

# EFFECT MESOHILIC

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**Submission date:** 08-Aug-2018 12:54 PM (UTC+0700)

**Submission ID:** 988397269

**File name:** 3\_STI\_Fajar-MF-Novia\_\_Effects\_of\_Mesophilic\_and\_Thermophilic.pdf (1.77M)

**Word count:** 4059

**Character count:** 21161

## Effects of Mesophilic and Thermophilic temperature condition to Biogas production (Methane) from Palm Oil Mill Effluent (POME) with Cow Manures.

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### ABSTRACT

Biogas is an environmentally friendly renewable energy source. Palm Oil Mill Effluents (POME) has the potential to produce biogas. However, the percent yield of biogas productivity is still not reach the optimum value due to the low conversion. The biogas productivity can be optimized by adding methanogen bacteria that will increase the methane production through the anaerobic fermentation process. This study aims to utilize cow manures as the source of methanogen bacteria in methane production from POME. Furthermore, this study specifically aims to obtain the optimum productivity condition of biogas production by the composition ratio of POME and cow manures to the amount of fermentation time at 35°C and 50°C for mesophilic and thermophilic bacteria, respectively. The ratio of POME and cow mature were A1 (100:0), A2 (80:20), A3 (70:30), A4 (60:40), and A5 (0:100). The highest yield of biogas production was A2 ratio using the thermophilic condition as well as 51.33% mol with the total solid decline of 73.43%, COD removal of 77.01%, and BOD removal of 70.02%.

**Keywords:** Biogas, Methane, POME, and Cow manure.

### INTRODUCTION

Malaysia and Indonesia is the biggest palm oil producer in the world. More than 80% of palm oil in the world comes from Indonesia and Malaysia. Every year there is an increasing in crude palm oil production, which means the increase in government revenue because more than 80% of palm oil is exported. However, the increasing of palm production oil is also means increasing **1** water consumption. To produce one ton of palm oil, about 5 tons of water required and most of the water will end up as waste water or Palm Oil Mill Effluent (POME) (N. Ma and A. S. H. Ong, 1985). Palm oil liquid waste has generated some sector such as condensate, clarification station, and hydro-cyclone or Palm Oil Mill Effluent (POME). POME is non-toxic waste residue, but it has high pollution due to its organic content with high BOD and COD values which showed in average as 18.000-48.000 mg/L, and 45.000-65.000 mg/L, respectively (Chin et al, 1996). The POME was treated through combined process adsorption and membrane filtration with optimum condition of 15.77% for POME concentration, 3.73 for pH, 0.5 bar trans-membrane pressure and 5 hours for filtration time would give significant effects in **1** reducing BOD, COD, TSS and turbidity (Said et al., 2016) The combination of three different pre-treatments with nanofiltration show a good results in removal of COD, TSS, colour and turbid-

**1** ity from POME. The combination adsorption and nanofiltration showed the highest reduction but the combination ultrafiltration and nanofiltration was much better in term of cost and operation times. On average, more than 90% of the parameter can be removed by the pre-treatment stage (Said et al., 2016).

In general, waste water from the ponding system will produce the greenhouse gas emission that is harmful to the **2** environment. The gasses generated in the anaerobic ponds include a mixture of methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ). Both gasses can be utilized as a renewable energy source. The potential biogas that can be produced from 600-700 kg POME approximately reaches 20 m<sup>3</sup> of biogas (Lacrosse, 2004). The components in biogas include 50 to 60 %  $\text{CH}_4$  (methane) gas, 30 to 40 %  $\text{CO}_2$  (carbon dioxide), and 5 to 10 % of  $\text{N}_2$ ,  $\text{O}_2$ ,  $\text{H}_2$  and  $\text{H}_2\text{S}$  gases. The methane which produced from livestock **2** manure contains the energy of 4800 to 6700 kcal/m<sup>3</sup>, while pure methane gas contains the energy of **8900 Kcal/m<sup>3</sup>** (Teguh et al., 2009). Biogas has an odourless and colourless properties which show the bright blue flame like LPG gas when it is burned. In the large scale, Biogas can be used as a generator of electrical energy, so it can be used as an alternative energy source that is environmentally benign (Harahap et al., 1980).

