Effect of temperature on methanogenesis stage of two-stage anaerobic digestion of palm oil mill effluent (POME) into biogas

B Trisakti*, Irvan, Mahdalena, Taslim, and M Turmuzi
Chemical Engineering Department, Faculty of Engineering, University of Sumatera Utara. Jalan Almamater Komplek USU Medan, 20155, Indonesia

Abstract. This study aimed to determine the effect of temperature on methanogenesis stage of conversion of palm oil mill effluent into biogas. Methanogenesis is the second stage of methanogenic anaerobic digestion. Improved performance of the methanogenesis process was determined by measuring the growth of microorganisms, degradation of organic materials, biogas production and composition. Initially, the suitable loading up was determined by varying the HRT 100, 40, 6, and 4.0 days in the continuous stirred tank reactor (CSTR) with mixing rate 100 rpm, pH 6.7-7.5 at room temperature. Next, effect of temperature on the process was determined by varying temperature at mesophilic range (30-42°C) and thermophilic range (43-55°C). Analysis of total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), and chemical oxygen demand (COD) were conducted in order to study the growth of microorganisms and their abilities in converting organic compound to produce biogas. Degradation of organic content i.e. VS decomposition and COD removal increased with the increasing of temperature. At mesophilic range, VS decomposition and COD removal were 51.56 ± 8.30 and 79.82 ± 6.03%, respectively. Meanwhile at thermopilic range, VS decomposition and COD removal were 67.44 ± 3.59 and 79.16 ± 1.75%, respectively. Biogas production and its methane content also increased with the increasing of temperature, but CO₂ content also increased. Biogas production at mesophilic range was 31.77 ± 3.46 L/kg-ΔVS and methane content was 75%. Meanwhile, biogas production at thermopilic range was 37.03 ± 5.16 L/kg-ΔVS and methane content was 62.25 ± 5.50%.

1. Introduction
Indonesia is the largest producer of palm oil in the world with a total production reached 33.5 million tons. Palm oil is produced from approximately 9.2 million hectares of oil palm plantations [1], which mostly located on the island of Sumatra and Borneo [2]. The process to extract oil requires significantly large amounts of water for steam sterilization of fresh palm fruit bunches (FFB) and clarification of extracted oil. As a result, palm oil mill (POM) produces large amounts of wastewater or commonly called palm oil mill effluent (POME). It is estimated in 2015 about 79.2-122.1 million tons or approximately 2.4 to 3.7 tons of POME was generated per ton of palm oil [3].

In an effort to reduce HRT, many researchers separate the anaerobic digestion process into two stages namely acidogenesis and methanogenesis stage [4]. Therefore, to improve the effectiveness of this process, the optimum operating conditions such as pH, temperature, and agitation rate of each
stage should be known [5,6]. Unfortunately, few data can be found in the literature about the effect of temperature on the growth of microorganisms in the methanogenesis stage. Therefore, this paper reported the effects of organic loading up (reduction of HRT) and temperature variations on methanogenesis stage or the second stage of two stages anaerobic digestion of conversion of POME to biogas.

Fresh POME taken from fat pit of POMs are brownish viscous mud with temperatures around 80-90°C, acidic (pH 3.8-4.5), and the concentration of organic compounds is high enough so the COD and BOD are also high [7]. POME contains large amounts of carbohydrates, protein, and fat with the composition 29.55, 12.75, and 10.21% respectively. In addition, there are also some macro and micro mineral compounds such as potassium (K), sodium (Na), calcium (Ca), iron (Fe), zinc (Zn), chromium (Cr), and others [8]. Therefore, POME can be utilized as substrate and nutrients for microorganisms in methanogenic anaerobic digestion for biogas production [9,10].

A two-stage process of anaerobic digestion was used in this study. In the first stage, the organic compounds were converted into volatile fatty acids (VFA) by hydrolysis, acidogenesis, and acetogenesis (commonly referred as acidogenesis stage) and subsequently the VFA was converted into biogas through methanogenesis stage [11–15]. Methanogenesis is the most important stage in the process of methanogenic anaerobic digestion because in this process occurs the slowest biochemical reactions.

Methanogenesis stage is greatly influenced by the operating conditions such as the composition of raw materials, feed flow rate (hydrolic retention time, HRT), temperature, agitation, and pH. Digester over loading, temperature changes, or the entry of a large amount of oxygen can result in termination of methane production [9,10].

