20</b>	88,87	Jabal Tsur

20	<b>f21</b>	75,99	Untung Suropati
21	<b>f22</b>	56,56	Al Maburur
22	<b>f23</b>	100,00	Bumi Rinjani Probolinggo
23	<b>f24</b>	39,43	Bakti ARtha Sejahtera
24	<b>f25</b>	95,33	Annisa Mukti
25	<b>f26</b>	43,99	Baktimakmur Indah
26	<b>f27</b>	98,34	Unawi Barokah
27	<b>f28</b>	90,33	Situbondo
28	<b>f29</b>	29,97	Bhakti Sumekar
29	<b>f30</b>	76,18	Jabal Nur

From Table 3, It showsd that there are only five BPRS that are efficient. It means only 15% islamic rural bank is efficeinct. The reseacrhrs applied output oriented assumption that there should a room for bank to be more efficiecent. This result should be taken with care as assumption of cost of production in BPRS may should follow different approach. From the initial result, we find that that BPRS from Pasuruan and Probolinggo is the most efficient one. The most inefficient is Magetan. We notice this as a result of its assets only on placement not loan. With same cost structure this finding confirm that output should be increased to enjoy economies of scale.

**Table 4 :** Efficiency and City of Origin

	City	var20
1	Gresik	78
2	Jember	36
3	Jombang	39
4	Kab Malang	54.5
5	Kediri	51
6	Kota Batu	66.5
7	Kota Malang	100
8	Lamongan	44
9	Magetan	30
10	Mojokerto	32
11	Pamekasan	49
12	Pasuruan	83.25
13	Ponorogo	57
14	Probolinggo	100
15	Sampang	39
16	Sidoarjo	79
17	Situbondo	90
18	Sumenep	30
19	Surabaya	76
	Total	64.31

## Conclusion

It can be concluded that BPRS as the banking industries provide special evidence. First of all, it is more unique in term of their role, business model, and business size. It was found that among these shariah rural banks are with the present software and, therefore, they have used better software for their financial report. In the initial report, this study revealed that the average efficiency is 64% with the minimum of 30% and maximum 100%. Only five banks can achieve efficiency (among 29 banks), in the investigation. From city of origin, it was found that the banks in Pasuruan and Probolinggo are considered to have the most efficiency. The following step found that the estimated cost efficiency are on a set of bank specific variables (such as size, equity to total asset, loan to total asset, problem financing).

It was found that the risk management being implemented can also increase the banks efficiency. In this case, it is advisaby that the shariah rural banks managers should do a rick management to improve their banks opertion and efficiencyt. Other big banks are not necessarily to be



considered their competitors. As long as they can manage the banks with minimizing their risk, they could be more even competitive and surviving.

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## **The effect of industrial diversification and geographic diversification on the practice of earnings management (An Empirical Study On Manufacturing Companies Listed On Indonesian Stock Exchange Year 2011-2014)**

<sup>1</sup>Dahlia, <sup>2\*</sup>Hasan Basri

<sup>1,2</sup> Department of Accounting, Faculty of Economic, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [p\\_haasan@unsyiah.ac.id](mailto:p_haasan@unsyiah.ac.id)

### **Abstract**

This research is conducted to address the prevalent issues regarding the effect of industrial diversification and geographic diversification either partially or simultaneously on the practice of earnings management. By utilizing purposive sampling techniques on the secondary data, the total of 80 sample studies are drawn from manufacturing companies listed on Indonesian Stock Exchange for the period of 2011-2014. Multiple linear regression analysis provided by SPSS 21.0 is also employed to test the hypothesis constructed within this research. The result of the multiple linear regression analysis presents evidence that industrial diversification and geographic diversification simultaneously have a significant effect on the practice of earnings management. Alongside, it also affirms that partially, industrial diversification and geographic diversification respectively has a significance effect on the practice of earnings management. These findings help to provide scholarly evidence on the implication of diversification strategies on the practice of earnings management to help financial statement users (investor, creditor, stakeholder, etc.), accounting standard setter, and regulators to assess the pervasiveness of earnings management within the company.

**Key words:** Industrial Diversification, Geographic Diversification, Earnings Management

### **Introduction**

Global volatility of economic condition that often triggers economic crisis indeed shakes up the existence of particular business in a country, either directly or indirectly. The 1998 and 2008 economic crisis for instance substantially exhibit an adverse impact of business climate in Indonesia, paralyzing most of economic activities due to a large number of companies that eventually gone to bankruptcy (Kartono, 2010). Undoubtedly, this impact has drawn the attention of managers to think of strategies on how to remain sustain and continuously develop in the middle of this unpredicted economic condition as well as emerging business competition. One of the prevalence strategies is that by doing diversification, either industrially or geographically as they are both believed to reduce the business risk of the company (Harto, 2005).

Rash of literatures argue that diversification strategies can improve the performance of companies' profitability (Farooqi, 2014; Satoto, 2009). However, this diversification, despite its ability to create multi-sources of revenue by expanding the business line, segment, and market share, demands a higher degree of organizational complexity which may create another unfavorable condition (El Mehdi and Sebuoi, 2011). Agency conflicts hypothesis argues that the company's degree of organizational complexity influences the ability of managers to alter and modify information as well as manipulate the earnings. Admittedly, this argument is reinforced by unveiling earnings management cases by industrially and geographically diversified companies, such as world class' company, Enron and Indonesian company, PT Kimia Farma (Arfan, 2006). Then, one might ask, does diversification create a favorable condition for the earnings management?

Considering the prospective unfavorable consequences of diversification strategies, this paper insist on seeking the empirical evidence related to the effect of industrial diversification and geographic diversification on the practice of earnings management. The findings of this research are then expected to provide scholarly evidence on the implication of diversification strategies on the practice of earnings management to help financial statement users (investor, creditor, stakeholder, etc.), accounting

standard setter, and regulators to assess the pervasiveness of earnings management within the company.

The remainder of this paper is organized as follows. Section 2 reviews the selected previous studies and formulates the hypothesis. Section 3 presents methodological framework for the basic of analysis. Section 4 discusses the empirical findings and their implications and finally section 5 provides the closing remarks of this paper.

## **Literature Review**

According to the agency conflicts hypothesis, the ability of managers to distort information and manipulate earnings depends on the company's degree of organizational complexity. Empirical research shows that diversified company is generally larger that they have more complex organizational structures, have less transparent operations and that their analysis poses difficulties to investors and analyst alike (Chang and Yu, 2004; Kim and Patzalis, 2003; Liu and Qi, 2007). There is, therefore, a company that is industrially and geographically diversified is supposed to have complex organizational structure as it deals with many business segments and divisions. With this regard, the detailed operation of each segment then is only known by the management and is hardly depicted to the shareholder.

There have been several studies conducted to examine the effects of industrially and geographically diversification on the practice of earnings management. Rodriguez and Hemmen (2010), who conducted a study in Europe, find that for less diversified companies, earnings management measured by discretionary accruals is less pronounced, whereas in relatively more diversified companies, discretionary accruals appears to be more pronounced. Lim et al. (2007), conducted a research in seasoned equity offering setting, also suggest that diversified companies are more aggressive in managing earnings than non-diversified companies. Farooqi et al. (2014) also confirm this view in their research on American companies.

Merits of literatures also suggest that there is a significant effect of geographic diversification to the practice of earnings management. In this regard, El Mehdi and Sebuoi (2011) who conduct a research on U.S companies find that earnings management increases with the level of geographic diversification. They provide evidence that for multinational companies, regardless of whether they operate in one or more business segments, they find income-increasing accruals. This finding is consistent with the view that the costs of geographic diversification outweigh its benefit. They also suggest that the aggressive manipulation in global diversified companies is motivated by high operating cash-flow volatility, high information asymmetry, and amplified operating risk.

Other similar research conducted by Chin et al. (2009) on Taiwanese company. They also agree that greater corporate internationalization is associated with a higher level of earnings management by way of greater asymmetry information and transparency decrement. However on the other hand, a number of studies provide different finding which is inconsistent with the above mentioned. The work of Jiraporn et al. (2008) provides empirical evidence that geographical diversification alone does not appear to impacts earnings management. Some other researchers even reveal that geographic diversification can help in mitigating the practice of earnings management (Farooqi et al., 2014)

Taking the literature review and previous findings as the point of departure, the researcher hypothesizes:

- Ha<sub>1</sub>: Industrial diversification and geographic diversification influence the practice of earnings management.
- Ha<sub>2</sub>: Industrial diversification positively influences the practice of earnings management.
- Ha<sub>3</sub>: Geographic diversification positively influences the practice of earnings management.

## **Research Method**

This study employed quantitative approach and is aimed at investigating whether or not there is an effect of industrial diversification and geographic diversification on the practice of earnings management. The data are drawn from IDX website, encompasses all listed manufacturing companies covering the 2011-2014 period. In this study, the earnings management is treated as the dependent variable, which is measured by discretionary accrual (DA) which is derived from the difference of total accrual (TA) and non-discretionary accrual (NDA). To measure discretionary accrual, the modified jones model is used. The reason why the researcher uses this model is that because this model is assumed to be the best model to investigate the earnings management and also give a strong result (Dechow et al., 1995). Further, this model has also been widely accepted in accounting literature to measure earnings management (Arfan, 2006).

Variable of industrial diversification and geographic diversification are treated as the independent variable. Variable industrial diversification (IHERF) is translated to the number of industrial segments or business segment owned and reported by company in the financial statement and specifically shown in the notes to the company's financial statement. We use the sales-based Herfindahl index as an alternative proxy for industrial diversification (Farooqi et.al, 2014). The Herfindahl index for the *i*th company in year *t* is computed as:

$$IHERF_{i,t} = \sum \left[ \frac{I\_SALES}{FSALES} \right]^2$$

where *I\_SALES* denotes the industrial segment sales for *i* company in year *t* and *FSALES* denotes the company's total sales across all reported industrial segments in that year. Variable geographical diversification (GHERF) is translated to the number of geographical segments owned and reported by the company in financial statements that are specifically shown in the notes to the company's financial statements. We also use the sales-based Herfindahl index as an alternative proxy for geographic diversification (Farooqi et.al, 2014). The Herfindahl index for the *i*th company in year *t* is computed as:

$$GHERF_{i,t} = \sum \left[ \frac{G\_SALES}{FSALES} \right]^2$$

where *G\_SALES* denotes the respective geographical sales for the company in year *t* and *FSALES* is the company's total sales across all reported geographical segments in that year. Further, to investigate the impacts of industrial diversification and geographic diversification on earnings management, this study employs multiple regression models of the panel data, as follows:

$$DA = a + b_1 IHERF + b_2 GHERF + \varepsilon$$

where *DA* is earnings management, *a* is constant term, *b*<sub>1</sub> and *b*<sub>2</sub> are the estimated parameters for industrial diversification (*X*<sub>1</sub>), geographic diversification (*X*<sub>2</sub>), and *ε* is the error term.

## Results and Discussion

### Descriptive Statistics and Hypothesis Testing Results

Table 4.1  
Descriptive Statistics Test Result

	N	Minimum	Maximum	Mean	Std. Deviation
IHERF	80	0.0000	1.0000	0.6434	0.2559
GHERF	80	0.1684	1.0000	0.5807	0.2515
DA	80	-0.0207	1.2648	0.3953	0.2554
Valid N (listwise)	80				

Source: Output SPSS 21.0 (2016)

Table 4.1 displays the descriptive statistics for the variable of industrial diversification (IHERF), geographic diversification (GHERF) and earnings management (EM) of the total 80 sample studies. The maximum values of both diversifications, either IHERF or GHERF is 1,0000, which means that there are companies that do not diversified at all or only have one single segment, either industrially or geographically. The minimum value of IHERF is 0.000 while GHERF is 0.1684. On average, the value of IHERF and GHERF is 0.6434 and 0.5807 with standard deviation of 0.2559 and 0.2515, respectively. In regards to earnings management, the maximum value is 1.2648 while the minimum is -0.0207. On average, the value of EM is 0.3953 with standard deviation of 0.2554.

Table 4.2  
F-Statistical Test  
ANOVA<sup>b</sup>

Model	Sum of Squares	Df	Mean Square	F	Sig.
1 Regression	1.400	2	0.700	14.367	.000 <sup>a</sup>
Residual	3.751	77	0.049		
Total	5.151	79			

a. Predictors: (Constant), GHERF,

Source: Output SPSS 21.0 (2016)

Based on F-Statistical test result displayed on table 4.2 above, it is revealed that the level of significance is 0.000 which is obviously less than 0.05 (5%). It infers that all the independent variables affect the dependent variable simultaneously. Thus, it can be concluded that the first hypothesis ( $H_{a1}$ ) stated that industrial diversification and geographic diversification simultaneously affect the practice of earnings management is accepted.

Table 4.3  
T-Statistical Test  
Coefficients<sup>a</sup>

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
1 (Constant)	-0.01	0.086		-0.117	-0.907
IHERF	0.203	0.097	0.204	2.091	0.040
GHERF	0.474	0.099	0.466	4.786	0.000

Source: Output SPSS 21.0 (2016)

Based on table 4.3, it is obvious that industrial diversification variable ( $X_1$ ) has a significance level of 0.040. As the level of significance is less than 0.05, thus it infers that ( $X_1$ ) has a significance influence on the dependent variable. Therefore, it can be concluded that the second hypothesis ( $H_{a2}$ ) stated that industrial diversification has a significance influence on earnings management is accepted.

Geographic diversification variable ( $X_2$ ) has a significance level of 0.000. As the level of significance is less than 0.05, so it means that ( $X_2$ ) has a significance influence on the dependent variable. Hence, it can be concluded that the third hypothesis ( $H_{a3}$ ) stated that geographic diversification has a significance influence on earnings management is accepted.

Table 4.4  
Coefficient of Determination Test  
Model Summary<sup>b</sup>

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.601 <sup>a</sup>	.272	.253	0.2207

a. Predictors: (Constant): GHERF, IHERF

b. Dependent Variable: DA

Source: Output SPSS 21.0 (2016)

Table 4.4 displays the result of R Square, i.e. 0.253. It means that 25.3% of the variance of earnings management can be explained by the independent variables of industrial diversification and geographic diversification. However the rest (100%-25.3% =74.7%) is explained by another variables which are not included within this research.

### *The Effect of Industrial Diversification on the Practice of Earnings Management*

The level of significance of industrial diversification variable ( $X_1$ ) is 0.04 and its regression coefficient is 0.203 as shown in table 4.3. As the level of significance is less than 0.05 (5%), and the regression coefficient is positive, it infers that industrial diversification has a positive significant influence on the dependent variable of earnings management. This result is contradictory with the work of Lupitasari and Marsono (2014) which stated that industrial diversification does not have a significant effect on the practice of earnings management. This contradiction might be caused by different objects and proxy used in the study. Lupitasari and Marsono (2014) conducted a study on mining and banking companies and measured the industrial segment by how many segments the company reported, while this current research is focus on the manufacturing companies and uses helfindahl index to measure the industrial diversification. On the other hand, the result of this research is supported by the work of Rodriguez and Hemmen (2010), Lim et al. (2007) and Farooqi et al. (2014). They all found that for less diversified companies, discretionary accruals is less pronounced, whereas in relatively more diversified companies, discretionary accruals appears to be more pronounced.

Finally, the result of this research also confirms the agency conflicts hypothesis which stated that the ability of managers to distort information and manipulate earnings depends on the company's degree of organizational complexity as caused by industrial diversification (Chang and Yu, 2004; Kim and Patzalis, 2003; Liu and Qi, 2007). Thus, the higher the level of industrial diversification carried by the company, the more likely the company to experience earnings management problem. To sum up, multiple regressions analysis of this study reveals that industrial diversification has a positive significant influence on earnings management. Within this regards, it means that  $H_{a2}$  is accepted while  $H_{02}$  is rejected.

### *The Effect of Geographic Diversification on the Practice of Earnings Management*

Geographic diversification variable ( $X_2$ ) has regression coefficient value of 0.474 at the significance level of 0.000 as shown in table 4.3. As the level of significance is less than 0.05 (5%), and the regression coefficient is positive, it infers that geographic diversification has a positive significance influence on the dependent variable of earnings management. This result is conflicted with the study conducted by Jiraporn et al. (2008) who provide empirical evidence that geographical diversification alone does not appear to impact earnings management practices. This conflicted result might be raised because of the difference of sample studies and year of observation between the two. Jiraporn et al. (2008) conducted a study on American companies for the period of 1994-1998, while this current research is conducted on companies listed on Indonesian Stock Exchange for the period of 2011-2014.

On the other hand, the output of this research is line with the research conducted by El Mehdi and Sebuoi (2011) who finds that earnings management increases with the level of geographic diversification. This finding is also consistent with the work of Chin et al. (2009) who agree that greater corporate internationalization is associated with a higher level of earnings management. Thus, it is inferred that again this current research agrees to accept the information asymmetry problem under agency conflict hypothesis proposed. Deployment of company's asset and operations across different geographical region increases the level of organizational complexity. This organizational complexity then increases the level of information asymmetry between the managers and the shareholders (Lupitasari and Marsono, 2014) and at the same time this also leads to transparency decrement (Chin et. al, 2009) which opens the door to the practice of earnings management. There is, therefore, multiple regressions analysis of this study agrees that geographic diversification has a positive significant influence on earnings management. Within this contention, it means that  $H_3$  is accepted while  $H_{03}$  is rejected.

## **Conclusions**

This study is aimed at seeking the empirical evidence of the effect of industrial diversification and geographic diversification on the practice of earnings management in manufacturing companies in Indonesia. The results suggest that industrial diversification and geographic diversification simultaneously and partially have a significant positive effect on the practice of earnings management on manufacturing companies in Indonesia. This evidence provides a new insights for the financial statement users, such as stockholders, potential investors and creditors in their decision making process. In determining the performance of the company, they should take into account the more risk of earnings management practice that bears from an industrially and geographically diversified companies.

In addition, there are also some limitations of this study that the researchers would like to acknowledge. Firstly, the independent variables within this study only explains 25.3% the variance of earnings

management. In this regards, the future research shall expand the independent variables that might have a stonger effects on the practice of earnings management, such as the ownership structure of the company or the legal protection of the country where the company is located. Further, the future research could also be conducted by expanding the number of sampled companies into other sectors or in other countries. The assosiation between diversification and earnings management may not hold in other sectors and countries. Different behaviors in different sectors and countries may reflect different pressure towards the practice of earnings management. In this regards, there is a need to examine again this effects to other sectors or countries.

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## The Relationship between Knowledge and Stress Felt by Teachers of Economics Implementing Kurikulum Tingkat Satuan Pendidikan

<sup>1\*</sup> Lisa Agustina, <sup>2</sup> Nor Aishah Buang, <sup>3</sup> Mohammad Hussin

<sup>1</sup> Faculty of Education, Universitas Serambi Mekkah, Batoh 23245, Banda Aceh, Indonesia;

<sup>2,3</sup> Faculty of Education, Universiti Kebangsaan Malaysia, 43600 UKM-Bangi, Selangor, Malaysia;

\*Corresponding Author: lisa\_unsyiah@yahoo.co.id.

### Abstract

The purpose of this study was to identify the relationship between knowledge and stress amongst teacher of economics in implementing *kurikulum tingkat satuan pendidikan*. This study used a questionnaire with 109 teachers from twelve middle schools in Aceh Besar, Indonesia, with overall reliability of 0.930. Descriptive statistical tests found that the level of knowledge of the teachers was at a moderate level with an overall mean value of 3.04. Meanwhile the level of stress was at a high level with an overall mean value at 3.56. An inferential statistical correlation test was used to test an hypotheses. The inferential statistical test found that there was a negative relationship between the level of knowledge and the level of stress. It can be concluded that the implementation of the curriculum gave the most teachers stress.

**Keywords:** curriculum, stress, knowledge.

### Introduction

Education is an important component for the development of human capital. Education is able to install new capacity into persons to learn hence producing more productive people. On the other hand, education is also a vehicle for expansion of systems and social mobility in society, both horizontally and vertically [Sulistyo 2007]. One important component of an education system is the curriculum. Therefore, since Indonesia provides free education, the government also prepares the curriculum [Mulyasa 2006]. Since 1980, Indonesia has changed the curriculum at least three times. *Kurikulum tingkat satuan pendidikan* (KTSP) is a continuation and development from the previous one, *kurikulum berbasis kompetensi* (KBK), and now give full authority to each school to develop a curriculum that is appropriate for that school's own characteristics and needs. The KTSP was created and established by teachers as was recommended by the Department of Education.

Development of the KTSP must be handled professionally with a good level of understanding in order to produce a curriculum with appropriate content and competency standards. Development of the curriculum requires not only the readiness of schools, but also the support of various stakeholders, especially the teachers. The role of teachers is very important as they are the major element that determines the success of the teaching- learning process, especially as protagonists and at the same time they play a major role in implementing the curriculum. Thus, in the early stages of curriculum change, the knowledge of teachers should be prioritized. According to Jadi [1993], teachers play an important role in addressing the implementation of curriculum reform, especially in terms of its spread in schools. This is because the teachers are the main driving force in the implementation of reforms in education. Furthermore, Nordin [1991] explained that a good education is the result of a good curriculum and good quality teaching as well. Whenever changes of curriculum occur, the teachers as educators must orientate themselves to the changes. Success of curriculum reform depends on the support, the readiness and the motivation of the teachers. They need more time, ability, knowledge, understanding and skills, otherwise, reforming curriculum will be difficult to perform effectively [Hurst 1981].

Lack of knowledge needed to prepare a curriculum, will make it difficult for teachers to implement the curriculum. Thus, plans prepared by teachers often do not relate to the philosophy and goals



of national education, and the objectives of the subjects. Malone and Howson [1987] and Avalos [1999] explained that failure of the teaching-learning process is due to lack of knowledge and skills. In order to develop teaching plans, teachers are required to have knowledge and understanding of the curriculum, either through manuals and/or training. In order to optimize the empowerment of teachers in preparing curriculum, however, facilities such as books and reference materials must be available. This is in line with the statement by Malone and Howson [1987], who has written that the processes of change will provide significant results if they are accompanied by an increase in materials.

According to Fattah [2000], improving the quality and the expansion of education requires at least three factors, namely: (i) availability of educational resources, such as educational personnel of good quality, low costs and ease of learning, (ii) quality teaching-learning processes that enable students to learn effectively, and (iii) quality of output in the form of knowledge, attitudes, skills and values. Furthermore, according to Karnadi of the State University of Jakarta, implementation of curriculum implies an increasing burden for teachers. Teachers are now expected to make curriculum for each subject. In fact, teachers are accustomed to follow curriculum set by governments. Now, however, the uneven quality of teachers in particular the diversity in the capacity for creativity of teachers is a difficulty in the program for all teachers to create their own curriculum. Thus, the current study investigates the relationship between knowledge and stress amongst teachers of economics at middle schools in Aceh Besar, Indonesia. It also aims to explore and understand problems experienced by teachers in implementation of curriculum. In particular, the current study was carried out to fulfill the following objectives: (i) identify the stage of knowledge of the sample teachers of economics, (ii) identify the phase of stress of the sample teachers of economics, and (iii) identify any relationship between knowledge and stress amongst the sample teachers of economics.

### **Literature Review**

Curriculum is at the core of education and has an influence on the entire educational system. For implementation of a curriculum, teachers must necessarily understand the concepts and objectives of the curriculum. This is because the teaching process can only be achieved if teachers have the appropriate knowledge. Teachers who have the right knowledge will be more willing to carry out teaching processes and will find it easier to make changes to a curriculum without experiencing stress. Government regulation no. 18 for the year 2007 states that one of the competencies required of teachers is professional competence. This includes the ability of a teacher to gain mastery of a subject broadly and deeply, such as having the ability to develop a curriculum. Teachers must know the objectives, principles and characteristics of a curriculum. There are five characteristics and professional skills that teachers have to develop, namely [Botung 2008]: (i) mastering the curriculum, (ii) mastering the materials, (iii) mastering the use of multiple-teaching methods, (iv) having a high commitment to the job, and (v) being disciplined. Teachers with a lack of knowledge will result in low quality education not in accordance with the standards of education [Sudarminto 2001]. The low quality of teachers appears from the following symptoms: (i) lack of mastery of the material taught, (ii) mismatch between the subjects studied and the facts the subjects to be taught, and (iii) lack of effective methods for teaching. Similar studies by Ahmad [1986] and Sulaiman [2003] have found that problems like lack of knowledge and skills about changes and innovations must often be one of the main reasons for failure in the implementation of curriculum. Teachers need to have the knowledge, skills and values to be taught every day.

Whatever we do, we cannot escape from stress. Stress experienced by a teacher usually cannot be separated from the situation of teaching-learning in school, whether in relation to students, school climate or work load. Jali [2001] has stated that stress may affect the quality of teaching and so influence productivity, achievement and efficiency of teaching. Stress may also be experienced by individuals whose skills are weak [Tang and Yueng1999]. Stress can be defined as uncomfortable, negative emotions such as anger, indecision, stress and disappointment caused by the work [Kyriacou and Sutcliffe 1978]. In this case, teachers facing stress are teachers who cannot control their emotions to change educational culture, that is not only to provide knowledge but also to educate people to become useful in the community. Teachers are forced to work overtime, provide teaching aids and attend courses or workshops throughout the week as they are forced to make adjustments for new teaching methods. Smith [1994] has stated that teachers will not be able to function with confidence if they are in a depressed state. Stress makes someone irrational, anxious, tense, unable to focus on work and fail to enjoy the feelings of joy or rewards for work done [Fun 2008]. According to a study by Pierce and Molly [1990], stress can be caused

by work overload, time pressures, problems with students, conflict between friends and/or other workers, high phase noise, lack of appreciation and a negative attitude towards rewards. While a study by Brown and Ralph [1991] has stated that teachers faced stress due to the introduction of new curriculum, classes which are not disciplined, malignant students, parent pressure, a big workload and poor management. Gold and Roth [1993] has also stated that one of the main stresses for teachers were rapid changes to the curriculum which contributed to their stress [Arshad 2003].

### **Research Method**

This research used an explanatory review method with a quantitative approach that aims to measure aspects related to implementation of curriculum, that is to measure the stage of teacher knowledge and the stress level of the teachers. This is a qualitative case study according to Marshall and Rossman [1999] and Tellis [2000], where the number of participants is not limited. In fact, the number of participants was 109 teachers of economics at middle schools in Aceh Besar district. Assessment used the whole sample because of the limited population. This size was included in the range suggested by Krejcie and Morgan [1970] which was based on the size of a sample to the population size. Participants were given specific codes to protect their identity and personal rights.

A Likert scale was used in the study questionnaire to measure several aspects related to the implementation of the curriculum. Data obtained via questionnaires was analyzed using statistical software. This study used descriptive statistics for identifying information about the demographic profile of the respondents, while inferential statistics were used for correlation. Descriptive analyses used were frequency, percent, mean value and standard deviation, while the inferential analysis used the Pearson correlation. This was used to determine the relationship between the variables. A level of significance of  $p < 0.05$  was used.

### **Results and Discussion**

Analysis of cross-tabulation showed that the level of knowledge of the teachers was at a moderate level. Only thirteen (12 %) of the 109 respondents had a high knowledge, fifteen respondents had poor knowledge and the remaining 81 had moderate knowledge. Five male teachers had higher knowledge and two had lower knowledge. While eight female teachers had higher knowledge and thirteen had lower knowledge. 38 teachers understand the goals of the curriculum, thirteen teachers understand the principles of the curriculum, and thirty teachers understand the characteristics of the curriculum. Similarly, a total of thirty teachers understand the objectives of teaching economics. However, six teachers stated that the principles in the curriculum are not suitable for the teaching of economics.

36 teachers understood the implementation of the curriculum, 38 teachers understood the implementation of the teaching materials program, but thirteen teachers stated that the annual teacher upgrading programs do not enhance the learning processes for teaching economics. A total of 35 teachers understood the implementation of the teaching program, and forty teachers understood the implementation of the lesson planning program. A total of ten teachers stated that the curriculum teaching approach is not effective for teaching economics, while 26 teachers stated that the curriculum teaching method can provide a self-learning method (learners' self-assessment). However, a total of six teachers stated that the teaching techniques in the curriculum are not suitable for economics. A total of forty teachers understood the implementation of teaching resource materials, 33 teachers understood the implementation of curriculum teaching aids, and 36 teachers understood the exercise of assessments.

Overall, the mean value for level of knowledge of all the teachers of economics was 3.04, which was at a moderate level. The findings found that many teachers lacked knowledge as they had never attended a training / workshop and did not have a curriculum handbook. The findings were supported by Sutrisno [2008] who stated that understanding the model curriculum was not fully followed by the teachers. Lack of socialization of the teachers was a key factor in curriculum implementation problems. In fact, the comprehensive understanding of a curriculum whether at school level, planning, implementation or system evaluation level is essential, especially for the perceptions of teachers as protagonists so that the curriculum can be implemented as stipulated. Socialization and workshops are needed to increase knowledge and understanding amongst the teachers so that the curriculum can be implemented effectively.

Analysis of the cross-tabulations showed that teacher stress was at a high-level. 72, 65 % of respondents had high stress and 37 (34 %) had moderate stress. 24 male teachers had high stress, and thirteen had medium stress. While 48 female teachers had high stress, and 24 had medium stress. A total of 57 (52 %) teachers felt their workload was too heavy. 59 teachers said they were bored with everyday tasks, but only six teachers never felt tired and lethargic. A total of 68 teachers said they were irritable when confronted with problems at school, and a total of 82 teachers said they got a headache when doing lesson plans. Only thirteen (12 %) teachers said they would not run into trouble when doing an instructional program for a new curriculum. While a total of 62 teachers do not have enough time for family, 65 teachers found it was difficult to complete work within the time available, and there were only fifteen teachers who did not feel stress when dealing with a lot of school work. A total of 49 teachers had difficult or fast breathing if they had too much work at school, 45 teachers always suffered loss of appetite when their workload was too much, 58 teachers found it hard to sleep lately because of too much work, and 47 teachers said their hands or feet felt cold when their workload was too much. 45, 41 % of teachers bring work home because of too much work at school.

Overall, the mean value for the level of stress was 3.56, which is a high level. These findings showed that many teachers experienced stress, caused by the administrative work that must be done for their schools such as planning instructions, while the teachers felt they did not have the right skills. Teachers had to do it although they did not understand the contents of the curriculum which is a liability as teachers must do the planning for their teaching work in class. For this reason, many teachers experienced stress due to having to perform tasks that they felt did not understand.

There was a significant relationship between knowledge and stress for the teachers of economics from middle schools in Aceh Besar, Indonesia, with a coefficient of  $r = -0.175$  (sign = .034 < 0.05). The negative relationship shows that the higher the knowledge, the lower the stress towards the implementation of curriculum. Conversely, the lower the knowledge, the higher the stress. This was due to if teachers not having the expertise to develop a curriculum, teachers felt overwhelmed by the task. This finding was supported by Aeria [1998] who found several factors influencing stress. These factors included overloaded workloads, lack of teaching materials, poor physical facilities and poor curriculum implementation factors such as lack of knowledge. This study found that the majority of teachers experienced stress when making a lesson plan, as they could not complete the work within the available time, even many teachers were forced to bring work home because of too much work at school. The study also found that one cause of stress experienced by the teachers was because of the introduction of a new curriculum that required teachers to perform tasks that were not in accordance with their previous work and understandings. This finding was also supported by Gold and Roth [1993] who stated that one of the causes of stress for the teachers of economics was changing the curriculum. This finding was also supported by Thomas *et al.*, [2003] who has said that lack of time and too high a workload were major causes of stress. Teachers now have to deal with changes in the field of education so that there is overload. In conclusion, this study showed that knowledge is very important in affecting teachers in schools in order to perform task assignments that can be carried out easily and effectively without any feeling of stress or being overwhelmed. The level of thinking and understanding affects the level of teacher educators in the face of changing curriculum.

### **Conclusions**

Descriptive statistics found that the level of knowledge of the teachers was at a moderate level with an overall mean value of 3.04. While the level of stress was at a high level with the overall mean value at 3.56. Inferential statistics found that there was an inverse relationship between knowledge and stress. It can be concluded that curriculum implementation knowledge had a very important impact on the stress of the teachers. Based on the findings, there are some recommendations to promote education and improve the quality of teachers' knowledge, and the government should make policies, namely: (i) send teachers for training, seminars and workshops, (ii) provide scholarships for teachers to continue their studies in order to improve teachers' knowledge and thinking, (iii) conduct comparative studies in other schools that are considered more advanced, (iv) complete facilities and a variety of media supporting learning activities, and (v) give awards to outstanding teachers and improve the welfare of teachers by providing additional income and provide exemplary, encouragement and arouse the conscience of teachers to be aware of their duties and responsibilities as teachers.

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## Honesty in Indonesian Literature

<sup>1</sup>B. B. Dwijatmoko, <sup>2</sup>B. Ria Lestari

<sup>1</sup>The Graduate Program in English Language Studies, Sanata Dharma University, Yogyakarta 55002, Indonesia;

<sup>2</sup>English Letters, Faculty of Letters, Sanata Dharma University, Yogyakarta 55002, Indonesia;

\*Corresponding Author: b.b.dwijatmoko@gmail.com

### Abstract

As a reflection of human experience (Meyer, 1997; Dubey, 2013), literature reflects the values which a society holds. Indonesian literature, therefore, may also reflect honesty, an important value in a society, in the literature of Indonesian people. This research is a corpus study on honesty in Indonesian literature. This study aims at knowing the meaning of honesty in Indonesia as it is reflected in its literature and identifying the motivations which drive Indonesian people to be honest. The data are sentences which use the word *jujur* 'honest', *kejujuran* 'honesty', and *sejujurnya* 'honestly'. The analysis reveals that honesty falls into two types, namely verbal honesty and behavioral honesty. The analysis also reveals the motivations that drive Indonesian people to talk and behave honestly.

**Key words:** Honesty, verbal honesty, behavioral honesty, motivations, Indonesian literature.

### Introduction

Honesty is one of the most important characteristics which people need to have (Van Lange & Kullman, 1994; Shanks, 2005). Frankel (2006, p. iii) states that honesty is important in "both in personal and business relationships" besides trust. People need to have honesty to gain trust from other people. Darminta (2007) states that honesty is essential for a mutual trust in human relation, and without truth and honesty life and personal relation will be ruined.

As a reflection of human experience (Meyer, 1997; Dubey, 2013), literature reflects the values which a society holds. Indonesian literature, therefore, may also reflect the concepts of honesty which Indonesian people believe and practice in their life.

This paper reports a study of honesty in Indonesian literary works. This study hopefully can reveal the meanings of honesty for Indonesian people and the motivations which drive Indonesian people to be honest or dishonest. The study is important in that its result may help people understand each other better, and it can be used as a reference for developing honesty.

### Research Method

This paper is a corpus study using the corpus website [www.corci.org](http://www.corci.org). It used a large number of works stored in the site (47 Indonesian novels and 323 legends and folktales). Despite the number of literary works, a search in the site using the key words *jujur* 'honest', *kejujuran* 'honesty', and *sejujurnya* 'honestly' only yielded 235 sentences which used the key words. All the sentences were, then, analyzed to achieve the research goals.

Using the principles of the grounded theory (Strauss & Corbin, 1998; Gray, 2004; Neuman, 2007), the research proceeded in three stages. In the first stage (open coding), the characteristics of honesty which appeared with the key words were studied and identified. This stage resulted in meanings of honesty. In the second stage (axial coding), the contexts of the meanings were identified. This stage produced the classification of the meanings of honesty and the features which differentiated one meaning from another. In the third or last stage (selective coding), the meanings were validated against more data and were organized to formulate the types of honesty. The three stages were also taken to identify the motivations that drove the speakers or characters in the literary works studied to be studied.

## Results and Discussion

The study of honesty in Indonesian literature reveals two major types of honesty and nine motivations that drive people to be honest. This section is going to discuss the two types of honesty and the motivations.

### Types of Honesty

Honesty in the culture of Indonesia as reflected in its literature can be differentiated into two major categories, namely verbal honesty and behavioral honesty. Verbal honesty can be differentiated further into four sub-types which show the meaning of honesty. Table 1 displays the types of honesty.

**Table 1.** Types of honesty

NO	Types	Meaning	Key Words			Total
			<i>Honest</i>	<i>Honesty</i>	<i>Honestly</i>	
1.	Verbal honesty	Factual	59	28	19	106
		Emotional	32	4	2	38
		Perceptual	25	2		27
		Sincere	14	1		15
2.	Behavioral honesty	Social norm	30	19		49
		Total	160	54	21	
Total						235

In the data, verbal honesty occurs in 186 sentences, and behavioral honesty occurs in 49 sentences. Verbal honesty can be differentiated into four sub-types, namely factual, emotional, perceptual, and sincere honesty.

In *factual honesty* (106 sentences), a speaker is honest if the talk agrees with a condition which underlies the talk. The talk also presents a marker of the presence of factual honesty like in text (1).

- (1) "Probably more," he answered with a low voice. "I don't know exactly"  
"Kugy Karmachameleon, you have to be honest now. *What is the problem?*"

The speaker in the second line demands that Kugy be honest. He thinks that Kugy knows more about the problem, and he asks him to tell the truth. The sentence *What is the problem?* in the second line is also the marker of the factual honesty.

*Emotional honesty* (38 sentences) occurs if the speaker is honest with his or her feeling. The talk matches the feeling which the speaker has. Emotional honesty is marked with the use of words such as *hati* 'heart', *suka* 'like', *kangen* 'miss (you)', and *cinta* 'love' like in the following text.

- (2) If I *like* Lasi, first of all, I have to be **honest** with myself. Then, go to hell with all the gossips.

The speaker is honest with his feeling. *I like Lasi* is marker of the presence of emotional honesty.

*Perceptual honesty* (27 sentences) occurs if the speaker is honest with what he thinks or has in his mind. Perceptual honesty is also accompanied with a marker which shows that the speaker is honest with his thought like *opini* 'opinion', *pikiran* 'opinion', *pendapat* 'opinion', *tanggapan* 'response', *merenungi* 'contemplating', and *memahami* 'understanding'.

- (3) "Sorry, Gy. If you really want me to talk **honestly**, that is my *opinion*. Nothing more, nothing less."

The word *opinion* is marker of perceptual honesty.

*Sincere honesty*, or just sincerity, occurs if the speaker does not have any personal interest. He only says or does something without hoping anything in return. Sincere honesty appears in the following text.

- (4) Deep in my heart, I have to say: Wiwik, you consider the man *honest*. That he is not the same as other men.

The man in (4) does not have any ill plan for Wiwik. He may approach Wiwik with a good intention or does not have the intention to make use of her.

The second major type of honesty is *behavioral honesty*. Behavioral honesty is the honesty which a person has in doing an action or activity. The following texts show behavioral honesty.

- (5) The night was the last time I saw her. The *gentle* and **honest** girl disappeared in the following day. She did her new tasks in farming fields far away from the capital.
- (6) All his older brothers are busy working to earn money for their living. Their father was a retired military personnel who was **honest** and *stuck to the principles* so that after the revolution he did not got anything.

The girl in (5) was an honest girl in her behavior so that when she suddenly disappeared, the narrator missed her. In (6), the father worked honestly so that after the revolution he did not have anything. Had he worked dishonestly such as receiving bribes or did some corruption, he would have got a lot money or wealth after the revolution.

Behavioral honesty occurs with another good trait presented explicitly or implicitly. In (5), the other good trait is her gentleness, and in (6) the other trait is his observance to the principles. The presence of the other trait confirms that the honesty performed has a wider scope that the agreement of a talk with some condition.

### **Motivations for Honesty**

Nine (9) different motivations can be identified from the honesty which the characters in Indonesian literary works exhibit. Table 2 displays the nine motivations and distribution.

**Table 2.** Motivations for honesty

NO	Motivation	Frequency
1.	Observing social norms	56
2.	Creating a better judgment	50
3.	Creating a mutual understanding	39
4.	Emphasizing characteristic	32
5.	Maintaining relationship and image	22
6.	Fear to other people	12
7.	Having a better life understanding	10
8.	Gaining trust	9
9.	Desire for a better life	5
Total		235

Each of the motivations occurs in a certain context which helps differentiate one motivation from another.

The first motivation, observing social norms, is the motivation which drives a person to behave honestly. The person behaves honestly because he is a good person who has a good personality and observes social norms. Text (7) clarifies the motivation.