The important factor influencing the fermentation process to produce biogas in an anaerobic digester is temperature (Santoso, 2010). Temperature plays an important role in the regulating of the metabolic reaction of bacteria. The ambient temperature which is higher than the tolerable temperature will cause the protein and the other cells components to denaturation which affect the die of cells. Similarly, if the temperature of the environment is

### Article History:

Received 9 November 2017; revised 2 January 2018; accepted 2 January 2018

http://doi.org/10.26554/sti.2018.3.1.19-25

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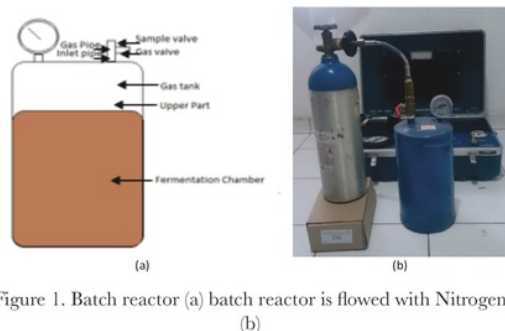


Figure 1. Batch reactor (a) batch reactor is flowed with Nitrogen (b)

below the tolerance limit, the nutrient transport will be inhibited which cause the stopping of cell metabolism. Thereby, the temperature of the anaerobic process will determine the process of organic matter and gas production. Some temperature conditions used to produce biogas in an anaerobic digester are psychrophilic conditions in which bacteria will live at temperatures (5-30°C). The mesophilic condition is the condition which the bacteria will live at temperatures 30-50°C whereas the thermophilic condition is a condition which the bacteria will live and reproduce at temperature 50-60°C (Gerardi, 2003).

The study aims to investigate how much the influences of temperature in the biogas production. The output of this research can provide a reference on the application of larger industrial scale and also provide the economic value added and quality of the waste.

## EXPERIMENTAL SECTION

### POME Sampling, added pH and Temperature

The Sampling of POME was carried out using methods according to SNI 6989-59-2004 (SNI, 2004). POME was taken at the inlet of wastewater treatment and the first anaerobic pond. The cow manure (CM) is obtained from cow farms in the Desa Air Batu, Banyuasin-Palembang, Indonesia. The sample was stored at 4°C and then analysed at the laboratory of PT. Sawit Mas Sejahtera. Before the fermentation process, some parameters such as pH, total solid sample, Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD) are measured as the initial condition.

### Sample Preparation

The sample preparation refers to Fadimtu et al (2013) with State the researcher that found the modification step. The samples are mixed between POME and CM with the predetermined ratio. The pH of the samples is adjusted at 6.8 – 7.5 (Sjafruddin, 2011). The sample is poured into a 5-liter batch reactor.

### Fermentation Process

Furthermore, The batch reactor is flowed with Nitrogen gas to eliminate the air and Oxygen (Free Oxygen) (Hamdani, 2013). The fermentation process is carried out in an incubator cabinet and incubated for 28 days at 35°C and 50°C for mesophilic and thermophilic respectively.

### Characterization of Sample and Biogas

Biogas Production and Sample identification are measured periodically at 7, 14, 21, and 28 days. The sample characterization is analyzed by standard environmental methods (APHA, 2005). The biogas production was measured using Gas Chromatography (GC).

## RESULTS AND DISCUSSION

### The formation of methane and carbon dioxide gas using mesophilic and thermophilic condition

The production of biogas under mesophilic and thermophilic condition for each ratio is shown in Figure 2 and 3. From the figure clearly seen that the mesophilic condition produce the higher biogas production.

The results showed that mesophilic and thermophilic condition produced different biogas production. (Demeyer et al., 1981) also reported that thermophilic condition would produce the higher methane gas compared to mesophilic condition. At thermophilic condition, the highest production of biogas occurred at A2 ratio and showed the higher production compared to the highest production of biogas in mesophilic condition occurred at A3 ratio. The high production of thermophilic condition produced 46.16% of methane in which the mesophilic condition only produced 35.35% methane in the highest production.

The condition of fermentation also influenced the production time of biogas. At thermophilic condition, the biogas could be produced at the second weeks whereas the mesophilic condition would produce at the third week. The condition of fermentation affected the temperature of fermentation in which the heat would cause the activity of bacteria was very fast and active. The charac-