Methanogenic microorganisms are generally more sensitive to temperature fluctuations compared to other microorganisms involved in anaerobic digestion process [16]. Methanogenic anaerobic digestion process has been reported to take place at several temperature ranges namely psychrophilic (below 25°C), mesophilic (25-45°C) and thermophilic (45-70°C) [9,17]. Temperature range commonly used in the process of methanogenic anaerobic digestion are mesophilic and thermophilic. Several researchers have reported that the mesophilic temperature optimum is in the range of 30-35°C and thermophilic temperature optimum is in the range 50-65°C [16,18].

### 2. Materials and Methods

#### 2.1. Materials

The feed was effluent from acidogenesis reactor where its characteristics are presented in Table 1. The acidogenesis reactor used fresh POME collected from “a fat pit of Adolina POM, Sumatera Utara, Indonesia” as feed. Methagenic bacteria as a starter were collected from 3000 l methanogenic anaerobic digester at the University of Sumatera Utara, Medan, Indonesia. Sodium bicarbonate (NaHCO₃) was used for adjusting the pH.

| Parameters                        | Units | Results          |
|-----------------------------------|-------|------------------|
| pH                                |       | 5.0 - 5.5        |
| Chemical Oxygen Demand (COD)      | mg/L  | 26,900 - 43,130  |
| Total Solid (TS)                  | mg/L  | 20,340 - 37,930  |
| Volatile Solid (VS)               | mg/L  | 18,980 - 36,980  |
| Total Suspended Solid (TSS)       | mg/L  | 11,480 - 18,440  |
| Volatile Suspended Solid (VSS)    | mg/L  | 10,860 - 16,920  |
| Volatile Fatty Acids (VFA)        | mg/L  | 5,622            |
2.2. Experimental Setup
The laboratory scale of continuous stirred tank reactor (CSTR) as fermenter is shown in Figure 1. The CSTR was EYELA model MBF 300ME with working volumes is 2 L. The fermenter comprised of an integrated on-line temperature and pH data recording system. No pH adjustment was applied to the fermenter. However, pH of the feed was controlled with sodium bicarbonate (NaHCO₃), before it was fed into the fermenter.

![Figure 1. Experimental set-up](image)

2.3. Data Collection
The research was conducted to determine the improvement of the methanogenesis process by measuring the growth of microorganisms, the degradation of organic materials, and production and composition of biogas. Initially, the suitable loading up was determined by varying the HRT 100, 40, 6, and 4.0 days with mixing rate 100 rpm, pH 7± 0.2, and room temperature. Then the effect of temperature on the process was determined by varying temperature at mesophilic range (30-42°C) and thermophilic range (43-55°C) with HRT 4 days, pH 7 ± 0.2, and agitation rate of 100 rpm.

Analysis and methods of data collection of the liquid and gas samples are presented in Table 2. Data collected from the liquid samples were pH, alkalinity, TS, VS, TSS, VSS, and COD based on the APHA standard methods for water and wastewater [19]. Meanwhile the volume and composition of biogas were collected for the gas samples.

Table 2. Analysis methods of data collection

| Parameters               | Units   | Methods     |
|--------------------------|---------|-------------|
| pH                       | -       |             |
| Alkalinity               | mg/L    | APHA 2320B  |
| Total Solid (TS)         | mg/L    | APHA 2540B  |
| Volatile Solid (VS)      | mg/L    | APHA 2540E  |
| Total Suspended Solid (TSS) | mg/L    | APHA 2540D  |
### Parameters

| Parameters                  | Units | Methods                        |
|-----------------------------|-------|--------------------------------|
| Volatile Suspended Solid (VSS) | mg/L  | APHA 2540E                     |
| Chemical Oxygen Demand (COD) | mg/L  | APHA 5220B                     |
| Biogas Volume               | ml/day| Gas meter                      |
| Biogas composition          |       |                                |
|    • H₂S                    | mg/L  | Gas detector tube (GASTEC, Type GV-100S) |
|    • CO₂                    | %     | Gas detector tube (GASTEC, 25~1600 ppm) |
|    • Methane                | %     | Calculated                     |

### Results and Discussions

#### 3.1. Profile of pH and Alkalinity during The Process

The pH and alkalinity profile is presented in Figure 2. The average pH at loading up, mesophilic, and thermophilic range were 6.89 ± 0.3, 7.02 ± 0.08, and 7.0 respectively. The pH was relatively constant at 7.0 as expected.