- (7) She has to get a punishment for her cruel allegation. I don't know what evils have changed Noura, who used to be **honest** and *kind* but now has changed into a slanderous heartless person.

Noura's honesty in (7) is part of her characteristics, and she is honest because that is the way of her life. She observes the norms of her society in her life.

The second motivation, *creating a better decision*, underlies the statement which a speaker says to the second person. He says the statement because he wants the second person to make a better judgment.

- (8) "... I want to hear the reason which comes from your heart, the **honest** and real reason, because you have sacrificed," Krining stared at the eyes of Aji in the bright light of the lamp. She loved the man who had shared his life with her for fifteen years and given her two children.

The woman in (8) wanted an honest reason because she was going to make her decision.

The third motivation, *creating a mutual understanding*, drives a speaker or character to talk honestly.

(9) "... You talk *honestly* to Mom," I said. "If you have a mistake, ask for an apology."

As can be seen in (9), this reason appears when there is a problem in communication or there is a problem between the speaker and the second person. In (9) the speaker had a mistake so that he does not feel comfortable to talk to his mother.

The fourth motivation, emphasizing a characteristic, emphasizes the characteristic which a speaker or character has. The characteristic can be presented explicitly or implicitly like in (10).

(10) Time went slowly as I was opening the e-mails in my husband's inbox one by one. **Honestly**, I do not feel comfortable doing that.

The use of *honestly* in (11) emphasizes the woman's honest characteristic. She is an honest person and does not want to sneak into someone else's mails although the person is her husband.

The fifth motivation, maintaining relationship and self-image, drives a speaker or character to talk *dishonestly*. He talks dishonestly because he does not want to ruin his relationship with the second person or because he wants to maintain his self-image like in texts (11) and (12).

(11) The man asked to himself. If I talk **honestly**, won't she break her heart?

(12) At that time, I could not tell **honestly** what happened to me in Fahri's room. I could not tell my own disgrace.

In (11) the narrator did not talk to the woman honestly because he did not want to break her heart, and in (12) the narrator talked dishonestly because she did not want to tell her own disgrace. Maintaining relationship and self-image only occurs in dishonesty.

The sixth motivation, fear to other people, drives a person to talk honestly because he is afraid to the person he is talking to like in (13).

(13) Ra Tanca answered the question with his hands closed to each other. Sri Jayanegara shivered when he heard the **honest** answer of Tanca, who did not have any dignity at all to him. Ra Tanca, however, was surprised when Jayanegara spit rudely to his face.

Ra Tanca answered Jayanegara's question honestly because he was afraid of Jayanegara. Jayanegara was a prince who could treat him as he liked, and it turned out that he spit on his face.

The seventh motivation, having a better life understand, drives a speaker or character to be honest in revealing nature and human condition. Text (14) exemplifies the motivation.

(14) When I entered the kitchen, I remember Sobar, my fellow journalist in Jakarta. He was a bright, **honest**, and brave journalist. He wrote critically and accurately.

Sobar wrote his report honestly because he wanted to present life as it was. His report could help other people understand life better.

The eighth motivation, gaining trust, drives a person to be honest because he wants other people to trust him. The motivation appears in (15).

(15) Sandy knew that the man was really serious. ... "I will be honest to you. When we left, I felt really messy for some days."

The woman said that she wanted to be honest because she wanted to gain attention. She wanted Sandy to believe her that she really missed him when he left.

The last motivation, desire for a better life, shows the drive a speaker or character has for a better life like in (16).

(16) But I do not need to hide it. Sometimes, honesty is still better than hypocrisy. Although not all people agree with me.



The desire for a better life occurs in a general statement like in (17). It does not refer to a specific context, and it shows that honesty is needed to have a better life.

The motivations to be honest can in general be differentiated into two classes. In the first class are the motivations which drive a person to be a better member of society, and in the second class are the motivations which drive a person for personal purposes. Observing social norms, creating a better judgment, creating mutual understanding, and having a better life understanding belong to the first class, and emphasizing characteristic, maintaining relationship and self-image, gaining trust, and desire for a better life belong to the second class.

### Conclusions

Honesty or dishonesty in Indonesia as reflected in its literary works can be differentiated into two major types, namely verbal honesty and behavioral honesty, and verbal honesty can be differentiated further into four sub-types, namely factual, emotional, perceptual, and sincere honesty. Both verbal and honesty, and its sub-categories, and behavioral honesty can be identified clearly, and it shows that the different types of honesty are important concepts which Indonesian people share well in their life.

The different positive motivations which drive Indonesian people to be honest shows that honesty is important for a better personality, better life understanding, better mutual understanding, and better society if its norms are well observed. The two negative motivations (maintaining relationship and self-image and fear to other people) indicates that honesty is still a problem for many people, but the hope for more-practiced honesty is still bigger as the number of occurrences of the two motivation is relatively low (13.6%).

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## **The Development of Senior High School Students' Worksheet Based on Chemo-Entrepreneurship (CEP) Approach on the Topic of Colloid**

\*Habibati, Zulfadli, Rizki Amalia

Chemistry Department, Faculty of Teacher Training and Education, Syiah Kuala University,  
Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: habibati581@yahoo.com

### **Abstract**

This study aim was to determine the feasibility of the LKS based on CEP approach and to evaluate the response of teachers and students against LKS-based CEP approach. The subjects were 4 chemistry teachers and 27 students of class XI IPA 3 SMAN 9 Banda Aceh that consists of 16 male and 11 female students. This research type was the Research and Development (R & D) with 4-D models, namely the define phase, design phase, develop phase, and the disseminate phase. The data was collected through LKS validity test, questionnaires, and observation. The research instrument used was the observation sheets of students' activities, attitudes and skills; the cognitive test, the questionnaire sheet of teachers' and students' response, and LKS. Based on the validation result from 2 validators, the feasibility result of LKS based CEP approach obtained was 94.6%, which can be interpreted very valid. The response of teachers to LKS based CEP approach can be interpreted very well, in which 84.4% gave positive responses and 15.6% gave negative responses. While the students' responses to LKS based CEP approach can also be interpreted very well, where 81% gave positive responses and 19% gave negative responses. Based on the results of this study, it can be concluded that LKS based CEP approach on the colloid topic was well received by the user so that it can be used as a learning material which can trigger the students to be entrepreneur.

**Key words:** Chemo-entrepreneurship (CEP), student worksheet, colloid.

### **Introduction**

The student worksheet (LKS) was one of the learning sources that can be developed by teachers as facilitator in the learning process based on the situation happened in the class (Wikhdah, Sumarti, & Wardani, 2015). LKS can be effective if it was design inovatively and interesting. Therefore, it can enhance the students' creative and critical thinking.

The curriculum of 2013 has philosophy that knowledge cannot be moved easily from teacher to their students. It requires the students to be more active and creative in sharpening their knowledge. This can be done, for example, by supporting the instructional process with LKS based on Chemo-entrepreneurship (CEP) approach. CEP approach is the approach that is related to the real life of the students in order to stimulate and develop them to be an entrepreneur. Besides getting the lesson, the students also have a chance to learn the making process of one ingredient to be one commercial product. Therefore, it is hoped that this approach can stimulate the students' creativity as they apply what they already learned in the class.

Colloid is one of the topics in chemistry lesson that are taught in semester 2 of class XI at Senior High School (SMA). Colloid contains theories related to real life experiences as it can be found in the form of paint, air, dust, etc. (Qudsiyah, Hadisaputro, & Sumarni, 2014). The colloid concept is also used in the business field such as in the production of jelly, jam, soy milk, etc. Therefore, CEP approach is suitable to be applied in the topic of colloid (Lelono & Saptorini, 2015).

Based on the observation and the interview results toward the chemistry teachers at SMAN 9 Banda Aceh (considered as SMAN that is focused on sports), it was found that, the majority of the students are less motivated to learn chemistry. This was due to they are more focus on achieving good performance in sport rather than in academic fields. SMAN 9 already has chemistry LKS based on 2009 curriculum which known as KTSP. This LKS may look uninteresting and less motivate the students to enrich their knowledge as it contains merely theories and questions to be answered especially in the

topic of colloid. As a result, the learning outcome of the students in the chemistry classes was not satisfactory (low). This was proved by the classical average value of the daily test on the lesson of colloid in the academic year of 2013-2014 was still below than the minimum completeness criteria (KKM) established at school for this subject that is 2.67. Therefore, it may be solved by designing LKS based on CEP approach.

Kusuma, Sukirno and Kurniati (2009) stated that by using CEP approach with the focus on green chemistry in the topic of acid-based solution, it can improve the students' outcome and life skills. Based on the research's result in cycle I, it was gained the average value and the mastery of students' life skills were 53,55% and 65% with middle criterion. At cycle II, these improved to good criteria with the average of 60,02% and 92,5% in a row. In addition, in cycle III, the average value of students' learning outcome and the mastery of students' life skills were 63,64%. Furthermore, the study results conducted by Kusuma and Siadi (2010) on the undergraduate subject of colloid showed that the undergraduate students mastery learning on this topic increase from 43% in cycle I to 50% in cycle II and to 86% in cycle III classically. The average score of undergraduate students' life skills in cycle I, II, and III were 38%, 55%, and 63% respectively. This was because of the implementation of learning source based on CEP approach. In addition, Rohmadi (2011) concluded in his research that the instruction by applying CEP approach with SETS vision in colloid topic could improve students' motivation to learn chemistry. This can be known from the study results in which, in cycle I, it was gained the average scores of affective, psychomotoric, cognitive, and students' activity were 80.55%, 71.52%, and 64.76% in a row. These increased to 88.89%, 76.83%, 75.51%, and 90.97% in cycle II. Therefore, it is important to conduct this research as many researchers found that CEP approach can assist the student to do better in learning chemistry as well as to be an entrepreneur.

### **Research Method**

This research's type used was Research and Development (R&D) with 4-D models. The research's subjects were four (4) chemistry teachers and 27 students of class XI IPA 3 which consists of 11 female and 16 male students at SMAN 9 Banda Aceh. These study subjects were collected through purposive sampling technique that is sample determination technique with certain consideration (e.g. research time and topic being studied).

The data was collected through the use of questionnaires, observation, and LKS validity test. The research instrument used was the observation sheets of students' activities, attitudes and skills; the cognitive test which was consisted of 5 multiple choice questions, the questionnaire sheet of teachers' and students' response, and LKS. Before these instruments were used, they were tested for their validation by 2 validators. The instruments' quality tested were test items, the observation sheets of students' activities, attitudes, and skills; and questionnaire sheet of students' response. After the data were obtained, they were analyzed by using qualitative (for questionnaire and LKS) and quantitative (for observation of students' activities, attitudes and skills) descriptive analysis techniques.

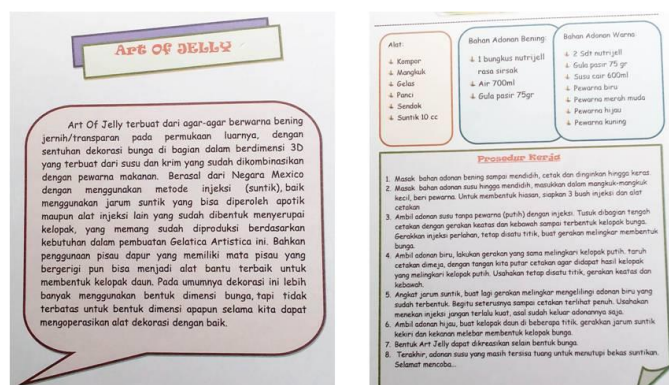
### **Results and Discussion**

#### **Define Stage**

This stage is aimed to determine and define the needs in the development of LKS which involves the analysis of problems emerged, students, concept, and preliminary design. The problem found in the LKS used at school was uninteresting as it only contains of topic's explanation and questions. In addition, the LKS's design do not attract students' attention as it does not have pictures and not colorful enough. Further, it still used KTSP curriculum which is not appropriate for 2013 curriculum. As a result, students become passive and less motivated to learn chemistry. In this stage is also determined the questions for teachers' and students' response toward LKS based CEP approach in colloid topic. The questions asked were arranged based on the rubric determined for collecting data.

#### **Design Stage**

This stage is used to design the learning source that is LKS based CEP approach. The design is made based on the weaknesses of the LKS used at school. It is hoped that the design LKS is the improvement of the recently used LKS. In the making process, first, LKS should be appropriate with the syllabi and learning implementation plan of colloid topic for 2013 curriculum. According to Rohaeti, Widjajanti and Padmaningrum (2009), in developing science subject LKS, it should contain of cover, title, learning purpose, clear and concise theories and concepts about science topic, questions, and conclusions. They also stated that LKS can be added with pictures and simple experiment. In this research, the LKS is also added with simple entrepreneurship experiment activities (see Figure 1).



**Figure 1.** Example of CEP experiment activity in the LKS

The CEP approaches LKS is divided into five parts, the two parts are contextual chemistry learning while the rest are entrepreneurship parts. In the first part of it, the students should identify the pictures about the types of colloid. The picture themselves related to the students' real world such as cheese, cloud, smoke, etc. The result of this part is that the students have an ability to identify the colloid types correctly. The second part of LKS contains an experiment about making simple colloid in which the students looked enthusiast to do and discuss its results. In here, the students did two experiments related to the making of colloid dispersedly and condensable.

The third until the fifth part of CEP approach LKS comprise of entrepreneurship experiment activities in which the students are asked to make art jelly, mango pancake, and soya bean milk with the teacher and researcher guidance. These experiments were chosen as they were related with the chemistry learning of colloid topic. The third part of LKS is about the making of jelly art. This experiment trains the students to be creative in creating painting forms inside jelly. Jelly art is related with the characteristics of colloid in which after doing the experiment they were asked to answer the questions related the characteristics of colloid. The two final parts are about mango pancake and soya bean milk. These part aims is to train the students the skills to make them. Furthermore, the students are also required to identify which materials or substances that are categorized as the types of colloid. In all parts of the experiments, the students are seen very active and enjoy the process. According to Prayitno, Dewi and Wijayati (2016), CEP approach if it is applied in the form of module as well as LKS, it can improve and equip the students with the life skills required in their future lives.

### Develop Stage

The aim of this stage is to obtain the learning source which was already revised based on the recommendation and consideration given by two validators. This stage involves LKS and questionnaires validation, trial and error at school, and setting the teachers' and students' questionnaire questions. Each of questionnaires has eight questions.

The validation result of CEP LKS showed that the average score of two validators' respond was 94,6%, which was considered as very valid and appropriate to be distributed to the students as a learning material. Meanwhile, the teacher and student's questionnaires validation result was 100%, which was assumed as very valid.

The trial and error of CEP LKS has done at SMAN 9 Banda Aceh towards 27 students of class XI IPA 3. They are students who have already learned the colloid topic before. They were also given explanation about what is meant by CEP approach LKS and then divided into 3 groups of 9 students. It was then followed by answering the questions and doing simple experiments. The experiences obtained might make them to be entrepreneur person.

### Disseminate Phase

This stage involved the distribution of CEP LKS and the questionnaires to the chemistry teachers and students in school in where the teachers were asked to validate and give feedback towards the CEP LKS. The teachers' and students' responds to the CEP LKS were obtained the average percentage of 84,4% and 81% respectively (as can be seen in Table 1 and 2), which were both considered as very good. As a result, the CEP LKS can be used as a data collection instrument.

**Table 1.** The teachers' response towards CEP approach LKS

No	The Respond Aspects	Respondent Answer Sum		Respond %	
		Yes	No	Yes	No
1	Have you developed <i>Chemo-entrepreneurship</i> (CEP) approach LKS to the topic of colloid?	0	4	0	100
2	Are you interested in CEP approach LKS that has been developed for colloid topic?	4	0	100	0
3	Has CEP approach LKS been appropriate with the indicators and the learning purposes for colloid topic?	4	0	100	0
4	Has CEP approach LKS used the language that is easily to be understood?	4	0	100	0
5	Can the content of CEP approach LKS help the students to understand better the colloid topic?	4	0	100	0
6	Can CEP approach LKS make you teach the colloid topic better?	4	0	100	0
7	Does CEP approach LKS appropriate to be used as a learning material for colloid topic?	4	0	100	0
8	Can the availability of CEP approach LKS make you be motivated to develop it for different chemistry topics?	3	1	75	25
Total percentage				675	125
Percentage average				84,4	15,6

Based on the table above, it can be seen that, for example, in question number 2, all the teachers gave positive responds. These were due to they can learn new knowledge on how to make a connection with being an entrepreneur person when learning colloid topic. Another example was the question number 8 in which three teachers gave positive statements whereas one teacher gave negative respond. This teacher said that almost chemistry topics have mathematical chemistry that may be difficult to be implemented to support the students to be an entrepreneur person in which they can produce something, sell it, and get benefit of it. This was in line with the statement of Nurmasari, Supartono and Sedyawati (2014) who said that the complex mathematical chemistry sometimes makes the students to experience difficulties in mastering the chemical concepts.

**Table 2.** The students' response towards CEP approach LKS

No	The Respond Aspects	Respondent Answer Sum		Respond %	
		Yes	No	Yes	No
1	Have you learned a chemistry topic with the used of <i>Chemo-entrepreneurship</i> (CEP) approach LKS?	0	27	0	100
2	Are you interested in CEP approach LKS that has been developed for colloid topic?	27	0	100	0
3	Has CEP approach LKS used the language that is easily to be understood?	27	0	100	0
4	Is the use of CEP approach LKS help you understand better the colloid topic?	27	0	100	0
5	Do you like better the way the teacher present colloid topic with the use of CEP approach LKS?	27	0	100	0
6	Are the pictures and colors in the CEP approach LKS make you interested to read it?	25	2	92,6	7,4
7	Can CEP approach LKS that has been developed improve your motivation to learn colloid topic?	23	4	85,2	14,8
8	Can learning colloid topic with the use of CEP approach LKS stimulate you to be an entrepreneur person?	19	8	70,4	29,6
Total percentage				648,2	151,8
Percentage average				81	19

Based on the table above, it can be concluded that the students have never been learnt a chemistry topic by using CEP approach LKS before. They also found that CEP approach LKS is an interesting LKS which can make them motivated to learn chemistry better especially in colloid topic as this LKS used the language that is easily to be understood and because of the way the teacher present the topic of

colloid. As a result, the chemistry learning experiences become meaningful for them. However, there were two students who did not like the way the LKS is presented in terms of its pictures and colors. This is due to they were more like direct experiences rather than read the colloid topic through LKS. Thus, they were less motivated to read it. Whereas, the majority of the students liked it as they said that the pictures presented are related to their real life experiences. In addition, they also said that CEP approach LKS comprises of simple experiment methods which are not difficult to be implemented. Therefore, they were motivated to read and to learn colloid topic through it. Last but not least, 19 of 27 students said that CEP approach LKS stimulates them to be an entrepreneur person while the rest of them (which were all men) said they were less stimulated as they had another dream (ambition) that was to be professional athletes. These results are in accordance with the research results of Rohmadi (2011) who noted that CEP approach with *Science, Environment, Technology and Society* (SETS) vision can improve students' outcome, performance, motivation, and proclivity in learning chemistry lesson. Further, Supartono, Saptorini and Asmorowati (2009) argued that CEP approach can make the students learning experiences of chemistry lessons more meaningful and joyful.

### Conclusions

Based on the research result, it can be concluded that the LKS based on CEP approach on colloids topic is proper to be used as teaching and learning source. As it is stated very valid by two validator who gave an average scores of 94,6%. Teachers' feedback can be interpreted very well as 84.4% gave positive responses while 15.6% gave negative responses. The students' responses to LKS based CEP approach can also be considered very well, where 81% gave positive responses and 19% gave negative responses.

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## SWOT Analysis: How Compact Curricular Agenda affects English Teaching and Learning Process

<sup>1</sup>\*Nyak Mutia Ismail, <sup>2</sup>Juliana, <sup>3</sup>Rusma Setiyana, <sup>4</sup>Hayatul Muna

<sup>1</sup>Department of English Language Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>2</sup>Nurse Academy of Kesdam Iskandar Muda, Banda Aceh 23121, Indonesia;

<sup>3</sup>Teuku Umar University, Meulaboh, 23651, Indonesia;

<sup>4</sup>STAIN Malikussaleh, Lhokseumawe, 24351, Indonesia;

\*Corresponding Author: nyakmutiaismail2010@gmail.com

### Abstract

A successful process of English teaching and learning has been considered as an ultimately major goal in Indonesian high-schools, including in integrated Islamic high-schools known with doubled-curriculum schools. To mention further, this agenda tightens students' academic activities as other subjects also bring about numerous high standard motives and goals. This study was conducted to analyze the effect of this compact curricular agenda towards the process of English Teaching and learning from the teacher's perspectives. The curricular agenda at SMP IT Az-Zahra Takengon was SWOT-analyzed regarding its effect to the process of English teaching and learning at the school. In significance, this study is expected to broaden the knowledge of stakeholders of high-school curriculum management in developing the curricular academic activities in more deliberately careful ways in the purpose of reaching the goal protocols. The method used in this study was mainly qualitative approach through interviews which solely sheds light on the description of the academic agenda in the congruence of English teaching and learning process through interviews with the English teacher at the school. The result showed that the strength is that it has risen the students' motivation whether in learning English or other subjects since the school provides various curricular activities, instead of monotonous ones, that loosen the students' boredom and tension. The weakness is that full academic agenda leads to the condition where lots of English lessons are left untaught from which we eventually see that it are a management failure. The opportunity from the agenda is that the students are exposed to multi-disciplinary material abundance. Finally, the thread is that realizing the students with low motivation in English would seemingly remove English into their blacklists as a result of exhaustion.

**Key words:** SWOT analysis, curricular agenda, English teaching and learning.

### Introduction

Recently, most schools have complied with the condition where the students should have a lot of academic activities, especially those schools with favorite-in-town predicate and private schools in order to achieve better students' achievement and higher-order thinking ability. Specifically speaking, private schools—including Integrated-Islamic schools—carry out double curriculum, from which the authors derived the definition for compact curriculum. It is run under the circumstances of both national curriculum and institution curriculum called Integrated-Islamic curriculum as it is one of the policy of *Jaringan Sekolah Islam Terpadu (JSIT)* or Integrated-Islamic School Network (Zahri, 2013).

SMPIT AzZahra Takengon is one of the schools that implement both 2013 National Curriculum and Integrated-Islamic curriculum for it is one of the schools under the *JSIT* network. Integrated-Islamic curriculum expands in term of teaching Islamic-characterized lessons such as *Tahfiz* and *Tahsin Quran* (Quran memorization and re-learning), *Hadits* memorization, leadership and social trainings, Islamic-character building classes, and outtings. Besides coping with national curriculum which includes English lesson, the students also have to be able to memorize *Quran* and *hadits*. This condition provides heavier activities for students and the authors wonder whether it is manageable for them. This is in line with Zahri's (2013) perception that the society see that *JSIT* schools have definite propensities in concentrating toward cognitive ability alone and induce the students to be burdensome. And burdens lead to stress. Further, Chen *et al.* (2014) add that academic burnout can also result from the unwell-being feeling and lack of competencies in any sorts of learning environment.

Concerning the analysis tool, SWOT Analysis, which is a straightforward framework that majorly done in evaluating an ongoing program in attempt to set a meaningful change and improvements (Orr, 2013), was used. According to Orr (2013), the *Strength* reveals out what the institution has successfully done; the *Weakness* refers to the desired improvements by the institution; *Opportunity* is the advantage that the institution could earn; and *Threat* focuses on the things that come as obstacles for the institution in running its program. *Strength* and *Weakness* are internally resourced, while *Opportunity* and *Threat* are rather external.

In the objective of this study, this compact curriculum application was analyzed using the SWOT-Analysis framework for which it examines the strengths, weaknesses, opportunities gained by the students, and threats faced by the students all the way through toward the English learning at SMPIT AzZahra, Takengon. While in significance, this study is expected to broaden the knowledge of stakeholders of high-school curriculum management in developing the curricular academic activities in more deliberately careful ways in the purpose of reaching the goal protocols.

### Research Method

The instrument used in this study was dominantly interviews. The interview with an English teacher at SMPIT AzZahra Takengon was arranged and she answered 7 questions concerning the curricular activities at the school. Ever since this school has just run its premiere year, so there is only one English teacher who handles only the 40 single-grade students who are divided into two classes, just to mention. The questions were formulated by the authors based on the SWOT principles in accordance with the curricular activities. The interview questions are as follows:

1. As an English teacher, do you agree with a school when it runs compact curriculum?
2. What are the merits for English teachers in a compact-curriculum school in concern to English teaching-learning process?
3. What are the drawbacks for teachers in a compact-curriculum school in concern to English teaching-learning process?
4. What opportunities can the students gain in a compact-curriculum school in concern to English teaching-learning process?
5. What is major risk for the students in a compact-curriculum school in concern to English teaching-learning process?
6. Are there students complaining the tight academic agenda affecting their English learning?
7. Do you on your own complain the tight academic agenda that affect your English teaching activity?

The process carried out in the researching process is as elaborated in the following. The authors came upon the English teacher to interview her concerning the curricular agenda at the school. The information from the teacher was written. After the interview, the teacher also related her experience and her viewpoints to the implementation of compact curriculum with its compact agenda as well. After the process settled, the authors thanked the teachers for the information she had shared. The data analysis was done through the three-step analysis including data reduction, data display, and data verification as suggested by Miles, Huberman and Saldana (2013).

### Results and Discussion

In this section, the result of this study which stems into four components are explicated. The first component is the strength of the curricular agenda toward the English teaching and learning (ELT) process; the second one is its weakness; the third and fourth are the external factors that predispose the ELT process in term of opportunity gained and threat confronted, respectively.

The foremost to mention is the strength. As pinned in the earliest section, the JSIT curriculum includes *Tahfiz and Tahsin Quran* (Quran memorization and re-learning), *Hadits* memorization, Islamic-characterized leadership and social trainings, Islamic-character building classes, and outtings to its instructional agenda. From the interview of question 1 and question 2, it was obtained that these additional lessons have emboldened the students' motivation whether in ELT or other subjects. The teacher noted that the students are very much delighted with assortedly additional activities they encounter at school especially the outting classes or better known as vacational classes. All teachers are obliged to take the students on a vacation class outside the school, to a recreational place in where they would learn about Islamic essence in accordance with nature (*Tafakkur*). Verily, the teacher regularly turned this activity into an English conversation class and the students have to express their amazement in English. In another occasion, the teacher also happened to tell the students several new words and expressions whilst they are doing the *Tafakkur*. The teacher noticed that during this sort of activity, their motivation in learning English elevates. The most probable reasoning for this is that in the



recreational area—outside the classroom, the students have less to worry about and feel more loose and open. Shia (2008) clarifies that this is seen as an intrinsic motivation and extrinsic motivation. Intrinsic motivation is where the students find a need to *mastery orientation* and *work-avoidant orientation*. In *mastery orientation*, the students render their motivation to task rather than to learning, however this leads to learning process though. Concerning to work-avoidance, the students do not have anything to accomplished through writing activity, which indeed they repel. Further, it also relates to extrinsic motivation where the students are motivated by *power* motivation, which is a desire to prove themselves to others and a feeling as a competent learner, with neither the feeling of rigor nor reluctance. In addition, based on the Krashen's Monitor Hypothesis, the language learner are easily learning and acquiring the language when their affective filter is low, and this promotes higher motivation afterward (Ellis, 1985).

Second, it is the weakness on the compact curriculum implementation toward the ELT process. From the answer for question 3, question 6, and question 7, the authors were informed that tight activities lead to the condition where quite a few of English lessons are left untaught. Since the teacher has to focus on many agenda, as all agenda are priority at the school. She has to teach *Tahfiz* and *Tahsin* as well, which means she has to memorize Quran for herself in advance. Besides, she is in charged to take the students to a recreational place once a month and still needs to arrange for the fun and appealing teaching scenario. Since interesting environmental learning created from interesting scenario would boost up students' motivation and promote the students' intrinsic motivation in language learning (Othman & Shuqair, 2013). This situation sometimes leads to a stressful situation or even burnouts. Administrative workload, along with academic workload, work environment, promotion and evaluation, and research funding, is a factor that can cause burnouts both in teachers and academes (Karabiyik, Eker & Anbar, 2008). In addition, Lou and Chen (2016) notify that burnout in English teachers is majorly caused by workload and teaching courses, students, management, teacher development procedures, and family.

Third, the component to be explained in this section is opportunity of compact curriculum implementation toward the ELT process. In asnwering question 4, the teacher clarified that the students can learn more abundance of materials from various activities. For example, when they do the *Tafakkur*, they could learn English lexis and expressions about nature and its conformation wiht Islamic tenets. Besides, when they are in leadership class or character building class, they also learn ample amount of vocabulary pertaining leadership and character building. The words and expressions are seemingly to be useful for them in the actual English classes. Likewise, Krashen's Input Hypothesis favors this condition where it states that the comprehensible input is likely to be acquired in a real situation without force and correction. It supplies the students not to learn immediately, but to learn when they are ready (Ellis, 1985).

The lattest is the threat. The teacher reported, in order to meet the answer for question 5, that the students are lethargic to activity that involves writing in the English class, including grammar where the students' ability in grammar is rather feeble. Nonetheless, they are prone to oral activities as mentioned earlier. Naturally, activities in English class that involve productive skills is more difficult than that of receptive. However, from the factual condition we learn that both writing and oral skills are productive skills. The authors are convinced that this situation happens because writing are more strenous that speaking since in speaking, the students can freely express their ideas and correct them as soon as the mistake is made and it never needs hectic editings and revisions. On the contrary, writing demands a very well-furnished text where the students have to invent ideas, contrast, concede, and wrap them into a desired forms of genre types thorough text interpretation and reflection (Hayes, 2003). This is in relation with work-avoidance motivation as the students are already replete with curricular agenda, so they avoid tied-up tasks. Still, there is no bond between their burnouts and English lesson mastery. This assupmtion is supported in a study by Palabiyik (2014) whose result states that there is no empirical correlation between academic burnouts and proficiency level.

The result of the SWOT Analysis on the implementation of compact curriculum toward the ELT process is intertwined in the following table.

**Table 1.** SWOT Analysis Result

No.	SWOT Principles	Result
1	Strength (Internal)	Various curricular activities elevate motivation.
2	Weakness (Internal)	The teacher stumbles into deficient time to finish teaching all of the English lessons (chapters).

3	Opportunity (External)	Students learn more of English words, phrases, and expressions from plentiful and heterogeneous materials.
4	Threat (External)	Students are too weary and they might develop the possibility to resent English.

In addition to the result drawn in the table above, the teacher also informed that the students' stress and joy come across the balance since the school does not offer dormitory life. So after a day full of tiring activities, they have chance to go back home and meet their family, and this is where the exhaustion is healed.

### Conclusions

In conclusion, the SWOT Analysis result has shown that the society's supposition that compact-curriculum schools are burdensome for most students is somewhat hypothetical. It depends from which point of view it is outlooked. It does not serve the drawbacks alone, but there are feasible potentials offered by such plenary curriculum instructions.

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## **Language Learning Strategies Employed by Successful and Less Successful Learners**

Chairina Nasir, \*Yunisrina Qismullah Yusuf, Raihan Zulfarlia

Department of English Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: yunisrina.q.yusuf@unsyiah.ac.id

### **Abstract**

This study investigated the language learning strategies used by successful and less successful learners in the first grade of a senior high school in Banda Aceh. Both groups of learners were categorized based on their scores on an English test. A number of 20 successful and 20 less successful first grade students were then selected as the respondents. A questionnaire was used to identify language learning strategies used by both groups of these learners. The questionnaire was adapted from Oxford (1990), which consisted 30 items related to six categories of language learning strategies: memory, cognitive, compensation, metacognitive, affective, and social. This data were then analyzed by using the independent sample t-test. The findings revealed that there was a significant difference between language learning strategies used by successful and less successful learners. The successful learners were found to use the language learning strategies more frequently than less successful learners. Thus, both groups of learners obtained the highest scores in the metacognitive strategy; this implies that these learners understood how to manage their own learning. The lowest scores obtained by the successful learners were in the affective strategies, whilst the less successful learners were in the memory strategies. Other strategies (compensation, cognitive and social) were also used by both groups of these learners. Hence, less successful learners did not use all of the language learning strategies very often; this is what affected them to become less effective in language learning. Therefore, it is suggested that teachers should train the less successful learners to apply more language learning strategies in their learning to improve their academic achievement.

**Key words:** Language learning strategies, successful learners, less successful learners.

### **Introduction**

Being successful in acquiring a language is the main purpose of learning a language. In order to be successful, there are many factors that can influence students' success in learning a language. Among them is the language learning strategies that can determine students' success in acquiring a language (Oxford, 2003). Oxford (1990, p. 8) said that "learning strategies are specific actions taken by the learners to make learning easier, faster, more enjoyable, more self-directed, more effective and more transferable to new situations". Similarly, Schmeck (1988, p. 5) defined learning strategy as the implementation of a set of procedures for accomplishing learning. Richards and Platt (1992, p. 209) further stated that learning strategies are intentional behavior and thought used by the learners during learning so as to better help them understand, learn, or remember new information. These definitions show that learning strategies are the steps taken by the learners to facilitate, help, and make them easier to achieve the goal of learning. The ways or steps students gain in learning will help them to better understand the target language. Based on Ellis (1994, p. 545), "there is a positive relationship between learning strategies and language learning process in learning". It means that students' learning strategies influence their learning process; it makes them easy to understand the material given because they use strategies in their learning. Furthermore, age, gender, individual difference, motivation, cultural background, etc., are also among the variables which affect the use of language learning strategies (Lee, 2010; Özmen & Gülleroğlu, 2013).

Considering the importance of language learning strategies in helping students in achieving their goals, this paper is to examine these strategies that are used by successful and less successful EFL (English as a Foreign Language) learners in a senior high school in Banda Aceh, Indonesia. The research question to this study is: Is there any significant difference between language learning strategies used by the

successful and less successful EFL learners? From this research question, the following hypotheses are formulated for this study:

- H<sub>a</sub>: There is a significant difference between language learning strategies used by successful and less successful learners.  
H<sub>o</sub>: There is no significant difference between language learning strategies used by successful and less successful learners.

It is expected that by knowing and understanding the language learning strategies used by successful and less successful learners, teachers can bring their learners' awareness in using language learning strategies and further train them to develop and to use various strategies in their learning to accomplish the goals of learning.

### **Literature Review**

Some studies, such as those conducted by Afdaleni (2013) and Özmen and Gülleroğlu (2013) found that there is a relationship between the language learning strategies used by the learners in learning a language to their achievement. Therefore, to bring students' awareness in the learning process can help them to be successful in the target language because their awareness in learning assists them to control their own strategies. This is in accordance with Ellis (1994, p. 549) who stated that "successful learners are thoughtful and are aware of themselves in relation to the learning process". Furthermore, identifying the language learning used by the learners is important because many learners are not aware of the strategies they use. Meanwhile, they need to know the kinds of language learning strategies that they might use in order to be more competent in solving tasks in class. Wenden (1998) further suggested that the different kinds of language learning strategies should be employed jointly to achieve success in learning because there is no good or bad strategy. Therefore, a learner should not focus only on one strategy.

SILL or (Strategy Inventory for Language Learning) can be used as the tool to identify the language learning strategies used by learners. This inventory was generated by Oxford (1990), in which she divided the language learning strategies into direct and indirect strategies. Direct strategies are the strategies used directly by the learners in dealing with a new language and they are memory, cognitive, and compensation strategies. Indirect strategies are the strategies used by the learners to manage their learning and they are metacognitive, affective, and social strategies. Moreover, the strategies in SILL cover all of the four language learning skills, which are listening, reading, speaking, and writing (Oxford & Nyikos, 1989). Many studies have used this instrument in investigating language learning strategies because the items do not show prejudice and are appropriate to most cultures (Oxford, 1996). Every strategy has its own advantage for different kind of tasks. For the six strategies of language learning strategies, their advantages are equal based on the task given.

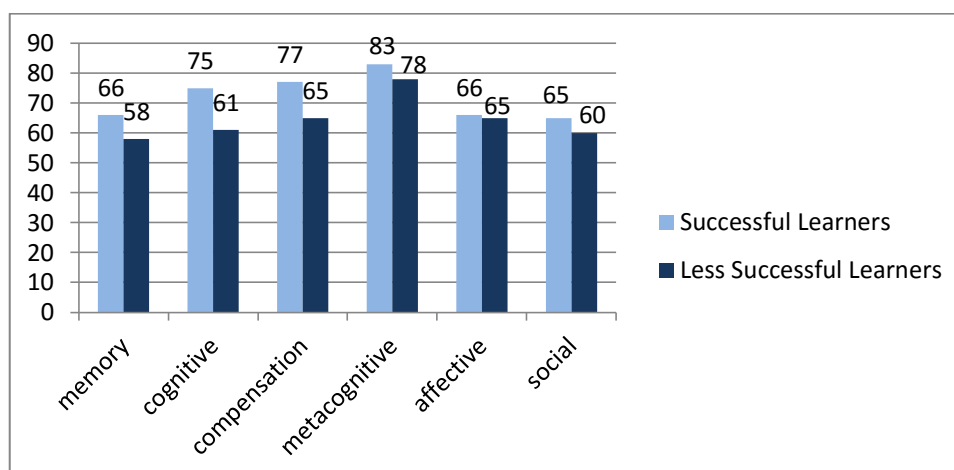
### **Research Method**

The respondents were selected randomly from a senior high school in Banda Aceh. The first grade students from three classes (with about 35 students from each class) were given an English test that had to be completed within 30 minutes. The test were 50 items and in the form of multiple choices which included reading and structure competences. The test was taken from the National Examination for the junior high school students from the previous year. The results were scored by using NR (Number Right) scoring. Only the right answers were counted, and the wrong answers were counted as zero. Then all of the correct answers were multiplied by two. After scoring was completed, a number of 20 students with the highest scores were categorized into the successful learners; they obtained scores above the KKM (Kriteria Ketuntasan Minimal) or standard value (within 81-100) determined by the school. Another 20 students with the lowest scores were categorized into the less successful learners; they obtained scores below the KKM (0-75). Since this is a comparative study, we selected 20 students from each group to have balanced data between successful and less successful learners.

One week after the test, the selected respondents were to fill in a close-ended questionnaire. The questionnaire was adapted from the Strategy Inventory for Language Learning (SILL) version 7.0 for ESL/EFL learners proposed by Oxford (1990). It consists of 30 statements related to the six categories of language learning strategies. For this research, the questionnaire was translated into Indonesian to ease the respondents in comprehending the statements. After the respondents filled in the questionnaire, their statements were evaluated on a five-point likert scale, ranging from (1) never true of me to (5) true of me. The data was finally analyzed by using the independent sample t-test to study the six categories of language learning strategies used by these two groups of learners.

## Results and Discussion

After the questionnaire scoring was done, the results in general which inquired about the language learning strategies of memory, cognitive, compensation, metacognitive, affective, and social of the successful and less successful learners are shown in Figure 1.



**Figure 1.** The average score of each category of language learning strategies used by the successful and less successful learners

Based on Figure 1, it can be concluded that for all of the six categories of language learning strategies, successful learners employ all the language learning strategies more often than less successful learners in every category.

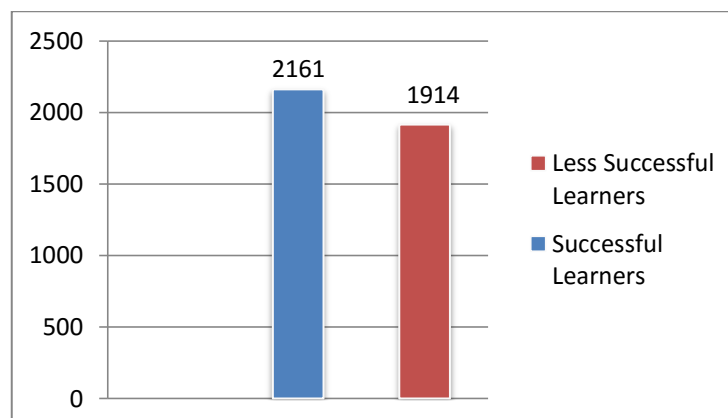
For the highest scores, both successful and less successful learners obtained the highest score for the same strategy, which are the metacognitive strategies. In this category, successful learners got the highest score for statement number 20 (*I have clear goals for improving my English skills*) which obtained the score of 90. This indicates that successful learners were confident of their goals to learn English while the less successful learners were less aware of it. For less successful learners, statement number 17 (*I pay attention when someone is speaking English*) obtained the highest score of 80. Therefore, the teacher's speaking skills greatly influenced their interest in learning.