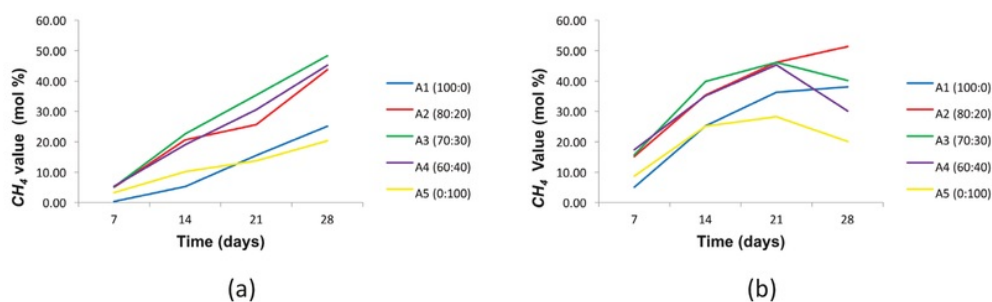
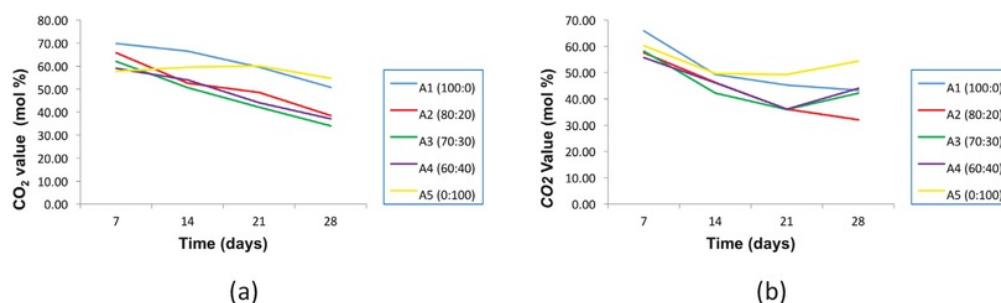


Figure 2. Biogas Production in (a) mesophilic (35°C), and (b) thermophilic Condition (50°C)

Figure 3. CO<sub>2</sub> production in (a) mesophilic (35°C), and (b) thermophilic condition (50°C)

teristics of thermophilic bacterial had faster growth compared to bacterial which growth in mesophilic condition (20°C-40°C) due to shorter cell membrane division in the process of breeding.

In the first week of the mesophilic reactor, gas production is not significantly different from the production of gas produced by the thermophilic reactor, but the gas begins to rise during the second week onwards, and the gas produced by the mesophilic reactor is lower than that of methane gas by the thermophilic reactor. This is in accordance with research that methane gas production began to increase in the second week (Fadimtu et al, 2013).

In the second week the production of methane gas at the thermophilic reactor increased compared to the mesophilic reactor and by the third week, the production of methane gas at the thermophilic reactor was higher (methane gas 46.16% in composition A2) than the highest mesophilic reactor production (35.35% methane gas in A3). This is because at high temperatures the activity of bacteria is very fast and active. The characteristics of thermophilic bacteria have faster growth compared to bacterial growth in mesophilic conditions (20°C to 40°C), cell membrane division in the process of breeding in shorter thermophilic bacteria.

At the end of the fourth week the mesophilic reactor generates the highest methane gas in the composition A3 (70:30) and at the thermophilic reactor in the composition A2 (80:20) produces the highest methane gas because of this composition the bacterial growth is faster so it is the optimum substrate composition to produce methane gas. Bacterial populations in thermophilic conditions can produce more enzymes and high enzyme concentrations can accelerate the rate of biochemical reactions in the hydrolysis

process.

The hydrolysis process is carried out by the hydrolysis bacteria in which the bacteria work to degrade the carbohydrate, fat and protein content of the substrate as the bacterial food. The result of chemical reaction produced by hydrolysis bacteria, which is the biochemical reaction which is catalyzed by the enzyme produced by hydrolysis bacteria in the form of organic and glucose products and CO<sub>2</sub> and H<sub>2</sub> compounds will be converted by acid bacteria into alcohol and acetic acid. A large number of anaerobic and facultative bacteria involved in hydrolysis and fermentation processes of organic compounds include *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Streptococcus*. Acidogenic bacteria (acid-forming) such as *Clostridium*, acetogenic bacteria (Acetate-producing bacteria and H<sub>2</sub>) such as *Syntrophobacter wolfei* and *Syntrophomonas wolfei*. At thermophilic temperatures, microorganisms such as Methanosarcinaceae on the substrate surface reach 70-100% more than in the lower layer or in the middle layer. At high temperatures (thermophilic conditions) cell division in bacterial proliferation is faster than cell division in bacterial proliferation under mesophilic conditions (Domaschko et al., 2010).

