Comparative start-up between mesophilic and thermophilic for acidified palm oil mill effluent treatment

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Abstract. Malaysia is one of the largest palm oil producers in the world and its most abundant waste, palm oil mill effluent (POME), can be used as a feedstock to produce methane. Anaerobic digestion is suitable for treating the POME for methane production due to its tolerance to high strength chemical oxygen demand (COD). This work emphasizes the study of conditions during the start-up of anaerobic digestion of acidified POME between thermophilic (55 °C) and mesophilic (37 °C). The pH of the digester was maintained throughout the experiment at 7.3±0.2 in 1000 ml working volume. The study showed that the thermophilic was much faster to stabilize on the 44th days compared with the mesophilic on the 52nd days. Furthermore, the thermophilic also indicates higher biogas production, which was 0.60 l/l/d compared with 0.26 l/l/d of mesophilic. This result can be supported by the COD removal of thermophilic which also higher.

1. Introduction
The palm oil that has been processed from mill will generate waste like POME which need to be treated because it can pollute the environment. Anaerobic digestion is an example of the available methods that can be used to treat organic waste such as POME, food waste, and sewage sludge to produce methane. According to [1], anaerobic digestion is better than aerobic system in the aspect of adaptability to environment change by not producing many solids, and using a low power for operation. For Malaysia, the current technology that had been applied to treat POME is by using a close tank. Until 2013, about 50% of palm oil mill still used the pond system to treat POME without capturing methane gas [2] probably due to large of land available in Malaysia that can be used for area of pond [3]. The performance of anaerobic digestion is influenced by many parameters. Among the parameters is operating temperature because it can affect the reaction of the methanogen. Generally, a methanogen grows in two temperature categories, mesophilic and thermophilic. Mesophilic indicates a temperature at 35–37 °C and thermophilic is usually between 55–60 °C [4]. A thermophilic digester has advantages like exhibit higher biogas production, pathogen destruction and substrate degradation while mesophilic is much easier to maintain and more stable [4]. [5] through the study on performance comparison between mesophilic and thermophilic anaerobic reactors had reported that the thermophilic at organic loading rate (OLR) of 15 kg COD/m³/d could produce 20.0 l/d of biogas which is higher than the mesophilic,
13.5 l/d. As for now, there is still no comparison have been made on acclimatization between mesophilic and thermophilic conditions. Other literature only focuses on one type of temperature condition during anaerobic digestion like [6] which investigated the start-up of methane production in mesophilic condition. By understanding the difference between mesophilic and thermophilic conditions, the performance and efficiency of anaerobic digestion can be improved. Therefore, the aim of this study is to determine the condition and performance between mesophilic and thermophilic during start-up period.

2. Material and methods

2.1 Seed Sludge and substrate
The seed sludge was collected from anaerobic digestion pond situated at Sime Darby palm oil mill Temamaram, Selangor, Malaysia. The substrate used in this study which is acidified POME was obtained from the effluent of [7] that used a biohydrogen fermenter fed with raw POME. The acidified POME had pH 5.14±0.05; COD between 44.3±3.7 g/l; volatile suspended solid (VSS) between 20.4±2.9 g/l; total suspended solid (TSS) 24.07±4.43 g/l and total nitrogen (TN) between 254±27 mg/l.

2.2 Analytical method
The liquid sample was subjected to analyses of pH, total alkalinity, and VSS based on [8]. Total nitrogen in acidified POME was determined by the method suggested by HACH. The volatile fatty acid (VFA) was analyzed by using high-performance liquid chromatography (Agilent 1200, California, USA). The HPLC system were run using a REZEX ROA column (Phenomenex, USA) and operated at a fixed flow rate of 0.6 mL/min. Furthermore, analysis of chemical oxygen demand was conducted by using a dichromate method operating with a COD analyzer (DR 2800, HACH). The biogas sample was collected and analyzed using gas chromatography (GC, model SRI 8600C, USA) by a helium ionization detector equipped with thermal conductivity detector. The biogas sample was injected into the GC with 40.0 °C oven temperature and pressure of 2.7 psi for 5 min. Helium gas with purity of 99.99% was used as a carrier gas with a flow rate of 25 ml/min.