For the lowest scores, successful learners obtained the lowest score in affective strategies for statement number 25 (*I talk to someone else about how I feel when I am learning English*) with the score of 51. This refers to discussing their feelings with someone else when learning, and this helps them to be better language learners (Oxford, 1990). For less successful learners, the lowest score was in the memory strategies for statement number 4 (*I remember new English words or phrases by remembering their location on the page, on the board, or on a street sign*) with the score of 49. This refers to applying images and sounds as a way to remember new information that has been heard or read in the target language and make a mental image of it.

Thus far, the findings are similar to the study conducted by Afdaleni (2013) where the samples from her study also used metacognitive strategies as the most in language learning. Accordingly, metacognitive plays an important role in language learning because when learners understand how to manage their own learning, language learning process can be faster (Anderson, 2002). Oxford (1990, p. 136) also has a similar point of view; she stated that "metacognitive strategies are essential for successful language learners, because many learners lose their focus and it can be regained by raising students conscious of using metacognitive strategies". These strategies then provide awareness to students of their lesson contents and help them plan their monitoring towards comprehension on the lessons learned (Fitrisia, Tan & Yusuf, 2015). Although metacognitive strategies are important for students' success, it will benefit less if learners only focus on one strategy. Therefore, these strategies should cooperate with others to make learning more efficient (Wenden, 1998).

Additionally, Figure 2 displays the total scores of the successful and less successful learners from the questionnaire. The numbers prove that the successful learners obtained higher scores (2161) than the less successful ones (1914). This implies that the successful learners used more language learning strategies than the less successful ones. This finding is in accordance with the study done by Afdaleni (2013) and Özmen and Gülleroğlu (2013) who also ascertained that learners who are categorized as

successful learners apply more language learning strategies with higher scores as compared to unsuccessful learners who use less language learning strategies or with lower scores.



**Figure 2.** The total scores of questionnaire of language learning strategies used by successful and less successful learners

After we calculated the scores of both groups of learners, we tested the normality of data. The result showed that successful learners obtained  $\alpha=0,941$  and less successful learners obtained  $\alpha=0,499$ . Since both groups of learners obtained values more than  $\alpha$  (0.05), this indicates that the two samples is of normal distribution. Afterwards, the test of homogeneity by using the Levene test also showed that the variance between the two samples is the same or homogeny ( $0.096 > 0.05$ ). Consequently, the difference between language learning strategies used by both groups of learners was done by conducting the t-test for independent samples. The means, standard deviation, and standard error deviation were calculated and the results are shown in Table 1.

**Table 1.** Independent samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Strategy	Equal variances assumed	,096	,758	3,263	38	,002	12,550	3,846	4,765	20,335
	Equal variances not assumed			3,263	37,964	,002	12,550	3,846	4,765	20,335

To find the significant difference, the t-score was compared to t-table, and sig. (2-tailed) was compared to  $\alpha$  (0.05). From Table 1, the t-score is 3.263, at df (degree of freedom) of 38 ( $n_1+n_2-2$ ), and the t-table score is 2.024 or  $3.263 > 2.024$ . This means  $H_a$  is accepted and  $H_0$  is rejected. The same result was also found by comparing sig. (2-tailed) to  $\alpha$  (0.05), where the sig. (2-tailed) is 0.002. Thus,  $0.002 < 0.05$ , and this means that there is a significant difference between the language learning strategies used by both groups of learners of the first grade senior high school students in Banda Aceh. It further infers that those successful learners used language learning strategies more frequently than less successful learners.

## Conclusions

From the findings of this study, it can be concluded that there is a statistical difference between successful and less successful learners in using language learning strategies. The difference is also at the frequency of using these strategies by both groups of learners. They both employed all of the six

language learning strategies, however, successful learners employed all more often than less successful learners. Both groups were found to obtain the highest scores in metacognitive strategies. Subsequently, the lowest scores for the successful learners were in the use of affective strategies, whilst the less successful learners were in the use of memory strategies. Hence, it is expected that teachers can teach less successful learners to apply more language learning strategies in their learning to improve their learning outcomes. Both groups of learners should also be trained to employ all strategies cooperatively to produce more resourceful learning effects.

This research was conducted with limitations, such as the number of respondents and the time constraint. Therefore, future related studies are suggested to enlarge the number of respondents from both groups of learners (i.e. successful and less successful learners). The trends in learning from these groups of learners specifically in each skill of language learning are also suggested to be examined. We believe that this would give a clearer impression to the main points of conclusion that has been drawn from this research.

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## **"Oke, any questions?" The Questioning Interaction in an EFL Classroom**

Fina Yanita, \*Yunisrina Qismullah Yusuf, Sofyan A. Gani

Department of English Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: yunisrina.q.yusuf@unsyiah.ac.id

### **Abstract**

This study examined the teacher's questioning interaction for meaning negotiation in an EFL classroom at a junior high school in Banda Aceh, Indonesia. Data was collected from an English teacher who was teaching seventh grade students. The instruments used were an observation sheet adapted from Arian (2004), a set of interview questions adapted from Skilton and Meyer (1992) and Beutel (2010), and recordings. The questioning interaction was analyzed following on the three questioning devices: comprehension check, clarification check and confirmation check (Long, 1983). The findings showed that comprehension check was the most dominant device applied by the teacher (30%), followed by clarification check (14.3%), and confirmation check (12.8%). The dominant use of comprehension check in the classroom reflected the typical interaction in the EFL context, in which there was much more negotiation of meaning going on between the teacher and students to gain understanding. All of these devices were indicators of the students' involvement in the classroom on negotiation and learning with the teacher. The result from the interview with the teacher further revealed that she must ask questions to know the students' understanding of what is being learnt in class. By being aware of this, upcoming directions on her teaching to reach her teaching objectives can be made more effectively.

**Key words:** Questioning, interaction, teacher, students, EFL classroom.

### **Introduction**

Being successful in acquiring a language is the main purpose of learning a language. The quality of a teacher's interaction with students plays an important factor in determining a teacher's success in his or her teaching. A teacher who fosters positive relationship with the students can create more conducive classroom environment for learning. The classroom interaction includes all of the classroom events, both verbal and non-verbal. The verbal interaction takes place because of the teacher and learners talk, while non-verbal interaction covers gestures or facial expressions by the teacher and learners when they communicate without using words (Tuan & Nguyen, 2010). Parrish (2004) finds that the language that teachers use in class, or "teacher talk," can have a tremendous impact on the success of interactions they have with students. Therefore, the classroom interaction is important, because it determines the learning opportunities the learners get, and how the teachers and learners together manage the classroom interaction and learning opportunities (Allwright & Bailey, 1991).

Additionally, learners also learn a language better through conversation and interaction (Masrizal, 2014). In view of that, Brock (1986) believes that an increase in the amount of classroom interactions will help foreign language learners learn the target language better, or in other words, increasing the language output will improve the language learning. One of the characteristics of teacher to students' interaction is through asking questions. According to Long (1983), there are three types of questions in term of teacher to learner interaction. Those consist of confirmation checks, comprehension checks, and requests for clarification. These devices refers to instances in an interaction in which the speaker and listener work together to determine that they are talking about the same thing (Farooq, 1998, p. 3). In other words, when the speaker carries out comprehension checks ('Know what I mean?') to determine whether he/she has been correctly understood, and when the listener requests clarification ('What do you mean, she's silly?') or confirms that he/she has correctly understood ('You stopped because you didn't learn anything?') (Nunan, 1989, p. 45).



A teacher's questioning is one of the manifestations on the merit of teaching. It plays an essential role in promoting students' knowledge construction and cognitive development. Therefore, it is deemed essential to conduct further investigation on the teacher's questioning interaction, especially in an EFL classroom. Hence, we would like to study a non-native English teacher's interactions through asking questions to her EFL students in class. This study intends to answer the following research questions:

1. What types of questioning interactions are used by the teacher in teaching her EFL students?
2. What are the teacher's views on using questions to negotiate meaning with her students?

### **Literature Review**

A number of studies have been conducted on classroom interactions between students and teachers. A study by Suhanna (2013) on senior high school students in an EFL classroom in Aceh, Indonesia, revealed that the teacher used a great amount of clarification request to her students, followed by comprehension check. Moreover, another study by Tsui (1991) on classroom interaction on ESL learners at an English medium school in Hong Kong found that clarification request as the most used interaction than other devices in the classroom, followed by confirmation checks.

Consequently, clarification check indicates learner's involvement in class because the questions require students to give alternative answers that help increase their understanding. From these studies, for the EFL learners, what follows is comprehension check (Suhanna, 2013), whereas for the ESL learners, it was followed by confirmation check (Tsui, 1991). Apparently, the situation of an ESL classroom did not require the teacher to use much of comprehension check since English is also a part of their lives; this is the contrary to the EFL learners where English is only employed in the classroom.

### **Research Method**

From three English teachers of the junior high school that was under study, only one of them consented to participate and be recorded for data of this study. She is coded as SZ in this paper. She has been an English teacher for the past 15 years and has received her teacher's certification.

The research instruments employed for this study were recordings, an interview and field notes. In this study, we had video recorded three classroom meetings of SZ with the 7th grade class that she was teaching; it consisted of 36 students. These recordings were later transcribed, analyzed and categorized for the three types of questions in term of teacher to students' interaction: confirmation checks, comprehension checks, and requests for clarification (Long, 1983). To study the most to the least devices employed by the teacher, a simple statistical formula was used to convert the frequency of occurrence into a form of percentage.

We further conducted an interview with SZ to gain additional information on her questioning practice in class. The interview questions were adapted from Skilton and Meyer (1992) and Beutel (2010) that asked about teacher's perceptions on the use of questions in negotiating meaning in the classroom. Thus, we had modified some of the questions to suit the context of this research. The questions we asked SZ were:

1. Do you think a teacher should ask questions in class? Why or why not?
2. In the classroom, how often do you ask questions?
3. How do these questions with students influence classroom learning?
4. What kind of questions in negotiating meaning do you often ask in the classroom?
5. What kind of questions in negotiating meaning do you rarely ask in the classroom?

The results from the interview were also transcribed. Then they were analyzed and processed by following the steps of data reduction, displays and drawing conclusion or verification (Miles & Huberman, 1994).

### **Results and Discussion**

The questioning interactions were used by SZ to improve the quality of her meaning negotiation in the classroom. The identified interactions were categorized into comprehension, clarification and confirmation checks. Table 1 displays the most to the least used devices by SZ in the classroom.

**Table 1.** The occurrence of the questioning interactions

<b>No</b>	<b>Devices</b>	<b>Frequency</b>	<b>Percentage</b>
1	Comprehension Check	21	52.5%
2	Clarification Check	10	25%
3	Confirmation Check	9	22.5%
	Total	40	100%

From Table 1, it can be concluded that comprehension check was the dominant device used by SZ in the classroom as an attempt to negotiate meaning with her students (52.5%), and it was followed by clarification check (25%) and confirmation check (22.5%).

The discussion with previous studies shows different findings obtained from this study. The study by Suhanna (2013) and Tsui (1991) found that clarification checks were the most used in the EFL and ESL classrooms, nonetheless, this study found comprehension check to be the dominant device used in class by the teacher. Most of the negotiation here occurred to help students gain more understanding on the subject being taught. Compared to Suhanna (2013), whose participants were senior high school students, the participant in this study were still in their first year of junior high (7th grade). They have just received the English subject that year; therefore, more negotiation on comprehension was needed compared to Suhanna's 11th grade senior high school students who have studied English for the past five years of their school education.

The discussion on the process of meaning negotiation in teacher to students questioning interactions in SZ's classroom is further elaborated below.

### **Comprehension Check**

Comprehension check, which occurred 52.5%, was the most employed device by SZ to check students' understanding. Its use is to avoid breakdown in conversation and as strategy to check whether or not the material presented by the teacher had been understood by the students (Boulima, 1999). In the following example from the recording, "E" refers to extraction, and "SZ" is for the teacher, S is for a student and Ss is for more than one students.

- (E1) SZ : *Sepuluh dari semua ini, boleh dibuat apa-apa saja kan tapi ada bentuk apostrophe-nya. Oke, understand?*  
 S : *Buat silsilah?*  
 SZ : *Tidak usah buat silsilah. Tidak usah buat silsilah lagi ya!*  
 Ss : (Students continue the exercise).
- (E2) SZ : *Kalau disuruh isi titik-titik, liat kalimatnya. For example, like this, misalnya ya kan 'she ... a doctor'. Ada disini dia kepunyaan 'doctor'? Kalau 'she' tidak bisa dia, dia biasanya singkatnya dengan kata ganti. Tidak ada dengan nama. Kalau nama sudah apostrophe pasti. Kalau dengan ini tidak boleh, dia kan kalau kepunyaan harus 'her'. Oke, any questions?*  
 S : Miss?  
 SZ : Yes?  
 S : What is the meaning of 'meanwhile'?

From the two examples above, we can see that the comprehension checks are taking place. In E1, 'understand' is to know the meaning of what someone is telling you. At the initial stage, the word 'understand' is a sense of assuring that the message delivered from SZ to the student is understood. That is why 'understand' is categorized as comprehension check because SZ needed assurance that the student had received the message correctly. Following her explanation, SZ then checked the students' comprehension by asking "oke, understand?" This is similar as in E2, where she asked her students "oke, any questions?" to further make sure that her students understood her before she moved on to the next topic.

### **Clarification Check**

Clarification check, which occurred 25%, was when SZ asked for clarification from the students' previous utterances. Following Long's framework on the definition of clarification check, therefore, it refers to the teacher's expression to elicit clarification of the students' preceding utterance (Boulima, 1999). Tsui (1991) informs that clarification check is one of the devices which are a better indicator of the learner's involvement in the interaction because in clarification check, students are given alternative answers that help boost their understanding. Below is an example taken from the interaction between SZ and her students.

- (E3) SZ : *Oke, Faza ...*  
 S : *Mention five family!*  
 SZ : *Family or families?*  
 S : *Families.*  
 SZ : *Yes.*

- (E4) SZ : '... the teachers have a lot of friends'. *Titik-titik itu, 'do' atau 'does'?*  
S : Do.  
SZ : 'Do', good! Muhammad Farhan! 'The teacher' *apa* 'the teachers'?  
S : The teachers.  
SZ : *Kalau* 'the teacher'?  
Ss : Does.

From the examples above, we can clearly see that clarification check took place. In E3, SZ wanted to correct the wrong sentence that the student made by asking, "family or families?". She then asked the students to think of what had just been said in order to get better understanding or answer. Similarly in E4, the question for clarification can be seen in "'The teacher' *apa* 'the teachers'?" This type of clarification check is done by repeating the two choices of answers to make sure that the student got the point of the answer. The student's response is considered as clarification.

### **Confirmation Check**

Confirmation check, which occurred 22.5%, was the teacher repeating students' utterances to ensure she had understood them. Based on Long, confirmation check employed by the teacher is in the form of repetitions in which the teacher immediately repeated the students' utterances to make sure that the utterances had been understood correctly (Boulima, 1999). In this study, the occurrences of such device are pictured in the next interactional events.

- (E5) S1 : 'What time is it?'  
S2 : 'It is eight fifty three'.  
SZ : 'Eight fifty three'?  
S2 : Oh... Mmm... 'Eight fifty three'.  
SZ : 'Fifty three'. Good. *Hamlul khair*.

In E5, we can see that SZ wanted a student to ask a question to another student according to the lesson, and the other student must answer the question. It was done by SZ to activate students' participation in learning. Here, SZ tried to confirm the answer given by the second student that was incorrect. However, she repeated the answer in the form of a question, 'Eight fifty three'? By doing so, it was expected that the student would be aware of his answer, and provide the correct one. This interaction was also important to point out the mistake made by the student, and SZ would also be more confirmed whether the student made the mistake deliberately (because he did not understand) or accidentally (because slip of the tongue).

- (E6) SZ : Yes, good. Now, 'My house ... a nice garden'. Syahla?  
S : Has.  
SZ : *Kenapa* 'my house' *itu dengan* 'has'?  
S : *Karena* 'it'.  
SZ : Very good.

In E6, SZ was the one who initiated a confirmation check. It was important for her to stress that the student's answer was correct and the student knew the reason. The student would gain better understanding by responding to his teacher's question. These features are in line with Long (Boulima, 1999) that the use of confirmation check is indicated by the use of repetition, which can be responded in simple answers as illustrated in the two interactions above.

### **The Teacher's Views on Using Questioning Interaction in the Classroom**

The interview conducted with SZ revealed several results. Regarding to the importance of asking questions and their frequency of use in the classroom, the result of interview indicated that SZ asked questions to her students about their understanding towards the materials being taught. As explained by SZ below in R1 (R refers to response), questions are needed to know whether the students are on the right track of learning or not. This is so that comprehension problems can be identified and reduced.

- (R1) *Yes, absolutely yes. I ask my students questions of their understanding about the material being taught and how much they have understood from what I have explained. So, if I don't ask about their understanding, I don't know how much they know, I don't know whether they understand my explanations...I, myself, should also know my way of teaching if there are students who still have trouble in understanding me.*

(R2) *Oh, I always ask questions in class because this is to know the depth of their understanding towards my explanation...about the material that I teach to them in the classroom. I can know their difficulties and this helps me find ways to overcome them.*

From R1, SZ's comment, "I, myself, should also know my way of teaching..." also reflected that she would find another approach if the students were still facing comprehension problems with her current way of teaching. As of frequency in R2, she always asked questions to recognize students' difficulties, and by realizing them, she could seek ways of solving them.

In relation to the influence of the questions on classroom learning, SZ asserted that:

(R3) *Em...I can get feedback from the student about the material that I teach in every meeting. So it is important that I obtain feedback from the class, and the point from the student herself.*

By asking questions, SZ emphasized in R3 that she can gain also feedback directly from the students about their knowledge. Consequently, these feedbacks were valuable for her to make a reflection on her teaching and find other ways that could help her improve her teaching.

Additionally, SZ informed that the common questions in negotiating meaning she often asked in the classroom were those that can determine whether she has been correctly understood (i.e. comprehension checks), and she did not use those that determined her to correctly understood her students (i.e. confirmation checks). Her reasons are as follows:

(R4) *Mm...there are general questions that I ask in the classroom, such as those that helps me know whether my students know what I am saying. By doing so, I can make sure that they achieve the goal of learning.*

In R4, SZ stated that she asked questions related to comprehension check to ascertain that they can accomplish the purpose of learning on a certain topic in the classroom. This is also confirmed from the data transcription that comprehension check was the most used by this teacher. It can be assumed that questions in comprehension can expose students' profundity towards the subject, and further enlighten the teacher that her goal of teaching has been reached for the day or not. That is why this type of questioning was the most used in her questioning interaction.

(R5) *Sometimes I forgot to ask questions such as those that help me reconfirm the students' answers because in my teaching and learning process, sometimes the class runs smoothly. I don't really ask questions such as "What do you mean?". Those questions are mostly asked by my students to me.*

Finally, R5 displays that SZ did not really ask questions that were related to clarifications, for example "What do you mean?" This is in line with the data transcription that confirmation and clarification checks were used lesser in the classroom. Presumably, it is SZ who provides clarification to the students instead of the other way around in her EFL classroom. This habit then caused her to reduce clarification checks in her questioning interaction.

## Conclusions

From the three types of questions in interactional modification devices (Long, 1983), comprehension check appeared to be the most dominant devices applied by the teacher (52.5%) in this study, which was followed by clarification check (25%) and confirmation check (22.5%). The dominant use of comprehension check in the classroom reflected the typical interaction in the EFL context, in which there was much more negotiation of meaning going on between the teacher and students to gain understanding. The result from the interview with the teacher further revealed that she must ask questions to know the students' understanding of what is being learnt in class. Yet again, questioning plays an important role in many instructional purposes because they elicit students' reflections and challenge students' understanding and engagement in the classroom (Adedoyin, 2010). By being aware of question types, teachers can prepare for upcoming directions in her teaching to reach her teaching objectives more effectively.

The limitation of this study is that we had only focused on the three types of questioning interactions by the teacher in a classroom. Therefore, the researcher suggests for future researchers to conduct further studies on this topic by investigating deeper into the other devices and obtain more data from teachers and students in various EFL classrooms.

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## **Testing Listening by Using Audio Aid and Animated Film**

\*Dian Fajrina, Syamsul Bahri, Mohammad Kholid

<sup>1</sup>Department of English Education, Faculty of Teacher Training and Education, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: dfajrina80@gmail.com

### **Abstract**

This study is a field research which is descriptive quantitative in nature. The purpose was to find out whether there are any differences in listening achievement between listening to audio aid and listening as well as watching to an animated film. The participants were 20 semester six students of English Education Syiah Kuala University. The instruments used in this study were two sets of test, one by listening to audio aid, and the other by listening and watching animated film. The result of the tests showed that the mean score of the animated film test was higher than the audio aid test. It was proven by t-score result where the t-score was 11.5, referred to the t-table at level of significance 5% with the degree of freedom (df) was 19. The result of t-score was higher than the t-table. In conclusion, the alternative hypothesis, which states that there is a different result on listening achievement between listening to audio aid and listening as well as watching animated film, is accepted. Therefore, it is suggested to the English teachers to use animated film as a media in teaching Listening Comprehension.

**Key words:** listening, audio aid, animated film.

### **Introduction**

Listening provides the input that serves as the basic for language acquisition and enables learners to interact in spoken communication (Brown, 2004, p. 249). However, gaining the comprehension in listening is not easy. For most Indonesian students, listening to English is very difficult since English has different grammatical rules from Indonesian language. Differences in phonology also play a role in the difficulties to understand most of English conversations. Flowerdew & Miller (1996) assumes that the problems of the learners in listening were on the speed of delivery, new terminology and concept, difficulty in focusing and the physical environment. Insufficient exposure to the target language also becomes an issue related to listener difficulties (Goh, 2000, p. 56).

One of the solutions to overcome the problems discussed above and to achieve the successful in listening, is the needed to involve technology as a creative media in teaching listening (Celse-Murcia (2002) cited in Barrani, Sayyedrezaie and Shojaie (2013, p. 117). There are a lot of media that can be used in teaching listening materials, among others are audio aid and animated film.

In this study, audio aid and animated film will be used to test the students' listening comprehension. The result of the test will be compared to find out whether there are any differences in students' score of listening comprehension.

### **Review of Literature**

#### **Listening**

Listening is the essential skill which is producing the basic for the successful communication (Liubiniene, 2009, p. 1). Listening is the instrument for children, young and adult people to increase knowledge and education, obtain information and improve their understanding of the words. Saricoban (1999, p. 13) states that listening is the ability to identify and understand what others saying. The concept of listening is to demand the listeners to be able to comprehend and follow what the speakers said, obtain information, increase knowledge and attitude and produce good communications. James (2006, p. 1) divides listening comprehension into two categories:

- 1) Lower levels of listening comprehension: This level included understanding only the facts that are explicitly stated in a spoken language.
- 2) Advanced levels of listening comprehension: This level included implicit understanding and drawing inferences from spoken language.

### **Factors Affecting the Listening Process**

Listening is a demanding process, not only because of the complexity of the process itself but also due to the factors that characterize the listeners, the speaker, the content of the message and any visual support that accompanies the message (Brown & Yule, 1999).

- a. The listener  
Interest in a topic increases the listener's comprehension; the listener may avoid the topics that are not interesting for them. A listener who is an active participant in a conversation generally has more background knowledge to facilitate understanding of the topic than a listener who is listening to a conversation on a recorded material. Furthermore, the ability to use negotiation skills, such as asking for clarification, repetition or definition of points that they do not understand, enable a listener to make sense of the incoming information.
- b. The speaker  
Idiomatic language and reduced forms make conversation more difficult. The extent to which the speakers use this language forms has an impact in listeners' comprehension. The more exposure the listener has to them, the greater his ability to comprehend. A speaker's rate of delivery may be too fast, too slow or has too many hesitations for a listener to follow.
- c. Content  
The content that is familiar is easier to comprehend than the content with unfamiliar vocabularies or which the listener has insufficient background knowledge.
- d. Visual support  
Visual support such as videos, pictures, diagrams, gestures, facial expressions and body languages, can increase comprehension if the listener is able to interpret it correctly.

### **Learning Listening through Audio Aid**

Audio aid in the form of stories or tales is an effective material for learner to develop their listening ability both in first and second language. Audio aid is often considered as traditional listening material as there are a lot of other advanced media that can make learning listening more complex. However, the role of audio aid in assisting listening learners until now does not need to be questioned.

Audio aid demonstrates a capacity to facilitate authentic engagement, allowing students to connect in various ways to the outside world, both as listeners and publishers. Listening through audio aid in the form of stories gives us valuable insights into the sense making component of learning (Engel, 2000).

### **Learning Listening through Film**

Kasper (2002) states that film facilitates learning in several ways, it is also providing learners with a graphic illustration of relevant content information. Integrating visual language like film in language learning will enable students to think in more complex ways (Horn, cited in Barrata and Jones, 2008, p. 16). Heron and Hanley (cited in Ismaili, 2013, p. 122) had conducted a research on using film in listening comprehension for EFL learners and they concluded that using film in EFL classroom offers background information that activates prior knowledge, which is essential in stimulating listening skill activities in the classroom.

Brodwell and Thompson (cited in Puspitasari, 2007, p. 25) proposed types of film as follow:

- 1) Documentary film  
A documentary film supports to present factual information about the world outside the film. As a type of films, documentaries present themselves as factually trustworthy. According to Bardwell and Thompson, there are two type of documentary films, they are;
  - a) Compilation films; produced by assembling images from archival sources.
  - b) Direct cinema; recording an ongoing event as it happens with minimal interference by the filmmaker.
- 2) Fictional film  
A fictional film shows imaginary beings, places or events. Yet, if a film is fictional, it does not mean that it is completely unrelated to reality. Fictional film are not always shown or implied to be imaginary, a typical fictional film stages its events; they are designed, planned, rehearsed, filmed and re-filmed. In fictional film, an intermediate portrays and depicts the agents, not photographed directly in documentary.
- 3) Animated film  
Unusual kinds of work that are done at the production stage distinguish animated film from live-action. Animated film is not continuously filming action in the real time, but it creates a series of images by shooting one frame at a time.
- 4) Experimental film

Some film producers start creating films that challenge orthodox notion of what film can show and how it can show it. Experimental film is made for several reasons, they are;

- a) The producers want to express personal experience or view point.
- b) The producers may also want to explore some possibilities of the medium itself,
- c) The experimental film may tell no story but they may create a fictional story that will usually challenge the viewer.

In this research, the writer used animated film to see the learners' achievement in listening and compare it with learners' achievement in listening through audio aid.

### Research Methodology

This study is a comparative study by using descriptive quantitative method (John, 2007, p. 2). The participants for this study was 20 semester six students of the English Education at Syiah Kuala University.

In this study, two tests were employed: first by using audio aid and followed by using animated film. The first test using audio aid was delivered to the students by asking them to listen and answer to four listening materials. After that the researcher gave another test by using animated film entitled "Avatar: The Legend of Korra". There were 20 test items of multiple choices with four options of each item. One correct item was scored 5 points. So if one manages to answer all the items correctly, he/she got 100 points.

The scores of the two tests were set up in the frequency distribution which was analyzed by using mean. To find mean, Usman and Akbar (2006: 90) suggested the following formula:

$$M = (\sum d)/N$$

M = mean

$\sum d$  = Audio aid test – Animated film test

N = number of scores

Next, to find out if there are any significant differences between the test given by using audio aid and by using animated film, t-score formula was applied (Arikunto, 2006, p. 349). The last step was to test the hypothesis by using the degree of freedom (df) and the critical value (tt).

### Results and Discussion

#### Results

The first test given was by using audio aid. In this activity, the writer delivered four listening materials to the participants of this study. The first material had 2 questions, the second had 6 questions, the third had 6 questions, and the last material had 6 questions. Overall the test had 20 questions. The participants only listened to each material once. The result of the test showed that the mean of the result of the participants' listening comprehension score was 55.5.

The second test was delivered by using animated film, "Avatar: The Legend of Korra". There were also 20 questions delivered to the participants after watching the animated film. The questions were related to the events, conversations and specific information found in the animated film. The mean of the result of animated film test was 70.

#### The Differences of Students' Scores on Audio Aid Test and Animated Film Test

Based on the mean of audio aid test and animated film test, the result shows the significant difference in students' achievement. The following table and diagram shows the differences between audio aid test and animated film test.

**Table 1.** Analysis of Audio Aid Test and Animated Film Test

Score	N	$\Sigma$	Alignment
Audio Aid	20	1150	55.5
Animated Film	20	1500	70
Gain		2610	130.5

The hypothesis must be proved by using the degree of freedom and the critical value (t-Value). In calculating degree of freedom, the writer used the following formula:

$$df = N - 1$$



$$df = 20 - 1$$

$$df = 19$$

The critical value for df 19 at the level of significance of 5% (0.05) was 2.093

From the statistical analysis of the t-test, it was found that the result was 11.5. The score was higher than t-Value at level of significant 5% (2.093). Therefore, the alternate hypothesis ( $H_a$ ) was accepted and the null hypothesis ( $H_0$ ) was rejected. It means that there was a significant difference in the results of the students' listening achievement when tested using audio aid and tested by using animated film.

### Discussion

Based on the result of the data analysis, alternative hypothesis was accepted. The mean of audio aid test was 55.5, meanwhile the mean of animated film test was 70.

There are some conclusions that can be drawn. In using audio aid as the listening material, there were some problems faced by the learners in answering the test. The problem was the native speakers' normal speed in speaking which the learners were not familiar with and also the native speaker unclear voice. The successful of listening comprehension also depends on many factors such as students' level of vocabulary in which here the writer still found that the learners were still lack of it. Moreover, the learners were still having difficulties in understanding the specific information from the materials. Therefore, the learners achievement were still poor or average in audio aid test.

After doing listening test through audio aid, the students were asked to do another listening test by watching animated film. The result showed that there were significant differences on the students' listening achievements.

Because animated film provides visual clues to understand the conversation, the students took benefit from the clue to deal with the native speakers' normal speed in speaking and the unclear voice. The visual clue in listening by watching animated film can also help the students in understanding some specific information from the material and guessing the meaning of a few unfamiliar vocabularies. As a result, the students can achieve more grades in listening test by watching animated film than by listening to audio aid.

### Conclusions

There was a different result on the students' listening achievements between tested by listening to audio aid and tested by watching animated film. This was identified that the t-test score (11.5) was higher than level significance at the degree of freedom 19 which was 2.093. It means that the alternative hypothesis which states that there is a different result on the listening achievement between students who are tested using audio aid and using animated film is accepted. Moreover, there was also a significant increase in the test result of the test given by watching animated film. This was identified from the increases of the students' achievement in the result of the test through animated film, the mean score of the test was 70. While in the result of the test by listening to audio aid, the mean score of the test was 55.5. It can be concluded that there was some significant differences between the results of the tests.

It can also be concluded that animated film can be a better learning media than audio aid. Even though audio aid provides clear conversations among the speakers, animated film gives an addition benefit in the form of visualization related to the conversations which can be utilized by students as the clues to answer the test. The result also showed that the listening achievement of the students tested with animated film was higher than the result of the test through audio aid.

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## **English Proficiency in Facing ASEAN Economic Community: an Opportunity or a Challenge?**

\*Iskandar Abdul Samad, Siti Sarah Fitriani

Department of English Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia;

\*Corresponding Author: iskandar.abdul.samad@gmail.com

### **Abstract**

The opportunity to compete among people of ASEAN countries in the era of ASEAN Economic Community (AEC) requires the Indonesians to have good English proficiency as a medium of communication to sell and buy products as well as to find proper jobs. Having good English proficiency gives opportunities to compete in this community. In contrast, those who do not have good English proficiency can be challenged to face this era. This study investigates the readiness of the Indonesian students, especially the Acehese undergraduate students, in facing the era of AEC that already started in the end of 2015. The investigation of this study is based on the students' English proficiency which is demonstrated from their achievement in the Test of English as a Foreign Language (TOEFL) conducted by Syiah Kuala University (Unsyiah), Banda Aceh, Indonesia. The data collected for the investigation include TOEFL score from 1916 undergraduate students undertaking the test at this university. This study also collected data from interview with one Unsyiah authority. The results indicated the level of challenge and opportunity of the Acehese undergraduate students to compete with people from other ASEAN countries in terms of their English proficiency.

**Key words:** English proficiency, Acehese undergraduate students, ASEAN Economic Community.

### **Introduction**

The ASEAN Economic Community (AEC) provides many opportunities for the people living in the ASEAN countries to reduce poverty through many possibilities for the free flow of services and investments, as well as goods (Soesastro, 2003). This highly competitive economic integration is also provided for all employment sectors. To be able to involve in this community, the ASEAN people are required to prepare themselves with appropriate as well as standardized skills and competencies. For example, an Indonesian nurse, who wants to seek for an opportunity to be a nurse in Malaysia, needs to prepare himself/herself with an international standardized skill and competency in health and medical field. Thus, different employment fields have different preparations in terms of skill and competency.

Moreover, each ASEAN country has a national language spoken by its people. To involve in this multi-language as well as multicultural community, the ASEAN people need to be able to use English as a medium of communication so that the economic integration in the AEC era can be realized. Having good English proficiency along with standardized skill related to a certain employment sector provides a higher opportunity for someone to compete with others in the AEC. However, in some ASEAN countries such as Indonesia, English is still considered as a foreign language where its exposure is still very limited. Hence, even if the people have been skillful enough to involve in the AEC in different sectors, without good English proficiency, the opportunity will turn into a challenge. Language can be a barrier in any economic integrations (Langhammer & Hiemmenz, 1990) happening across the globe, that can challenge people to involve in the community. According to Yue (2011), language proficiency is one of the requirements in the economic integration of AEC which will constrain and impede mobility. Aldaba (2013) reported that language –in this case English– is the biggest barrier in the economic integration for the people living in ASEAN countries where English is not a medium of instruction. Meanwhile, having good English proficiency in the economic integration of AEC is beneficial for all sectors of employment and trading. For example, Supakankunti and Herberholz (2012) revealed the reasons that Malaysia has strong opportunities in medical and health services sector in the ASEAN; one of those is due to the use of English in the services.

In sum, previous studies have emphasized the importance of having good English proficiency in all sectors to provide opportunity for the ASEAN people to compete in the AEC. Building from this issue, this study looked at another point of view of investigation in relation to English proficiency, which is by finding out the readiness of the Indonesian Acehese undergraduate in facing the AEC. Hence, this study proposed this research question: — In relation to English proficiency, to what extent are the Acehese undergraduate students from different majors ready to compete in the ASEAN Economic Community?

It is expected that this study provides information especially for undergraduate students of different majors about their level of readiness to seize the opportunity given by the economic integration in the AEC which is seen in terms of their English proficiency. When English proficiency is seen as a challenge, it is also expected that this study arises the students' awareness to increase their English proficiency in order to involve in this community, because language is not an absolute barrier and it can be surmounted (Langhammer & Hiemmenz, 1990).

## **Literature Review**

### **ASEAN Economic Community (AEC)**

Developed and developing countries around the world have started regional integration since 1990s, for example, there is a Regional Trade and Investment Agreement (RTIA) which is formed in 1994 by the US, Canada and Mexico (Asher & Srivastava, 2003). There are also some regional trading arrangements, for example, AFTA (ASEAN Free Trade Area) among Southeast Asian countries for the exception of East Timor (Asher & Srivastava, 2003). The ASEAN Economic Community (AEC) is also a form of regional economic integration which was proposed in Bali when the ASEAN leaders attended the Bali Summit in 2003 (Urata & Okabe, 2010). This integration was started in the end of 2015 and to be achieved in 2020. The era of AEC held among ASEAN countries including Thailand, Myanmar, Laos, Vietnam, Malaysia, Singapore, Indonesia, Philippines, Cambodia and Brunei provides the people of these countries for the opportunity to carry out free flow of investments, skilled labors, services and goods (Dee, 2009; Hew & Sen, 2004). This study assumes that this favorable circumstance can only be achieved by the ASEAN people who are skillful and competent, to enable them to compete for the opportunity. Language competence as one of competencies needed by these people has turned into an important aspect in the AEC (Yue, 2011).

### **English Proficiency**

English proficiency is widely known as an ability of someone to use English for various purposes. This proficiency is realized when he/she includes in the activities of reading, listening, speaking and writing. Within these activities, for example in speaking, he/she needs to convey a message or information which is understood by interlocutors, listeners or audiences. In other words, the communication must be meaningful and understandable. Proficiency in English can be measured using English proficiency tests such as Test of English as a Foreign Language (TOEFL) and International English Language Testing System (IELTS). This is done to indicate the level of proficiency of someone to use English in different professional levels, for example, English proficiency for routine travel needs and social demands, formal and informal conversations, and education and work requirements. This study focuses on the English proficiency of undergraduate students for engaging in education and work requirements, which can be measured using TOEFL. This study assumes that their English proficiency is important for them to involve in the ASEAN Economic Community.

Currently, some studies have focused on investigating the relation of English proficiency of migrant employees and laborers working in other countries to their careers as well as to effective services. For example, Trajkovski and Loosemore (2006) investigated the extent to which the inability of migrant workers in interpreting information about workplace hazards communicated by supervisors and co-workers affects in occupational and safety risk. Dutschmann (1994) reported that migrant workers need to fulfill some requirements set by the country where they are working important for them to earn money; one of them is to understand the language spoken by the incumbent population. In line with this, Kossoudji (1998) informed that language proficiency must be taken into account as one of earning determinants for immigrant workers. This implies that the deficiency to use language while working overseas can be a constraint for the earnings (Dutschmann, 1994). Similarly, Dutschmann and Fabbri (2003) also reported that migrant workers with language proficiency have positive opportunity for employment overseas. They also argue that these workers can lose earnings without English fluency. Most previous studies investigated the relation between language proficiency and employment success of the workers working in other countries who already become immigrants. This present study, however, looked at the Indonesian undergraduate students' readiness (represented by the Acehese undergraduate students) in facing AEC where they have opportunities to search and get employment opportunities in other ASEAN

countries. In other words, this study focuses on the readiness – in terms of English proficiency – of the people that are not considered immigrants yet.

### **Acehnese Undergraduate Students**

Acehnese –for this study– are the people who are originally from Aceh province, Indonesia, who also live in Aceh, or those who live in Aceh for employment or education even if they are not originally from Aceh. Undergraduate students are those who are studying for first degree at a university. They already graduated from high school, but are not graduate students because they do not graduate yet from their first degree. Acehnese undergraduate students, therefore, are the Acehnese who are undertaking their first degree at a university. For this study, the Acehnese undergraduate students are those studying at universities in Aceh province, Indonesia.

### **Research Method**

This is a descriptive qualitative study aimed at investigating the Acehnese undergraduate students' readiness in facing the AEC in terms of English proficiency as one of the competencies needed in this economic integration. Qualitative study provides rich data (Day, 1997) to deeply describe the focus of the investigation through, for example, conducting interviews and collecting relevant documents (Newby, 2010). Hence, this study collected the Acehnese undergraduate students' TOEFL score that they undertook in the Language Center of Unsyiah, Banda Aceh, Indonesia. This study also interviewed one representative of Unsyiah, who is eligible to provide information regarding its students' English proficiency in relation to their readiness in AEC, and the efforts organized by the university to help its students increase their English competence.

### **Participants**

To investigate the Acehnese undergraduate students' English proficiency in general, the participants chosen need to be heterogeneous, representing the whole undergraduate students from different fields of study. Hence, this study involved 1916 undergraduate students studying in different fields of study of different universities in Banda Aceh, who undertook TOEFL test at Unsyiah Language Center. This study also involved HH (a pseudonym) from Unsyiah in the interview.

### **Research Instruments**

This study included the use of two instruments: review of documents and an interview guide. In the review of documents –in this case students' TOEFL score–, this study indicated the level of readiness of the Acehnese undergraduate students in facing the AEC. Moreover, the interview guide consisted of open-ended questions. The interview was conducted in Bahasa Indonesia to provide opportunity for the interviewees to express their perspectives without any constraints in terms of language. An audio recorder was used during the interview sessions.