According to Dhadse et al (2012), which successfully obtained 8 bacterial isolates from cow manure. Four of the 8 isolates are methanogenic bacteria and 4 other isolates are nonmethanogenic bacteria. The methanogenic bacteria include *Methanobrevibacter ruminantium*, *Methanobacterium formicum*, *Methanosarcina frisia*, and *Methanotherix soehngenii*. While the nonmethanogenic bacteria include *Clostridium*, *Propionibacterium*, *Bacteroides*, and *Peptostreptococcus*.

While in Divya et al (2014) mentioned that there are 9 species of bacteria successfully isolated from cow manure in anaerobic

Table 1. Kinetic reaction of methane gas production to ratio between POME and Cow manure in mesophilic condition

Material		Code	Time (hour)					Kinetic reaction rate
Ratio			0	7	14	21	28	
POME	Cow manure		Parameter					
			CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	
			k	k	k	k	k	
100	0	A1	0	-0.17	0.12	0.13	0.12	y = 0,0076x - 0,0682
80	20	A2	0	0.23	0.22	0.15	0.13	y = 0,0030x + 0,1104
70	30	A3	0	0.23	0.22	0.17	0.14	y = 0,0028x + 0,1085
60	40	A4	0	0.24	0.21	0.16	0.14	y = 0,0028x + 0,1105
0	100	A5	0	0.17	0.17	0.12	0.11	y = 0,0025x + 0,0789



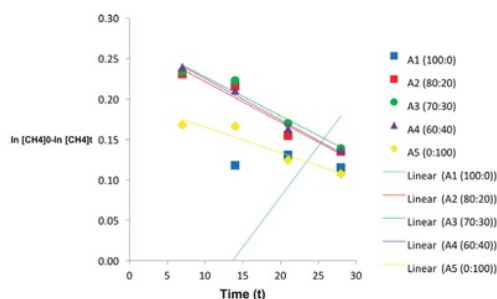


Figure 4. Kinetics reaction of methane production from POME and Cow manure on mesophilic condition (35°C)

decay conditions. Six of the 9 isolates are hydrolytic bacteria (*Bacteroides nordii*, *Clostridium perfringens*, *Prevotella bivia*, *Porphyromonas asaccharolytica*, *Ruminococcus gnavus*, *Lactobacillus acidophilus*), 1 isolates are *acetobacter syzygii* bacteria, and 2 isolates are methanogenic bacteria (*Methanobacterium formicicum* and *Methanosarcina siciliae*).

In addition, temperatures also affected the activity of microorganisms in the substrate conversion. The substrate conversion activity will be obtained at the maximum when it is at the optimum temperature of fermentation (I. Angelidakia and Kaparaju, 2007). At the end of the fermentation, the highest percentage of methane gas production in the mesophilic reactor was 48.33% in the A3 ratio whereas the highest methane production in the thermophilic reactor was 51.33% in A2 ratio.

The percentage of CO<sub>2</sub> productions in both conditions showed significantly less productivity. The highest CO<sub>2</sub> production was 34.10% in the A3 ratio in mesophilic condition while the thermophilic condition produced only 32.10% in the A2 ratio.

In general. The first order reaction is a reaction whose speed is directly proportional to the concentration of one compound.



$$\frac{d[CH_4]}{dt} = k[CH_4]$$

The results give the value of CH<sub>4</sub> concentration at various times. If the concentration at t=0 is CH<sub>4</sub>₀ and at t = t is CH<sub>4</sub>ₜ, then the integration is

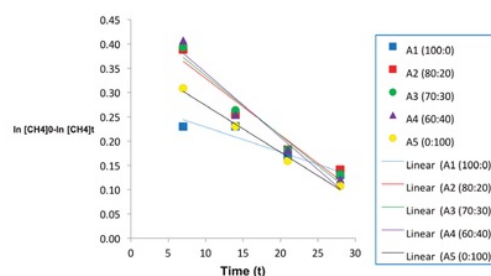


Figure 5. Kinetic reaction of methane production from POME and Cow manure on thermophilic condition (50°C)

$$\int_{[CH_4]_0}^{[CH_4]_t} \frac{d[CH_4]}{[CH_4]} = k \int_0^t dt$$

$$-\ln \frac{[CH_4]_t}{[CH_4]_0} = kt$$

$$\ln[CH_4]_t = -kt + \ln[CH_4]_0$$

Then the k constant can be calculated by the formula given:

$$\ln[CH_4]_0 - \ln[CH_4]_t = kt$$

Table 1 and figure 4 the kinetics reaction of methane gas to the ratio between POME and Cow manure in mesophilic condition.

Table 2 and figure 5 presented the kinetics reaction of methane gas to the ratio between POME and Cow manure in thermophilic condition.

