Effect of Agitation on Acidogenesis Stage of Two-Stage Anaerobic Digestion of Palm Oil Mill Effluent (POME) into Biogas

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Abstract. The acidogenesis stage in two-stage anaerobic digestion of palm oil mill effluent (POME) was studied in a continuous stirred tank reactor (CSTR). This research investigated the effect of agitation rate on the growth of microorganisms, the degradation of organic substances, and volatile fatty acids (VFA) production and composition. Initially, the suitable loading up was determined by varying the HRT 6.7, 5.0, and 4.0 days in a 2 L CSTR with agitation rate 50 rpm, pH 6.0 ± 0.2, at room temperature. Next, effect of agitation on the process was determined by varying agitation rate at 25, 50, 100, and 200 rpm. Analysis of total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), and volatile fatty acids (VFA) were conducted in order to study the growth of microorganisms and their abilities in converting organic compound to produce VFA. The highest growth of microorganisms was achieved at HRT 4.0 day with microorganism concentration was 20.62 mg VSS/L and COD reduction was 15.7%. The highest production of total VFA achieved was 5,766.61 mg/L at agitation rate 200 rpm, with concentration of acetic acid, propionic acid, and butyric acid were 1,889.23; 1,161.43; and 2,725.95 mg/L, respectively. While degradation VS and COD were 16.61 and 38.79%.

1. Introduction
Indonesia is currently 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]. 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 [2].

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 COD and BOD of POME are also high [3]. 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 [4]. Therefore, POME can be utilized as substrates and nutrients for microorganisms in methanogenic anaerobic digestion for biogas production [5].
POME as feedstock utilization of biogas through methanogenic anaerobic digestion process has been widely reported by the researchers. Furthermore, it has been applied at commercial scale of several POMs in Malaysia and Indonesia [6]. However, the hydraulic retention time (HRT) of the process is still high enough, so that high investment are needed to build this process [7].

In an effort to reduce HRT, many researchers separated the anaerobic digestion process into two stages namely acidogenesis and methanogenesis stage [8]. 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 [2,9,10].

A two-stage process of anaerobic digestion was conducted 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). Subsequently the VFA was converted into biogas through methanogenesis stage [11–15]. Environmental factors that influence the acidogenesis stages are substrate, inoculum, pH, temperature, and HRT [2,16,17]. Low pH and short HRT are conditions favored by acid-forming bacteria to grow and may inhibit the growth of microorganisms forming methane [18]. Therefore, control of HRT is needed to help multiply the microorganisms of hydrolysis and acidogenesis [19] and will increase the production of VFA [20].

Continuous stirred tank reactor (CSTR) is the most commonly digester used in methanogenic anaerobic process where the substrate agitation in the digester is necessary in the formation of biogas [21]. The purpose of agitation is to enhance microorganisms and substrate contact and distribution, ensures uniform pH and temperature, prevents deposition of denser solids at the bottom and flotation of lighter solids at the top, and helps to release biogas bubbles. Therefore, this paper reported the effects of agitation rate on acidogenesis stage or the first stage of two stages anaerobic digestion in conversion of POME to biogas.

2. Materials and Methods

2.1. Materials
Fresh POME was collected from a fat pit of Adolina POM, Sumatera Utara, Indonesia, and stored in a clean 1000 L container. It was transported to the laboratory, mixed well, put in 1-liter plastic bottles, and stored at 4 °C until further use. Fresh POME characteristics are shown in Table 1. Acidogenic bacteria as starter were collected from acidification pond of WWTP of POM at Torgamba Sumatera Utara, Indonesia. Sodium bicarbonate (NaHCO₃) was used for adjusting the pH.

| Parameters                          | Units | Results       | Methods         |
|-------------------------------------|-------|---------------|-----------------|
| pH                                  | -     | 3.70 – 4.70   |                 |
| Chemical Oxygen Demand (COD)        | mg/L  | 48,300        | APHA 5220B      |
| Total Solid (TS)                    | mg/L  | 13,420 – 37,020 | APHA 2540B    |
| Volatile Solid (VS)                 | mg/L  | 10,520 – 31,220 | APHA 2540E    |
| Total Suspended Solid (TSS)         | mg/L  | 2,080 – 27,040 | APHA 2540D      |
| Volatile Suspended Solid (VSS)      | mg/L  | 1,920 – 25,800 | APHA 2540E      |
| Oil and Grease                      | mg/L  | 6.25          | APHA 5520B      |
| Protein                             | %     | 0.53          | Kjeldahl        |
| Carbohydrate                        | %     | trace         | Lane Eynon      |
2.2. Experimental Setup
The experimental setup is shown in Figure 1. The main equipment are feed tank (2), continuous stirred tank reactor (CSTR) as fermenter (4), screw pump (3), and discharge tank (9). The CSTR is EYELA model MBF 300ME made in Japan with working volume is 2 L. The fermenter was equipped with 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 (NaHCO$_3$), before it was fed into the fermenter.