The pH was maintained by adding sodium bicarbonate into the feed. Consequently, alkalinity profile fluctuated during the process but it is still in the range where the methanogenesis process takes place i.e. in the range of 2,500 – 5,000 mg/L [17]. The average alkalinity value at loading up, mesophilic, and thermophilic range were 3,067.31 ± 569.90 mg/L, 3,213.04 ± 349.10 mg/L, and 4,440.91 ± 451.88 mg/L respectively.

![Figure 2](image.png)

**Figure 2.** Profile of pH and alkalinity during the process

#### 3.2. Effect of Temperature on the Growth of Microorganisms

Microorganism growth is described by the change of volatile suspended solid (VSS) concentration in the fermenter. The VSS profile during the process is presented in Figure 3.

The average values of VSS at loading up, mesophilic, and thermophilic range were 21,691.89 ± 1,238.61, 19,875.73 ± 1,766.07, and 22,170.14 ± 1,281.13 mg/L, respectively which indicated that the number of microbes increased with the increase of temperature.
3.3 Effect of Temperature on the Degradation of Organic Materials
Effect of temperature on microbial activity can be observed from VS decomposition and COD removal during the process as presented on Figure 4. The average values of VS decomposition at loading up, mesophilic, and thermophilic range were 53.90 ± 3.95, 51.56 ± 8.30, and 67.44 ± 3.59%, respectively. While the COD removal at loading up, mesophilic, and thermophilic range were 87.28 ± 3.63, 79.82 ± 6.03, and 79.16 ± 1.75%, respectively, both indicating that microbial activity increased with the increase of temperature.

3.4 Effect Temperature on Production and Composition of Biogas
Effect of temperature on production of biogas per volatile solid decomposed during the process is presented in Figure 5. The average biogas production at loading up, mesophilic, and thermophilic
range were 18.90 ± 8.11, 31.77 ± 3.46; and 37.03 ± 5.16 L/kg-ΔVS, respectively. Biogas production is increasing with increase of temperature. Similar result was reported by previous researchers [20–24]. This is in accordance with the growth of microorganisms and degradation of organic materials which also increased when the temperature was increased.

![Figure 5](image1.png)

**Figure 5.** Biogas production during the process

The amount of H$_2$S, CO$_2$, and CH$_4$ contained in the biogas produced during the process is presented in Figure 6. The amount of H$_2$S in the biogas produced was in trace amount except at loading up it was 0.30 ± 0.30 L/kg-ΔVS. The average values of CO$_2$ at loading up, mesophilic, and thermophilic range were 3.75 ± 2.57, 9.88 ± 3.23, and 13.97 ± 1.98 L/kg-ΔVS, respectively. The average value of CH$_4$ at loading up, mesophilic, and thermophilic range were 15.08 ± 6.62, 22.30 ± 5.77, and 23.39 ± 4.82 L/kg-ΔVS, respectively.

![Figure 6](image2.png)

**Figure 6.** The amount of H$_2$S, CO$_2$, and CH$_4$ contained in the biogas produced during the process.
Biogas production increased with increasing temperature, but the amount of CO\textsubscript{2} in the biogas also increases. The CO\textsubscript{2} and CH\textsubscript{4} values at mesophilic range were 31.43 ± 12.55 and 68.57 ± 12.56% respectively, while at thermopilic range; they were 37.75 ± 5.60 and 62.25 ± 5.50% respectively.

4. Conclusions
Degradation of organic content ie VS decomposition and COD removal increased with increasing temperature. In mesophilic range, VS decomposition and COD removal were 51.56 ± 8.30 and 79.82 ± 6.03%, respectively. Meanwhile at thermopilic range, VS decomposition and COD removal were 67.44 ± 3.59 and 79.16 ± 1.75%, respectively. Biogas production and its methane content also increases with the increasing of temperature, but CO\textsubscript{2} content also increases. Biogas production at mesophilic range was 31.77 ± 3.46 L/kg-ΔVS and methane content was 68.57 ± 12.56%. Meanwhile, biogas production at thermopilic range was 37.03 ± 5.16 L/kg-ΔVS and methane content was 62.25 ± 5.50%.

Acknowledgment
This research was supported by Hibah Penelitian Unggulan Perguruan Tinggi No: 017/SP2H/LT/DRPM/II/2016, date February 17, 2016.