#### Effect the fermentation condition on the change of substrate pH

The condition of fermentation showed a quite significant difference in the pH change of the substrate. In the mesophilic condition, the pH of substrate did not significantly change along with the increasing of fermentation time, whereas in the thermophilic condition, the pH of substrate positively correlated to the increase of fermentation time. Figure 6 showed the pH value of substrate (a) at the mesophilic condition, and (b) thermophilic condition.

In the beginning, the pH value of the substrate decreased as a result of the acidification process. According to (Carneiro et al.,

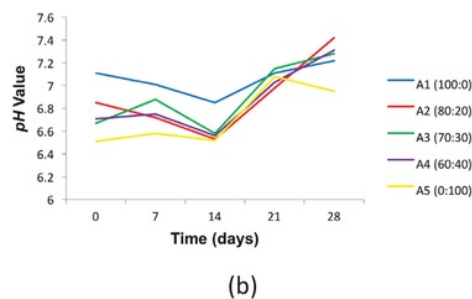
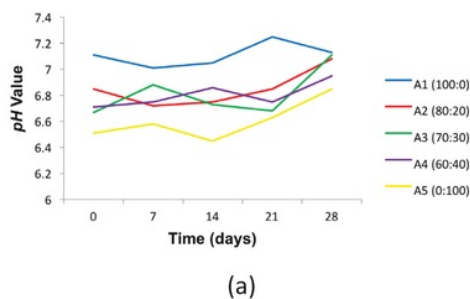
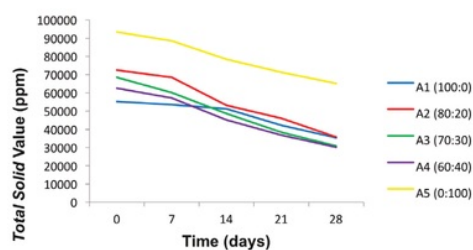


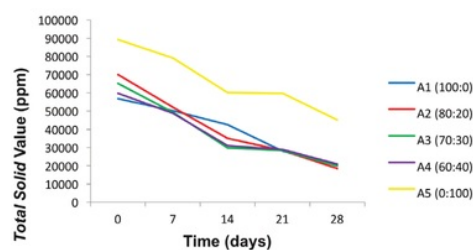
Figure 6. The pH value of Substrate at (a) mesophilic condition, and (b) thermophilic condition.

Table 2. Kinetic reaction of methane gas production to ratio between POME and Cow manure in thermophilic condition

Material		Code	Time (hour)					Kinetic reaction rate
Ratio			0	7	14	21	28	
POME	Cow manure		Parameter					
			CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	CH <sub>4</sub>	
			k	k	k	k	k	
100	0	A1	0	0,23	0,23	0,17	0,13	y = 0,0029x + 0,1121
80	20	A2	0	0,39	0,25	0,18	0,14	y = 0,0002x + 0,1895
70	30	A3	0	0,39	0,26	0,18	0,13	y = 0,0011x + 0,1784
60	40	A4	0	0,41	0,25	0,18	0,12	y = 0,0008x + 0,1835
0	100	A5	0	0,31	0,23	0,16	0,11	y = 0,0009x + 0,0148



(a)



(b)

Figure 7. Total Solid Conversion on (a) Mesophilic, and (b) Thermophilic condition

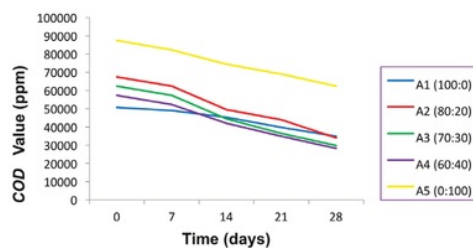
2008) the acidification was a process of converting the organic molecules as the hydrolysis process. The acidification products were the volatile fatty acids which had characteristics as acidic molecules such as acetic acid, propionate acid, and butyrate acid. After acidification process, the fatty molecules would be converted to methane by methane-bacterium in the methanogenesis process. The substituting of fatty acid to methane would increase the pH value of substrate in neutral or alkaline condition (Gerardi, 2003).