2.3 Experimental set-up and operation
In this experiment, the anaerobic digestion of both mesophilic and thermophilic were performed with the sequencing batch mode in a 1000 ml working volume in Schott Duran bottles. Before the digestion started, nitrogen gas was purged to allow the process to operate under anaerobic conditions and then, the bottles were closed tightly. The bottles were incubated in a water bath shaker with the temperature at 55 °C and 37 °C with an agitation rate of 80 rpm. The anaerobic digestion began with HRT 30 days, with OLR between 1.1–1.3 g COD/l/d and the feed was provided for every two days manually. Both mesophilic and thermophilic operations stopped as soon as they reached steady state where the biogas volume, pH and VSS had less than 10% variation.

3. Result and Discussion

3.1 Start-up between mesophilic and thermophilic
Start-up period is important in anaerobic digestion because start-up operation that operates effectively allows the methanogen to grow well in the new environment. [9] has suggested that the start-up operation must be started with a low OLR to avoid overloading of organic matter. Based on this explanation, the selection of a low OLR (1.1–1.3 g COD/l/d) for this study is important to allow the methanogen to grow effectively and avoid overloading of organic matters that can lead to anaerobic digestion failure. This study also exhibits good performance of COD reduction and biogas production as a proof of successful start-up.
Figure 1. The range of biogas volume of thermophilic and mesophilic during the acclimatization period.

Figure 2. The range of pH variation of thermophilic and mesophilic effluent during the acclimatization period.

Figure 3. The range of COD of thermophilic and mesophilic effluent during the acclimatization period.

Figure 4. The range of VSS of thermophilic and mesophilic effluent during the acclimatization period.

Figure 5. The range of VFA of thermophilic and mesophilic effluent during the acclimatization period.

Figure 6. The range of total alkalinity of thermophilic and mesophilic effluent during the acclimatization period.
3.2 The biogas production variation upon start-up operation

The production profile of biogas for both mesophilic and thermophilic conditions as illustrated in Figure 1 shows that the production increased from the initial anaerobic digestion until the biogas production attained stability at the end of the acclimatization period. [10] reported that the increase in biogas production could be explained by the methanogen that was already active in this period and had started consuming the volatile fatty acids. The high microbial activity could accelerate the rate of breakdown and thus contributed to greater biogas production. In this study, we have observed that the thermophilic condition registered 2.5 times more biogas production than did the mesophilic. An underlying reason for this observation may be the higher degradation rate of organic matter caused by the thermophilic condition than by the mesophilic condition [11]. This study has successfully demonstrated that the selection of thermophilic conditions would favor biogas production compared to mesophilic conditions. Furthermore, at the end of the acclimatization period, the thermophilic and mesophilic conditions registered stable biogas production, as observed from the 44th and 52nd days onwards, respectively. Thus, the system has reached stability with less than 10% variation. The results are also consistent with those by [6], who reported the biogas production increased until the insufficiency of the substrate became the limiting factor for the methanogen to thrive. However, [6] elucidated only the mesophilic condition (37°C) and it took about 45 days to completely acclimatize. In comparison, the results from this study have suggested that the mesophilic condition took much longer time, 52 days to acclimatize than that of [6] which is 45 days. The reason could be the difference in the OLR used by [6] was 3.4 g COD/l/d which was more than twice than that in this study (1.2 g COD/l/d). It is believed that the operating at a high OLR will result in higher concentrations of food being fed into the digester, thereby augmenting the biogas production.