References
[1] Wright T and Rahmanulloh A 2016 Oilseeds and Products Annual Report 2016
[2] Jaenicke J, Rieley J O, Mott C, Kimman P and Siegert F 2008 Determination of the amount of carbon stored in Indonesian peatlands Geoderma 147 151–8
[3] Trisakti B, Manalu V, Irvan, Taslim and Turmuzi M 2015 Acidogenesis of Palm Oil Mill Effluent to Produce Biogas : Effect of Hydraulic Retention Time and pH Procedia - Soc. Behav. Sci. 195 2466–74
[4] Jung J-Y, Lee S-M, Shin P-K and Chung Y-C 2000 Effect of pH on phase separated anaerobic digestion Biotechnol. Bioprocess Eng. 5 456–9
[5] Veeken A, Kalyuzhnyi S, Scharff H and Hamelers B 2000 Effect of pH and VFA on Hydrolysis of Organic Solid Waste 6 1076–81
[6] Polprasert C 2007 Organic Waste Recycling - Technology and Management (London: Iwa Publishing)
[7] Zinatizadeh A A L, Mohamed A R, Najafpour G D, Hasnain Isa M and Nasrollahzadeh H 2006 Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed film bioreactor Process Biochem. 41 1038–46
[8] Salihu, A. and Alam M z. 2012 Palm oil mill effluent: a waste or a raw material? J. Appl. Sci. Res. 8 466–73
[9] Seadi T A, Rutz D, Prassl H, Köttner M, Finsterwalder T, Volk S and Janssen R 2008 Biogas Handbook (Esbjerg: University of Southern Denmark)
[10] Irvan, Trisakti B, Sosanty F and Tamiuchi Y 2016 Effect of Discontinuing Sodium Bicarbonate on Fermentation Process of Palm Oil Mill Effluent Asian J. Chem. 28 377–80
[11] Olvera R and Lopez-lopez A 2009 Biogas Production from Anaerobic Treatment of Agro-Industrial Wastewater Biogas ed S Kumar (Shanghai, China: InTech China) pp 91–103
[12] Li W W and Yu H Q 2011 From wastewater to bioenergy and biochemicals via two-stage bioconversion processes: A future paradigm Biotechnol. Adv. 29 972–82
[13] Blonskaja V, Menert A and Vilu R 2003 Use of two-stage anaerobic treatment for distillery waste Adv. Environ. Res. 7 671–8
[14] Demirer G N and Othman M 2008 Two-Phase Thermophilic Acidification and Mesophilic Methanogenesis Anaerobic Digestion of Waste-Activated Sludge Environ. Eng. Sci. 25 1291–300
[15] Wong Y-S, Teng T T, Ong S-A, Norhashimah M, Rafatullah M and Lee H-C 2013 Anaerobic Acidogenesis Biodegradation of Palm Oil Mill Effluent Using Suspended Closed Anaerobic Bioreactor (SCABR) at Mesophilic Temperature Procedia Environ. Sci. 18 433–41

[16] Schunurer A and Jarvis A 2009 Microbiological Handbook for Biogas Plants (Malmö)

[17] Zupan G D and Grilc V 2012 Anaerobic Treatment and Biogas Production from Organic Waste Management of Organic Waste ed S Kumar (Shanghai: InTech China) pp 3–28

[18] Verma S 2002 Anaerobic Digestion of Biodegradable Organics in Municipal Solid Wastes (Columbia University)

[19] APHA 1999 Standard Methods for the Examination of Water and Wastewater (Washington, DC)

[20] Choorit W and Wisarnwan P 2007 Effect of temperature on the anaerobic digestion of palm oil mill effluent Electron. J. Biotechnol. 10 376–85

[21] Bolzonella D, Fatone F, Di Fabio S and Cecchi F 2009 Mesophilic, thermophilic and temperature phased anaerobic digestion of waste activated sludge Chem. Eng. Trans. 17 385–90

[22] Bouallagui H, Rachdi B, Gannoun H and Hamdi M 2009 Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors Biodegradation 20 401–9

[23] O-Thong S, Boe K and Angelidaki I 2012 Thermophilic anaerobic co-digestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production Appl. Energy 93 648–54

[24] Ventura J-R S, Lee J and Jahng D 2014 A Comparative Study On The Alternating Mesophilic And Thermophilic Two-Stage Anaerobic Digestion Of Food Waste J. Environ. Sci. 26 1274–83