#### Total Solid Conversion of Mesophilic and Thermophilic Condition

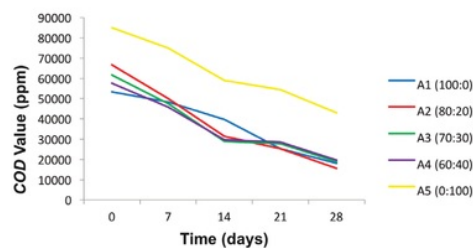
The change in total solids contents was one of the indicators of the biomass conversion contained in POME. The measurement

of the total solid content was calculated until the 28<sup>th</sup> day of the fermentation process. In the reactor with the mesophilic condition, there was a decrease of total solid content from A1, A2, A3, A4, and A5 composition of 34.76%, 50.14%, 54.78%, 51.63%, and 30.33%, respectively. In the other hand at the thermophilic condition, the decrease of total solid content from A1, A2, A3, A4, and A5 were 64.62%, 73.43%, 69.54%, 64.88%, and 49.46%, respectively. The decreasing of total solid was caused by the degradation of organic compound in the hydrolysis process. The highest decrease in total solid content was generated at the thermophilic condition in ratio A2 with a decreasing value of 73,43%.

#### Effects of the fermentation condition to the Chemical



(a)



(b)

Figure 8. The COD values on (a) Mesophilic and (b) thermophilic condition.

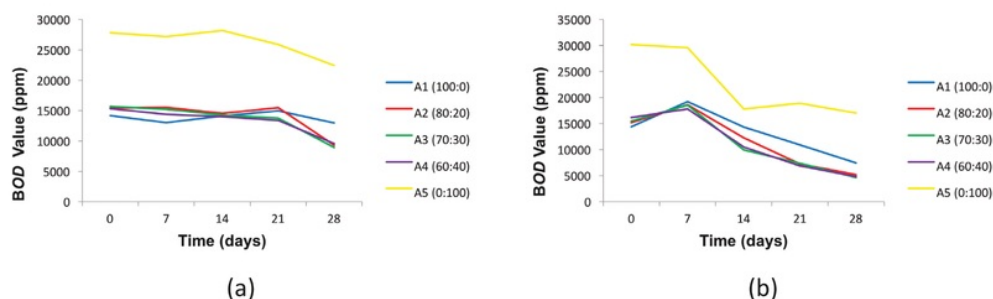


Figure 9. The BOD values on (a) Mesophilic and (b) thermophilic condition.

### Oxygen Demand (COD)

Figure 7 showed the value of COD in various ratios between POME and Cow manure. The COD value tended to decrease with the increase of the fermentation time. The COD values were calculated until the 28<sup>th</sup> day of the fermentation process. The COD value in the A1, A2, A3, A4, and A5 ratios were 31.20%, 49.44%, 52.07%, 50.80%, and 28.60%, respectively. In the other hand, the COD value of the A1, A2, A3, A4, and A5 ratios in thermophilic condition were 64.53%, 77.01%, 69.83%, 65.75%, and 50.81%. The highest decrease in COD values was obtained in thermophilic reactors with the A2 composition which showed the decrease of 77.01%.

This study proved that the organic substances present in the POME could be almost degraded by the microorganism that operates the batch reactor. The length of contact time between microorganisms to POME would give the optimum condition to degrade the organic compounds contained in POME so that causing the decrease of COD values (Ambar et al., 2004)

### Effects of the fermentation condition on the Biological Oxygen Demand (BOD)

Figure 8 showed the BOD values in various ratios between POME and Cow Manure. The BOD value tended to decrease with the increase of the fermentation time. The BOD values were calculated until the 28<sup>th</sup> day of the fermentation process. The BOD value in the A1, A2, A3, A4, and A5 ratios were 8.29%, 39.81%, 43.06%, 37.25%, and 19.33%, respectively. In the other hand, the BOD value of the A1, A2, A3, A4, and A5 ratios in thermophilic condition were 48.26%, 66.01%, 69.57%, 70.22%, and 43.58%. This condition was caused by the degradation of organic material in the hydrolysis process. The highest decrease in BOD values was obtained in thermophilic reactors with the A2 composition which showed the decrease of 70.02%.

### CONCLUSION

The conclusions that could be obtained from this study include: The highest methane gas production was obtained from the A2 ratio in the thermophilic condition in which the highest methane gas production was 51.33 % mol. Both mesophilic and thermophilic condition showed the positive correlation between the pH value of substrate and the fermentation time. The highest de-

crease of total solids, COD, and BOD was obtained in the A2 ratio at the thermophilic condition that is equal to 73.43%, 77.01%, and 70.02%, respectively.

### ACKNOWLEDGEMENT

The author is grateful to Universiti Sriwijaya (UNSRI), PT. Sawit Mas Sejahtera and PT. (Pupuk Sriwijaya) PUSRI for supporting through complete this research.

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