### **Data Analysis**

Two types of data collected by this study were analyzed in different ways. The students' TOEFL scores were listed in a table by ordering them from the lowest to the highest ones. These scores were then classified into three divisions. The first division is for the TOEFL scores below 450. Based on one of requirements set by Unsyiah, students with TOEFL scores less than 450 are not eligible to follow thesis examination and graduate from the university. Some other universities in Aceh have also expected their students to achieve a certain TOEFL score to graduate. The second division is for the TOEFL scores between 450 and 497. Students with the TOEFL scores included in this second division are eligible to graduate from Unsyiah and from other universities in Aceh, but not yet eligible to pursue further education overseas which is 5 based on the American Exam Services website (2014). In other words, students who got TOEFL score within this second division are not yet ready to involve in international communities, especially in the economic integration. The third division is for the scores of TOEFL equal to and above 500. Accordingly, the level of readiness of the students in involving in the AEC is seen from these three divisions. This study assumes that students are ready to involve in the economic integration in terms of English proficiency only if their language competence allow them to interact with the international community. For this assumption, this study classifies students' readiness into two different levels: ready and not ready. The undergraduate students who achieve TOEFL scores above 500 are classified into the ready level, and below 500 into not-ready level. From these divisions, this study indicated the number of undergraduate students in Aceh who are ready and not yet ready to involve in the AEC in order to represent the readiness of the Acehnese undergraduate students in general to involve in the economic integration.

The audio recording obtained from the interview was transcribed into a written form. To treat the written transcriptions, this study then followed the stages suggested by Miles, Huberman and Saldana (2012)

to condense which is to reduce any unimportant information, to display which is to organize the important and related information into a visible organization, and to verify the conclusion of the information related to the topic of the investigation. Because the interviews were conducted in Bahasa Indonesia, hence, some quotes from the interviews presented in this paper were translated into English.

## Results and Discussion

### **Test of English as a Foreign Language (TOEFL) Scores**

This study aims at investigating Acehese undergraduate students' readiness to involve in the AEC by looking at their TOEFL scores. Based on the analysis of 1916 undergraduate students' TOEFL scores from Unsyiah, they can be divided into three different groups as seen in Table 1 below.

**Table 1.** The divisions of students' TOEFL scores from Unsyiah

Number of students	TOEFL scores	Level of readiness
8	500 and up	Ready
53	450 – 497	Not ready
1855	Under 450	Not ready

Table 1 indicates that only eight students (or about 0.42% from 1916 students) from different fields of study are ready to involve in the AEC. The other 1908 students are not yet ready for the economic integration.

### **Interview with an Unsyiah Authority**

From the interview with HH, this study indicated two things: a requirement set by Unsyiah, and some efforts carried on by Unsyiah to help students achieve the requirement. These are related to English proficiency and TOEFL. Unsyiah has added one requirement for its students to achieve an expected TOEFL score for undertaking thesis defense examination at the end of their study program as mentioned in the following response:

*Unsyiah expects its students to achieve 450 of TOEFL score as one of requirements to undertake thesis defense examination. Later on, this score expectation will be raised to 470.*

The above response also shows the importance of having good English proficiency which can be demonstrated from students' TOEFL score. Furthermore, HH adds the reason Unsyiah sets the TOEFL requirement as well as the importance of this for the AEC.

*TOEFL is an indicator that students are competent in English. The goal of increasing students' scores in TOEFL is to prepare them to compete in the international community, such as the AEC.*

The interview results also revealed two programs carried out by Unsyiah to help its students to be more competent in English and increase their TOEFL score. The first one is a foster brother program (called in this university as UP3BI), and the other one is English unit (called as MKU). The foster brothers and sisters involved in UP3BI program are Unsyiah alumni. The conduct of UP3BI is mentioned by HH in the following quote.

*In their very first semester, Syiah Kuala University tries to develop a sense of awareness among students about the importance of Test of English as a Foreign Language (TOEFL). This sense is established through socialization of TOEFL from the department level of each major until faculty level. The development of this awareness is realized through a foster brother program (UP3BI) involving Unsyiah alumni as volunteer trainers. This program is provided for two semesters, in the first and second semester of students' study program.*

Besides developing students' awareness regarding the importance of TOEFL, HH further mentioned that the foster brothers and sisters as volunteers in UP3BI program also help students to understand the generic features of the TOEFL test. This understanding is believed to make it easier for the students to be familiar with the aspects involved in the test. As stated by HH:

*By having high senses of awareness, the students are expected to understand the generic features of the TOEFL test, for example, they know the number of sections, the number of questions, the way to answer questions and strategies to answer questions in the real TOEFL test. This understanding helps the students to undertake the real TOEFL test.*

After following the UP3BI program in the first semester, the students in this university continue the same program in the second semester. In this second semester as well, they start to enroll in English unit (MKU). Thus, in the second semester, they undertake both UP3BI and MKU as mentioned in HH's response:

*UP3BI is followed by students in the first semester prior to undertaking MKU in the second semester. When students enroll MKU in the second semester, they also follow the second round of UP3BI.*

MKU is a unit that focuses in preparing students to increase their English proficiency which is measured in the TOEFL test.

The responses given by the Unsyiah authority in the interview indicate that this university has realized that its students still need help to increase their English proficiency, firstly for graduating from the university, and secondly for applying further education overseas and at the same time involving in an international community such as the AEC. Therefore, Unsyiah has initiated the conduct of UP3BI program and MKU unit to help its students improve their English proficiency.

Having standardized English proficiency is one of competencies needed by the ASEAN people to involve in the AEC (Aldaba, 2013; Yue, 2011; Langhammer & Hiemmenz, 1990). The language proficiency along with standardized skills needed enable the people to seize the opportunities available for trading and employment. However, this language proficiency can also be a challenge for the people to involve in this economic integration if the level of the proficiency is below standard. The results of the analysis conducted to the Acehese undergraduate students' TOEFL scores have indicated that the number of them who are ready to engage in AEC is much lower than those who are not yet ready. Based on the data taken from Unsyiah, the percentage of those who are ready is less than 1%. The other 99% of the students is not yet ready. The result demonstrates that the big opportunities given by the AEC for the students' future career in the ASEAN countries cannot yet be balanced with sufficient level of English competency. In other words, it can be said that these big opportunities have now become a challenge for the students due to their lower level of English proficiency.

According to the results of interview with an Unsyiah authority, the university has added one additional requirement a few years ago for its students to achieve a minimum of 450 for TOEFL score to enable them to graduate from the university. One of the reasons to set this requirement is to encourage students to learn English more so that they can reach the score and afterwards continue further studies overseas. However, this study assumes that the TOEFL score of 450 is not sufficient for the students to compete in the AEC or involve in another international community. This study suggests that the expected score can be increased into at least 500. Hence, the students will be more motivated to learn English especially when the students are informed the benefit of having standardized English proficiency in relation to the opportunities to become immigrant workers (Dustmann and Fabbri, 2003; Kossoudji, 1998; Dustmann, 1994) especially in the AEC era. Unsyiah has also shown its efforts to help its students from different fields of study improve in English proficiency by providing UP3BI program for two semesters and MKU unit for one semester, focusing on TOEFL preparation.

### **Conclusion and Suggestion**

To conclude, the majority of Acehese undergraduate students are not ready yet to be involved in the AEC which is due to their lack English proficiency. The language proficiency which can provide opportunities for students to engage in this economic integration has now become a challenge for them. Unsyiah's efforts to help its students improve in English proficiency need to be appreciated. However, this study assumes that it takes some more times for the majority of Acehese undergraduate students from different fields of study to be able to involve in the AEC.

Some implications of this study are drawn. Firstly, students' awareness about the importance of having standardized English proficiency especially in the AEC era needs to be developed, even when they are still in school levels. Secondly, it is important for English teachers from schools to university levels to investigate students' difficulties in mastering English and find out solutions for the students to decrease the difficulties, and at the same time increase their English proficiency. Thirdly, schools and universities are required to undergo some programs to help students in this regards. Lastly, formal and informal higher education institutions and language trainings specifically in Banda Aceh are suggested to help their students increase English proficiency in accordance with international standard.

This study is open for criticisms and suggestions especially for its limitations, for example, the readiness of students in facing the AEC was only investigated from their TOEFL scores which were taken from one formal institution, which is Unsyiah. Accordingly, this study recommends further researchers to conduct a similar investigation which collect data from different formal institutions in one province or in some provinces in Indonesia.

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## **Investigating the Language Choice of Acehnese Intermarriage Couples in the Home Domain**

\*Zulfadli A. Aziz, Bukhari Daud, Windasari

English Department, Faculty of Teacher Training and Education, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: zulfadli.aziz@unsyiah.ac.id

### **Abstract**

This study aimed at investigating Acehnese intermarriage couples choosing Bahasa Indonesia as medium of communication in the home domain and discovering the influences of their language choice on the survival of their mother tongues. Interview guide and questionnaire were applied as the instruments in this qualitative study which was conducted in Banda Aceh with five Acehnese intermarriage couples who use Bahasa Indonesia to interact in the home domain. Results of interview showed that there were some reasons those couples prefer to Bahasa Indonesia which are: (1) to accommodate spouse; (2) to be convenient; (3) to expand social network; (4) Bahasa Indonesia is community language; (5) Bahasa Indonesia is the national language; (6) Bahasa Indonesia is the medium of instruction; (7) Bahasa Indonesia is the dominant language; (8) Bahasa Indonesia is a neutral choice; (9) the couples are less motivated to use the indigenous languages. In addition, the results of questionnaire revealed that the intermarriage couples' language choice might promote linguistic suicide; that is, the parents do not pass their mother tongues to the children. The linguistic suicide may cause the children to own Bahasa Indonesia as their first language or mother tongue resulting in an inability to own their parents' mother tongues.

**Key words:** Intermarriage, language choice, language in home domain.

### **Introduction**

Marriage is a relationship in which two people are allowed to be unified. Most of marriages are endogamous; that is, the marriage within the same ethnic, social, and religious group. However, there are also people who marry the members of different social group or community with different background. The difference between spouses can be in terms of ethnic or race, nationality, religion, socio-economy, etc. The different ethnic and nationality usually mean different culture and language. This situation then will result in the couple to be exogamous (Dow, Reed & Woodcock, 2013), which is known as the mixed marriage or intermarriage. The mixed marriage which is specifically referred to in this study is a marriage between two people coming from different ethnic groups with different indigenous languages as their mother tongues. Therefore, the variety which is focused in this study is on their mother tongues while the other diversities are not the concentration. These marriages are usually found in multilingual or multicultural sites as mentioned by Schmidt and Makey (1971).

Aceh is one of the provinces in Indonesia that may promote intermarriage since there are some indigenous languages which represent the various cultures exist. In other words, Aceh is a multilingual province that is also multicultural. Each spouse of an intermarriage couple brings their own background of language into the marriage while they need to understand each other in the new relationship. Therefore, it is necessary for them to choose the medium of their communication.

Such differences of the couple may provide the advantages and disadvantages as well. On the other hand, if that parent uses both of their mother tongues in the home domain, it will enrich their children to be bilingual, one person speaking one language (Bourguet & Plaha, 2016). Besides, if one of the two languages is neglected, it will cause that language to be shifted by the children. Moreover, there are the parents deciding not to expose both of them. It is the best chance of language shift to occur on the next generation.

Dumanig and David (2011) express that the mixed marriage couples which have different linguistic and cultural backgrounds usually implement such strategies to interact with their spouse and their children at home. One of the strategies which can be applied by the couple is selecting only one language which

is understandable for both wife and husband. The language selected then will also be the language taught to the children. This strategy is usually the most preferred one for especially the young couples.

Bahasa Indonesia has served as both national and official language, and it is a dominant language in Indonesia and important to be acquired. It has been selected by many Indonesian couples to be exposed to their children. This, therefore, may be an alarming condition in term of existence of their ability to use mother tongues which in turn will also threaten the survival of those indigenous languages in Aceh. The situation under which the children are not exposed to the parents' mother tongues may end with an inability to own the languages. It means that the language will be shifted on their generation and so forth. The decrease of the number of speakers of a language may possibly put it under threat.

Indeed, this study attempted to investigate the reasons Acehnese intermarriage couples in Banda Aceh choose Bahasa Indonesia as the preferred means of communication in which they interact with family members in the home domain. In addition, it is also important to examine the effects of their decision to speak Bahasa Indonesia on their own mother tongues' survival.

## **Literature Review**

### ***Language Choice of Intermarriage Couples in Home Domain***

Language choice in this study refers to the preferred languages of mixed marriage couples in the home domain. One of the studies about language choice of intermarriage couples was carried on by Dumanig (2010). He examined the language choice in mixed marriages of Malaysians and Filipinos in term of the couples' language choice in interactions in the home domain and investigated the reasons of it. Each spouse speaks two or more languages such as the mother tongue, English, Malay and other languages. The result of the study showed that the dominant language used by intermarriage couples was English with some switching to local language and Filipino. They had some reasons to select English. First, it is to accommodate spouse. The couples need to understand each other by using the understandable language for both of husband and wife. Second, it is to be convenient. The couples try to speak in a language they feel most proficient in. This helps them to provide the clear message they would like to convey and prevent the misunderstanding. Last, using dominant language helps the couples to expand their social network. The couples usually choose the language that may benefit them and the family.

Those intermarriage couples of Acehnese people also need to select certain language to interact with each other at home. They need to compromise the languages they have to expose to the children as well. The difference of couple's mother tongues actually is not an obstacle to run their household since they tended to use Bahasa Indonesia to interact with each other in the home domain.

Bahasa Indonesia which is a dominant language is an inevitably preferred language. The study of Alamsyah *et al.* (2011) has shown that the parents of Acehnese who are the native speakers of Acehnese tend to use Bahasa Indonesia at home. The finding of the research showed that the role of Bahasa Indonesia which is not only used as the community language, but also as the medium of instructional process at schools, lets the parents believe in the significance of acquiring it. Bahasa Indonesia seems very important for the children that could help them comprehend the school courses well. Besides, those children might interact and communicate well with others by using this national language. Then according to the participants of the research, Bahasa Indonesia is considered more trendy and modern than Acehnese. It helps people show prestige and financial advance or success. In addition, Bahasa Indonesia acts as the neutral choice of various dialects of Acehnese. It functions as the neutral language to prevent the misunderstanding of differences among Acehnese dialects.

### ***The Effect of Preferring to Use Dominant Language in Mixed Family Homes***

What are the influences of using dominant language on the survival of indigenous languages in mixed family homes? The intermarriage couples decide not to use their mother tongue anymore in a family when they engage in a new relationship with spouses coming from different linguistic background. It means that they select another language as a result of linguistic compromise, whether consciously or unconsciously. This situation can inevitably facilitate the language shift although there is no guarantee that the endogamous marriage may always save the couple's mother tongue. In short, most frequently, intermarriage results in language shift of the couples (David & Daelwis, 2011).

In addition, intermarriage parents not only tend to use dominant language to interact with spouse, but they also expose only that language to the children. Thus, the parents stop passing their each mother tongue to the children. This phenomenon is known as "Linguistic Suicide" (Beck & Lam, 2006). In short, the speakers' need of gaining better life and prestige which is usually affected by the empowerment of dominant language is one big reason behind their effort to move from their indigenous languages to this

dominant one. Linguistic suicide results in an inability of the children to own their parents mother tongues, thus, the shift of those languages may occur in the children's generation that promote them to be monolingual.

Besides, the continuous increase of speakers of dominant language may in turn result in language loss of minority languages by the mixed marriage couples. Therefore, the choice of the dominant language to be used at home can be a significant factor towards the abandonment of indigenous or minority languages (Dumanig, 2010).

### ***Threatening the Survival of Indigenous Language***

Crawford (1995) classifies some criteria of endangered language. First, the number of language speakers is declining. When speakers keep decreasing in number, it means that the language is under threat, meanwhile; if the speakers number is stable, then the language still survive. Second, fluency in the language increases with age, as younger generations prefer to speak another, usually, a more dominant prestigious language. The children tend to be reluctant to speak indigenous language. Third, usage declines in "domains" where the language was secure, for example, in religious places, cultural observances, schools, and most important is at home. The numbers of parents that fail to teach the language to their children keep growing. The parents should be aware of the importance of maintaining their indigenous languages instead of passing only the dominant language to the children.

### **Research Method**

A qualitative approach was used in this study with the participants were five Acehnese intermarriage couples who already had children. They were selected as the participants as they are mixed marriage couples coming from different speech communities with different first languages or mother tongues. Meanwhile, they decided to use Bahasa Indonesia as the means of communication at home. Here is the table showing the couples' mother tongues (L1):

**Table 1.** The couples' mother tongues (L1)

<b>Couples</b>	<b>1<sup>st</sup> couple</b>	<b>2<sup>nd</sup> couple</b>	<b>3<sup>rd</sup> couple</b>	<b>4<sup>th</sup> couple</b>	<b>5<sup>th</sup> couple</b>
Husband's mother tongue	Aneuk Jamee	Gayonese	North Acehnese	Aneuk Jamee	Gayonese
Wife's mother tongue	North Acehnese	Greater Acehnese	Tamiang	West Acehnese	Greater Acehnese

Interview guide and questionnaire were the instruments used to obtain the data. All of the participants were firstly distributed questionnaire regarding their first language and language use to examine the effects of their decision to speak Bahasa Indonesia on their own mother tongues' survival. The following day, they were interviewed in term of their views of why they prefer Bahasa Indonesia as the medium of communication at home. This process of data collection was conducted in about two weeks long from July 19-August 1, 2016. To analyze the collected data, this study utilized the process suggested by Miles, Huberman and Saldana (2013) involving three steps: data condensation, data display, and drawing conclusion.

### **Results and Discussion**

#### ***The Reasons of Preferring Bahasa Indonesia***

Based on the data obtained from the interview, it shows that there are some reasons of why Bahasa Indonesia is preferred by Acehnese mixed marriage couples in home domain. The first one is that the couples decide to use Bahasa Indonesia in order to accommodate spouse. The differences of their linguistic backgrounds result in the couples deciding to implement such strategy to accommodate spouse; that is, those spouses determine the language which is understandable for both husband and wife. Holmes (2001) revealed that Bahasa Indonesia spreads well all over Indonesia as the neutral choice to unite people from various cultures with different mother tongues.

Second, Bahasa Indonesia is preferred since the couples feel more proficient in it. Thus, the mixed marriage couples keep using Bahasa Indonesia in home domain because they have good proficiency in it to be more convenient. By communicating in Bahasa, they prevent the family members from being confused with the message they need to convey. Besides, Bahasa was the language with which they communicated when they (the husband and wife) firstly met. Thus, it can be summarized that the language they usually used before getting married also influence the language choice to interact in home domain in addition to the fact that Bahasa Indonesia is the community language. As Paauw (2009) stated that Bahasa Indonesia is a standard language which is used in government, education, and mass

media. Thus, Bahasa Indonesia is mostly used and spoken that in return may let people feel more convenient and confident to use it in daily life.

Third, Bahasa Indonesia may help the couples to expand their social network. The couples imply that everywhere their children later will be they absolutely need Bahasa Indonesia to speak with others. Those three reasons were summarized by Dumanig (2010) of why the intermarriage couples in Malaysia preferred English which is dominant language there.

In addition, the couples live in urban area and in a community where Bahasa Indonesia is the medium of communication. Besides, the children need to be able to speak Bahasa Indonesia since they have to interact with their friends and neighbors who come from different speaking communities. In short, Bahasa Indonesia is the community language. Dumanig (2010) argued that people determine one language as the identity in their community; thus, it unavoidably influences the language choice of intermarriage couple living neither in husband's nor wife's speech communities.

Next, Bahasa Indonesia is national language which has been promoted as the medium of instructional process at school as well. Therefore, the intermarriage couples tend to expose only Bahasa Indonesia at home to the children regarding its significance. The previous study of Alamsyah et al. in 2011 also found out this reason behind the preference of Bahasa Indonesia by Acehese parents.

The sixth reason is that Bahasa Indonesia is dominant language. Among other languages, Bahasa Indonesia is the most influential one in Indonesia. It is used everywhere, especially in formal activities. Thus, the status of one language determines whether or not it is chosen and used as Dumanig (2010) states that, whether it is dominant or weak, the language status influences the speakers to speak it or not.

Then the characteristic of Bahasa Indonesia which may neutralize the varieties of regional languages in Indonesia becomes one of the reasons of why it is preferred to be used in mixed family homes. Alamsyah et al. (2011) also found out that Bahasa Indonesia is a neutral choice among various dialects of Acehese. In this case, it acts as one that may neutralize the different mother tongues of the mixed couples. Holmes (2001) also argued that Bahasa Indonesia may be the neutral choice for those whose mother tongues are different. Thus, the couples do not need to learn and acquire new languages to be united with spouses.

Last, Acehese mixed marriage couples are less motivated to use the indigenous languages or their mother tongues. As aforementioned that intermarriage couples tend to select the languages that benefit them, they do not see any advantages of using their first languages at home. For example, Gayonese is not either used as a medium of instruction or when someone looking for job. Therefore, Bahasa Indonesia that plays those roles is preferred, especially by intermarriage couples. Moreover, all the family and relatives, whether husband's family or wife's family, are able to speak Bahasa Indonesia. This situation facilitates both of spouses to keep on using Bahasa Indonesia although they are in indigenous language speaking community. It is unnecessary for them to master the spouse's language because their relatives and in laws use Bahasa Indonesia with them. Thus, what Dumanig and David (2011) state "the presence in laws influences the language choice of the son or daughter in law" does not make any sense in this case.

### ***The Influences of Bahasa Indonesia on the Survival of Acehese Indigenous Languages***

The results of questionnaire showed that there was a visible indication of the influences of Bahasa Indonesia preferred by Acehese intermarriage couples toward the survival of local languages. The phenomenon of 'linguistic tsunami' (Aziz & Amery, 2015), the situation when dominant language, in this case national language, affects the indigenous language speakers to prefer using dominant language at home resulting in threatening the survival of their own languages, is found in this study.

What David and Daelwis (2011) concluded in their research that intermarriage may facilitate language shift plays no role in this study. Here, instead of shifting to Bahasa Indonesia, those spouses of Acehese intermarriage couples decided to be bilingual or multilingual. They still use their mother tongues in daily life with their friends or colleague and relatives. It means that their first language ability is not either influenced by their language choice at home, Bahasa Indonesia. Hence, they may also avoid the language loss. Keeping in touch with homeland is one of the factors that prevent them from losing mother tongue as revealed by Holmes (2001).

On the contrary, unfortunately, those couples decided to expose only Bahasa Indonesia to their children that promote them to be monolingual. This linguistic suicide phenomenon, the situation when the parents stop passing their mother tongues and prefer another dominant language to their children, is in line with the explanation of Majidi (2013) that mentioned a number of influential factors of shifting to dominant language such as Bahasa Indonesia has the values which other indigenous languages do not have: economic and social ones. In this case, it may be implied that the intermarriage couples do not shift from the beginning of marriage; however, they move to dominant Bahasa Indonesia to be exposed to the children. This decision results their children to own Bahasa Indonesia as their mother tongue without any knowledge and skill of parents' mother tongue.

Another finding of questionnaire is that Acehnese intermarriage couples have positive attitude toward their mother tongues that those indigenous languages must be preserved, on one hand. On the other hand, they do not pass those languages to the children. There is a gap between what those participants state and what they act. This result supports what Aziz and Amery (2015) revealed "But positive attitudes alone may not be sufficient to save a language because sometimes there might be a sizeable gap between what people say and what they do." In fact, the language choice of Acehnese intermarriage couples whose domiciles are in Banda Aceh may endanger those indigenous languages serving as their mother tongues since the children are unable to speak them which results in decreasing number of younger speakers that put those languages under threat.

### **Conclusion**

It has shown that the Acehnese mixed marriage couples prefer to use Bahasa Indonesia in home domain. By using Bahasa Indonesia in Acehnese mixed family homes may facilitate linguistic suicide and language shift on children's generation that of indirectly may put the local languages in Aceh under threat. Indeed, intermarriage is not the only factor that may threaten indigenous languages; however, it is one of the ways that may lead people to promote the endangered languages. At the end, it is the individuals' choice to whether preserve or endanger the indigenous languages.

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## Questioning in Teacher Talk

<sup>1</sup>\*Cut Aulia Makhsum, <sup>2</sup>Siti Sarah Fitriani, <sup>2</sup>Usman Kasim

<sup>1</sup>SMA Negeri 6 Lhokseumawe, Aceh, 24375, Indonesia;

<sup>2</sup>Department of English Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia;

\*Corresponding Author: zataullia@gmail.com

### Abstract

Asking questions is one of components in teacher talk. Some types of questions are posed by teachers to assess students' understanding of learning materials. This paper presents the results of the investigation of the types of questions used by English as Foreign Language (EFL) teachers in teaching English for the second graders of a senior high school. The subject was a female teacher who teaches English at SMA Teuku Nyak Arif Fatih Bilingual School, Banda Aceh. This qualitative study was conducted employing six classroom observations (480 minutes) focusing on four types of questions (factual, empirical, productive and evaluative) based on Moore (2007). The results revealed that factual questions appeared to be the most dominant type employed by the teacher (59.9%), followed by evaluative (16.6%) and empirical (12.9%). Productive questions were the least occurrence from all types of questions applied, which is 10.7%.

**Key words:** Questions, teacher talk, classroom interaction.

### Introduction

Teachers' language or teacher talk plays an important role for implementing language teaching and achieving teaching goals. Ellis (1986) concludes that the language used in the classroom by the teacher to second language learners is adjusted to teacher talk. Therefore teacher talk is teachers' use of specific language features in addressing students in the classroom. The most important thing is that teacher talk is useful for language acquisition processes because the quality of teacher talk will determine the comprehensible input and students' output (Richards & Lockhart, 1996). Teacher talk is categorized into seven aspects, they are accepting feelings, praising or encouraging, accepting or using students' ideas, asking questions, lecturing, giving directions, and criticizing or justifying authority. Commonly, asking question is one of the aspects of teacher talk that is mostly used by teachers to facilitate interaction in the language learning (Brown, 2001; Richards & Lockhart, 1996; Nunan, 1991; Chaudron, 1988). More than half of the time in the classroom talk is run under question-answer exchanges (Richards & Lockhart, 1996). Chaudron (1988) mentions that a teacher's question is the main tool to get students' participation stimulate students' oral response and assess students' progress. In the classroom, a teacher is a key person to stimulate interaction. Interaction or turn talk can occur if teachers are able to encourage students to participate in a language classroom. Therefore, employing strategies to encourage students to talk during teaching-learning processes is important. Some scholars (e.g. Brown, 2001; Richards & Lockhart, 1996; Nunan, 1991) conclude that asking questions is one of a teacher's strategies to get students talked and participated in the classroom lesson.

In the Indonesian EFL context, the important function of teacher questions has been formulated in the 2013 curriculum (K13) involving the emphasis on the scientific approach (observing, questioning, associating, experimenting and networking) in learning. Teacher question is the second step of five main learning activities in K13, within which, teachers are expected to be able to address good criteria of questions in order to guide students learning and answer the questions well. Teacher question is expected to encourage students to actively learn and develop questions, encourage students' participation and develop their ability to think creatively and critically (Kementerian Pendidikan dan Kebudayaan, 2013, p. 157). Principally, the main basic competence expected from learning language at the senior high school level is to be able to analyze the text rather than understand or recognize it. In other words, students are required to extend their thinking to be more critical and creative in the learning process. To this end, teachers have to provoke students' thought by asking more thought-provoking questions or higher order thinking skills questions. Higher order thinking skill questions require students to be able to analyze critically, think creatively and to evaluate including the process of analysis, synthesis and evaluation (Barjesteh & Moghadam, 2014; Bloom, 1956).

Some previous studies have investigated teacher questions in teacher talk. Faruji (2011), by conducting eight observation sessions, found out types of questions posed by an Iranian English teacher who teaches students studying at a language institute in Iran. The analysis of the question types was based on Moore (2007). Moreover, Mahmood and Rana (2006) involved Japanese EFL elementary students and a teacher to look into the level and types of questions used by the teacher to monitor students' progress in learning language.

The framework used to analyze the teacher questioning is also based on Moore (2007). Similar to these two studies, this present study also used Moore's (2007) types of question to analyze the teacher questions. However, in this study, the process of teaching and learning in the classroom relied on questioning process as the second step of five main learning activities in the classroom. The greater the degree of requirement of K13, the greater the efforts are expected from the teacher to achieve the learning goal. Furthermore, in an isolated area like Aceh, there has not been much research so far emphasizing questioning in teacher talk, especially in a senior high school level. Accordingly, this study proposed this question: "What types of questions are used by the teacher in EFL classroom?" To find out the frequency of each question type, this study proposed the other question: "What is the frequency of different types of questions used by the teacher in the EFL classroom?" These two research questions will hopefully fulfill a need for existing research studies in teacher talk in Aceh, Indonesia.

### **Literature Review**

This study will discuss some theoretical backgrounds related to teacher talk, teacher's classroom question and the description about types of teacher's questions used in the classroom interaction.

#### **Teacher Talk**

Allwright and Bailey (1991) simplify a talk as one of the main ways to deliver information to student, and a talk is also utilized in the classroom to control students' behavior. So, teacher talk is defined as the use of specific language features by teachers in addressing second or foreign language students in the classroom. Classroom is the place where students can get more comprehensible input of language learning. Harmer (2007) argues the language input that low-level students get from outside of the classroom will commonly incomprehensible. Consequently, students need a model to provide the language which is comprehensible for them. Harmer (2007) therefore suggests that teachers must be a model to provide comprehensible input for students.

Occasionally, teachers adjust the classroom talk differently to different levels of students' proficiency as what Henzl (1979, cited in Ellis, 1986) studies in term of adjustment in pronunciation where the teacher used standard pronunciation for low-level students. Chaudron (1988) advises that teachers should modify their talk in the area of phonology, lexis, syntax and discourse in addressing classroom processes. Opposing to Gaise's finding, Nunan (1991) suggests that teachers need to try to elaborate the language rather than make it simpler.

Nunan (1991), Yan (2006), Yanfen and Yuqin (2010) conclude that teacher talk plays two important roles in teaching language; firstly, teacher talk is sources of language input or valuable input of language exposure, and secondly, teacher talk can serve as communicative sources to generate interaction during classroom instruction. The role of communicative function of teacher talk is to maintain and promote facilitation in learning interaction including types of questions the teacher ask, modification they use when talking to learners, and the way the teacher react to students' mistakes (Lei, 2009). The part of classroom talk considered as a talk for communicative function is found in Mahmood and Rana's (2006, p. 25) study such as, "what is the lesson now?" "What is date today? May I go to drink water"? Kind of input and interaction from teacher talk is mostly important for the learner. Beller (2008) concludes that the quality and quantity of language acquisition is very demanding on language input; the input about the language that students hear and accept from that they can learn a language. Also, Ellis (1986) argues that successful outcomes of learning depends on language features used by the teacher and types of interactions taking place in the classroom, no matter it is a subject lesson or a language lesson.

So, quantity and quality of language input is relied on teacher talk or language features used by the teacher in the classroom interaction. Teacher talk has been assumed as an important part in learning a language which is aiding for learners' input.

#### **Questions in Teacher Talk**

One important variable of teacher talk is teacher's questions. Questioning is employed by teachers to create classroom interaction with the students during teaching-learning processes. Questioning in



teacher talk is considered to have a potential effect on learners' comprehension, and has been hypothesized to be important for Second Language Acquisition (SLA) (Ma, 2008).

Teacher's question is the main aspect of language teaching which affects students' participation in classroom negotiation (Farahian & Razaee, 2012). Teachers usually initiate an interaction by asking questions, explaining activities and giving directions (Yanfen & Yuqin, 2010). Some studies show that asking questions in the classroom is one of the most dominant strategies used by teachers (Farooq, 1998; Yu, 2010; Yanfen & Yuqin, 2010).

Teacher's questions serve as language input for students because questions require responses from students. When giving the response, learners also contribute to interactions (Ellis, 2008). Other benefits of questioning in the classroom which is more pedagogic are like encouraging and maintaining students' interest, encouraging students to think and focus on the lesson, checking students' comprehension, prompting and clarifying (Richards & Lockhart, 1996). Essentially, teacher's question plays a crucial role for classroom interaction. And interaction can help students acquire comprehensible input.

More than half of the time in the classroom talk is run under question-answer exchanges (Richards & Lockhart, 1996; Farooq, 1998). Normally, teachers spend a day to ask about 300-400 questions in the classroom (Darn, 2008). According to Darn (2008), teachers need to consider some effective types of questions, for example, teachers have to minimize the use of yes/no questions, ask balance of display and referential questions, use open-ended questions (divergent) to encourage opinions, elaboration and discussion. So, questions which are categorized as higher order thinking skills are highly suggested to be employed in the classroom.

Therefore, questioning has the main role for both language acquisition and maintaining students' participation in the classroom talk. Instead of creating an interactive environment in the classroom, teacher's questions are employed to measure students' progress about the lesson.

### ***Types of Teacher's Questions***

A number of researchers have classified and identified questions in term of types and functions. Long and Sato (1983) classified questions based on types and functions into display and referential. In recent years, teacher's questions have drawn researchers' attention to study display and referential questions in EFL context (e.g. Farooq, 1998; Shomoossi, 2004; Yan 2006; Özcan, 2010; Meng & Wang 2011; Hamiloğlu & Temiz, 2012; Farahian & Rezaee, 2012; Al-Zahrani, 2014). Display questions are defined as questions used which the answer is already known by the teacher (Yan, 2006). On the other hand, referential questions refer to questions that the teachers do not know the answer (Yan, 2006).

Another researcher like Barnes (1969) differentiates types of questions into closed and open questions. Closed question refers to narrow questions, and it is usually has one existing answer. On the other hand, open questions may have more than one answer. They call for students to think creatively and imaginatively. This classification is very useful to language teachers as it can relate to types of questions with students' language output (Pearl, 1995). Additionally, Richards and Lockhart (1996) group questions into three categories such as procedural, convergent and divergent. Procedural questions more focus on classroom procedures, routines and management; while convergent questions are distributed in order to recall students' knowledge particularly to support similar students' response about the topic. In contrast, divergent questions are delivered to get dissimilar students to respond with long statements, and to encourage students to use their own information rather than recall previous lessons.

Bloom (1956) determines six cognitive levels of classroom questions which are widely used for educational purposes. They are knowledge, comprehension, application, analysis, synthesis and evaluation. The six types of teacher's questions by Bloom (1956) had been studied by other researchers in EFL context (e.g. Brown, 2001; Adibah, 2012; Barjesteh & Moghadam, 2014). Brown (2001) adopts and modifies six categories of classroom questions based on Bloom into seven types, they are: knowledge, comprehension, application, inference, analysis, and synthesis and evaluation questions. Brown (2001) defines inference questions as a conclusion made by the teacher that is not directly stated in instructional materials. Another useful classification of teacher questions was developed by Moore (2007). Not only Brown (2001), Moore (2007) also modifies six cognitive questions from Bloom (1956) into four types of classroom questions namely factual, empirical, productive and evaluative. Four types of questions based on Moore (2007) had been studied by other researchers in other EFL contexts (Mahmood & Rana, 2006; Faruji, 2011).

Factual questions aim to simply recall information from students which refers to knowledge or comprehension questions. Empirical questions try to ask student to analyze or integrate the information given, it includes application or analysis. The question requires a lot of thinking, but once thought out, and the answer is a single correct answer. Productive questions do not have a single correct answer. This question requires students to think creatively and imaginatively to produce something unique. They are open-ended and broad which ask students to go beyond the simple recall of information. The last one, evaluative question, is used to make judgments and give an opinion to evaluate an idea or product. Like productive questions, they are often open-ended. However, they are more difficult to answer than productive questions because they ask for some internal or external criteria which are established to evaluate and judge on something.

Having reviewed different classifications of classroom questions, the one proposed by Moore (2007) is employed to the present study, considering that the classification is modified based on Bloom taxonomy which is the most useful ones for educational purposes instead of language teaching. In the context of Indonesian curriculum, Bloom taxonomy is used to classify questions that have to be addressed during teaching processes by teachers (Kementerian Pendidikan dan Kebudayaan, 2013). Mostly, teachers are familiar with six levels of questions based on Bloom taxonomy. The hierarchy of cognitive levels is also helpful for teachers to ask questions differently to different levels of thought.

### **Research Method**

The purpose of this study is to find out and describe the process of classroom questions used by the teacher. Therefore, the descriptive qualitative which is a naturalistic inquiry was employed in this study in order to describe and to understand the classroom process as what they are. This study was conducted at SMA Teuku Nyak Arif Fatih Bilingual School, Banda Aceh. The participant was a female English teacher teaching fourteen Grade 2 female students. In teaching, the teacher used Student's Book and Work Book 3.

This study attempted to analyze the teacher talk particularly teacher's questions used by an Acehnese EFL teacher during 840 minutes sessions of teaching-learning processes. The teaching-learning process was videotaped and then transcribed for the purpose of data analysis. During the classroom observation, observation sheets and field notes were also utilized.

### **Data Analysis**

To treat the data from observation, this study followed steps suggested by Miles, Huberman and Saldana (2014): data condensation, data transcription, data display and conclusion verification. To analyze the types of questions posed by the teacher, this study consulted Moore's (2007) list of question types as discussed in the literature part of this paper. To calculate the frequency of each question type used by the teacher, this study manually count the number of each type of question found in the analysis.

### **Findings and Discussion**

This study found that the teacher in her talk used the four types of questions mentioned by Moore (2007). In addition, the teacher has different purposes when posing different types of questions to her students. These are discussed below.

#### **Factual Question**

In this study, the teacher used factual questions mostly to check students' understanding or comprehension about the material given, vocabulary and other topics related to students' background knowledge. This type was used to ask about grammar, listening and reading topics. Meanwhile, questions related to vocabulary include definition of words, terms and idioms from the reading and listening topic. Questions about the topic related to students' background knowledge is a question asked by the teacher in order to give opportunity for students to link their background knowledge to the topic.

In the present study, the examples of factual questions related to the material given, vocabulary and students' background knowledge are as follows:

- [E1] So, all of them are in what tenses?
- [E2] Ok, and then number two who was the first person to travel more than 100 kilometers in a car?
- [E3] Number one is apologize, what is number two? [E4] Trunk, trunk, what is it?
- [E4] Trundling, what does it mean?
- [E5] You can call me anytime if you need to talk. What does it mean? Bottom of my heart...
- [E6] Who has boyfriend here?
- [E7] Yes, do you know The Kite Runner?

### **Empirical Questions**

There are two forms of empirical questions found by this study. They are application questions and questions related to analyzing particular information. Based on the finding of this study, the teacher applied more application questions than analyzing information questions. The example is as follows:

- [E8] OK, so what is the difference between limp and hobble? [E10] Kate, what happen to her?  
[E9] To be yes, we have to be....and the 'to be', how is the 'to be'?  
[E10] How many sentences can you make using this chart?

### **Productive Questions**

The occurrences of productive questions found by this study included questioning on students' ideas and experiences, describing something and students' action toward something. Mostly, productive questions about students' experiences are related to students' background knowledge. Below is the example:

- [E11] And then, what kind of holiday is it? What do you think?  
[E12] Heart, ok, what can you relate to heart? What can you relate to heart?  
[E13] Susi Air ya, I heard there is no stewardess, and you can talk to the pilot, have you ever been in SUSI Air? How was there (in Susi Air) tell us?  
[E14] Ok, so have you ever felt something like this?  
[E15] What if you make mistake to your parent, then how will you apologize?  
[E16] And then (pause) so what do you do? Ohh what about when it is, it was the first time for you to be on the plane? How do you solve your feel down?

### **Evaluative Questions**

Evaluative questions found by this study can be categorized into two purposes. Firstly, the questions demanded students to give their preference about something.

Secondly, the question was aimed to ask students' wish or hope about something. Some examples of evaluative questions about preferences and wishes are shown in the following:

- [E17] Marking, which marking do you like?  
[E18] Ok, by the way, what about you? Ok, in which place do you prefer to live? Town, or a big city?  
[E19] What do you wish Ririn for this city, what do you wish? Before we go for the next one, you want to help Ririn Balqis?  
[E20] Ok, exploit, complete the sentence with your own ideas. What would you do if you had 3,000 pounds for a holiday?