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

2.3. Data Collection
Data collected during the experiment were pH, alkalinity, TS, VS, TSS, VSS, COD, and VFA, in accordance to the American Public Health and Association standard methods (APHA) for water and wastewater [22] as presented in Table 2.

| 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      |
| Volatile Suspended Solid (VSS)    | mg/L  | APHA 2540E      |
| Chemical Oxygen Demand (COD)      | mg/L  | APHA 5220B      |
| Volatile Fatty Acids (VFA)        | mg/L  | APHA 5560B      |

3. Results and Discussions
3.1. Effect of HRT reduction
Microbial adaptation and growth of the starter with VSS 8,060 mg/L were carried out by reducing the HRT or organic loading up. During loading up, a series of continuous experiments were conducted by
using various feed flow-rates of 298, 400, and 500 ml of POME per day, which relate to HRT of 6.7, 5.0, and 4.0 days. The fermenter was controlled at room temperature, and mixing rate 50 rpm. The pH was kept constant at 6 ± 0.2. The experiment was conducted in intermittent operation mode, where fresh feed and effluent where flown in every 4 hours or 6 times per 24 hours. pH, alkalinity, TS, and VS were analysed every day, while TSS and VSS were analysed in every three days, while the COD and VFA were analysed after the stable condition achieved. Results for organic loading up have been reported in detail [2].

The pH value slightly fluctuated but remained constant at 6, because the addition of sodium bicarbonate in the feed. As a result, large fluctuation in the value of alkalinity occurred, although it was still in the range where the acidogenesis process take place i.e. in the range of 542 – 3.580 mg/L [23,24]. The pattern of microbial growth is significantly influenced by the slightest change in pH during the process. The highest concentration of microbes achieved at HRT 4 days was 20,620 mg VSS/L, although this was the shortest HRT. The decrease of COD indicated the occurrence of microbial growth but the expected decrease COD is not too large, because the desired product of acidogenesis process is VFA.

3.2. Effect of Agitation on The Acidogenesis

Effect of agitation on acidogenesis process was studied by conducting a series of experiments by varying the agitation rate at 25, 50, 100, and 200 rpm, pH 5.5 ± 0.2, temperature of 55°C, and HRT 4 days. The experiment was conducted in intermittent operation mode, where fresh feed and effluent where flown in every 4 hours or 6 times per 24 hours. The pH was maintained by adding sodium bicarbonate (NaHCO₃) in the feed. The pH, alkalinity, TS, and VS from the feed and effluent were analysed every day, while the TSS, VSS, COD and VFA were analysed after the stable data was achieved. The results of experiments are presented in a graph, showing the profile of pH and alkalinity, the growth of microorganisms, the degradation of organic substances, VFA production and composition, and the VFA-alkalinity ratio.

3.2.1. Profile of pH and Alkalinity during The Process The profile of pH and alkalinity during the process are presented in Figure 2. The average pH at 25, 50, 100, and 200 rpm were 5.41 ± 0.12; 5.41 ± 0.05; 5.43 ± 0.14; and 5.52 ± 0.16, respectively. The pH was relatively constant at 5.5 ± 0.2 as expected.

![Figure 2. Profile of pH and alkalinity during the process](image-url)
The pH was maintained by adding sodium bicarbonate into the feed. Consequently, alkalinity profile fluctuated during the process but it was still in the range where the acidogenesis process take place i.e. in the range of 542 - 3580 mg/L [23, 24]. The average alkalinity value at 25, 50, 100, and 200 rpm were 2,466.67 ± 385.76; 2,513.33 ± 258.06; 2,518.75 ± 424.61; and 2,320.00 ± 544.06 mg/L respectively.

3.2.2. Effect of Agitation on the Growth of Microorganisms

Microorganism growth is described by the change of volatile suspended solid (VSS) concentration in the fermenter. Effect of agitation on the VSS is presented in Figure 3.

![Figure 3](image)

**Figure 3.** Effect of agitation rate on VSS

The average values of VSS at 25, 50, 100, and 200 rpm were 2,466.67 ± 385.76; 2,513.33 ± 258.06; 2,518.75 ± 424.61; and 2,320.00 ± 544.06 mg/L, respectively which indicated that the number of microbes increased with increasing of agitation rate.

3.2.3. Effect of Agitation on the Degradation of Organic Substances

Effect of agitation 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 25, 50, 100, and 200 rpm were 16.61 ± 4.12; 16.82 ± 7.70; 15.62 ± 4.77; and 16.61 ± 6.11%, respectively. While the COD removal at 25, 50, 100, and 200 rpm were 28.80, 36.44, 20.22, and 28.79%, respectively, both indicating that the highest microbial activity was achieved at agitation rate 200 rpm.

3.2.4. Effect of Agitation on VFA production

Effect of agitation on VFA production and composition is presented in Figure 5. The highest VFA production was achieved at agitation rate of 200 rpm where the production of acetic acid, propionic acid, butyric acid, and the total were 1,889.23; 1,161.43; 2,725.95; and 5,776.61 mg/L respectively. Although the production of propionic acid at 200 rpm was large i.e. 1,161.43 mg/L, but the value was still below 1,500 mg/L. According to [25], if the value of propionic acid is > 1500 mg/L, then it will disturb the next stage (methanogenesis stage).
The effect of agitation on the VFA-alkalinity ratio is presented in Figure 6 where the highest ratio was achieved at agitation rate of 200 rpm i.e. 2.49. The VFA alkalinity ratio is to describe the stability of process acidogenesis where it is assumed stable if the VFA-alkalinity ratio is greater than one [25].
4. Conclusions
The highest growth of microorganisms was achieved at HRT 4.0 day with microorganism concentration was 20.62 mg VSS/L and COD reduction was 15.7%. The highest production of total VFA achieved was 5,766.61 mg/L at agitation rate 200 rpm, with the concentration of acetic acid, propionic acid, and butyric acid were 1,889.23; 1,161.43; and 2,725.95 mg/L, respectively. While VS decomposition and COD removal were 16.61 and 38.79%, respectively.

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