From those types of questions found from the observations, they were calculated manually afterwards and displayed in Table 1. The table confirms the most dominant type of questions occurred in the classroom to the least one. Hence, the question frequency can be made in percentage as shown in the following table.

**Table 1.** The frequency of types of questions

Types of questions	Code	Frequency	Percentage
Factual	Q1	184	59.8 %
Empirical	Q2	40	12.9 %
Productive	Q3	33	10.7 %
Evaluative	Q4	51	16.6 %
Total		308	100 %

From the table above, factual questions appeared to be the most type of question used by the teacher in the classroom (59.8%). This finding is in line with Faruji's (2011) finding. He found that factual questions were the mostly used questions by the teacher. 52.71 % of the questions were shown to be factual which mostly related to asking the meaning of the word. Moreover, another study conducted by Mahmood and Rana (2006) has proved such questions occurred in the highest number compare to other types of questions. It indicated the questions more focus on the knowledge level.

Furthermore, evaluative questions appeared to be the second dominant type employed by the teacher. It reached 16.6%. This is followed by empirical (12.9%). The material-oriented questions indicated that evaluative questions appeared to be more dominant due to the topic of second conditional in the first

and the second meeting. This finding is supported by Faruji's (2011) finding. Evaluative questions are also the second highly used types of questions which were used with proportion of 27.13%. Faruji (2011) concludes that the use of these types of questions was to get the learners to participate in the classroom.

Empirical questions appeared to be the third highly used type of questions due to grammar topics during the fifth and the sixth observation. On the contrary, Faruji (2011) found that empirical questions were used as the lowest number in his study compare to other types of questions. While, the least type of question applied by the teacher based on this study was productive question, which merely occurred 10.7%. The teacher described that such questions were barely appeared in grammar and vocabulary-based topics. This phenomenon is similar to Mahmood and Rana's (2006) finding on four types of questions. They found out in their comparative studies in 2001 and 2003 that factual and evaluative questions were increased. However, empirical and productive questions were decreased. A decrease in empirical and productive questions indicated a decrease of higher order thinking skill questions.

It can be concluded that the classroom interaction portrayed the more factual questions. This situation remains unchanged, if the students mostly use their lower order thinking skills to answer factual questions. Factual questions are derived from knowledge and comprehension questions based on Bloom taxonomy. Bloom (1956), Sadker, Sadker and Zittleman (2011) and Barjesteh and Moghadam (2014) grouped knowledge and comprehension questions into lower order thinking skill questions. Yet, the student's English proficiency is well enough to produce more response in the target language since their exposure to the target language is full time during the lesson.

### Conclusions

Based on the analysis of data obtained from the classroom observation, factual questions appeared to be the most dominant types of questions employed by the teacher (59.8%) which was followed by evaluative (16.6%). Empirical (12.9%) and productive (10.7 %) questions were the least one among four types of questions employed. This finding is in line with Chaudron's (1988) statement that the characteristic of classroom question is display questions. Factual and display questions are questions which only require simple recall information. The teacher used these questions to know students' understanding.

Although most of the questions used appeared to be factual questions, the interaction between the teacher and students seemed to be interactive. The teacher seemed to encourage students' participation to respond the questions. So, more responses and participations were coming from the students. Almost all of them actively participated in learning during the lesson. Productive and evaluative questions were posed by the teacher in order to open more discussions, to give more chances for students in expressing personal ideas and to evaluate each other's ideas.

Nevertheless, this study is not without limitation, for example, this study only observed one teacher teaching in a homogenous classroom. Therefore, this study invites other researchers to conduct a similar study involving more teachers teaching students who are heterogeneous in nature.

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## **The Effect of Indirect Corrective Feedback in Reducing Error on Students' Writing**

<sup>1</sup>\*Endah Anisa Rahma, <sup>2</sup>Siti Sarah Fitriani

<sup>1</sup>University of Teuku Umar, Meulaboh 23681, Indonesia;

<sup>2</sup>Department of English Education, University of Syiah Kuala, Banda Aceh 23111, Indonesia;

\*Corresponding Author: endahdarussalam89@gmail.com

### **Abstract**

This experimental study aims to investigate the application of Indirect Corrective Feedback in reducing errors on students' writing recount text. The subjects of the study were 60 tenth grade students of SMAN 1 Meulaboh chosen by random sampling. True experimental research was employed involving experimental group which was given Indirect Corrective Feedback and control group which was not given feedback. The instrument used was writing tests and the data were analysed by using Statistical Package for the Social Science (SPSS). This study focuses on giving Indirect Corrective Feedback on students' writing recount in terms of grammatical features of the text. Therefore, there are nine error aspects mainly analysed, those are: verb, noun ending, spelling, word form, word order, pronoun, conjunction, missing and unnecessary word. The findings indicate that Indirect Corrective Feedback helps students to reduce errors in nine aspects. It is shown from the decrease of mean number of error in the post-test ( $\bar{x}$  = 12.17) which was lower than in the pre-test ( $\bar{x}$  = 27.07). It was also proven from errors performed in the pre-test as much as 388 and in the post-test as much as 250 from nine error categories. The results revealed that there was the reduction of error in the post-test. The finding confirmed that there is a significant difference between the results of post-test from both groups. It means that Indirect Corrective Feedback significantly improved the students' writing achievement by the reduction of errors.

**Key words:** Indirect Corrective Feedback, error, writing.

### **Introduction**

English is taught in Indonesian formal education as foreign language. Therefore, some difficulties are common in learning English. One aspect of teaching and learning process is writing. Hence, writing is the skill needs to be learnt by the students. In the context of the teaching English in senior high school in Indonesia, particularly for first grade students, the students are expected to be able to write a simple recount text based on its context and understand the social function, text structures and grammatical features of the text. Teaching writing for senior high school aims to develop the students' competence in writing various types of texts from functional text to different text genres (Syllabus Diknas, 2013).

The difficulty in writing can come up from lack of understanding about grammar. Besides, the students were not interested in learning writing because they made the same error. From the writing task collection, it was shown that teacher did not give error correction to the students' writing task. Teachers just put cross mark on the error part without providing the correct form. Most of them give composition assignment without any marks of correction to the students' work and no discussion of error in the classroom before or after giving the work back to the students (Hartono, 2010). In this situation, this present study assumes that teachers need to apply new technique that can utilize students' mistake by using correction code on students' writing so that they can improve their writing easily, which is called as corrective feedback.

Using corrective feedback helps the learners to improve students' grammatical accuracy of their text (Ferris, 2003). Students who wish to compose well in writing need to help in understanding and avoiding mistake in their writing since they need ways to know whether they are on track or not. The error correction can be done by providing correction symbol or by locating the error that is called Indirect Corrective Feedback (Ferris, 2003). Riddel (2001) stated that teacher can use correction symbols to students on their writing and teachers can underline the errors to signify the mistake and write the symbol for these mistakes. The students can correct the mistakes by themselves. Many teachers believed that feedback should be provided indirectly through the use error correction codes because this gives

students the opportunity to look up their errors (Corpuz, 2011). Therefore, this study investigated to the use indirect corrective feedback in students' recount text to reduce errors on their writing. The research question posed by this study is "Is there any significant difference between the students who are given Indirect Corrective Feedback and those who are not given Indirect Corrective Feedback in terms of grammatical errors?"

## **Literature Review**

### **Writing**

According to Nunan (2003, p. 88), writing is the process of thinking to invent ideas, thinking about how to express into good writing, arranging ideas into statement and paragraph clearly. It means that when learners want to write a composition well; they have to organize idea as well. In the context of the teaching English in senior high school in Indonesia, particularly for first grade students, they are expected to be able to compose descriptive, recount and narrative text. The focus of this present study is recount text. According to Derewianka (1990, p. 14), recount is the unfolding of a sequence of events over time.

### **Error**

Dulay, Burt, and Krashen (1982, p. 70) define errors as the flawed side of learners' speech or writing, which deviates from some selected norm of mature language performance. In other words, error occurs because the learners do not know what is correct, and thus it cannot be self-corrected. Grammatical error categories have been identified by Ferris and Robert (2001) into five categories, they are considered verb errors, noun ending errors, article errors, wrong word and sentence structure errors. In this study, the aspect of errors focus on grammatical feature of recount text which was adapted from Ferris and Robert (2001), Dewerianka (1990) and Butt, Fahey, Feez, Spinks and Yallop (2003). Therefore, there are nine aspect of errors mainly focus; verb, noun ending, spelling, word from, word order, pronoun, conjunction, missing and unnecessary word.

### **Feedback**

In addressing grammatical errors on students' writing, teacher can use two types of strategies, Direct Corrective Feedback (DCF) and Indirect Corrective Feedback (ICF) (Ferris & Hegdcock, 2005; Hendrickson, 1984; Lalande, 1982).

Direct Corrective Feedback (DCF) is done by providing the correct form (Ellis, 2009, p. 99). In other words, DCF is the provision of correct answer in response to students' error (Lee, 2008, p. 67). Indirect Corrective Feedback refers to the situations when the teacher marks the errors have been made but the teacher does not supply the correct form so that the learners diagnose and correct the error (Lee, 2004). Coded ICF refers to locate the errors and the types of errors that are marked. In this study, the researcher used coded ICF.

## **Research Method**

### **Research Design**

This is experimental research which consists of two classes, chosen as the sample by using random sampling technique. One class was chosen as experimental group which was given IDF on writing recount, whereas another class was chosen as control group which was not given error correction.

### **Participants**

The participants of the present study contained 60 X/MIA students of SMAN 1 Meulaboh. They were randomly assigned into two groups (one experimental and one control group). Each group consists of 30 students.

### **Data Collection Technique**

To answer the research question, the researcher analyse by test. Therefore, there were pre-test and post-test in this study. There were five meetings which included pre-test, treatment and post-test. In the pre-test which conducted on the first meeting, the students were given the topic about "Idul Adha Holiday" with the length of words 100. While on the treatment conducted from second meeting to fourth meeting, the students were given with code after they completed a simple recount text about "My best holiday". Then the tasks were revised by them and recollect by researcher to be given second feedback.

Next meeting, the writing task with second feedback was returned and the students did second revision. Post-test was conducted the last meeting. Students were given the topic about "unforgettable experience".

## Result and Discussion

The result from the statistical data analysis is presented in Table 1 as follows:

**Table 1.** Statistic result summary from pre-test of Control and Experimental Group  
**Independent Samples Test**

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Pre-test	Equal variances assumed	.512	.477	-1.188	58	.240	-2.800	2.358	-7.519	1.919
	Equal variances not assumed			-1.188	57.472	.240	-2.800	2.358	-7.520	1.920

The result from Table 1 shows that t-counted from the pre-test of both groups is -1.18. T-table for df = 58 at the level significance 5% ( $\alpha = 0.05$ ) is 1.68. The result shows that  $t_{\text{counted}} < t_{\text{table}}$  ( $-1.188 < 1.68$ ). Therefore,  $H_0$  is accepted and  $H_a$  is rejected. This has indicated that there is no significant difference in the pre-test results between the two groups. Meanwhile, Table 2 shows that the statistical result of post-test from both groups.

**Table 2.** Statistic result summary from post-test of Control and Experimental Group  
**Independent Samples Test**

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Post-test	Equal variances assumed	.824	.368	1.823	58	.01	-2.333	1.639	-5.615	.948
	Equal variances not assumed			1.823	56.557	.01	-2.333	1.639	-5.616	.950

Table 2 shows that t-counted from the post-test of both groups is 1.823. The result of t-table with df = 58 and significance 0.05 is 1.68. Hence,  $t_{\text{counted}}$  is higher than  $t_{\text{table}}$  ( $1.823 > 1.68$ ). Therefore,  $H_0$  is rejected and  $H_a$  is accepted. This finding indicates that there is a significant difference between the result of the post-test in the experimental and control groups. It means that there is a progress of



students' writing recount text in the experimental group in terms of error reduction after the treatment is given. This following table shows that the description of error aspect in pre- and post-test.

**Table 3.** Total error aspect and reduction from the Experimental Group

Error Aspect		Pre-test	Post-test	Frequency of reduction
Verb	Verb Tense	64	40	24
	Verb form	48	16	32
Word order		48	22	18
Word form		77	61	16
Spelling		48	34	14
Pronoun		34	22	12
Noun ending	Plural marks	2	4	-2
	Possessive	27	14	13
Unnecessary word		21	13	8
Missing word		26	23	3
Conjunction		1	0	1
Total		388	250	139

The table above shows list of total error aspects from pre-test and post-test in experimental group. The table shows that verb is the dominant error and the most reduced error. Meanwhile, conjunction is the lowest error. The following is total error and reduction from control group.

**Table 4.** Total error aspect and reduction from the Control Group

Error Aspect		Pre-test	Post-test	Frequency of Reduction
Verb	Verb Tense	80	42	38
	Verb form	43	38	5
Word order		55	21	34
Noun ending	Plural Mark	8	7	1
	Possessive	35	2	27
Pronoun		37	8	28
Spelling		46	32	14
Unnecessary word		19	6	13
Missing word		31	22	9
Word from		66	74	-8
Conjunction		0	0	0
Total		417	255	204

The table above show list of total error aspects from pre-test and post-test in control group. The table also shows that verb is the dominant error and the most reduced error. Word form is aspect that does not have improvement since there is the higher error found on post-test.

### Conclusion and Recommendation

According to the result analysis, there was a significant difference in writing achievement between the students who are given Indirect Corrective Feedback and those who are not given Indirect Corrective Feedback ( $1.823 > 1.68$ ). It can be concluded that the implementation of Indirect Corrective Feedback in assessing students' writings reduces the students' errors in writing recount text in terms of grammatical errors: verb, noun ending, word form, word order, spelling, pronoun, conjunction, missing and unnecessary word, compared to students who are not given this type of correction feedback. However, the present study had some limitations in terms of grammatical error aspects. There were two grammatical aspects that did not have improvement between pre- and post-test: word form and plural mark (noun ending). The researcher did not teach extensively about the aspects since the recount text mainly focus on verb, pronoun, and conjunction aspects Hence, this study recommends further studies to include the teaching of all aspects in grammatical features of a recount text when teaching writing to students.

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## **Biochemistry Concept Level of Difficulty Profile of Prospective Biology Teachers' Perception**

\*Hafnati Rahmatan

Department of Biology Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia;

\*Corresponding Author: hafnati\_rahmatan@unsyiah.ac.id

### **Abstract**

This descriptive study deals with the prospective teachers' difficulties in understanding the biochemistry concepts to obtain a figure of biochemistry difficulty profile. The subjects of this research are 42 2nd semester biology students in at LPTK in Aceh Province. The data needed for this study are collected by using an open and closed questionnaire referring to the Likert scale. The data are statistically processed. The results showed that the most difficult biochemistry topics include the topics on the catabolism of carbohydrates (27.3%), catabolism of lipids (16.8%), and catabolism of protein (14.5%). The difficulties in mastering the concepts are sought to be due to their using a lot of chemical formulas and chain reactions that hindered the students to fully understand them. These concepts will be easier to understand and attractive by using computer technology. The results show that biochemistry is important for the prospective teachers. In terms of this, it is highly recommended to prepare good material, learning, and authentic assessment that provide challenges and improve students' capabilities that can be applied in real life situations. Besides, it is important to do continuous and periodic assessment on this matter.

**Key words:** Level of difficulty, biochemistry concepts, prospective biology teachers.

### **Introduction**

As an agent of learning, teachers should have five professional competences. One of them is mastering the subject's material, structure, concepts, and scientific mind-set that support students in learning the subject. Professional competence is further elaborated for teachers in each respective subject of learning. One of 14 professional competencies that should be possessed by a biology teacher is to understand the concepts, laws, and theories of biology and its application in a flexible dimension (Permendiknas No. 16, 2007).

The Institution of Teacher Training (LPTKs) is an institution that produces teachers, one that prepares the students of prospective teachers to have four competences including pedagogic, personality, professional, and social competences. The professional competence of a biology teacher is attained from the Expertise Course (MKK). One of the courses included in MKK is Biochemistry. Biochemistry is one of the basic courses that are a basic and compulsory subject that should be taken by the students of prospective biology teachers in the institute. This course is intended to help students understand the chemical processes which are going on in the living things.

Biochemistry is the science of the structure, organization and function of the molecular processes of living beings. Biochemistry learning can be divided into three parts, namely: 1) the chemical structure of organic matter and the relationship between its structure and function, 2) metabolism, which is the overall chemical reaction that occurs in the organic material, and 3) molecular genetics, which studies the chemical structure of organic materials including the structure and function of macromolecules such as studies of the structure and function of carbohydrates, amino acids and proteins, lipids and nucleic acids. The study of metabolism covers the material such as enzymes, catabolism and anabolism of carbohydrates, proteins and lipids, while the study of molecular genetics includes DNA (chromosome structure and gene), DNA replication and transcription, and protein synthesis and regulation (Lehninger, 2008).

The results of analysis of the biochemistry syllabus on some LPTK show the lectures' goal that only emphasizes the aspect of concept understanding. On the other hand, the author's experience in handling the course for about  $\pm$  11 years using a strategy that focuses on the concept explanatory attributes utilizing various media and learning sources have not showed satisfactory results. The outcomes

achieved are restricted only on the mastery of the defined concepts. Meanwhile, the ability to understand abstract concepts and the relationships between concepts are difficult to achieve. When dealing with abstract concepts, such as metabolic reactions, students tend to develop alternative definitions to understand them.

Furthermore, if faced with the stage of chemical reactions in metabolism, students tend to just memorize the stages of the reaction, but the meaning of each phase is not well understood and difficult to understand, along with the occurrence of some common misconceptions. This misconception occurs due to students' inability to directly observe all stages of metabolic reactions at the molecular level. This is in line with a research by Meir *et al.* (2005) which suspected that the misconceptions that occur were due to the inability to directly observe the process of diffusion and osmosis at the molecular level.

Based on the results of research and field experience, the most difficult concepts mastered by high school teachers in class XII is carbohydrate catabolism or respiration (49%) (Hamidah *et al.*, 2009). The same result is also showed by many high school biology teachers from various regions in Indonesia. The teachers of class XII mention that the most difficult materials to explain are enzyme, carbohydrate catabolism, anabolism of carbohydrates, the linkage between the catabolism and anabolism, the linkage between the metabolism of carbohydrates, fats, and proteins. Likewise, in terms of teaching materials and students' mastery of the subject, the difficulties that occur are mainly on the metabolism (Hamidah & Nuryani, 2008).

Understanding the abstract concept is a great difficulty and challenge for either school or college students, which mostly lead to misconceptions. The limitations of media that were used in delivering lessons of abstract concepts have motivated some researchers to present the biochemistry material by utilizing computer technology in the form of animation or visualization as that of Roberts *et al.* (2005) with the physical models of three-dimensional molecular visualization and computer programs to help students understand the abstract concepts better and Ouyang *et al.* (2007) who present the lecture material with the aid of biochemistry multimedia device.

Based on the categories that make biochemistry considered as a difficult subject, it is necessary to consider how teachers teach and how students learn biochemistry. Both of these are closely related to the learning methods used in the lectures in class. In spite of the fact that there are many ways to create an effective teaching, all of those require three particular concerns: 1) the material being taught, 2) the most appropriate strategies to teach the subject, and 3) how students learn (Xiaoyan, 2003).

Thus, from the description above, it becomes compulsory for us to develop a biochemistry course program by knowing the prospective biology teachers' perception on how the biochemistry course profile is in terms of its material characteristics and way of learning.

## **Method**

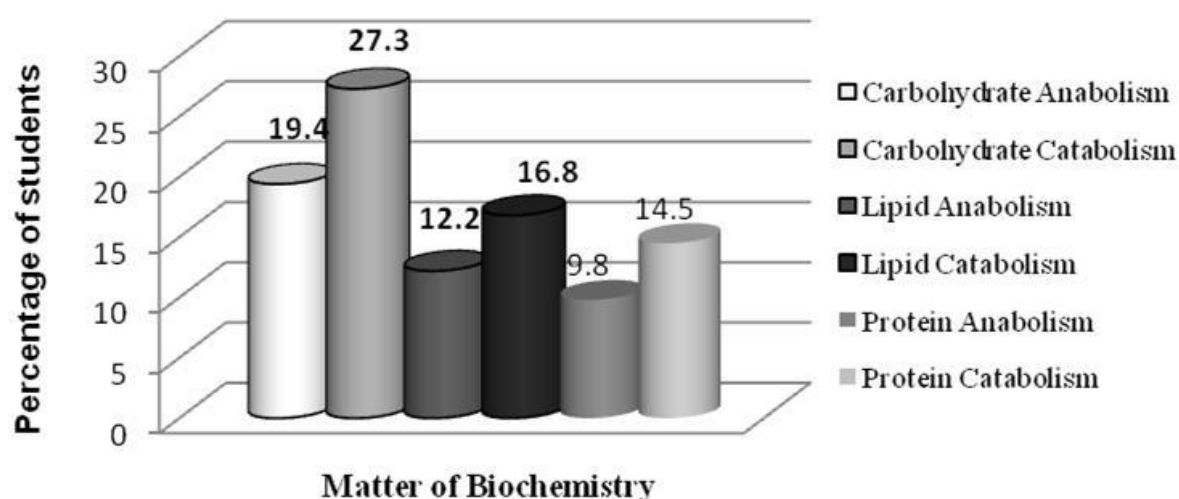
This research is a descriptive study that concerns about the biochemistry material identification. Identification includes materials that are considered difficult by students and the things underlying their difficulty, and how the learning strategies that are expected by the students of prospective teachers so that the biochemistry materials in concern can be attractive and easy to understand.

The study involved 42 students of biology education class from batch 2009/2010 in an LPTK in the province of Aceh as the research subject. The data for this study is retrieved by using an open and closed questionnaire using Likert Scale. Research data both in the form of qualitative and quantitative are presented descriptively. Then, the data obtained are presented in graphical form, analysed and interpreted to see the prospective biology teachers' perception on the biochemistry course profile.

## **Results and Discussion**

### ***Level of Difficulty of the Biochemistry Subject on Concept Level***

Based on the results of the analysis of questionnaires given to students, the writer obtained the results presented in Figure 1.



**Figure 1.** Percentage of students' difficulty of biochemistry materials

Based on the results presented in Figure 1 it can be explained that the difficulty level of biochemistry subject from the students' point of view occurred on the topic of carbohydrate catabolism with percentage of 27.3%, carbohydrate anabolism topic with the percentage of 19.4%, lipid catabolism topic with the percentage of 16.8%, protein catabolism topic with the percentage of 14.5%, lipid anabolism topic with the percentage of 12.2%, and the topic of protein anabolism with the percentage of 9.8 %.

On the carbohydrate catabolism topic, the students stated that the thing that makes it difficult on this topic is the use of many complicated chemical reactions. In this topic, students should understand the stages that occur in each reaction pathway including the substance structures, enzymes, coenzymes and cofactors involved. In addition to that, this topic is considered difficult because students need to link one stage of catabolism to other stages which are taught at separately.

On the topic of carbohydrate anabolism, a deeper understanding of how the mineral nutrients and molecules enter into the plant is needed. Moreover, in order to understand how water and nutrients enter the plant, students need to understand the anatomical structure of the plant, especially the root. Another concern is that the number of nutrients needed by plant is too much, along with their functions and deficiency symptoms.

Whereas, on lipid catabolism topic, the difficulty was due to its chemical reaction with a long carbon chain. In this topic, students also need to understand many reaction chains even though not as many as in carbohydrate catabolism and anabolism topics. Additionally, students must understand the energy produced by a variety of lipids.

The lipid anabolism topic is also considered difficult although it is not as complicated as the lipid catabolism. This is because many chemical formulas and carbon chain in various lengths to form various fatty acids are used in this topic. Students also need to know the enzymes and coenzymes involved in the process.

The topic of protein catabolism is also considered difficult. In this topic, students have to remember many kinds of amino acids produced to be converted into intermediate compounds in the primary metabolism process to produce energy. Therefore, this topic is considered difficult since there are a number of precursors for the primary metabolism which are important in forming various amino acids that the students need to know.

### **Biochemistry Learning**

The results show the prospective teachers students' questionnaires analysis to find out the biochemistry learning is presented in Table 1. The information in Table 2 shows nine things considered important by the prospective biology teachers in response of how biochemistry learning should be. Among all the nine things, over 50% agreed that learning biochemistry will be easier with the aid of computer animation. Furthermore, it is necessary to relate their learning with everyday life and thus with the example they can presume in daily life.

**Table 1.** Responses of the prospective biology teachers on biochemistry learning (%)

No	Statement	Number of Student	Percentage
1	Using computer animation	25	59.52
2	Associated with everyday life and provided an example in life	21	50.00
3	Using a practical method	18	42.85
4	Discussion with questions and answers	17	40.47
5	Discussion with a problem	12	28.27
6	Using a learning innovation	12	28.27
7	At the end of the quiz	10	23.80
8	Given examples of cause and effect	8	19.04
9	There should be a summary / hand-out	7	16.66

In learning biochemistry, students need a detailed and profound fundamental understanding since this subject is the basis for learning the development in other courses such as Plant Physiology, Animal Physiology, Genetics, Microbiology, Biotechnology, Nutrition and Health Sciences, Agriculture and Forestry.

### Conclusion

Based on the results showed above, it can be concluded that three topics considered difficult in biochemistry are carbohydrate metabolism (27.3%), lipid metabolism (16.8%), and metabolism of proteins (14.5%).

According to the students, these learning topics become difficult because they require an understanding of the structure of the compounds, enzymes, coenzymes and cofactors that are required for chemical reactions and phases in a chemical reaction.

To facilitate the understanding of biochemistry learning, the students believe in some aspects that will enable them to understand the subject better, including: the use of computer animation in learning, given the association and examples in the daily life, using a learning method in form of discussion and question and answer, 8 discussion with a problem, given laboratory activities, given *hand out* and finally quizzes.

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## **Problem-Based Learning Associated by Action Process Object Schema Theory in Mathematics Instruction**

Achmad Mudrikah, \*Luki Luqmanul Hakim

Nusantara Islamic University, Bandung 40286 Indonesia;

\*Corresponding Author: achmadmudrikah@yahoo.co.id

### **Abstract**

Problem-Based Learning (PBL) Associated by Action Process Object Schema (APOS) Theory were implemented to 26 prospective mathematics teachers in the mathematics for senior high school students course. It was hypothesized that the six steps of PBL are appropriate with mental constructions of APOS that make students can reach the objectives of the course. Five main questions on recorded semi-structured interviews were applied to get the information about the implementation of PBL associated by APOS theory and students' ability to create mathematics instruction in senior high school by using PBL associated by APOS theory. The data strongly supported this model in confirmatory factor analyses. The data also revealed the hypothesized differential relations between teacher cognitions and teacher education.

**Key words:** Problem-Based Learning; action process object schema; Mathematics instruction.

### **Introduction**

In an effort to develop education towards a better quality, Indonesia has set 2013 curriculum as the curriculum that should be implemented in every level of education. There are principles emphasized in order to achieve the quality that has been designed in curriculum documents (2014). The principles presented entirely associated by the facility to be provided by a teacher against students in the learning process. Overall this principle should be a guideline in implementing the learning process including mathematics learning.

Teachers, as person who perform the learning activities, are expected to apply these principles using appropriate models of learning. One model of learning that is recommended in the above regulation is a problem-based learning model. In addition, the learning mode used in the 2013 curriculum is directed learning through a scientific approach and indirect learning. Directed learning is done to produce an immediate impact in the form of knowledge and skills, while indirect learning to produce companions impact that develop values and attitudes.

On the basis of the implementation of the curriculum, teachers need to anticipate it by implementing quality learning through their attention to various aspects, primarily related to students. The main supporting aspects related to students in mathematics learning, in terms of how mathematical concepts can be obtained and understood by the students.

Initial studies with high school math teachers in Bandung Regency and the math teachers of vocational schools in Karawang show that math teachers tend to better prepare students only in answering exam questions. They are less concerned about the efforts to convey mathematical concept to students as a whole. This is evident when teachers were asked how their efforts to teach the concept of limit function and derivative function. The teachers tend to teach the concepts separately without any connection. This has resulted in lack ability to understand the concept of limit function and derivative function as a whole of the students and even the teachers themselves.

The existence of various policies in the field of mathematics education would certainly impact on efforts to prepare prospective teachers of mathematics itself. One of the courses that prepare mathematics teachers to teach mathematics in high school is mathematics for high school course. Prospective mathematics teachers through this course is expected to be early to know the strategic efforts that must

be implemented to improve the quality of mathematics learning in line with expectations in the curriculum document.

## **Literature Review**

### **Problem-Based Learning**

Problem-Based Learning (PBL) is a learning approach that can challenge the students to make them think visible. PBL is known as a progressive active-learning and learner-centered approach where unstructured problems (or simulated real-world complex problems) are used as the starting point and anchor for the learning process (Tan, 2004, p. 7). The process of learning in PBL according to Delisle (1979) is organized into the following steps: connecting with the problem, setting up the structure, visiting the problem, revisiting the problem, producing a product or performance, and evaluating performance and the problem. Through these steps, PBL can make the students find themselves, asking questions and answering others posed by their peers.

Research on problem-based learning at all educational levels have been carried out and managed to prove that this model runs quite effective. Padmavathy and Mareesh (2013) states that by adopting PBL in teaching mathematics, teacher can create a number of students who are creative, critical decision makers, problem solvers, entirely is needed later in the world of competition. PBL teaching strategies according to Padmavathy and Mareesh (2013) had an impact on the content of the knowledge presented in the more widely opportunities. The students learn the content with more engagement and increase their active participation, motivation and their interest in mathematics. This of course can lead the students have positive attitudes towards mathematics.

Abdullah, Tarmizi and Abu (2010) stated that the group of students who learn mathematics using a PBL models can use heuristic problem solving of Polya more effectively. As a result they were able to demonstrate mathematical communication skills better, and can work together better than students who study mathematics with conventional learning.

Meanwhile, a similar thing was also expressed by Fatade, Mogari and Arigabu (2013) which states that PBL can make students more creatively, act in a focused, rational thinking and dealing effectively with a friend in their group in mathematics. The use of PBL can also make the teachers know when and how they use scaffolding for learning. The results of the above study reinforce earlier findings about the use of PBL in teaching mathematics. Schmidt, Loyens, Van Gogg and Paas (2007) stated that PBL was compatible with the person's cognitive architecture that is being studied, because the elements of learning in PBL allows for flexible adaptation of coaching and cognitive call. Meanwhile Hmelo-Silver, Duncan and Chinn (2007) states that the PBL approach involving students, with the application of appropriate scaffolding, in doing exercises and concepts that will increase the understanding of the construction of knowledge, known as a learning process.

For the use of PBL in college students' mathematics learning, Triantafyllou and Olga (2013) states that the use of PBL will enable teachers to combine constructionism pedagogical ideas with PBL, to teach mathematics in a wider context, and to adopt a student based strategy. On the other hand, it is expected to help students assimilate mathematical knowledge and Overcome Reviews their deficiencies regarding mathematics. Thus, the use of PBL could fit in college especially for prospective teachers of mathematics.

### **Action-Process-Object-Schema Theory**

Talking about the theory of Action-Process-Object-Schema (APOS) will not be separated from Piaget's Reflective Abstraction as the underlying theory. Reflective Abstraction according to Piaget (in Arnon *et al.*, 2014) is: 1) the major mechanism of mental constructions in the development of thinking; and 2) the mental mechanisms that occur when the overall structures of mathematical logic developed in one's mind. Furthermore, according to Piaget (in Arnon *et al.*, 2014) Abstract Reflective contains two parts: 1) the reflection in the sense of a) awareness and thinking contemplative about content and operations on the content, b) recognized to reflect a variety of content and operation of a the cognitive level lower



to a higher cognitive level; and 2) the higher cognitive level will be constructing and reorganizing content and operations acquired and will be content on the operations itself to be applied as new operations.

Thus, the development of knowledge about an object, either mental or physical, according to Piaget requires both the object and a subject who acts on the object. Someone will receive the properties of objects that are not derived from these objects, but derived from the way he or she acts to objects in the form of operations conducted against them. This suggests that abstract ideas are the most common and useful according to the concept of Reflective Abstraction, obtained from a variety of phenomena instead of taking a picture pickup.

Stages of construction and reorganization of content and operations on reflective abstraction has been raised by Dubinsky, being very close to the present mathematical ideas (Arnon *et al.*, 2014). Dubinsky's thought, related to Piaget's reflective abstraction, can be applied to understand the concept of limit function. When students, for the first time, construct knowledge about  $\Delta x$  that is read "delta x" with the meaning "change in x" and  $\Delta y$  that is read "delta y" with the meaning "change in y" constructed as operations that transforms the meaning of "changes in x" and "change in y". This transformation occurs when students are able to determine  $\Delta x$  and  $\Delta y$  on two points, say point A ( $x_1, y_1$ ) and B ( $x_2, y_2$ ) and determining the gradient of the line through A and B both for points A ( $x_1, y_1$ ) and B ( $x_2, y_2$ ) and at points A (2, -5) and B (1, 4). Then at a higher stage, the meaning of  $\Delta x$  and  $\Delta y$  will be content on the line gradient concept that will construct new operations and leads to an understanding of the concept of limit function, when "changes in x" increasingly reduced through the attribution of the concept on the bowstring a curve  $f(x)$ .

From the example above, it can be seen that the concept of reflective abstraction, what is referred to as an object is the concept of "change in x" and "change in y". Objects will have the properties when given the command to determine the value of both the points yet to be determined and specified coordinates. These properties according to Piaget (in Arnon *et al.*, 2014) owned by the action is not just owned the object just so it certainly cannot be separated from the subject of the action.

While students are doing an action seeking a "change in x" and a "change in y" by the coordinates of two points that are known, then the action is termed by Piaget (in Arnon *et al.*, 2014) as a material action. Furthermore, students will try to determine whether the line gradient formed by two known points or lines will be as a bowstring or in the form of a tangent curve  $f(x)$ . Continued activity is called by Piaget as the activity translates to the success of the actions of material determine "change in x" and "change in y", into understanding the operations seek gradient of the line and determine the basic concept of limit function. Understanding of the operations seek gradient of the line and determine the basic concept of limit function is called Piaget (in Arnon *et al.*, 2014) as a system of operations that have been interiorized.

In addition, students also acquire the rules to be able to acquire basic concepts limit function which will have implications for future activities. A translation of the material to the action of understanding of the operations and the understanding of the rules is what was intended as a Reflective Abstraction.

Dubinsky (in Arnon *et al.*, 2014) interpreted "material actions" as actions undertaken by a subject and an external part of the subject. While the "system" in the phrase "system operations" that have been interiorized as a scheme in this case, is the concept of limit function as a gradient of a tangent curve. When this concept has been conceived so that students can later be applied to the higher operating as understand the concept of the derivative function, he will be transformed into an object. This transformation occurs through a mental mechanism known as encapsulation.

The general framework in the form of content and operations on this item by Arnon et al. (2014) directs the operations of their own to be the new content. These operations rests on a foundation for more differences subtle, such as the difference between the material acts and interiorization, which bears the distinction between mental structures of action and process as well as how the mental mechanisms, as interiorization and encapsulation, The overall lead to the formation of different load conception progress: Action→Process→Object→Schema known today as the APOS theory.

In APOS theory, interiorization in Piaget's abstractive reflection is called the "Process". "Shifting" the term interiorization into the mental mechanisms of APOS theory, by reconstructed an external thing, namely the physical form of the Action into the subject's mind into a Process (interiorized operation). The Process itself is an internal action in the form of mental construction that similar with Action, but overall occurs more in mind than external subject (Arnon *et al.*, 2014).

Detailed information about how the APOS theory emerged based on Piaget's reflective abstraction and the development of intelligence that pay attention to the statements about the schema, thematization of schemas, and coordination of the schemas, also described by Arnon *et al.* (2014). This was done by having regard to the concept of positive integers which is described by Piaget throughout the period of development of this concept.

In applying this schema, a child using a schema of 1-1 correspondence (constructed before or simultaneously) to explain that the two sets of a particular set has the same unit or use a set of schema inclusion, to explain that a set have more (or less) members than the other set. The final understanding lay on the foundation of the schema development of seriation by the student's ability to imagine a sequence of sets such as:  $\{1\}$ ,  $\{1 + 1 + 1\}$ ,  $\{1 + 1 + 1 + 1\}$ , ...

With these constructions, the child called the sets as one, two, three, four, and so on. And also call their position in seriation as the first, second, third, fourth, and so on. Finally, the two schemas namely classification and seriation are being thematized and then coordinated in the formation of a new schema. A key step in the coordination emerge, when the child realizes that set with four members on the row above is also the fourth set on the line. The resulting scheme is the concept of positive integers. Piaget noticed throughout constructing this to be used as examples of reflective abstraction.

In the example above, the Actions are: a) transforming the physical objects by taking two sets of small objects, b) calculate numbers in one set, then the other set, and c) summing up the two results to obtain the total number of objects; and d) repeating the action in reverse order from two sets to see that the same of total amount. Meanwhile, their objects are: numbers (integers that are represented by a set of physical objects). The action applied to these objects is the summation, and the properties (operation, not a number) are commutative properties.

Coordination according to Piaget is an action of the two schemas. This is a very common usage of the term coordination includes various construction using two schemas, such as the one following the other, or back and so forth between the two schemas to use part of that one and then the other. In an effort to do this, schemas must be thematized in advance, which means made into objects (as the processes being encapsulated into objects) on the state of the action of coordination can then be applied.

The statement of a mentally transfer of an individual from the Actions to Processes, and from Processes to Objects, emerges clearly in the Piaget's discussion (in Arnon *et al.*, 2014) concerning the development of cognitive functions. Piaget's comments could be interpreted as referring to the function as a mapping that is originally an action and then processes and further to the objects. Dubinsky (in Arnon *et al.*, 2014) interpreted the types of these steps as a description of cognitive development that began with the Actions that interiorized into Processes and then encapsulated into Objects to the condition of actions will be applied. This is an example of Piaget's reflective abstraction, about the development of the Action to Process and then to Object and then to the Schema, the progress of which is the main part of the APOS theory.

According to Arnon *et al.* (2014), Piaget's reflective abstraction formed the antecedents to APOS theory –the mental structures of Action, Process, Object, Schema, and the mental mechanisms of interiorization, coordination, reversal, encapsulation, and thematization– as well as their formation into the developmental of APOS progression. This progression is illustrated in Figure 1. The figure shows that Actions operate on Objects; Actions are interiorized into processes (internal action); Processes are encapsulated into Objects; and Objects will be de-encapsulated back on Processes whence they came. The entire system is part of a Schema.

#### SCHEMA OF FIGURE 1

Figure 1. APOS Theory (Arnon *et al.*, 2014)

#### **Problem-Based Learning Associated by Action-Process-Object-Schema Theory**

The principle of problem-based learning process that is based on APOS theory (Action-Process-Object-Schema) run on 5 steps that are part of the establishment of the action, process, object and schema, namely: (1) connecting with the problem, (2) setting up the structure, (3) visiting the problem, (4) revisiting the problem, (5) producing a product or performance, (6) evaluating performance and the problem.

Connecting with problems and structuring them (steps 1 and 2) were categorized as Action. This happens because when students are encountered to the steps 1 and 2, they still commit the material

action to the object being characterized by these actions. Visiting the problem step (step 3) is categorized as an interiorization of mental mechanism, and in the condition that the Actions and Processes are happen. This step is categorized as interiorization because its results will be the understanding of the issues that are internal in students' mind. Revisiting the problem step as an attempt to complete problem that has been defined and the conclusion of the alternatives solutions (step 4) is a step of reversal or coordination. When it became reversal, it will go back into action and process, while when the definition has been refined and agreed, then the alternatives to solving the problems will be the encapsulation step.

In this encapsulation step, it is obtained a product or the ability and their abilities and the abilities and the problem (step 5 and 6) which will eventually become the object. This step will also be a de-encapsulation when the resulting product cannot be understood after the test. Cycle is ongoing in order to obtain a scheme of which it considers most appropriate solution and can be generalized. Thus it can be said that the Problem-Based Learning has a learning steps which can be associated with APOS theory.

### **Mathematics for Senior High School Students Course**

Mathematics for Senior High School Students Course is the Course that has the purpose to deepen the mathematics material that will be taught to high school students while at the same time gave the students an understanding of how to provide understanding to the senior high school students. Problem-based learning that is associated by APOS theory is expected to be understood by students as one of the steps that can be used to understand the lecture material given. Besides that, it is expected the students are able to use PBL based on APOS theory related to the practice of teaching at the school. In general, this course will contain the following things:

1. Deepening the concepts that must be mastered by students and will be taught to high school students.
2. Searching the prerequisites material that must be owned by the students and creating concept maps relating to the material.
3. The problems typically encountered by teachers related to the concepts that will be given to students and the effort to solve them.
4. Determining learning scenarios that can solve problems by using problem-based learning approach through the activities that based on the APOS theory. In these activities, students are grouped by sub subject.
5. Delivering the presentation of results of group discussions.

### **Aim and Questions Addressed**

Aim of this research is to know how far PBL associated by APOS theory can implemented in Mathematics for Senior High School Students Course. Besides that, it was needed to know about students' ability to create mathematics instruction in senior high school by using PBL that associated by APOS theory.

To reach the aims observed the information about: 1) how the students connecting and developing structure of the problem related to Action step in APOS theory; 2) how the students visiting the problem related to interiorization step in APOS theory; 3) how the students revisiting the problem, complete the defined problems, and conclude various alternative of problem solving related to reversal and coordination steps in APOS theory; 4) how does the students' encapsulation step was done when they obtain the capability and evaluating it; 5) how could the students be in the stage of having new object as well as a schema related to APOS theory.

### **Research Methods**

Based on the aim of the research, students are given lectures of mathematics high school course by using PBL which is based on APOS theory. Furthermore, at the end of the mid semester, it was applied semi-structured interviews to 26 prospective mathematics teachers that will teach in secondary school. Interviews focused on five main questions: (i) what to do when going to solving the initial problem?; (ii) what is developed to understand the problem and relate it to the concept being studied?; (iii) when there is an error in concluding concept, what to do? (iv) how to conclude that the answer to the problem that posed is right? (v) Any phase obtained after completing a variety of questions? Answers were audio taped to guarantee a better and more reliable data transcription.

After the transcription, it was applied content analysis. The sample integrated 19 female and 7 male students for undergraduate degrees in mathematics education, in a Bandung West Java private university.

## Results

After the content analysis of the five questions and considering the objectives of the study, the following results were obtained.

**Table1.** Students' answers to Q1: What have you done, when solving the initial problem?

Categories of answers	Frequency	(%)
Associating and structuring the problem on the known mathematical concept	18	69.23
Only associating the problem the problem on the known mathematical concept	5	19.23
Only structuring the problem	2	7.69
Unable to recognize the intended mathematical concept.	1	4.34

The analysis of the question Q1 (Table 1) reveals some interviewees who had not done two next steps of PBL. But, the majority of students (69.83 %) do so.

**Table2.** Students' answers to Q2: what have you developed to understand the problem and associate it with the mathematical concepts being studied?

Categories of answers	Frequency	(%)
Reviewing the problem to acquire mathematical concepts and how to teach it for high school students	15	57.69
Reviewing the problem only to acquire mathematical concepts	4	15.38
Reviewing the problem only to know how to teach mathematics for high school students	3	11.54
Directly making conclusions about solving problems	3	11.54
Unable to make a conclusion	1	4.34

In question Q2, 22 prospective teachers referred to review the problem to understand and associate it with mathematical concepts. As presented in table 2, 15 of them (57.69%) consider that they review the problem to acquire mathematical concepts and to know how to teach the concepts for high school students, 4 of them (15.83%) review the problem only to acquire mathematical concepts, and 3 of them (11.54%) review the problem only to know how to teach the mathematical concepts for high school students.

**Table3.** Students' answers to Q3: when there has been an error in concluding concept, what to do?

Categories of answers	Frequency	(%)
Revisiting the problem to find appropriate concept and alternative	14	53.84
Specify some other alternative solutions, without revisiting the problem	5	19.23
Revisiting the problem but did not have another alternative	3	11.54
Creating a new answer without alternative	3	11.54
Didn't do anything	1	4.34

In question Q3, 17 prospective teachers referred to revisit the problem. As presented in table 3, 14 of them (53.84%) consider that they revisited the problem to find appropriate concepts and alternative, 3 of them (11.54%) review the problem only to acquire mathematical concepts, and 3 of them (11.54%) review the problem only to know how to teach the mathematical concepts for high school students.

**Table4.** Students' answers to Q4: how do you conclude that your answers to the problems are proper?

Categories of answers	Frequency	(%)
After re-evaluation and believed to be true	16	61.54
After re-evaluation but not sure to be true	5	19.23
Immediately convinced without doing evaluation	4	15.38
Not evaluated and not convinced	1	4.34

In question Q4, 21 prospective teachers referred to reevaluate the answers to the problems. As presented in table 4, 16 of them (61.54%) consider that they reevaluated the answers and believed to be true and 5 of them (19.23%) reevaluated the answers but not sure to be true.

**Table 5.** Students' answers to Q5: What are the stages that you learn after solving the problem?

Categories of answers	Frequency	(%)
Knowing the problem based on the concept that once owned (Action); Relating the owned concept by the new concept to be understood; (Interiorization-Process); Testing owned new concept (coordination-reversal), Testing initial conclusion (encapsulation-de-encapsulation); and conclude a new concept (Object)	15	57.69
Knowing the problem based on the concept that once owned (Action); Relating the owned concept by the new concept to be understood; (Interiorization-Process); and conclude a new concept (Object)	5	19.23
Relating the owned concept by the new concept to be understood; (Interiorization-Process); Testing owned new concept (coordination-reversal); and conclude a new concept (Object)	3	11.54
Knowing the problem based on the concept that once owned (Action) and conclude a new concept (Object)	2	7.69
Conclude new concept (Object), without recognizing the problem, testing prior conclusion, and relating the owned concept by the new concept.	1	4.34

In question Q5, 20 prospective teachers said that they had taken the steps (Action – Process – Object), which are contained in the APOS theory. As presented in table 5, 15 of them (57.69%) consider that they knew the problem based on the concept that they owned (Action); They Related the owned concept by the new concept to be understood; (Interiorization-Process); they tested owned new concept (coordination-reversal), they tested initial conclusion (encapsulation-de-encapsulation); and then they concluded a new concept (Object). 5 of them (19.23%) have done all the steps that are part of APOS theory, except testing owned new concept (coordination-reversal).

### Conclusion

With this research we may conclude that Indonesian prospective mathematics teachers acknowledge the importance of PBL associated by APOS theory to understand the concepts of mathematics as well as to teach them. They recognized that the mental mechanisms and the mental structures described in APOS theory, they have felt themselves. With convinced about this, they can better prepare themselves before actually practicing their profession as a mathematics teacher.

Teaching mathematics using the PBL approach will make teachers more focused in knowing the mental mechanisms and the mental structures mentioned in APOS theory, and predicted is happening to students in the classroom. As a result, the math teacher would be able to make the right decisions in solving problems that occur in mathematical instruction in the classroom. The prospective teachers who have known this would have a very complete provision to make them professional mathematics teachers.

The lecturer who teaches mathematics for high school students' course to the prospective mathematics teacher using PBL approach associated by APOS theory, would make the lecturers have two main targets, namely: 1) the prospective teacher students are able to understand mathematical concepts; and 2) the prospective teacher students are able to teach math concepts to students with appropriate learning steps.

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## Developing Numeracy Skills by Using Numbers Lottery Game

\*Dinny Mardiana, Achmad Mudrikah, Nurjanah

<sup>1</sup>Mathematics Education, Nusantara Islamic University, Bandung 40286 Indonesia;

\*Corresponding Author: achmadmudrikah@yahoo.ac.id

### Abstract

This study aimed to describe: mathematics instruction by using numbers lottery game, numeracy skills of children after participating in instruction, and values that develop during the mathematics instruction. The method used is a qualitative research with the type of case study. Research subjects were the children of B1 class in one kindergarten in Bandung city, headmaster, and teachers who teach in the classroom. The data required are obtained through: observation, interviews, and document analysis. In order to obtain a conclusion, the data collected were processed using interactive analysis. The results showed: numbers lottery game happened naturally according to the abilities of each child, so the children excited to add and subtract numbers obtained through shaking; numeracy skills of children on six indicators measured were at the level of good or very good; and values that developed during the mathematics instruction were at the level began to develop or habituated.

**Key words:** Numeracy skills, Kindergarten, numbers lottery game, values.

### Introduction

Early childhood education is a pre-school education which introduces the climate and culture of the school, as a foundation to develop the potential of children optimally by using education, when they were aged 0 to 6. This is in accordance with *UU Pendidikan No 20 tahun 2003 bab 1 pasal 1 butir 14*, "Early childhood education is a development efforts aimed at children from birth to the age of six years are accomplished by providing stimulation of education to help the growth and development of the physical and spiritual so that children have the readiness to enter further education". Kindergarten is one means formal education which very important for a young child. Kindergarten has two characteristics: first its function as a preparatory event for the children to enter the school; second as a garden, it is expected to provide comfort and pleasure for children.

Early childhood characterized by curiosity and a desire to experiment. The high curiosity will be met if the kids receive stimulation, motivation, or directives in accordance with their development tasks. It's a loss if we do not take advantage of the high curiosity of children by not teaching them math concepts, whatever the reason. Vygotsky says "Children have their own preschool arithmetic, which only myopic psychologists could ignore" (in Sarama, 2009, p. 3). Of course, learning of mathematics for children kindergarten must be adapted to the principles of preschool education, which is learn while playing and play while learning. Fedriyenti (2012) conducted a class action research entitled "Capacity Building of Early Childhood Mathematics by Using Smart Clock Game in Taman Kanak-Kanak Pembina, Kecamatan Barangin Sawahlunto. The conclusion was by playing smart clock the mathematical abilities of children increased from cycle to cycle.

Meanwhile, according to *UU SISDIKNAS No. 20 tahun 2003*, "education is a conscious and deliberate effort to create an atmosphere of learning and the learning process so that learner is actively developing his potential to have the spiritual power of religion, self-control, personality, intelligence, character, and skills needed by him, society, nation, and state". Providing early childhood education is one way to establish the characters and their personalities for the foreseeable future. Thus, early childhood education should be organized in a holistic manner, not only emphasize the cognitive aspects, so that children could develop good values.

Based on the background that has been described, researchers are interested in doing research on the application of one of the games as mathematics instructional media in kindergarten, with the title "Developing Numeracy Skills by Using Numbers Lottery Game". The formulations of the problem in this research are: 1) How to use numbers lottery game as a learning tool to count? 2) How numeracy skills

of children after participating in the mathematics instruction by using numbers lottery game? 3) What develops values when children learning mathematics by using numbers lottery game?

### **Research Method**

The research approach is the qualitative research, "Qualitative research is characterized by its aims, the which relate to understanding some aspect of social life, and its methods which (in general) generate words, rather than numbers, as the data for analysis", Brikci and Green (2007, p. 2). The research method is case study, in accordance with the opinion of Neale, Thapa, and Boyce (2006, p. 3), "a case study is a story about something unique, special, or interesting stories can be about individuals, organizations, processes, programs, neighborhoods, institutions, and even events".

The case study is divided into the four stages, namely: 1) Defines and designed the study, 2) develop instruments and collect data, 3) Analyze the data and draw conclusions, and 4) prepare reports. The first stage is to define and design the research by reviewing the development of theories or concepts to determine the case and designing research procedures. Development of theory and concepts used to formulate research questions. The second stage researchers made preparation of instruments and validation, followed by data collection. Analyze the data and draw conclusions are the third stage. This stage is used to check the correctness, relevance or significance of the concept or theory that has been developed or underlying research. The final stage is writing, disseminating and publication the results. The report is addressed to the providers of funds and institutions where researchers serve.

### **Location and Subjects**

Location of the research is one of state kindergarten in Bandung. Research was conducted in the second semester of school year 2015-2016, precisely in April-May 2016. The subjects in this study were children in B1 class consisting of 8 girls and 9 boys. Besides the children, other research subjects are the headmaster and the teachers who teach in the class.

### **Data Collection and Analysis**

Data required is collected through observation, interviews, field notes and document analysis. Observation is done since children enter kindergarten until they go home, not only when children and teachers play numbers lottery game. Interviews were conducted to headmaster and teachers, to complete data in kindergarten learning, especially mathematics. Field notes is used to record the findings during the research, which is not documented in the observation sheets. The documents taken for the analysis are: syllabus, daily program plan, and students' workbooks. There are two types of observation results: about numeracy skills, and about values. Numeracy skills data were analyzed based on the criteria applied in kindergarten curriculum appropriate in 2004. Those data measured quantitatively by 1-4, and qualified as: less, enough, good, very good. Value that appears was analyzed based on the quantity and quality of the appearance; measured quantitatively by the percentage, then qualified to categories: not seen, emerging, began to develop, habituated. The data from interviews and documents analysis used to complete the discussion.

Overall, the analysis technique used is the technique of interactive analysis. The step consists of several components that related to each other: collection, reduction, presentation, and conclusion/verification of data. Data reduction is the process of selecting, determining focus, simplify, summarize and remodel the existing data in the field record. Once the data is reduced, the next step is the presentation of data analysis. Presentation of data directed to the data reduction results organized and systematically arranged. Furthermore, researchers compile the relevant data as a set of structured information, which gives the possibility of making conclusions and taking action.

### **Results and Discussion**

The instruction happened from 07.30 a.m. until 10.00 a.m. Children who came faster could play to take advantage of facilities such games: seesaw, swing bench, round bowls, or super slide. Separate mathematics learning is usually done in the area of mathematics. Nevertheless, before entering the area of mathematics teachers almost always teach various math concepts at the beginning of daily learning. Various activities provide an introduction to mathematical concepts are: 1) count the children who were present and who have not attended by using a finger; 2) by using a finger to compare the number of children who were present and who is not present; 3) sang together about numbers and counting; 4) compares the size between the classroom and schoolyard, etc.; 5) examine the objects that exist in the classroom and school environment, then mentioning their geometric shapes, 6) answer teacher's question about the addition or subtraction of numbers 1-20, who else could answered could start the game, or go out of the classroom to break or go home.



### **Numbers Lottery Game as a Learning Tool**

Numbers lottery game is quite simple, requiring only two pieces of glass covered with paper and contains rolls of paper with numbers. Before playing, the children were divided into groups consisting of four children, guided by a teacher. To illustrate the course of the game, the following will be presented the dialogue between teacher and children of the Apple group. The names are written here are fictitious names.

Teachers prepare two cups, each containing 10 rolls of colored paper that is labeled with the number 1, 2, 3, ..., 10. The teacher shuffles the first glass, then pulled out roll of paper from the glass, and put it in Tita's hand.

- Teacher : Open the scroll Tita! What's your number?  
*Tita unrolled, then said, 4 Mam!*
- Teacher : Correct. Write down the number you got on book your workbook, Tita!  
*The teacher shuffles the second glass, then pulled out roll of paper from the glass, and keeps it on Fikri's hand.*
- Teacher : Fikri open the paper roll! What's your number?  
*Fikri unrolled, then said, 8 Mam!*
- Teacher : Correct. Fikri, write the numbers you got on your workbook!  
*The game resumed after Fikri finished writing.*
- Teacher : Tita, who gets the bigger number: you or Fikri?  
Fikri : Me
- Teacher : Quiet Fikri, now Tita's turn to answered  
Tita : Fikri
- Teacher : Tita, how much  $8 + 4$  ?  
Tita : 11 Mam !
- Teacher : Try to count again dear! 8 in the mouth, four in hand  
Tita : 8, (then held up four left fingers, and she started counting: 9, 10, 11, 12 till all of her finger is calculated). Finally she answered 12 Mam!
- Teacher : (clapping her hand) Exactly, good Tita, 100 for you.  
*All of the children in the apple group clapped hands.*  
*The teacher reminded Tita and Fikri to write: " $8 + 4 = 12$ " in their workbooks.*

The learning process occurs naturally and fun. Next Dafa's and Rani's turn, the last two apple's group member remaining. The shaking process was until numbers writing in the workbook run fast. Moreover, Dafa and Rani can add 3 and 9 (numbers they got) correctly in a short time, without command. Dafa and Rani also agreed, that 9 is more than 3. This advantage is used by teacher to learn the subtraction operation by using numbers lottery game.

- Teacher : Dafa, can you count  $9-3$ ?  
Dafa : Yes, the result is 6 Mam!
- Teacher : Try to explain to your friends, use your fingers boy!  
*Dafa held up nine fingers, then he counted backwards: 8, 7, 6.*  
*Teachers give praise to Dafa, and rewarding applause for all children.*

Teachers give opportunities for replaying to several children who could not smoothly add and subtract. Of course, the difficulty level of the game set by the teacher, such that all the children can play with pleasure, and they can learn math according to their respective capabilities. Nothing to lose, because children who have high ability given the higher challenge than his friends who have the low ability. After providing a sort of reinforcement and reflection, numbers lottery game ended.

The game is repeated on the next meeting. Glass was filled with 20 rolls of paper with the numbers: 1, 2, 3, ... 20. Children learned how to sum and subtract numbers 1-20. Sometime teachers have to shuffle up to three times to help children for getting small number, so they could easily add or subtract that numbers. This does not apply for Nanda, the smartest boy in the class. Nanda has no problem to operate numbers 1-20. He already mastering to count numbers to 100, not only in addition and subtraction, but also multiplication.

It can be concluded, numbers lottery game in kindergarten was naturally done according to the abilities of each child. Everyone excited and enjoyed the learning process to add and subtract numbers 1-20. This situation is achieved because teachers implement clear rules, and determine the proper destination. This can be explained by the Oldfield opinion (Wiersum, 2012, p. 23), "mathematical games are

'activities' which: involve a challenge, usually against one or more opponents; are governed by a set of rules and have a clear underlying structure; normally have a distinct finishing point; have specific mathematical cognitive objectives".

### Numeracy Skills

Indicators of numeracy skills used in this study is that the child can: 1) pronounce numbers 1-20 sequentially, 2) make a sequence of numbers with objects 1-20, 3) pair numbers symbol with objects 1-20, 4) state the result of additions with objects 1-20, 5) state the result of subtraction with objects 1-20, 6) distinguishes two objects which are equal and not equal, more than and less than. Assessment is done refer to the criteria applied in kindergarten appropriate in curriculum 2004, namely: 4 = 76% - 100% = Very good, 3 = 51% - 75% = Good, 2 = 26% - 50% = Enough, 1 = 0% - 25% = Less. Numeracy skills of kindergarten children after participating mathematics instruction used Numbers lottery game as media will be presented in Table 1.

**Table 1.** Numeracy skills of kindergarten children after participating Mathematics instruction by using Numbers Lottery Game

No	Assessed Aspect	Result				Note
		1	2	3	4	
1	pronounce numbers 1-20 sequentially				v	Very Good
2	make a sequence of numbers with objects 1-20				v	Very Good
3	pair numbers symbol with objects 1-20			v		Good
4	state the result of additions with objects 1-20			v		Good
5	state the result of subtraction with objects 1-20			v		Good
6	distinguishes two objects which are equal and not equal, more than and less than				v	Very Good

Of the six indicators measured, three indicators at the level of Good, and three indicators at the level of Very Good. This achievement is higher than the performance demanded by the curriculum 2004, namely: "count/mention the number order 1-20, count (recognize) concept of numbers by objects up to 10, making the sequence of numbers 1-10 with objects, connecting/pair numbers symbol with objects up to 10 (children are not told to write), differentiate and create a collection of objects which: the same amount, not the same amount, more than, and less than; state the results of addition and subtraction with objects up to 10, estimate next order after seeing the shape of more than three sequential patterns, For example: red, white, and blue; imitate the patterns using a variety of objects"

### Values Growing During The Instruction

Character education has a role and functions that are strategic for the whole nation and state of Indonesia. According to Darmu'in (2013, p. 46), "content of character values in kindergarten curriculum accordance with the Permendiknas number 58 of 2009 contained in all aspects of development, both in terms of habituation and in aspects of basic capabilities, but textually the values of these characters more listed in the habituation aspect, namely the development of habituation is an activity performed continuously in daily life, so that students become accustomed to doing good behavior".

This study did not specify any values that will be developed. During the study, it seems that many good values were emerging and develop naturally. Furthermore, based on the quantity and quality of the appearance, these values were scored using the criteria by Darmu'in (2013, p. 62). There are four criteria, namely: "BT= *Belum Terlihat* (Not Seen), if students do not show early signs of behavior that is expressed in the indicator; MT= *Mulai Terlihat* (emerging), if students have started showing early signs of behavior that is expressed in the indicators but have not been consistent; MB = *Mulai Berkembang* (began to develop), if students already exhibit a variety of behaviors expressed in indicators and began to consistently); and MK=*Memiliki Karakter/Membudaya* (Having Character/habituated), when children constantly exhibits behavior indicators expressed in a consistent manner. The values that developed during mathematic learning are presented in table 2 below.

**Table 2.** The values that developed during the instruction

No.	Values developed	Score	Note
1.	Obediently follow the rules	MK	Have Character/habituated
2.	Critical	MB	Began to develop
3.	Autonomy	MB	Began to develop
4.	Confidence	MB	Began to develop
5.	Care	MK	Have Character/habituated
6.	Patient	MK	Have Character/habituated
7.	Respect for others	MK	Have Character/habituated
8.	Concentration	MB	Began to develop

During follow mathematics instruction by using lottery numbers game, there are eight values observed emerging and developing in children's. All eight values are: Obedient to follow the rules, critical, independent, confident, caring, patience, respect for others, and concentration. Four of the eight values got the highest score, which is habituated; while four others got began to develop score. It can be concluded, learning mathematics by using numbers lottery game can help foster good values in children's kindergarten.

This is in accordance with the opinion of Peng and Nyroos about mathematics instruction and Davies opinions about the advantages of using the game. Values in effective mathematics lessons from students' perspective are: "personalized help, explanation, quietness, collaboration, sharing, strictness, concentration; explanation, independence, relaxation, quietness, fun" (Peng & Nyroos, 2012, p. 418). Meanwhile, educational benefits of using games in instruction according to Davies are: "provide a meaningful learning situations, support students to build a positive attitude such as providing opportunities for students, motivate students to learn, build a self-concept and developing positive attitudes towards mathematics, increase learning by adding more formal activities, create more interaction between students, give students opportunities to self-assessments, and improve students problem solving skills. It consider as "interactive learning tasks for both school and home and allow students to operate at different levels, and make students can work independently" (Al-Mashaqbeh & Al Dweri, 2014, p. 135).

### Conclusions

Based on the research findings and the results of data analysis, the researchers concluded: 1) Numbers lottery game happened naturally, according to the abilities of each child, so the children excited to add and subtract numbers obtained through shaking; 2) Numeracy skills of children on six indicators measured were at the level of good or excellent; and 3) Values that developed during the instruction were at the level began to develop or habituated.

### Acknowledgement

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## **Students' Character Development and Lecturer's Teaching Profile in Introduction to Elementary Mathematics Class Using Logical Mathematics Materials That Based on Character Education**

Nurjanah, Usep Kosasih, \*Dinny Mardiana

Department of Mathematics Education, Faculty of Teacher Training and Educational Sciences  
Nusantara Islamic University, Bandung 40286, Indonesia;

\*Corresponding Author: [dinnyalaudin@gmail.com](mailto:dinnyalaudin@gmail.com)

### **Abstract**

This study is following up previous one about the development of Introduction to Elementary Mathematics course materials using character education approach; which had resulted in a material about mathematical logic using character education approach which is valid, practical, and effective. The subjects are freshmen of the Department of Mathematics Education, Faculty of Teacher Training and Educational Sciences, Nusantara Islamic University, Bandung, Indonesia. This study employed qualitative action research design with practical action research type. The action research was carried out in five classroom sessions where every session was divided into four practical action research steps as follows: identifying focus area, collecting the data, analyzing and interpreting data, and developing an action plan. All classroom sessions are carried out by referring to the main materials that were previously developed; and they all implemented contextual approach based on character education stages: moral knowing, moral feeling, and moral actions. This study used the following instruments to gather the data: observation over students and instructors' activities, questionnaires, and interview guidelines. This study has successfully found that both students' character development and instructor's teaching profiles dynamically improve when the instruction session implement materials that integrated character components into mathematical logic materials.

**Key words:** Mathematical logic, character, teaching profile, practical action research.

### **Introduction**

Adversities that present in Indonesian community are basically direct results of poverty and ignorance which later lead to high rate of unemployment. In addition, other indicators such as crime rates, drug abuse, terrorism, mobbed rallies, and corruption are also increasing. Things get worse when those misconducts are not only conducted by low class and uneducated people, but also by those who have more proper education and possess public official status. Meanwhile, adolescents, the generation to whom the future of this nation heavily relies upon, are not only conducting misdemeanor behavior, but also felonies that cause many loss of life. In short, moral conditions of this nation are in lowest level nowadays.

Those poor conditions have drawn attention of government which then took initiatives to prioritize character development in the development plan of the nation. As the consequence, every step taken to develop the nation must bear efforts to give positive impacts on characters. As an institution considered being the most responsible to the issues of character, the Ministry of National Education has urged all schooling levels to implement and integrate character education in their programs. The character education includes a focus on intellectual curiosity and polite behaviors that are implemented through habituation and intervention. Thus, it is expected that the program could result in a school culture that nurture the development of good characters.

There are various notions of character, one of them according to the Government of the Republic of Indonesia, "Characters are special values (knowing the value of kindness, willing to take good actions, realizing it in real life, and giving impacts to surrounding environment) that are planted and exhibited through actions" (Pemerintah Republik Indonesia, 2010, p. 7). Meanwhile, with respect to character education, Public Schools of North Carolina (2006, p. 2) said that "Character education is a national movement creating schools that foster ethical, responsible, and caring young people by modeling and teaching good character through emphasis on universal values that we all share."

In relation to the development of nation's characters and cultures, the Ministry of Education, Kementrian Pendidikan Nasional (2010, p. 6) claims that "the process of values development have become the foundation of characters and it requires sustainable actions, integrated to all subjects (Citizenships, History, Geography, Economics, Sociology, Anthropology, Indonesian language, Social Studies, Science, Mathematics, Religion Classes, Physical Education, Arts, and Sports)." Clearly, character development is not merely a responsibility of Citizenship and Religion teachers; rather, it needs to be developed across all subjects.

The aforementioned conditions have triggered the researchers to develop learning materials that integrate character education into mathematics. In a research and development process in 2012-2013, a study has successfully developed a mathematical logic learning materials that integrate character education. The materials will be used in the first semester to the freshmen. This study expects that the students will have awareness of noble characters since the first day of study; and they will keep up the good character and develop it further with other noble characters through studying mathematics.

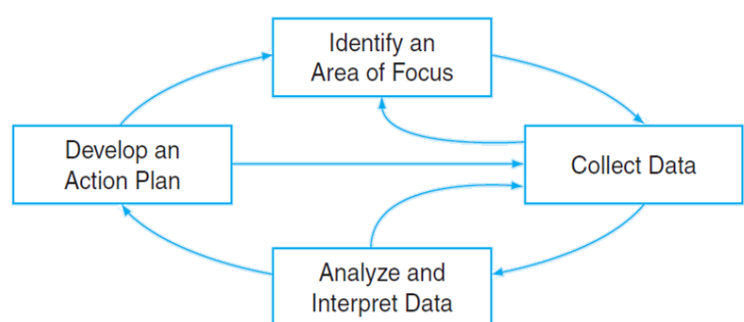
The learning materials have been tested for their validity, practicality, and effectiveness. Mathematical logic materials based on character education approach have explicitly integrated character of honest, discipline, and hard work. In general, the integration of character education in mathematical logic learning materials include the character of honest and discipline that integrated in: statements and their truth, operations on statements, and also in argument and deductive method topics. Meanwhile, the character of hard work was integrated in the argument and deductive method topic.

In 2015, the researchers continued the study by doing followed up research intended to answers the following questions: 1) How were the characters of students who learned Introduction to Elementary Mathematics using mathematical logic materials? 2) What profile of a lecturer teaching that could develop good character in students?

## Research Method

Answers to the questions will be described in more qualitative ways. The objective of qualitative research is "to describe and possibly explain events and experiences, but never to predict" (Willig, 2008, p. 9). The qualitative research method being used was referred to action research design. According to Mills (Creswell, 2012, p. 577), "action research designs are systematic procedures done by teachers (or other individuals in an educational setting) to gather information about, and subsequently improve, the ways their particular educational setting operates, their teaching, and their student learning". More specifically, the type of action research used is practical action research which according to Creswell, (2012, p. 579), "is a research when teachers seek to research problems in their own classrooms so that they can improve their students' learning and their own professional performance. Teams composed of teachers, students, counselors, and administrators engage in action research to address common issues such as escalating violence in schools. In these situations, educators seek to enhance the practice of education through the systematic study of a local problem."

In general, there were three phases in this study: preparation, implementation, and report writing. Preparation phase started before the formal class session began; implementation phases was conducted in five stages/lectures, and the last phase was report writing. The implementation stage refers to the 4-step practical action research, as seen in the picture below



**Figure 1.** Picture of Practical Action Research (Mills, in Cresswel, 2012, p. 581)

Identification of focus areas was conducted in the planning stage where the objectives of the research were being formulated. Data collection was then collected in every session by using observation sheets equipped with **video recording** and questionnaires in the end of the fifth session. In the end of every session, lecturer and observer made discussions intended to analyze and interpret the data. Reflections

over the analysis results led to another step of action and identifying a new focus area. The four steps were taken respectively until the fifth session, and the study was concluded by composing the report.

### **Instruments and Data Analysis**

The data was collected through questionnaires, observation sheets, and interviews. These varied method of data collections intended to get more detail and accurate information about the topic being investigated. Questionnaires and observation sheets for students were developed to capture the character the students developed when studying Introduction to Elementary Mathematics. Meanwhile, observation sheets for lecturer's activities were intended to capture the profile of teaching of the lecturer. In the observation sheets, there was a blank space intended to put anecdotal record that captures the behaviors that indicate students' or lecturer's characters. As a complement, interviews were held to get more accurate data.

Based on the results of questionnaires and observation, this study analyzed the students' character; and conclusion was drew by referring to concepts identified Supinah and Parni (2011) as follows: Not Seen, meaning that the students have not yet indicated early signs of characters indicators described; Began to being Seen, meaning that the students have begun to exhibit early signs identified by indicators; Started to Develop means that the students have exhibited behaviors in accordance to indicators; Developed means that the students have frequently exhibited the behaviors that are consistent with the indicators; and Habituated or become norms means that the students have continuously exhibited the behaviors that consistent with the indicators.

The analysis on the lecturer's profile was conducted by referring to the steps taken in Contextual Teaching and Learning and included three steps of character education identified by Lickona (1991), they were respectively: moral knowing, moral feeling, and moral action. The lecturer was considered to complete one step of learning activity when he has reached at least 75% of all indicators that has been previously determined.

### **Setting and Subjects**

This study took place in the Faculty of Teacher Training and Educational Sciences, Nusantara Islamic University, Bandung, Indonesia. The subjects were freshmen in the Department of Mathematics Education in Academic Year 2015-2016.

### **Finding and Discussion**

In classroom, there are two lecturers: one acts as instructor and the one as observer. After the class is over, both lecturers held a post-observation discussion to criticize about the class session and identify both students' character and lecturer's profile of teaching. In the sections to follow this paper will provide more thorough discussion about the findings by referring them to the previously formulized problems.

### **Students' Character**

The following table presents the overall analysis results of the observation sheets over students' characters in every session and it has been cross-analyzed with the data taken form questionnaires and interviews.

<b>Table 1.</b> Students' character in every class session			
	<b>Honest</b>	<b>Discipline</b>	<b>Hard Work</b>
Session 1	Not seen	Not seen	Not seen
Session 2	Started to develop	Seen	Not seen
Session 3	Started to develop	Started to develop	Started to develop
Session 4	Developed	Developed	Started to develop
Session 5	Become norms	Become norms	Become norms

In general, the three characters integrated in mathematical logic material have been improved in students from one session to others. This is due to the fact this study situated character education based on Lickona's (1991) principle as follows: moral knowing, moral feeling, and moral action respectively. This principle identifies that the honest character developed in the first session keep developed in the next sessions until it reaches the highest level as become norms or habituated in the fifth session.

The character of honest has been integrated in statements and their truth topic was introduced in the first session. By then, the students have already in the stage of moral knowing condition. In the second session, students were introduced to enlightening story about the greatest Prophet Muhammad PBUH, a great example of honesty and one who possess the noblest character of all. Students are also accustomed to read and appreciate the meaning of Asmaul *Husna* before the session began. These efforts had brought the students to the stage of moral feeling. In the following sessions, students were conditioned to act honest when doing assignments and tests. Here, the students have arrived to the stage of moral action. The same approach was also implemented to the character of discipline and hard work. The more immediate one character being integrated, the bigger chance the students will have to possess the character in higher level.

In addition to the three characters that were explicitly integrated into mathematical logic material, the study also found another three characters: curiosity, self-confidence, and respect to others. The students developed the three characters in themselves. This can be explained by the opinion of Sam and Ernest (1997) which describe that the values about mathematics education include accuracy, systematicness, rationalism, accuracy, being analytical, rationalism; compassion, integrity, moderation and gratitude; curiosity, thriftiness, patience, trust and creativity. Although the findings of this study do not exactly confirm to Sam and Ernest explanation about character education, they consistent with Dede's (2006, p. 86) claim that "teaching mathematics educational values may show difference results according to countries, cities, school types, and grades."

### Lecturer's Teaching Profile

The following table presents observation results over activities conducted by lecturer in every session:

<b>Table 2.</b> Observation results over activities conducted by lecturer in every session					
	<b>S1</b>	<b>S2</b>	<b>S3</b>	<b>S4</b>	<b>S5</b>
Teaching using contextual learning scenarios and based on character education principles	Very good	Very good	Very good	Good	Good
Become the model of character education	Good	Very good	Very good	Very good	Very good

*Note: S means session*

Of the five sessions, only in two sessions did the lecturer marked as "Good" for his teaching activities, and one time for character education modeling. The lecturer was marked as "Very Good" for his teaching activities in three sessions, and in four sessions for character education modeling. This achievement was gained due the well-prepared instruction scenarios that included teaching materials and modeling. Another thing that supported this successful implementation of character education was that the university provides supportive environments that have helped in shaping students' noble characters as it has become the major ideas of the establishment of the university. Another important finding was the way how the lecturer used impromptu moments to integrate character values into his teaching.

The findings of this study are consistent with Sabandar's (2011, p. 11) explanation that "it is expected that mathematics characteristics will also have impacts in forming good characters of the learners, especially when the teachers as well as the learners are ready to notice a right momentum in modeling and in forming the intended good characters. At the same time those characters identified in the process of learning should be well communicated by the teachers to the learners continually."

### Conclusion

The study drew conclusions that Introduction to Elementary Mathematics delivered using contextual basis and referred to character education integration into mathematical logic could improve students' characters in the following ways:

1. Students' characters develop through the following paths: honesty and discipline develop into become norms or habituated level and hard work develops in the level start to develop. In addition to the three characters intentionally developed, this study also found other characters developed, they were: curiosity, self-confidence, and respect to others.
2. Lecturer's teaching profiles that could improve students' character were teaching using contextual approach in which she implemented impromptu moments to integrate character education into her teaching. Specifically, the use of learning materials to develop character followed the following scenarios: moral knowing (knowing the value cognitively), moral feeling (understanding the value affectively), and moral action (implementing the value in real action). Moral knowing was achieved



by integrating character education into certain chapters in the materials. Moral feeling was integrated through reflection on every step taken to implement moral knowing that was strengthened by the story of the noblest human being ever, Prophet Muhammad PBUH, and the reading of *Asmaul Husna* before the session began. Meanwhile, moral feelings were achieved through providing models or examples of character by the lecturer and consistently adhere to the rules that governed the teaching and learning sessions.

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## The Principles of Law to Resolve Disharmony of Regulations in the Calculation of Financial Loss to the State

Ronald Hasudungan Sianturi, Rizkan Zulyadi, Rahmayanti

Department of Law, Faculty of Law, Prima Indonesia University, Medan, North Sumatera  
20113, Indonesia;

\*Corresponding Author: hasudungan\_r@yahoo.com

### Abstract

Upholding the rule of law remains a serious challenge in Indonesia, that law enforcement should be carried out based on the principle of legality, included in the calculation of financial loss to the state in law enforcement against corruption. Certainty calculation of losses to the state is difficult to be achieved because of disharmony some regulations in the definition, the process and subjectivity in determining the method of calculation of losses to the state. Conflicts of some regulations makes law enforcement became ineffective, inefficient and inconsistent. This research aims to investigate the principles of law in the calculation of financial loss to the state to overcome conflicting regulations. This research is juridical normative. The data used are related regulations and court verdicts. This research use statue approach and conceptual approach. This research found that disharmony of regulations in the calculation of financial loss to the state can be solved with *lex superiori derogat legi inferiori* and *lex specialis derogat legi generali*.

**Key words:** Disharmony of regulations, financial loss to the state, calculation, corruption.

### Introduction

Corruption is an extraordinary crime because it inhibits the destination country to prosper citizens (Prasetyo, 2012). Corruption resulted in losses to the state so that the state loses the ability of the economy to prosper citizens (Agbiboa, 2012). As a state law, criminal law enforcement in Indonesia has the primary objective to restore the economic capacity of the country in its people prosper by prioritizing the return losses to the state through criminal restitution (Setiabudhi & Artha, 2014). Corruption compensation should be determined based on the principle of legality, which must be in accordance with the amount of state financial loss calculated by competent authorities, the appropriate procedure legislation with due regard to the substance of the right (Hadjon, Djatmiati, Addink & Berge, 2011).

In practice, the calculation of losses to the state are controversial because of disharmony stakeholder perspective on calculation of financial loss to the state such as: (i) the scope of the state finances; (ii) that institution calculating the financial losses of State authority; (iii) the existence of the state financial losses in post indemnification of state financial; (iv) the financial loss calculation method different countries.

Disharmony of regulations concerning the calculation of financial loss resulting state of legal uncertainty in the eradication of corruption that need to be harmonized with the principles of law in Indonesia. Harmony legislation is one of the requirements of the legal system in the eradication of corruption effectively, efficiently and consistently.

### Research Method

#### Procedure

This research type is juridical normative by using secondary data consist of (i) the primary legal materials in the form of legislation (ii) secondary legal materials from previous studies and books. The data analysis approach to legislation and approach to the concept.

### Results and Discussion

#### **Disharmony of Regulations in the Calculation of Financial Loss to the State**

Disharmony of regulations in the calculation of financial loss to the state consists of (i) the scope of state finances; (ii) that institution calculating the financial losses of State authority; (iii) the existence

of the state financial losses in post indemnification of state financial; (iv) the financial loss calculation method different countries as follows:

#### *The Scope of State Finances*

The scope of state finances became controversial because of the difference in status of state assets set aside. Law No. 17 of 2003 on *Keuangan Negara* stated that state assets set aside a part of state finances, while Law No. 40 of 2007 on *Perseroan Terbatas* stated that the status of state assets have been separated to the limited liability company is not a part of state finances, but belonged to a limited liability company as a legal entity. Some cases become controversial because of the difference in perspective on the status of state financial losses, among credit lending from PT. Bank Mandiri to PT Cipta Graha Nusantara case (2005), the bioremediation Chevron case (2012), the credit lending from BNI 46 to PT Bahari Dwi Kencana Lestari case (2013).

#### *Institutions that Authorities Calculate the Loss of State Finances*

Indonesian regulations do not declare explicitly the institution having authority to calculate the loss of state finances. The court verdicts by using the calculation of financial loss to the state by the agency that has the authority of financial control, among others Controlling System of Government Internal (BPKP, Inspectorate General, Inspectorate Provincial Inspectorate District / City), the Supreme Audit Agency (BPK) and Law Enforcement (Police, The Prosecutor and Corruption Eradication Commission). Problems agencies that have authority in the calculation of financial loss to the state if there are differences in the calculation of financial loss to the state by each of these institutions.

#### *The Existence of State Financial Losses after the Return of the Country's Financial Losses*

The existence of the state financial losses in the country's financial losses after the return of a controversy because of a conflict between the two laws. Law No. 15 of 2006 on Supreme Audit Agency which states that compensation losses to the state removing elements while Law No. 31 of 1999 on Corruption Eradication stated that the payment of compensation does not remove corruption.

#### *The Different Method of Calculating the Financial Losses to the State*

The calculation of losses to the state can be done through five methods of calculating losses to the state, namely: (1) Total loss with some adjustments, (2) The difference between the contract price with the cost of purchase or cost of production, (3) Difference between the contract price with the price or value of a specific benchmark, (4) Acceptance that the rights of the country but not remitted to the state treasury, (5) expenses that are not in accordance with the budget, used for personal interests or certain parties (Tuanakotta, 2009). Selection of an impact on the calculation method of the difference in losses to the state, but until now there is no provision regulating the parameters / requirements for using a particular method so heavily influenced by the element of subjectivity from officers who calculate financial losses of the state.

#### *The Principles of Law in Indonesia*

The principle of regulation stipulated in Law No. 12 Year 2011 on the Establishment of legislation that is *lex superiori derogat legi inferiori*, the principle of *lex specialis* and *lex posterior derogat legi principle priori*. The principle of *lex superiori derogat legi inferiori* reflected in Law No. 12 of 2011 on the Establishment of legislation that declared unenforceable rules and regulations in accordance hierarchy consists of (1) Indonesia Constitution, (2) the MPR (3) law / government decree (4) government regulations (5) presidential decree (6) provincial regulation and (7) district Regulation. The principle of *lex specialis derogat legi generali* states that are specific regulations overriding the rules of a general regulations. This principle is reflected in Article 63 Point 2 of the Code of Law Criminal Law which states "If a deed entered in a rule of criminal common, regulated in the rules of criminal special, then just the special ones that are applied." This principle can only be used if both rules are in the same position on legislation hierarchy. The principle of *lex posterior derogat legi priori* states that the new regulations put aside the old rules equivalent. The principle of *lex posterior derogat legi priori* can be seen from the legislation, which generally states that are still applicable legislation is not contrary to the old regulation (Sian, 2015).

If there are differences methods in calculation of loss to the state carried out by the authority by legislation, then the determination of the competent institutions calculate the financial loss to the state should be based on the principles of the legislation, namely the principle of *lex superiori derogat legi inferiori*, principle of *lex specialis derogat legi generali* and principle of *lex posterior derogat legi priori*.

### **The Principles of Law to Resolve Disharmony of Regulations in the Calculation of Financial Loss to the State**

The principle of *lex superiori derogat legi inferiori*, *lex specialis derogat legi generali* and *lex posterior derogat legi priori* in the calculation of financial loss to the state are:

#### *The Scope of the Country's Financial Losses*

Legal principle in determining the scope of state loss is *lex superiori derogat legi inferiori* which are limited liability finance financial legal entities as stipulated in Law No. 40 of 2007 on Perseroan Terbatas throughout the financial wealth is not included in the separated state as stipulated in Law No. 17 of 2003 on *Keuangan Negara*.

#### *Institutions that Authorities Calculate the Loss of State Finances*

The legal basis of the authority of institutions to calculate the financial loss to the state is (a) Government Internal Control Systems (BPKP, Inspectorate General, Provincial Inspectorate, Inspectorate District / City) based on Government Regulation No. 60 of 2008 on *Sistem Pengendali Intern Pemerintah*; (b) Supreme Audit Agency (BPK) based on Law No. 15 of 2006 on *Badan Pemeriksa Keuangan*; (c) The Law Enforcement based on Law No. 2 of 2012 on *Kepolisian Negara Republik Indonesia*, The Attorney based on Law No. 16 of 2004 on *Kejaksaan Republik Indonesia* and The Corruption Eradication Commission based on Law No. 30 of 2002 on *Komisi Pemberantasan Tindak Pidana Korupsi*. Legal principle in determining the authority calculating the financial losses the state is *lex superiori derogat legi inferiori* hereinafter through *lex specialis derogat legi generali*. The calculation of losses to the state carried out by the Commission which has specialized in combating corruption. Prosecutors and police can do all the calculations are not executed by the authority of the Commission. The Supreme Audit Agency can perform calculations in connection with the state financial losses that are administrative violations, while the BPKP, Inspectorate General, Provincial Inspectorate, Inspectorate District/Municipal authorities can perform all calculations are not executed by the Supreme Audit Agency.

#### *The Existence of the State Financial Losses after the Compensation Payment*

Legal principle in determining the existence of state loss in post indemnification of state finances is *lex specialis derogat legi generali*. The payment of state financial losses originated from corruption case specific provisions of an administrative violation. Indemnification of the State finances caused no corruption does not remove a criminal offense as stipulated in Law No. 31 of 1999 on *Pemberantasan Tindak Pidana Korupsi*. Returns the state financial losses caused by administrative violation resulted in the abolishment of the State financial losses as stipulated in Law No. 15 of 2006 on Audit Board of the Republic of Indonesia.

#### *The Method of Calculating the Financial Losses of Different Countries*

Legal principle in determining the method of calculation of financial loss to the state is a *lex specialis derogat legi generali*. Losses to the state of expenditure on procurement of goods / services can be accomplished by (1) total loss with some adjustments, (2) The difference between the contract price with the cost of purchase or cost of production, (3) The difference between the contract price the price or value of a specific benchmark by taking into account the value of benefits from the procurement of goods / services. Losses to the state from spending apart from the procurement of goods / services using the expenditure method that does not comply with the budget. Financial losses from reception using the method acceptance to the Government but not remitted to the state treasury.

### **Conclusions**

The principles of law to resolve disharmony of regulations in the calculation of financial loss to the state consists of *lex superiori derogat legi inferiori* hereinafter and *lex specialis derogat legi generali*; the principles of law in determining the scope of state loss is *lex specialis derogat legi generali*; the principles of law in determining the authority calculating the financial losses the state is *lex superiori derogat legi inferiori* hereinafter through *lex specialis derogat legi generali*; the principles of law in determining the existence of state loss in post indemnification of state finances is *lex specialis derogat legi generali*; the principles of law in determining the method of calculation of financial loss to the state is a *lex specialis derogat legi generali*.

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## **Legal Instruments for the Protection of Migrant Workers by ASEAN and Indonesia National Law**

\*Jelly Leviza, Ningrum Natasya Sirait, T. Keizerina Devi

Faculty of Law, University of Sumatera Utara, Medan 20155, Indonesia;

\*Corresponding Author: levizajelly@gmail.com

### **Abstract**

The existence of ASEAN as an international organization with legal personality was referring to the birth of the ASEAN Charter, 2007. The Charter became effective on December 15, 2008 that Indonesia has ratified by Law No. 38 Year 2008. The Charter emphasizes the commitment of regional cooperation within the framework of the ASEAN Community based on three pillars: political security, economic and socio-cultural. The three pillars are interrelated for the purpose of peace, stability and prosperity in the ASEAN. One area that was agreed by ASEAN is legal protection for migrant workers. Legal protection for migrant workers is important because of some cases of abuse and torture by the employer, as in the case of Siti Hajar 2009. For the Indonesian legal protection for migrant workers is urgent because the number of migrants is increasing every year and it is the Government's obligation to protect its citizens wherever existence. ASEAN, in the regional level already has legal instruments for the protection of migrant workers namely the ASEAN Declaration of the Protection and the Promotion of the Rights of Migrant Workers. While at the national level, Indonesia has a number of legal instruments related, that is Law No. 13 Year 2003 and Law No. 39 Year 2004 on the Placement and Protection of Indonesian Workers Abroad. Based on the above, this paper will examine first, the protection of the rights of migrant workers within the framework of the ASEAN agreement and the second will examine the comparison between the protections of the rights of migrant workers in ASEAN level with Indonesia national law. The goal is to determine the harmonization and synchronization of the legal protection of migrant workers in ASEAN perspective and the perspective of Indonesian national law.

**Key words:** Legal instrument, protection, migrant workers, Indonesia.

### **Introduction**

ASEAN Charter reflects the commitment of its member to make regional cooperation more based on law within the framework. This cooperation was based on the ASEAN Community by 2015 that which consists of three pillars: political and security pillar, economic pillar and socio-cultural pillars. For Indonesia, the ASEAN Charter was ratified by Law No. 38 Year 2008 on the Ratification of the Charter of Association of Southeast Asian Nations. (Secretariat Director General of Cooperation for ASEAN, 2012). One of the important agreements in ASEAN is the protection for migrant workers contained in the ASEAN Declaration of the Protection and the Promotion of the Rights of Migrant Workers. The issue of protection of migrant workers is important for Indonesia because Indonesia has the largest migrant workers in ASEAN. The large number of migrant workers of Indonesia has raises a lot of problems as well, such as wrong treatment and abusive employers, for example in the case of Siti Hajar that occurred in 2009, the threat of the death penalty for migrant workers in 2015 amounted to as many as 281 persons, 59 of them have been executed. Therefore normative studies are needed to determine the legal protection of national and regional laws of ASEAN for migrant workers.

### **Research Method**

This article uses the method of normative law to examine norms that applicable contained in national legislation and in international agreements. Normative Study conducted from the legal protection of migrant workers in the national and regional level and then looks at the legal implications of both.

## **Results and Discussion**

### ***The Protection of Migrant Workers' Rights in ASEAN Framework***

The issue of migrant workers is different from the issue of skilled workers within the framework of the ASEAN Community by 2015. Migrant workers were include in two pillars, the pillars of the ASEAN Political Security Community (APSC) and the pillars of the ASEAN Socio-Cultural Community (ASCC), while skilled workers were on the pillars of the ASEAN Economic Community (AEC).

In the context of the political pillars of security, protection of the rights of migrant workers, among others, posted on the ASEAN Human Rights Declaration. The general principle of the ASEAN Human Rights Declaration states that: "The rights of women, children, the elderly, persons with disabilities, migrant workers and vulnerable and marginalized groups are part of human rights and fundamental freedoms are inherent, integrated and inseparable". On October 9, 2013 adopted the ASEAN Declaration on Strengthening Social Protection which contains the principle that all people, especially the poor, are at risk, the disabled, the elderly, drop out of school age, children, migrants and other vulnerable groups, are entitled to have equitable access to social protection where it is a basic human right (Taufik, 2014).

In the context of socio-cultural pillar, the protection of migrant workers are in the ASEAN Socio-Cultural Community (ASCC) Blueprint. Furthermore, the protection and promotion of migrant workers are described in the core element included in Item C2 on "Protection and Promotion of the Rights of Migrant Workers". Point 28 of Item C2 above stressed that the protection of the rights of migrant workers must be based on two things: first in accordance with the laws, regulations and national policies of ASEAN member countries, both in accordance with the Declaration on the Protection and Promotion of the Rights of migrant workers (ASEAN Declaration on the protection and Promotion of the Rights of migrant workers) in 2007. the 2007 Declaration is the basis of the ASEAN agreement for the protection of migrant workers.

ASEAN Declaration on the Protection and Promotion of the Rights of Migrant Workers in 2007 contains a number of rights for migrant workers such as:

1. The fundamental rights and dignity of migrant workers and their families without violating the laws, regulations and policies in the recipient country.
2. Welfare right.
3. The right to access information, training and education, access to justice, social security services that are carried out in accordance with national law, bilateral and multilateral agreements.
4. The right for wages payment and sufficient access to jobs and decent living conditions
5. Migrant workers who are victims of discrimination, abuse, exploitation, violation, have adequate access to the legal system and the courts of the receiving country.
6. The right to obtain legal assistance when migrant workers were arrested or jailed by using diplomatic channels between the sending and receiving countries.

Given the above declaration is a form of international treaties that contain an agreement between the member states, it can be seen the obligation of the host country and the obligations of the sending country. Rights for migrant workers are: access opportunities to have jobs and livelihoods; the right to be facilitated from recruitment, preparation for deployment overseas, and the protection of migrant workers when abroad and repatriation and reintegration into their countries of origin;

ASEAN Foreign Ministers in 2007 has established the ASEAN Committee on Migrant Workers (ACMW) which has the duty to carry out the above-mentioned Declaration. ACMW dividing the work into four: improving the protection and promotion of the rights of migrant workers to exploitation and ill-treatment, strengthen the protection and promotion of the rights of migrant workers by improving government work migration in ASEAN countries, regional cooperation to combat trafficking in ASEAN, develop "ASEAN Instrument" on the Protection and Promotion of the Rights of Migrant Workers. ACMW make a report on the Senior Labor Officials Meeting (SLOM) (<http://humanrightsinasean.info/asean-committee-migrant-workers/about.html>).

Some legal issues related to the implementation of the 2007 Declaration of the above are:

1. The political declaration by the heads of state or government will require operational mechanism, therefore the next ASEAN leaders mandated the ASEAN Instrument as a follow up to the Declaration, but unfortunately there are different interpretations of what is meant by "instruments" outlined in the Declaration Cebu 2007. Indonesia and some ASEAN countries that send their employees (Indonesia, the Philippines, Cambodia, Laos, Vietnam and Myanmar) interprets the "instrument" above as an agreement that is legally binding, but instead for some other ASEAN countries that are state receiver (Malaysia, Singapore, Brunei Darussalam and Thailand), "instrument" is nothing more

than just some guidelines that are not legally binding. (<http://www.thejakartapost.com/news/2012/05/03/an-asean-way-protecting-indonesian-migrant-workers.html>)

2. ASEAN countries that send their employees (sending states) insisted that the scope of application of the instrument should include undocumented migrant worker (migrant workers are undocumented) in view of the migrant workers are human and have human rights so that all ASEAN states are obliged to respect the human rights of the as stipulated in the ASEAN Human Rights Declaration. Contrary to the views above, receiving states (recipient countries) argued that the issue of human rights for undocumented migrant workers was more to the legal issues in the national level. (<http://thediplomat.com/2015/01/the-need-for-an-asean-treaty-on-migrant-workers/>).
3. Sending countries argued that "instruments" should include family members of migrant workers, while the host country refused to approve the scope of family members.
4. The sending State found ASEAN regional instruments should include the issue of human rights as contained in the International Convention on the Protection of the Rights of All Migrant Workers and Members of Their Families as well as the ASEAN Human Rights Declarations, otherwise the group receiving countries tend to narrow the scope of the human rights of migrant workers far from what is stipulated in the above two international instruments.

### ***Protection of the Rights of Migrant Workers According to the Indonesia National Legislation***

The rights of migrant workers in an atmosphere of national law can be seen in a variety of laws and regulations in force, from the Constitution (UUD 1945) and the various legislations, as can be seen below.

1. The 1945 Constitution of the Republic of Indonesia  
Article 28 regulates the rights to earn a living, the right to health, right to information and the right to social security. Article 27 paragraph 2 also ensures that everyone has the right to obtain decent and humane work.
2. Law No. 39 Year 2004 regarding the Placement and Protection of Indonesian Workers Abroad, has establishes a number of rights for migrant workers: the right to work abroad; the right to obtain correct information about the job market overseas and procedures placement of migrant workers abroad; the right to receive services and equal treatment in the placement abroad; the right to obtain the freedom of religious belief and conviction as well as the opportunity to run a religion and worship in accordance with her faith; the right to earn wages in accordance with prevailing wage standards in the country of destination; acquired the rights, opportunities and equal treatment of foreign workers earned more in accordance with the regulations and legislation in the country of destination; obtain legal protection in accordance with the legislation on measures that can lower a status and dignity as well as the violation of the rights that have been established in accordance with the legislation during the placement abroad; obtain protection guarantee the safety and security upon return to place of origin; and obtaining the agreement of the original work. Law No. 39 Year 2004 also set of some obligations for migrant workers namely: comply with the legislation either in the country or country of destination; comply with and carry out its work in accordance with the employment agreement; pay a service charge placement of workers abroad; and notify or report the arrival, presence and returning to the Indonesian Representative in the country of destination.  
Law No. 39 Year 2004 has shortcomings, among others because it is more focused on the issue of the placement so that aspect of protection and legal assistance be excluded (Krustiyati, 2013), therefore it is up to now the Commission IX of the House of Representatives continues to do a revision of the law. (<http://nasional.kompas.com/read/2015/09/30/02145991/Komisi.IX.DPR.Jamin.Revisi.UU.Berikan.Perlindungan.terhadap.TKI>).
3. Law No. 21 of 2007 on the Eradication of Trafficking in Persons also regulates the protection of migrant workers.
4. Law Number 13 Year 2003 on Manpower does not regulate comprehensively on the protection and rights of foreign laborers working in Indonesia, the protection and rights of foreign workers the same labor Indonesian citizens. Such rights are: the right to work and rest periods (Article 77-85), the right to the protection of health and safety as well as moral and morality (Article 86), equality of treatment in accordance with human dignity and religious values , the right to earn that meet a decent livelihood for humanity (Article 88), the right to social security (Article 99), the right to form and join trade unions / labor unions (Article 104), the right to practice their religion required by religion (Article 80) and the right to obtain work safety protection (Article 87). However, Indonesia imposes restrictions for foreign workers in Indonesia.
5. Some areas in Indonesia have local regulations (regulations) that deal with migrant workers, unfortunately, one study found that most of the regulations that deal with migrant workers (an



estimated 80% or more) they are 'extractive' rather than protect, because they actually charge additional fees (Farbenblum *et al.*, 2013).

In addition to the legislation above, the law that comes from ratification also part of the Government's efforts to protect the rights of migrant workers, such as Law No. 6 of 2012 on Ratification of the International Convention on the Protection of the Rights of All Migrant Workers and Members of Their Families.

### **Migrant Workers Rights in ASEAN and Indonesian National Legal Perspective**

Normatively legal protection for migrant workers have a base ranging from the national level, regional (ASEAN) to multinational (ILO conventions). Some fundamental rights for workers / migrant workers such as the right to work and a decent living, the right to information, right to welfare and others can be found in all three levels above.

ASEAN itself through the 2007 Declaration on the Protection and Promotion of the Rights of Migrant Workers has guaranteed protection by taking into account and respect the laws and national policies of its members. Indonesia, for example through Law No. 13 Year 2003 on Manpower (Chapter VIII Article 42) has restrictions for foreign workers (migrant) in Indonesia. Restrictions by the Indonesian national law of course must be respected by the others of member countries of ASEAN and its migrant workers. Instead the Government and Indonesian migrant workers must also respect the national laws and policies of member countries of ASEAN, especially of the recipient country. In fact in the position of the sending country, the Government of Indonesia would have to be more preoccupied with national laws and policies recipient countries. Malaysia and Singapore, for example as a receiver for migrant workers still do not agree that "ASEAN instrument" made legally binding on the grounds that they had to have its own policy in his country related to the setting of migrant workers. Besides Malaysia cannot accept that the protection of migrant workers are also given to the families (<http://www.voaindonesia.com/a/negara-asean-belum-sepakat-soal-perlindungan-buruh-migran/2842676.html>), whereas if it referred to international law and standards that exist so that protection should also include the families of migrant workers.

### **Conclusions**

Protection of migrant workers within the framework of ASEAN are in two pillars include the pillars of politics and security and socio-cultural pillars. Then there is the ASEAN Declaration on the Protection and Promotion of the Rights of Migrant Workers in 2007.

A thing that must be resolved is the difference in perception between sending countries and receiving countries of migrant workers. The impact of that difference is the absence of a legally binding agreement for the protection of migrant workers in ASEAN. Indonesia in its capacity as the sending country should take an active role in negotiating the importance of such legal instruments referred to above.

As for the national scope, it is important to make the revision of Law No.39 of 2004 so that there is better protection for Indonesian migrant workers who work in other ASEAN countries. Another thing that is important is the provision of legal aid. If migrant workers from Indonesia decided to escape because they could not receive harsh treatment from the employer then he will be arrested because, according to the contract of employment if they are out of the house they are considered illegal. Last but not least important is the Indonesian government should give attention to human trafficking and the threat of the death penalty for Indonesian migrant workers

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## **Harmonization of ASEAN Investment Law on the Perspective of Indonesian National Investment Law**

\*Jelly Leviza, Ningrum Natasya Sirait, T. Keizerina Devi

Faculty of Law, University of Sumatera Utara, Medan 20155, Indonesia;

\*Corresponding Author: levizajelly@gmail.com

### **Abstract**

ASEAN's decision to accelerate the implementation of the ASEAN Economic Community (AEC) by 2015 based on the Cebu Declaration, 2007. ASEAN then create a blueprint that describes the steps that must be reached in the AEC in 2015 such as the elimination of taxes and tariffs based on sectors which have been agreed, and all the factors of production such as labor and capital are allowed to move freely crossed the line of ten member countries through the common market. In the field of investment, ASEAN has an ASEAN Comprehensive Investment Agreement (ACIA) to conduct a review of the Framework Agreement on the ASEAN Investment Area (AIA) and ASEAN Investment Guarantee Agreement (IGA). The establishment of ACIA is to encourage a more liberal investment environment, transparent, competitive and facilitative. Indonesia has been preparing the investment regime under ASEAN for example by creating a variety of legal instruments to further open the influx of foreign investment and ensure equal treatment of foreign investors to domestic investors as desired by the ACIA. The problem today is that besides the existence of ACIA as regional investment agreements under the ASEAN there is also a multilateral investment agreement under the WTO (TRIMs). In most small scale there is also Bilateral Investment Treaty (BIT). That condition is still added to the national investment regimes of each country, including for example the Indonesian national investment laws. Based on the above, this paper will examine the ASEAN agreement on investment (ACIA) and the Indonesia national legal arrangements. Then it will also examine Indonesian Government policy on BIT with respect to the ACIA. The purpose of this study was to explain the relevance of Indonesia's national investment law harmonization with ASEAN Agreement and to determine the relevance of the BIT with the ACIA.

**Key words:** AEC 2015, investment, ACIA, BIT, Indonesian Government.

### **Introduction**

ASEAN Community in 2015 has been in force by the end of 2015, Indonesia as one of the ASEAN Countries has to do a lot of researches in many fields based on become ASEAN regional agreement. Regarding the ASEAN Economic Community (AEC), investment is area that needs to be studied more. Legal product on investment areas in ASEAN is known as the ASEAN Comprehensive Investment Agreement (ACIA). It signed by the Government of Brunei Darussalam, the Kingdom of Cambodia, Republic of Indonesia, the Lao People's Democratic Republic, Malaysia, the Union of Myanmar, the Republic of the Philippines, the Republic of Singapore, the Kingdom of Thailand and the Socialist Republic of Viet Nam in Cha-am, Thailand, on 26 February 2009. ([http:// www.asean.org/news/asean-secretariat-news/item/asean-comprehensive-investment-agreement-acia-enters-into-force-creating-a-stable-and-predictable-business-investment-environment](http://www.asean.org/news/asean-secretariat-news/item/asean-comprehensive-investment-agreement-acia-enters-into-force-creating-a-stable-and-predictable-business-investment-environment)).

Meanwhile in Indonesia, now, arrangement of investment is based on the Law No.25 of 2007 coupled with Bilateral Investment Treaty (BIT) made by the Government of Indonesia with a particular country. Today there are three regimes of investment law in Indonesia, ranging from national law (Law No. 25 of 2007 on Investment), international law in the scale of bilateral (Bilateral Investment Treaty) and international law at the regional scale (ACIA). Legal consequences come up from the three legal regimes is important to be studied to synchronize in order to provide legal certainty for both foreign investors and domestic investors.

### **Research Method**

The study used the method of normative legal research that focuses on the study of the norms of law contained in the legislation. The legal norms applicable in this context are the investment legal norms contained in national law and international. Normative study conducted from product investment laws

national, bilateral and regional to see harmonization of the legal implications of these three legal regimes investments.

## **Results and Discussion**

Harmonization of ASEAN Comprehensive Investment Agreement (ACIA) with the Indonesian National Investment Law Investment Law No.25 / 2007 is a product of the national investment law that replaces two previous legal products, i.e. the Law No.1 / 1967 on Foreign Investment and the Law 8/1968 on Domestic Investment. Whereas, the Law No.25 / 2007 have comply the investment principles contained in the ACIA, as can be seen below:

### *The Principle of Liberalization in Investment*

This principle can be seen from Dictum Considering letter c of Law No.25 / 2007 states that to accelerate national economic development and realize the political and economic sovereignty, Indonesia need to enhance investment from potential economic into real economic strength by using capitals within the country or abroad. (Mukti Fajar, 2015, p. 4). Furthermore, this principle can also be seen in Article 12 of Law No. 25/2007 which stated that "All business sectors or types of business open to investment activity, except the areas of business or type of business that is declared closed and open with conditions". The area of business or type of business that is declared closed and opened with conditions stipulated in Presidential Regulation of the Republic of Indonesia Number 76 Year 2007, concerning Criteria and Requirements Making of Business Closed and Opened Business with the requirements in the Investment Sector. It further implemented in Presidential Decree Republic of Indonesia Number 36 Year 2010 concerning List of closed Business and Opened Business Area with requirement in the field of Investment. The principle of liberalization in line with Article 2 of the ACIA which states that "This Agreement shall create a liberal, facilitative, transparent and competitive investment environment in ASEAN ..."

### *National Treatment Principle*

Equal treatment in the context of national treatment to the Law No. 25 Year 2007 on Investment is the guarantee of equal treatment of both governments towards foreign investment and domestic investment as set out in Article 4 paragraph (2). This principle in the ACIA contained in Article 5 (1) and (2), namely: "1. Each Member State shall accord to investors of any other Member State treatment no less favorable than that it accords, in like circumstances, to its own investors with respect to the admission, establishment, acquisition, expansion, management, conduct, operation and sale or other disposition of investments in its territory". 2. Each Member State shall accord to investments of investors of any other Member State treatment no less favorable than that it accords, in like circumstances, to investments in its territory of its own investors with respect to the admission, establishment, acquisition, expansion, management, conduct, operation and sale or other disposition of investments". It should be noted that the equal treatment is desired in Law 25 of 2007, is limited by national interests. One form of national interest is listed directly in Article 4 paragraph (2) c. In other words, equal treatment between foreign and domestic is not applicable when the national interest requires the protection of the micro, small, medium and cooperative.

### *Most Favored Nation (MFN) Principle*

MFN principle contained in Article 6 paragraph (1) of the Act No.25 / 2007 where the government should give equal treatment to all investors originating from any country that conduct investment activities in Indonesia in accordance with the provisions of the legislation. Exceptions to the application of the MFN principle in Law No. 25/2007 mentioned in Article 6 paragraph (2) that the treatment referred to in paragraph (1) shall not apply to investors of a country that gained privileges based on agreements with Indonesia. The elucidation of Article 6 paragraph (2) mentioned understanding of these privileges as a customs union, free trade area, common market, the monetary union, similar institution, and the agreement between the Indonesian government and seven foreign governments associated with certain privileges in the administration of capital investment (The MFN principle in Article 6). The ACIA is loaded on the principle of National Treatment and MFN above has actually been adopted by the ASEAN countries and the Government of Indonesia since ratified the Agreement on Establishing the World Trade Organization (WTO). Ratification for Indonesia made through Law No. 7 of 1994 which with the ratification, the government no longer would provide protection for all economic players both local and foreign (Yue & Tan, 1996, pp. 4-14).

### *Principles of Protection*

This principle is reflected by Article 10 of Law No. 25/2007 which states that the company's investment should meet the needs of labor should give priority to employment of Indonesian citizens, however, fixed capital investment company is entitled to use the expertise of foreign nationals to certain positions

and expertise in accordance with the provisions of the legislation. Besides mentioning that investment companies are required to improve labor competencies for Indonesian citizens through vocational training in accordance with the provisions of laws and regulations. Lastly, Article 10 stipulates that the investment company employing foreign workers is required to conduct training and transferring technology to the work force Indonesian citizens in accordance with the provisions of the legislation. Principles facilitate or provide incentives to investors who require expansion or new investors given the specific conditions under Article 18. These facilities (incentives) can be administered in the form of a reduction in various taxes or customs duties. These things are generally arranged in a number of Minister of Finance. Furthermore, facilitation for obtaining permits foreign workers under Article 23 which is substantively similar to the one contained in Article 22 of the ACIA.

### **Agreement on Investment in the Framework Agreement BIT and Presence ACIA**

Applicability ACIA has brought changes to the member states, historically the majority of member states to make the investment agreement on a bilateral basis by taking models commonly used in the world, including agreement model bilateral investment used United States, Canada, a model North American Free Trade Agreement (NAFTA), or the United Nations Conference on Trade and Development (UNCTAD). Clause national treatment and most-favored nation in the ACIA more profitable investor compared with bilateral agreements that made Indonesia. Meanwhile, a clause on fair and equitable treatment and full protection and security is more favorable to investors under a bilateral agreement setting. ACIA is still unclear, especially since there is no regulation on the implementation of the ACIA and bilateral agreements. There are no explicit arrangements, whether bilateral agreements will remain in effect or be terminated (Protect Foreign Investors, Indonesia Requested End of the Investment Agreement, as accessed on <http://m.hukumonline.com/> On February 24, 2016 14:00 AM GMT). However, there was a view that the obligations born of BIT remain relevant for Indonesia despite the enactment of ACIA (Losari, 2013, p. 5).

### **Indonesian Government Policy on BIT related to ACIA's presence**

Indonesian Government Policy on BIT is doing the review process within three steps: first, reviewing the existing international investment agreements, second, reassessing the existing BIT and last, developing a new model of BIT. In these efforts, the Government of Indonesia involves academics, national and international lawyers, non-governmental organizations (NGO), UNCTAD and experts from various countries and international institutions to contribute their perspective.

The first step taken by Indonesia is stopping 17 of 64 BIT. This incremental approach is taken to avoid the implications that may potentially damage the position of Indonesia. The second step, every single BIT dissected to find the most problematic provisions such as the 'scope' and 'definition of investment', 'Most Favorite Nation Treatment principle', the principle of 'National Treatment'. Assessment is primarily to examine the provisions which provide protection to investors and the impact on government policy space. The third step is the development of the Model Treaty.

### **Conclusions**

International investment agreements within the framework of the ACIA are something that must be obeyed by the Government of Indonesia. Indonesian National Investment Law is sufficient to accommodate the principles in the ACIA without immolating the national importance.

Governments need to reexamine the provisions of the BIT that give wide and much opportunity for foreign investors there by limiting the sovereignty and independence in determining the national development policy. Some of the BIT agreement is disrupting Indonesia, for example in the case of Bank Century. In this case, the lawsuit is based on the BIT between Indonesia and UK.

ACIA is not able to wipe out the obligations within the BIT that made between the Government of Indonesia and other countries. Termination of BIT can be carried out according to the collective agreement and applicable international law.

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## **Post-Conflict Peace Education to Build Sustainable Positive Peace in Aceh**

\*Suadi Zainal

Department of Sociology, Faculty of Social and Political Sciences, University of Malikussaleh Aceh, Lhokseumawe 24355, Indonesia;

\*Corresponding Author: [suadisostro@yahoo.com](mailto:suadisostro@yahoo.com)

### **Abstract**

This study aims to analyze the post-conflict peace education model developed by the Aceh Government to realize a peace culture as the efforts to establish sustainable positive peace in Aceh. Many experts believe that peace education is able to reduce or even eliminate the violence and foster a peace culture. For this instance, Aceh had implemented peace education which initiated by UNICEF during the conflict. Many senior high schools in Aceh region had been assisted by UNICEF to implement peace education by providing training to the teachers. After the conflict period the same thing also has been implemented by the Department of Culture and Tourism of Aceh Government. However, this study found that the implementation of peace education at schools has not implemented in accordance with the concepts of peace education developed by peace experts and international organizations such as UNESCO. Therefore, this study will analyze the peace education developed in Aceh to build sustainable positive peace.

**Key words:** Aceh Government, peace education, peace culture, sustainable positive peace.

### **Introduction**

The Memorandum of Understanding (MoU) between the Government of Indonesia (GoI) and the Free Aceh Movement (GAM) signed on August 15, 2005 has taken place for ten years, but it does not mean that peace agreement implemented as a whole. Even some principles of MoU interpreted into Law of Governing Aceh have been opposed to MoU (CMI, 2012). In these conditions Aceh Government is obligated to manage Aceh as well as possible to avoid Aceh return to violence. It needs to transform all aspects of violence, direct violence, structural violence, and cultural violence. Therefore post-conflict peace building program should be able to transform culture of violence to culture of peace. Hence, the peace in Aceh is not only absence of war, but all forms of violence threatening people's lives can be deducted or eliminated. Thus, sustainable positive peace is realized, the people are free from all forms of wants and fears.

For those, one aspect that should be reformed is education system, because it has impacts on conflict and peace too. It may be a driver of conflict (fuelling grievances, stereotypes, xenophobia and other antagonisms), although the conflict also harm the education system, such as destruction of physical infrastructure of schools, and decreased capacity as a result of teachers fled or targeted by violence. Then, education also can be a way to prevent, mitigate, and transform conflict and build peace. These all need the capacity of education – the content, the structures, the processes of delivery, and the capacity of stakeholders are able to address the proximate and root causes of conflict within the society and realizing a peaceful future for the country, even global.

Based on this, the roles of education to be analyzed before, during and after conflict in order to understand the roles of education according to conflict transformation and peace building. Thus, for case Aceh, post-conflict peace education system should be examined to build a culture of peace in Aceh due to gain sustainable positive peace.

### **Research Method**

This paper used primary and secondary data sources. The primary was collected through interviews with relevant informants of Aceh Government. While the secondary sources were collected through academic books, research articles, mass media and other relevant texts. These all carried out by qualitative approach, with data analyzed in descriptive and analytical methods referring to interactive analysis developed by Miles and Huberman (1984), that involved data collection, data reduction, data display, and drawing conclusion/verifying.

## Results and Discussion

Many concepts of cultural peace suggested peace education as a way to build cultural peace. UNESCO (2000) states, countries and civil society need to promote a culture of peace; one way is through peace education. For this, Askandar (2006) suggested that peace education should be developed at all levels of education.

Related to peace education in Aceh, according to Head of Aceh Education Department (Anas Adam) that peace education had been discussed and debated among its stakeholders, mainly education officers of Aceh government, but until now there is no a decision and a clear implementation mechanism. Even some teachers believe that religious education and moral beliefs (*aqidah-akhlak*) covered peace education. So it's no need to add subject in the curriculum that burden students. In addition, peace education can be taught through a subject Local Content (*muatan lokal*), such as Aceh Cultural History. It depends on school headmasters' understanding and consideration how Local Content Subject should be elaborated and collaborated. They have the autonomy to determine local content subject (Interviews, March 29, 2014). In addition, Irwandi Yusuf (former Aceh Governor) said that the religious education and Pancasila if internalized and practiced by people, it were enough for education and culture of peace. For example, in practice to create a culture of peace, he embraced all groups in developing Aceh, such as "Militias against us (GAM) during the conflict also involved in the construction and they have been cared" (Interview, 5 April 2014). For this, BRA (Aceh Reintegration Agency) provided reintegration assistance to them although they were not mentioned in the Peace Agreement (MoU Helsinki). However, the things regard to a culture of peace (via change of mind) BRA formally did not conduct it through education (Interviews, Nur Juli, 5 April 2014).

Previous facts showed peace education conducted was not comprehensive, because of the peace education curriculum consist of all phases of peace, peacemaking, peacekeeping, peace building and peace preventing, it is impossible to be taught via religious education, moral theology, and Pancasila. Particularly, to teach and train skills to students. Tinker (2016) stated that peace education had developed into many variant fields, including study on the causes of war and other forms of direct violence and strategies to reduce or eliminate them; structural and cultural violence. Hence peace education adapted programs to address violence. It involved many focus, including anti-nuclearism, international understanding, citizenship, global education, environmental responsibility, communication skills, conflict resolution, critical pedagogy, life skills, democracy, coexistence and gender equality, human rights awareness, peace building, and tolerance of diversity. By doing so, hoped succeeding generations will learn how to deal with conflicts non-violently and to eventually sustain a culture of peace (Tinker, 2016). For that, Smith (2010) stated that in societies that experienced violent conflict, education has important role in longer-term, post conflict development to help successive generations understand the violent conflict that took place within their own society and potentially contributes towards future peace building.

In addition, Anas Adami said, till now peace education has not been a primary program at Aceh Education Department. The main program is the implementation of the National Curriculum 2013 and increasing the quality of education. However, peace education is under consideration (discourse), because it is a priority of Aceh Government Program. It may be integrated into the subjects of Social Sciences later on, such as environmental education integrated in the Natural Sciences. By this way, it does not add the subject for students (Interviews, March 29, 2014).

To integrated peace education with others curriculum, it is also one strategy suggested to implement peace education, but it need good capacity of teachers for doing it. All teachers of subjects should be trained firstly about peace education. If not so, it is impossible to be realized. Harris and Morrison (2003) stated that school curriculum often includes a range of topics related to peace education, such as; Cultural diversity or multicultural education; Human rights education; Sustainability or environmental education; Development education; Global education; International education. According to Teachers without Borders (2010) these topics can be incorporated across the curriculum – for example, social sciences, mathematics and the sciences, literature and language. These are considered as peace education when they address issues of conflict and violence, social justice, inequity, human diversity and global problems (Reardon, 1999).

However, those realities indicated that peace education does not become an important affair in public policy makers of Aceh Government although International NGO, such UNICEF had undertaken it before peacemaking by training teachers. Hence, the campaign, promotion and training on peace education conducted by the Civil Society Organizations or social workers could not be implemented maximally (Somerpes, 2011). Except from that, Aceh Culture and Tourism Department had built the capacity of



teachers, community leaders, and youth about education and culture of peace. It was conducted through a training of them in all districts / cities in Aceh (Interviews, Fauziah, 28 March 2014). Particularly for school teachers, it was organized a training to cultivate the culture, character and manners of the nation. The goal is to improve the skills of teachers installing the values into students; religious, honesty, tolerance, discipline, hard work, creative, viable, democratic, curiosity, nationalism, patriotism, honoring achievement, friendly, love peace, like to read, environment care, social care and responsibility. These values expected to be integrated with each subject by every teacher, from primary school to secondary school (Dokumen Dinas Kebudayaan dan Pariwisata Aceh, 2012). These things are not enough for peace education, it does not just learning values, but many other aspects should be taught to students, such as skills of conflict management, transformation and peace building, and understanding of sustainable development to meet human needs.

In addition, at universities level, the implementation of peace education is separated from the educational curriculum, except in certain disciplines, such as sociology and political science that teach conflict theory and conflict resolution. In addition, they established peace and conflict research unit or department<sup>1</sup> to organize seminars and training for academics, NGOs, and social workers who invited to attend program or to run a discussion with the stakeholders about the phenomenon of violence as an early warning<sup>2</sup>. While for all their students, peace education is not a main target of the program although since January 2010 they had launched Curriculum of Conflict Resolution Education to be developed at nine universities in Aceh. It was initiated by the Center for Conflict Resolution and Peace Study of UIN Ar-Raniry (AntaraNews, January 29, 2010). Whereas peace education curriculum had been prepared in 2001 by academicians of UIN Ar-Raniry and Unsyiah collaborated with UNICEF - Peace Education Program. As a pilot project of peace education was conducted at 96 Senior High Schools in 6 districts / cities (Banda Aceh, Aceh Besar, Bireuen, Aceh Pidie, Aceh Utara, Aceh Timur) (Ashton, 2002).

As a follow up, a new University of Tengku Umar established a Peace Research Unit and Peace Club in December 2013, and has held seminars for peace community leaders, members, and students. Then on 29 to 30 April 2014 was organized peace education for UTU Peace Club members and Kelap Peace Members of Universiti Sains Malaysia (USM). The subjects and courses of training delivered directly by a specialist of Asean Peace (Askandar, 2006) (Observation, 29-30 April 2014).

However, Aceh Government supported peace education conducted by NGOs for societies or the younger generations and students in Aceh. For an example; Aceh Peace Generation<sup>3</sup> Program implemented by USAID-SERASI since 2008 for the young generations in Aceh, taught 12 basic values of peace, namely; self-acceptance, prejudice, tribalism, religious distinction, gender distinction, economic status distinction, distinction collection, understanding diversity, understanding conflict, reject violence, recognize mistake, and forgiveness. For this, Irwandi Yusuf as Aceh Governor stated a foreword in publishing a book of 12 values of peace, and said that these values should be taught to students and it may reach out to all groups. Thus, expected all parties should support the implementation of the peace education program (Lincoln & Amalee, 2010). In addition, the Government of Japan in collaboration with UNICEF had promoted peace education through Acehnese tradition and ethics education for intermediate learners at 90 schools in 23 districts / cities by providing a grant to the NGO Peace Education Program, approximately US \$ 86.198 in September 10, 2007 at the BRA Office, Banda Aceh (Kedutaan Besar Jepang, 2007).

Even though, peace education in the formal school (from the elementary school to high school) is still limited in Aceh Government wishes and discourse until now. Even the scope of the planned study is very narrow, "The history of the Acehnese struggle to fight colonialism and the struggle to reach Memorandum of Understanding as Peace Agreement". It expected no later than next year, peace education including MoU Helsinki might be taught in schools (Abdullah, 2014). It strongly relevant to Wenger (2014) explained that peace education including the history of Aceh and the new conflict resolution are very welcomed by teachers and students, because the educational history has been taught was dominated by the history of Java. Besides that, the educational curriculum in Aceh must be in

<sup>1</sup> UIN Ar-Araniry (Center for Conflict Resolution and Peace Studies), Syiah Kuala University (Center for Peace and Conflict Resolution Studies), Malikussaleh University (Aceh Peace Consultative Management), and the University of Teuku Umar (Conflict and Peace Studies Unit).

<sup>2</sup> Center for Peace and Conflict Resolution (CPCRS), Unsyiah held a Roundtable Discussion on Early Prevention of Aceh Conflict (29/11/2012). Attended by 12 participants, involving Kesbangpol Linmas, Bappeda, Peace Consultant, Centre for Peace Studies Unsyiah, IAIN, JKMA, and Forum LSM (The Globe Journal, 29 November 2012).

<sup>3</sup> Aceh Peace Program in Aceh has actually been commenced in 2007 in Sabang. It was supported by the American Friends Service Committee (AFSC) Indonesia, in collaboration with the Ministry of Social Affairs of the Republic of Indonesia. The result, a local network, Meutuah Children Centre (MCC) was established (AFCS, 2008).

accordance with the national education system and curriculum of Indonesia. Therefore Aceh has less autonomy to manage education in accordance with the current social and political contexts.

## Conclusions

Aceh Peace Agreement has been a decade, but Aceh Government has not made the regulations governing the implementation mechanisms of peace education in formal schools, whether separately from other subjects or integrated. Religious education, moral theology and Pancasila are considered representing the peace education. Besides that, the universities in Aceh who understand peace education, they do not act a movement to implement peace education through all sciences disciplines formally, but they do it informally for some students who are interested in it. The Center for peace studies in universities prefer to conduct studies or seminars for outsiders of various elements or components of society.

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# **POSTER PRESENTATION**

**Science and Engineering**

**Life Science**

**Social Science**

**AAC Dayan Dawood, Darussalam-Banda Aceh, Indonesia  
October 4-6, 2016**

## Identification of Mineral of Jades from Nagan Raya Aceh, Indonesia by using XRD and SEM-EDX Techniques

<sup>1\*</sup>Julinawati, <sup>2</sup>Surya Lubis, <sup>3</sup>Irfan Mustafa

<sup>1,2,3</sup>Department of Chemistry, Faculty of Mathematics and Natural Science, University of Syiah Kuala, Darussalam, Banda Aceh, 23111, Indonesia

\*Corresponding Author: juli\_fuadi@yahoo.com.

### Abstract

XRD and SEM-EDX techniques can be used to identify of mineral in a variety of stones including the jades. The samples of the jades was obtained from Nagan Raya Aceh. Based on data of XRD and SEM-EDX, they showed that Jades Nagan raya Aceh belonged to a mineral of silicate. The types of minerals in the Jade of Nagan Raya respectively is grossular ( $\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$ ) from the Garnet group, which is a mineral of nesosilicate, the other type mineral is a calcium magnesium ferro silicate, ( $\text{Ca}_{10}(\text{Mg},\text{Fe})_2\text{Al}_4(\text{SiO}_4)_5(\text{Si}_2\text{O}_7)_2(\text{OH},\text{F})_4$ ), from the vesuvianite group, which is a mineral of sorosilicate and the actinolite, ( $\text{Ca}_2(\text{Mg},\text{Fe})_5(\text{Si}_8\text{O}_{22})(\text{OH})_2$ ), from the amphibole group which is a mineral of inosilicate. XRD and SEM-EDX are the analysis techniques that can identify the types of mineral and the results obtained more quickly and accurately.

Keywords: XRD, SEM-EDX, Jades, Mineral, Nagan Raya, Aceh.

## Removal of Naphthol Blue Black Dye from Aqueous Solution by Adsorption on Titania Pillared Bentonite

Surya Lubis\*, Sheilatina, Vicky Praja Putra

Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: suryalubis@unsyiah.ac.id.

### Abstract

The adsorption of naphthol blue black dye onto titania pillared bentonite has been studied. Titanium tetra isopropoxide in hydrochloric acid environment was used to synthesize titania pillared bentonite. X-ray Diffraction (XRD), scanning electron microscope energy dispersive X-ray Analysis (SEM EDX) and nitrogen adsorption-desorption isotherms were used to explore the characteristics of the natural and titania pillared bentonite. The adsorption process of naphthol blue black was found to be dependent on solution pH, initial dye concentration, the amount of adsorbent and contact time. The optimum pH, adsorbent dose, initial dye concentration and contact time were found to be 3, 0.2 g, 20 mg/L and 120 minutes, respectively. The adsorption capacity for the dye on titania pillared bentonite was 1.207 mg/g. Adsorption of naphthol blue black onto  $\text{TiO}_2$  pillared bentonite followed the Langmuir and Freundlich isotherms and the value of the correlation coefficient of Langmuir isotherm is higher than Freundlich isotherm value.

Keywords: pillared bentonite, naphthol blue black, adsorption isotherms

## **River Water Quality Analysis Near Illegal Gold Mining Area in Aceh Jaya District**

<sup>1</sup> Saiful, <sup>2</sup>Abduh Ulim, <sup>3</sup>Asri Gani

Department of Chemistry, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia

<sup>2</sup>Department of Land Resource Conservation, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

<sup>3</sup>Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: s.saiful@fmipa.unsyiah.ac.id

### **Abstract**

Illegal gold mining has contributed greatly towards the socio-economic development of the country but raise a question about its negative impacts to surface water and drinking water quality. This is caused by the discharge of mining waste into rivers after amalgamation process. In this research the water quality of rivers affected by artisanal gold mining was evaluated based on seven parameters and nine sample stations. Water samples have been taken and analyzed from three main rivers in the district of Aceh Jaya. River Kr. Teunom, Kr. Panga and Kr. Ligan have taken samples of the sample water covering upstream, median and downstream. The results showed that the physical water quality in Kr. Teunom, Kr. Panga, and Kr. Ligan are still in line with river water quality standards by Government Regulation No. 82 of 2001. Based on the chemical parameters of the river water conditions Kr. Teunom, Kr. Panga, and Kr. Ligan have found mercury (Hg) in 2016 with varying degrees. However, the levels are still below the quality standard in accordance with Government Regulation No. 82 of 2001 for Class I water and the Regulation of the Minister of Health No. 492/Menkes/Per/IV/2010 on drinking water quality requirements. The presence of this mercury should be wary because it can accumulate in the food web in the long term. Cyanide levels in all samples of river water are lower than the quality standard for drinking water.

Keywords: water quality, mercury, gold mining, drinking water

## **The effect of cellulose particles from oil palm empty fruit bunch on mechanical properties and the crystallinity of chitosan-cellulose composites**

Rahmi

Department of Chemistry, Faculty of Sciences, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: rahmi@fmipa.unsyiah.ac.id.

### **Abstract**

A chitosan-cellulose composite has been successfully prepared by solution casting. Cellulose particles were produced by hydrolyzing oil palm empty fruit bunch with hydrochloric acid. Several compositions were prepared by varying the chitosan:cellulose ratio of the composites. The structure and the properties of composites were investigated by Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD) and mechanical performance measurement. FTIR spectra confirmed that cellulose particles were incorporated into the chitosan matrix. Tensile test results showed that the contents of chitosan and cellulose influenced the mechanical properties of composites. The composite with 10 wt% cellulose particles had the highest tensile strength. The X-ray diffraction patterns indicate the crystallinity index of composites decreased with addition of cellulose particles. This low crystallinity is important for metal ions removal in water treatment.

Keywords: chitosan, cellulose, composite, crystallinity

## Using Bayesian Inference to Analyze the Phylogenetic of Dipterocarpaceae Family

<sup>1</sup>Essy Harnelly, <sup>2</sup>Muhammad Subianto, <sup>3</sup>Mirna Yunita

<sup>1</sup>Biology Department, Mathematics and Natural Science Faculty, Syiah Kuala University, Indonesia, Banda Aceh, 23111

<sup>2</sup>Department of Informatics, Mathematics and Natural Science Faculty, Syiah Kuala University, Banda Aceh, 23111

<sup>3</sup>Department of Informatics, Mathematics and Natural Science Faculty, Syiah Kuala University, Indonesia, Banda Aceh, 23111

\*Corresponding Author: [msubianto@gmail.com](mailto:msubianto@gmail.com)

### Abstract

Dipterocarpaceae is one of the largest plant family which has more than 500 member of species. This family mostly used for timber plant such as for housing, making ship, decking and main material for making furniture. In Indonesia we have many species of Dipterocarpaceae which has morphological similarity and difficult to recognize in the field. This research will analyze phylogenetic of Dipterocarpaceae based on chloroplast *matK* gene. The aim of this research is to use Bayesian inference to analyze the phylogenetics of Dipterocarpaceae family. This research used chloroplast *matK* gene instead of morphological characters. Delineating the phylogenetic using chloroplast gene has many advantages such as; the character is more stable and not easily influenced by the environment. The analysis steps are collecting data, modification of the stucture sequence name, sequence alignment, constructing tree by using Bayesian method, evaluating and analyzing phylogenetic tree. The result showed that *Dipterocarpus* genus is not from *Dipterocarpeae* tribe.

Keywords : *Dipterocarpaceae*, phylogenetic, bayesian inference

## Removal of Cadmium from groundwater Using Aceh Natural Zeolite

<sup>1</sup>\*Sri Mulyati, <sup>1</sup>Cut Raziah, <sup>1</sup>Sofyana, <sup>1</sup>Syawaliah

<sup>1</sup>Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [sri.mulyati@unsyiah.ac.id](mailto:sri.mulyati@unsyiah.ac.id).

### Abstract

The presence of Cadmium-containing waste in groundwater is a problem that needs to be handled exclusively as it can bring issues to human health. Adsorption is one of the processes that can be exploited to eliminate this metal. The ability of Banda Aceh natural zeolite in removing cadmium content in solution has been studied in a batch process. The removal of cadmium by zeolite adsorbent influenced by contact time, dosage of adsorbent, pH, and initial concentration of the sample solution. The most optimal conditions were obtained at operating conditions as follows; Contact time: 40 minutes, initial pH of the samples: 6, adsorbant dosage: 20 g/L and concentration of initial sample of 40 mg/L. The result of the study suggests that the isotherms of the adsorption found to follow the Freundlich model, meanwhile the reaction kinetics followed the pseudo second order.

Keywords: Adsorbtion, natural zeolite, Cadmium .

## **Purification And Characterisation Of Thermostable $\alpha$ -Amylase From Jaboi Sabang Isolat**

\*Febriani<sup>1</sup>, Rayyana<sup>1</sup>, Mildatul Ulya<sup>1</sup>, Frida Oesman<sup>2</sup>, and T.M. Iqbalsyah<sup>1</sup>

<sup>1</sup>Chemistry Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh,

<sup>2</sup>Pharmacy Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh

\*Corresponding Author: : [febriani@unsyiah.ac.id](mailto:febriani@unsyiah.ac.id), and [t.iqbalsyah@unsyiah.ac.id](mailto:t.iqbalsyah@unsyiah.ac.id)

### **Abstract**

$\alpha$ -Amylase (EC3.2.1.1) is a group of enzymes that catalyses the hydrolysis of  $\alpha$ -1,4 glycosidic linkages in starch into simpler sugars such as dextrin and glucose with an endo mechanism. The goal of this study was purified and characterized thermostable  $\alpha$ -amylase, including temperature, pH and molecular weight of enzyme. The enzymes was purified by ammonium sulfate fractionation and anion-exchange chromatography (DEAE Sepharose Fast Flow). The results showed that, fraction IV of ammonium sulfat purification (60-80% saturation of ammonium sulfate) gave the highest specific activity of 19.16 (U / mg) with a purity level of 2.62 times higher than the crude extract. Purification by anion exchange chromatography (DEAE Sepharose Fast Flow) on ammonium sulfate fraction IV has a specific activity of 143.56 (U / mg) with enzyme purity of 19.63 times higher than the crude extract. The  $\alpha$ -amylase has an optimum activity at temperature of 75°C, pH 8 with a molecular weight of 12.19 kDa. These results suggest that  $\alpha$ - amylase produced by isolates Jaboi Sabang were an alkali thermostable and a low molecular weight.

Keywords :  $\alpha$ -amylase, anion-exchange chromatography, isolate Jaboi Sabang

## **Effect of Zeolite Adsorbent on Patchouli Oil Quality Parameter**

\*Suraiya, Bastan Arifin, Muhammad Faisal

Master Program of Chemical Engineering, Technology and Management of Environment Syiah Kuala University

\*Corresponding Author: [Suraiya@che.unsyiah.ac.id](mailto:Suraiya@che.unsyiah.ac.id)

### **Abstract**

Aceh is one of region producing patchouli oil in Indonesia. Commonly, patchouli oil farmers in Province of Aceh are using simple equipment composed by used drums, so the patchouli oil produced was containing a lot of iron element that darken the color of patchouli oil. This study aims to determine the effect of time and amount of zeolite adsorbent used in the adsorption process on patchouli oil quality parameters. The process of removing impurities from patchouli oil can be done by sorption method (adsorption). This work used zeolite adsorbent. Zeolite was activated with 1 N HCl for 60 minutes, and then dried in the oven at a temperature of 600°C for 120 minutes. The volume of patchouli oil used was by 100 mL at stirring rate of 100 rpm with zeolite adsorbent size by 80 meshes. Zeolite mass was varied to 1, 2, 3, and 4 grams with various adsorption time of patchouli oil by 15 minutes, 30 minutes, 45 minutes, and 60 minutes to determine the most influential variable between zeolite weight and adsorption time. Patchouli oil produced was getting clearer as the increase of adsorbent mass and adsorption time. Zeolite adsorbent could adsorb Fe ion from patchouli oil until the iron content became 3.765, 2.704, 2.125, and 0.647 ppm from its initial concentration by 8.946 ppm. The acid number of patchouli oil before adsorption process was by 8.947 ppm. The acid number of patchouli oil after adsorption has fulfilled the SNI standard which is by 4.488. Patchouli alcohol area in the initial patchouli oil was by 30.84% and has increased after adsorption by using zeolite to be 38.25%.

Keywords: Patchouli, iron (Fe), Zeolite, adsorb, patchouli alcohol

## **The Removal of Phosphate from Laundry Waste Using Combined Zeolite Adsorption And Cellulose Acetate Ultrafiltration Processes**

<sup>1</sup>\*Cut Meurah Rosnelly, <sup>2</sup>Hisbullah, <sup>3</sup>Fuadi Harun

<sup>1</sup>Department of Chemical Engineering – Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

<sup>2</sup>Department of Chemical Engineering – Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

<sup>3</sup>Departement of Agrotechnology – Faculty of Agriculture, Universitas of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: [cutnelly@ymail.com](mailto:cutnelly@ymail.com)

### **Abstract**

Laundry industry may create problems to environment due to high phosphate content in its wastewater. Membrane ultrafiltration is one of separation technologies promising to be implemented to handle liquid waste. In this work, a combination of process of zeolite adsorption and membrane ultrafiltration in laundry wastewater treatment in term of phosphate removal has been studied. The aim of this work is to study the performance of the proposed process and emphasis is given to observe the level of flux and rejection in membrane used. The variation of the trans-membrane operation pressure of 1, 2, and 3 bar is applied. The result shows that the process proposed perform satisfactorily. The highest flux is 202,474 L/m<sup>2</sup>.h which is obtained at the pressure of 3 bar. The maximum rejection of 98% is obtained at the pressure of 1 bar and the concentration of phosphate in the wastewater can be reduced from 15,33 mg/L to 0,3 mg/L.

Keywords: laundry waste, adsorption, zeolite, cellulose acetate membrane, ultrafiltration

## **Structure Formation of Polyethersulfone-Nano Carbon Membrane Prepared with Difference Polymer Solutions**

<sup>1</sup>\*Nasrul Arahman

<sup>1</sup> Department of Chemical Engineering, Faculty of Engineering, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [nasrular@unsyiah.ac.id](mailto:nasrular@unsyiah.ac.id)

### **Abstract**

Membrane technology have been widely developed and applied in various industries, particularly in the separation technology. This technology has many advantages such as energy saving, high product purity, low operation cost, save in environment, and simple in application. Researcher are now developing the membrane module with the best quality in application for separation industries. The objective of this research is to investigate the effect of three difference solvent such as (N-Methylpyrrolidone, Dimethylformamide, Dimethyl sulfoxide) on the morphology of polyethersulfone membrane. The effect of addition of membrane pore forming agent (Nanocarbon) was also studied on the hydrophilicity and filtration performance of resulted membrane. The morphology of fabricated membrane was observed by scanning electron microscopy (SEM). Surface functional group and surface hydrophilicity of membrane were respectively analyze by Fourier transform infrared spectroscopy (FTIR) and hidrophilicity test by water contact angle meter. The filtration performance of membrane was designed by single module of dead-end filtration method. The different types of solvent effect on the membrane morphology, where the membrane has a finger from the longest to the shortest row is PES/DMSO, PES/DMF, and PES/NMP. The addition of 0.05 wt% of nanocarbon in the polymer solution brought about the increases of water permeability and hydrophilicity properties of resulted membrane. The results showed that, the PES/DMSO+Nanocarbon membrane system gave the highest water permeability ( $L_p = 14,7565 \text{ L/m}^2\cdot\text{hour}\cdot\text{bar}$ ), and types of membrane with the best hidrophilicity properties is PES/NMP+Nanocarbon (contat angle = 71,58o).

Keywords: additive, pore forming agent, palm oil shell, polyethersulfon, nanocarbon



## **Analysis Of Chitosan Addition Toward Physical And Mechanical Properties And Bioplastic Degradation Based On Cassava-Peel Starch**

<sup>1</sup>\*Umi Fathanah, <sup>1</sup>Mirna RahmahLubis, <sup>1</sup>Cut MeurahRosnelly

\*Chemical Engineering Department, Engineering Faculty, Syiah Kuala University

\*Corresponding Author: [umi\\_fathur@yahoo.com](mailto:umi_fathur@yahoo.com)

### **Abstract**

The increase of plastic use in daily life causes environmental pollution because of plastic load in land. Synthetic plastic is hard to be degraded so that it is raw material needed for bioplastic fabrication i. e. its starch. Cassava peel is one of solid wastes that still contain relatively high starch so that it is potential as raw material of biodegradable plastic. So far cassava peel waste has not been utilized optimally, it is just used as ensilage, and it is still problematical with cyanide content or cassava peel that is discarded without result. This research aims to increase economical value of cassava peel waste as raw material of plastic fabrication whose existence is still abundant in Indonesia. The fabrication of cassava peel starch is done by hydrolysis cassava peel with water. Furthermore, sedimentation is done for 24 hours and continued with drying process at temperature of 70°C for 3 hours, until starch powder of cassava peel is obtained. The next step is bioplastic fabrication process that is done by mixing starch, chitosan, and glycerol as plasticizer. Bioplastic fabrication is done at gelatinous temperature as much of 80°C. Chitosan addition is done at variation of 0; 10; 20; 30; and 40 (% w). Whereas glycerol addition as plasticizer is done with concentration variation of 20; 30; 40; and 50%-w. Research result indicates that mechanical property i. e. optimum value of tensile strength is obtained at value of 40.99 MPa with chitosan addition as much of 30% and glycerol concentration 30%. The highest percentage is obtained as much of 58.23% with addition of 0% chitosan with glycerol concentration of 30%. The highest young modulus is obtained as much of 1.99 Mpawith chitosan addition as much of 40% and glycerol concentration of 40%. Physical property of bioplastic resulted is obtained from the best test of water-absorption power (swelling test) at 30% chitosan addition and glycerol concentration of 20% i. e. 21.66%. Result of SEM (Scanning Electron Microscopic) at top section indicates that starch-chitosan mixture seems relatively homogeneous. Cross section indicates plastic structure and pores formed. Functional group analysis by using FTIR (Fourier Transform Infra Red Spectrophotometer) indicates the existence of carbonyl, carboxyl, and ester group. This matter indicates that bioplastic resulted does not produce new compounds so that groups contained in bioplastic could be degraded in soil. Whereas biodegradability test toward environment indicates that bioplastic could be degraded completely in soil for 24 hours.

**Keywords:** bioplastic, cassava peel, degradation, mechanical property, physical property

## **Land Suitability for Palm Oil in Tripa Peat-Swamp Forest, Aceh Province (Indonesia)**

<sup>1</sup>\*Sufardi, <sup>1</sup>Sugianto, <sup>1</sup>airul Basri, <sup>1</sup>Syamaun A. Ali, and <sup>1</sup>Khairullah

<sup>1</sup>Department of Soil Science, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;

\*Corresponding Author: [sufardi.usk@gmail.com](mailto:sufardi.usk@gmail.com)

### **Abstract**

This research aims to assess the suitability of Tripa Peat-Swamp Forest (TPSF), Aceh Province, Indonesia for palm oil plantation. The criteria of suitability for palm oil plantation according to the technical manual of Research Headquarter for Agricultural Land Resources (2014) were utilized for 16 land mapping units. Field observations were conducted to obtain the parameters such as soil types, thickness and maturity of the peat, as well as the type of land used in order to obtain the land mapping units. The results show that more than half (52%) of TPSF area are not suitable for palm oil plantations. The main limiting factor is the land condition that is easily inundated, the thickness of the peat layer > 200 cm, and the maturity of the peat-fabric. The areas which are suitable for palm oil plantations are divided into two subclasses

i.e. moderately suitable ( $S_2$ -rc) 8.97% and marginally suitable ( $S_3$ -nr, fh) 43,03%. The limiting factors on the use of land for palm oil cultivation on TPSF areas are nutrient retention, soil fertility, and flooding.

Keywords: Peat swamp forest, land characteristics, crop cultivation.

## **Demand Elasticity and Projected Consumption of Raskin in Aceh (Almost Ideal Demand System Approach)**

Suriani<sup>1\*</sup>, Diana Sapha<sup>2</sup>, Cut Zakia Rizki<sup>3</sup>

Economics and Business Faculty of Syiah Kuala University, Banda Aceh, Indonesia

[\\*Corresponding author: scsani.2009@gmail.com](mailto:scsani.2009@gmail.com), [suriani@unsyiah.ac.id](mailto:suriani@unsyiah.ac.id)

### **Abstract**

This research aims at supporting government programs to reduce poverty in Aceh province by examining the *Raskin* program (a subsidized rice program). *Almost Ideal Demand System (AIDS)* model is utilized to explain quantitatively and qualitatively the demand elasticity of poor households' expenditure (RTSPM) and the projections of *Raskin* consumption. To examine the elasticity of households' expenditure, a number of variables are taken into account, among others, the price of subsidized rice, the price of non-subsidized rice, and the price of meat/fish/eggs/milk. The research results shows that out of six commodities spending on non-subsidized rice, meat, fish, subsidized rice, and eggs makes up mainly the consumption patterns of the poor. Based on the values of coefficient estimates and  $R^2$ , spending on rice influences largely household's expenditure. This means rice is a primary staple for Acehese people so that the program is of great benefit to help the poor to meet their food needs. However, spending on milk does not influence households' consumption patterns since they do not consume milk in large portion of their total expenditure. Moreover, with the variable of natural log of expenditure, respondents' spending on rice is very influential. Based on the own-price elasticity of demand, the total amount of rice expenditure remains constant at any projected price levels and is elastic. Conversely, the cross price elasticity of rice demand does not change and is inelastic. It can be concluded that, under the *Raskin* program, rice commodity is still affordable for the poor though its price increases. Additionally, the *Raskin* program for the poor is of great use as they can meet other food needs since the government subsidizes rice. Their monthly income also can be diverted from rice to other staples such as meat, milk, eggs and fish. However, since this program is only temporary, it could not improve the welfare of the poor if they only depended merely on the *Raskin* program. Therefore, the government need to provide another supporting programs to improve the poor's skills in order for them to increase their income. This means that the *Raskin* program is a government's poverty reduction program that could not be implemented separately and need to be supported by other real sector programs.

Keywords: elasticity of demand, consumption projections, the *Raskin* (a subsidized rice government's program), AIDS model.

## Physical Quality of the Meat of Aceh Cattle

\*Al Azhar, Triva Murtina Lubis, Razali Razali, Sugito Sugito

Faculty of Veterinary Medicine, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

\*Corresponding Author: alazhar@unsyiah.ac.id

### Abstract

Aceh Cattle is one of genetic biodiversities of Indonesian beef cattle that has special position in Aceh peoples. This beef cattle has become the primary source of red meats mostly consumed by the Acehnese. Although many farmers in Aceh raise the cattle and the numbers of cattle slaughtered per year progressively increase, studies on both physical quality and nutritional values of the meat of Aceh cattle are very limited and not comprehensive. In the study presented here, physical quality of aceh cattle meats was determined using standard methods. The results showed that pH, meat color, fat color, marbling, tenderness, cooking loss, water holding capacity (WHC) of the meat of aceh cattle were  $5.60 \pm 0.15$ ,  $2.75 \pm 0.50$ ,  $4.00 \pm 0.00$ ,  $1.00 \pm 0.00$ ,  $3.75 \pm 0.30$ ,  $49.70 \pm 3.24\%$ ,  $50.51 \pm 18.24$  mgH<sub>2</sub>O and  $16.84 \pm 6.08$  %mgH<sub>2</sub>O, respectively. Based on physical quality data obtained it can be concluded that meat of local Aceh cattle has good quality, but is not recommended for short time cooking

**Key words:** aceh, marbling, WHC, beef cattle, quality

## A Local Isolate of PIs 80 Capable of Producing A B-Lactams Antibiotic

Teuku M. Iqbalsyah<sup>\*1</sup>, Fani Sartika<sup>1</sup>, Yusniar<sup>1</sup>, Nurdin Saidi<sup>2</sup> and Febriani<sup>1</sup>

<sup>1</sup>Biomolecule Application Research Group, Chemistry Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia

<sup>2</sup>Natural Product Chemistry Research Group, Chemistry Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, Indonesia

Corresponding authors: t.iqbalsyah@unsyiah.ac.id, febriani.tahar@yahoo.com

### Abstract

Some strains of thermo-halophile have previously been isolated from an underwater hot springs in PriaLaotSabang, north of Weh Island Aceh. Some strains were capable of producing antibiotics. The goal of this short study was to determine the group of antibiotic produced by one of the strains, PLS 80 isolate, which was a Gram-negative bacteria. The results of Kyrbi-Bauer diffusion test showed that the supernatant from PLS 80 fermentation, grown in TSB medium for 112 hours, gave an inhibition zone against *E. coli* ( $\phi$  14mm) and *S. aureus* ( $\phi$  9mm). Inhibition zone test of fractions of the supernatant showed that the ethyl acetate fraction gave a strong inhibition zone against *E. coli* ( $\phi$  23mm) and *S. aureus* ( $\phi$  14mm) while fractions of n-hexane and methanol showed no inhibitory activity. We used two positive controls of gentamicin and cephalazolin. Gentamicin, an antibiotic containing the aminoglycoside group, gave an inhibition zone against *E. coli* and *S. aureus* of 12.5mm and 13.5mm, respectively. Meanwhile cephalazolin, a  $\beta$ -lactams antibiotic, gave an inhibition zone against *E. coli* and *S. aureus* of 15mm and 18mm, respectively. Ninhydrin, iodine vapour and iodine potassium-iodine indicated positive results. The results predicted that the antibiotic produced by PLS 80 belongs to  $\beta$ -lactams group.

**Key words:** PLS 75, PLS 80, antibiotic,  $\beta$ -lactam, Kyrbi-Bauer, *E. coli*, *S. aureus*

## **Geochemical Study On Geothermal Systems In Upflow And Outflow Manifestations Zone, Seulawah Agam, Aceh Besar**

Muhammad Yusuf<sup>1</sup>, Muksin Alatas<sup>1</sup>, Subhan<sup>1</sup>, Andi Lala<sup>1</sup>, Ghazi Mauer Idroes<sup>2</sup>, Fajar Fakri<sup>4</sup>, Marwan<sup>3</sup>, Muhammad syukri<sup>3</sup>, Saiful<sup>1</sup>, Rinaldi Idroes<sup>1,4,\*</sup>)

<sup>1</sup> Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia

<sup>2</sup> Department of Chemical Engineering, Faculty of engineering, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia

<sup>3</sup> Department of Geophysics Engineering, Faculty of engineering, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia

<sup>4</sup> Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia

\*) *Corresponding Author:* rinaldi.idroes@unsyiah.ac.id

### **Abstract**

Indonesia's geothermal resources are among the largest in the world, around 40 percent of the total geothermal resources worldwide with a total potential energy of 28,617 MW. Geothermal energy is one of the alternative energy world that can be developed and can be renewed. This is an alternative source of energy that is environmental friendly and highly prospective compared with fossil energy. One of the potential is Seulawah Agam geothermal field with upflow (Ie Jue) and outflow (Ie Seu-um) manifestations zone. This study will be analyzed quantitatively in the form of geochemical analysis of geothermal field, includes the elements that are dissolved in the geothermal fluid such as cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ), anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HNO}_3^-$ ), isotopes  $^{18}\text{O}$ , and deuterium contained in the manifestation of the upflow and outflow zone of Seulawah Agam, Aceh Besar. Interpretation of data based on the geothermometer (Na-K, Na-K-Ca, Na-K-Mg, K-Mg and isotopes) and geindicator ( $\text{Cl-SO}_4\text{-HCO}_3$ ) analysis using liquid chemistry plotting spreadsheet version 3 powell geoscience method Ltd.3 September 2012 by Powell & Cumming, to determine the temperature of the reservoir, fluid types and source of the fluid in the manifestation of the upflow and outflow zone.

**Key words:** Geothermal, Geochemical, Geothermometer, Geoindicator, Seulawah Agam

## Isolation and Screening of Proteolytic Lactic Acid Bacteria from Civet (*Paradoxurus hermaphroditus*)

<sup>1\*</sup>Murna Muzaifa, <sup>1</sup>Anshar Patria, <sup>2</sup>Febriani, <sup>3</sup>Amhar Abubakar

<sup>1</sup> Department of Agricultural Product Technology, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia;

<sup>2</sup> Department of Chemistry, Faculty of Mathematics and Natural Science, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia;

<sup>3</sup> Department of Animal Husbandry, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh, 23111, Indonesia

\* Corresponding Author : murnamuzaifa@unsyiah.ac.id

### Abstract

Kopi Luwak (civet coffee) is an exotic Indonesian coffee made from coffee berries that have been eaten by civet (*Paradoxurus hermaphroditus*). Because of the scarcity, kopi luwak is considered to be the most expensive coffee in the world. An alternative for kopi luwak production is employing an *in vitro* fermentation to mimic natural fermentation on the digestive system of civet. Unfortunately, scientific information about microbial digestion of civet is still limited. This study focused on enumeration and screening of proteolytic lactic acid bacteria from civet feces. The isolation was carried out by using MRS agar followed by screening using skim milk. The results showed that the lactic acid bacteria count was  $5,8 \times 10^8$  CFU/ml. Three different colonies (ICF 1, ICF 2 and ICF 3) were isolated and subjected to Gram and catalase tests. All isolates showed Gram positive and catalase negative. The result confirmed that they were lactic acid bacteria. Two isolates (ICF 1 and ICF 3) showed proteolytic activity and isolate ICF 1 showed the largest zone of clearance of 15 mm. Further investigation is needed to identify and select of lactic acid bacteria performance for artificial kopi luwak fermentation.

**Key words:** lactic acid bacteria, kopi luwak, proteolytic

### Introduction

Coffee is a very popular beverage that is consumed world-wide. After oil, coffee is the most internationally traded commodity. The economies of many countries depend upon coffee production for stability and growth (Iscaro, 2014). Drinks made of coffee are always pleasing and stimulating and hence become the favourite of many people. Good quality coffee flavour has been described as pleasant sensation, a balanced combination of flavour, body and aroma in the absence of faults (Mori et al, 2003; Buffo et al., 2004; Sunarharum et al, 2014).

Coffee is consumed because of its typical smell and flavour. The differences in coffee processing can impact coffee flavour. In bean coffee processing, some processing techniques are applied to the harvested coffee fruits to green bean. The techniques are dry processing (natural), wet processing (washed) and semi-dry processing (semi washed) (Clarke and Macrae, 1985; Duarte et al, 2010). There is certain "specialty" style of coffee processing that is produced using uncommon processing methods, "kopi luwak". This coffee is one of the most popular coffee. It has been produced from the coffee beans which have been digested by a certain Indonesia cat- like animal, Asian palm civet or luwak (*Paradoxurus hermaphroditus*, called then kopi luwak or civet coffee (Marcone, 2004).

Asian palm civet are native to regions within and around Asia. They naturally live in temperate and tropical forests, but in developed areas they also found in parks, suburban gardens, plantations and fruit orchards. Where these civets choose to live depends mostly on availability of food and the presence of areas they can rest in (Nowak, 1999). The processing of kopi luwak is unusual, civet eats the premier coffee berries. During the digestion process on intestinal civet, coffee beans are not digested by the civet. The beans hence excrete with civet feces. Farmer collected the feces, washed and dried (Cuang-Hoang, 2012). Kopi luwak is very hard to find and claimed as the most expensive coffee in the world. Due to the scarcity and the high price of kopi luwak, farming of kopi luwak started to rise tremendously

in the last few years but the business is also widely repudiated as it relates to animal rights (Cuang-Hoang, 2012, Jumhawan et al, 2013).

Coffee bean that was fermented naturally in the gastrointestinal tract of civet changed the chemical composition of coffee bean and gave specific taste and odor of coffee due to the enzymatic process and bacteria found in gastrointestinal tract of luwak (Marcone, 2004). The coffee fermentation is characterized by presence of different microorganisms. It should be possible to produce artificial kopi luwak through controlled fermentation using indigenous civet microorganism as starter culture. Unfortunately, scientific information on microbial digestive of civet is still limited.

Lactic acid bacteria is one of the common bacteria found in gastrointestinal tract. It's well known that lactic acid bacteria is the most important bacteria used as starter cultures in fermentation. Apart from general demands for starter cultures from the view of safety, technological effectiveness and economics, numerous specific aspects have to be considered when selecting strains for the different food fermentations. Therefore selection criteria for lactic acid bacteria depend on the type and the desired characteristics of the final product, the desired metabolic activities, the characteristics of the raw materials and the applied technology (Buckenhuses, 1993). The objective of the present study was to isolate and to screen proteolytic lactic acid bacteria from civet feces.

## **Materials and Methods**

### ***Sample Collection***

This research was explorative study on isolation and screening of proteolytic lactic acid bacteria from civet feces. Civet feces was obtained from a coffee farming in Jejem, Central Aceh District, Aceh Indonesia. Sample was collected in sterilized sampling flask and was brought to Microbiology Laboratory with ice box. The sample was kept in the freeze at -4°C until the experiment was conducted.

### ***Isolation and enumeration of lactic acid bacteria***

Lactic acid bacteria were isolated from civet feces by taking one loop of the feces samples into pepton and shakes for 15 minutes on the shaker. About 1 ml of the sample was added to 9 ml of sterile distilled water and this suspension was serially diluted. About 0.1 ml from each dilution was spread plated onto sterile MRS (Man Rogosa and Sharpe, Merck Germany) agar plates and incubated at 37 °C for 24 hours. Enumeration was made in petri dishes containing from 30 to 300 cfu. Average of triplicate plates were taken to express the lactic acid bacteria count as cfu (colony forming unit) per ml of sample (Jini et al, 2011). Different and single colony types were picked up and purified by repeated streaking. Pure cultures were submitted to Gram and catalase test to confirm as lactic acid bacteria (Sharpe, 1979; Leveau et al., 1995). The ones that had Gram positive and catalase negative selected, sub cultured onto slants media and maintained for screening and identification.

### ***Screening of proteolytic lactic acid bacteria***

Selected isolates from samples were spot inoculated on 1% skim milk agar plates and incubated at 37°C for 24 hours. The formation of clear zone (halo) around the colonies indicated the proteolytic activities of bacteria resulting from milk protein hydrolysis. The diameter of halo formation was measured in mm. Skimmed milk agar plate assays allow principally for qualitative determinations of protease activity. The hydrolysis zone produced on the casein agar could be related to the amount of protease produced by microorganism (Vermelho et al. 1996).

### ***Data Analysis***

All data obtained from the microbial analysis represented in table form. Data analyzed descriptively.

## **Result and Discussion**

Civet feces obtained by collecting from coffee farmers adjacent to the forest. The feces found under large trees or among banana trees and shrubs under the coffee plant on different form (Fig 1). Feces were sampled is still intact and wet.



**Fig 1.** Civet feces obtained on different form

Lactic acid bacteria count of civet feces was reported in Table 1. On average, lactic acid count bacteria on this study was  $5.8 \times 10^8$  cfu/ml. This was slightly higher than lactic acid bacteria count enumerated by Fauzi (2008),  $3.0 \times 10^8$  cfu/ml. Lactic acid bacteria are part of the normal microorganism, the ecosystem that naturally inhabits the gastrointestinal and genitourinary tracts, which comprised by a large number of different bacterial species with diverse amount of strains (Aureli et al, 2011; Barinov et al., 2011).

**Table 1.** Lactic acid bacteria count on civet feces.

Sample	Colony count (cfu/ml)
1	$4,5 \times 10^8$
2	$7,9 \times 10^8$
3	$5,0 \times 10^8$
Average	$5,8 \times 10^8$

Based on morphological assesment, three different colonies were isolated. The colony characteristic of isolates showed in Table 2. The isolates were submitted to Gram and catalase test. The result showed that all of isolates were Gram positive and catalase negative. It confirmed that they are lactic acid bacteria (Table 3).

**Table 2.** Colony Characteristics of Isolates

No	Isolates	Colony characteristics			
		Form	Elevation	Colour	Size (mm)
1	ICF 1	circular	raised	milky white	1,0
2	ICF 2	circular	raised	light milky white	1,0
3	ICF 3	circular	raised	milky white	2,0

**Table 3.** Cell Characteristics of Isolates

No	Isolates	Cell form	Gram staining	Catalase
1	ICF 1	Cocci	+	-
2	ICF 2	Rods	+	-
3	ICF 3	Cocci	+	-

All isolates were evaluated for their potential to produce protease. Isolation of proteolytic lactic acid bacteria was carried out using MRS agar media and further screened for protease production on skim milk agar plates. Results of screening protease production showed that two isolates (ICF 1 and ICF 3) were able to produce protease (Tabel 4).

**Table 4.** Screening of proteolytic lactic acid bacteria

No	Isolates	Detection of Proteolytic	Clear zone (mm)
1	ICF 1	+	15
2	ICF 2	-	-
3	ICF 3	+	10

+ : presence of clear zone around colony

- : absence of clear zone around colony



**Fig 2.** Clear zone of isolate ICF 1

Isolate ICF 1 showed largest zone of clearance of 15 mm (Fig 2). Formation of clear zones around the colonies was considered as indication of protease production. Bacterial proteases are generally used to break down oligopeptides into amino acids. That was one of the important criteria of a lactic acid bacteria isolates could be used as a starter (Sanz et al., 1999). Lactic acid bacteria is best known as starter culture due to their versatile metabolic characteristics such as acidification activity, proteolytic activity and synthesis of metabolites (Chintas, 2001; Cheriguene et al., 2006).

The next step in this research is to identify of the proteolytic lactic acid bacteria by molecular and to analyze the lactic acid bacteria performance as starter on coffee fermentation (artificial kopi luwak fermentation).

### Conclusions

The average of lactic acid bacteria count on civet feces was  $5,8 \times 10^8$  CFU/ml. Three different isolates were confirmed as lactic acid bacteria. Among all isolates, 2 isolates showed proteolytic activity. Further investigation is needed for the selection of performance as starter on coffee fermentation (artificial kopi luwak fermentation).

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