3.3 The pH variation upon start-up operation

As can be seen from Figure 2, under the thermophilic condition, the pH declined from 7.33 to 6.70 at the start of the operation until the 12th day; likewise, the mesophilic condition, it declined from 7.40 to 6.60. [10] reported that at the early stage of anaerobic digestion (1st day to 10th day), the pH declined from 6.95 to 5.11, as might be attributed to the prevalence of VFA during the beginning of the digestion. Then, the mesophilic and thermophilic conditions registered a rise in pH on the 16th days and on the 12th days, respectively. The pH of thermophilic has shown significant rise compared to mesophilic. It is believed that the higher pH produced under the thermophilic condition is a result of the higher rate of degradation of nitrogenous compounds. This study has demonstrated that the final pH achieved from both mesophilic and thermophilic is suitable for anaerobic digestion, which can yield satisfactory biogas production and COD removal. The results herein are in agreement with [6] and [12], both of which demonstrated that the methanogen was only active when the pH is suitable for anaerobic digestion (7.35±0.15). Methanogen is sensitive to pH changes. Non-ideal pH out of the neutral condition will impair biogas production.

3.4 The COD variation upon start-up operation

In the early stage, the COD removal under both thermophilic and mesophilic conditions decreased: the former declined from 40.7 to 12.4%, and the latter from 43.6 to 17.3%. The excess in VFA concentration was observed to retard the anaerobic digestion and eventually increase the COD effluent. This can also be supported by the elevation in VFA concentrations for both mesophilic and thermophilic conditions in the same period. Subsequently, the COD removal under both thermophilic and mesophilic conditions increased from the 12th and 16th days onwards respectively as demonstrated in Figure 3. The COD removal was increased until stabilize at the end of the start-up operation. It is believed that the methanogen had already been active by this stage and started to consume the substrate, as also evidenced by the diminution of the VFA effluent. At the end stage of anaerobic digestion, COD removal did not
fluctuate considerably; a COD removal of 64.5±0.2% was registered under the thermophilic condition whereas and a COD removal of 58.5±0.1% was registered under the mesophilic condition. This finding is also in agreement with [5], whose study on the treatment of POME revealed a similar trend: higher COD removal (94±1%) under the thermophilic condition than under the mesophilic condition (92±1%). The high COD removal that had been achieved at thermophilic condition seem to have a good impact in anaerobic digestion because a high COD removal at thermophilic proportional to the high biogas that had been produced compared to mesophilic.

3.5 The VSS variation upon start-up operation
Figure 4 indicates that the VSS under the thermophilic and mesophilic conditions decreased at the beginning of the experiment. It also can be seen that at the beginning of start-up, both mesophilic and thermophilic only had small differences in VSS. Time was required by the methanogen to become active prior to its consumption of the VFA. When the anaerobic digestion reached the 44th day, the VSS under the thermophilic and mesophilic conditions was in a narrow range of fluctuation that marked the final stage of acclimatization period. Under both the thermophilic and mesophilic conditions, the VSS registered less than 5% variation. [12] found a similar trend of VSS and TSS during acclimatization, in which the VSS decreased before it started to grow. From the authors results, the VSS decreased from 21900 mg/l to 17200 mg/l until the 21th day of operation. The high concentration of VSS at the end of the anaerobic digestion found by [12] indicated favourable anaerobic digestion which also supported by the high COD removal. The thermophilic condition registered a higher concentration of VSS than did the mesophilic counterpart with a 11.1% difference. [13] have reported that the VSS for the mesophilic condition (19,875.73 ± 1,766.07 mg/l) was lower than the thermophilic condition (22,170.14 ± 1,281.13 mg/l). Both studies also reached an agreement in which the low VSS associated with the thermophilic condition corresponded to high residual VFA [14]. Other results that can validate this statement is the high pH and biogas production exhibited by the thermophilic condition which showed better performance of the methanogen at a higher temperature.

3.6 The VFA variation upon start-up operation
The concentrations of VFAs under the thermophilic and mesophilic conditions rose from the start of the digestion until the 12th day and 16th day respectively based on the result from Figure 5. The high concentration of the VFA at the early stage of anaerobic digestion was due to the hitherto inactivity of the methanogen and its not consuming the VFAs. The same trend has been reported by [15], in whose study the VFA was accumulated from the beginning of the start-up until it reached 970 mg/l. The high concentration of VFAs is not suitable for anaerobic digestion because the presence of the low pH could hinder anaerobic digestion. Furthermore, another possibility that causes the accumulation of VFA is by the wash out of VSS from the system that will lead to acidification [16]. Given the favourable biogas production and COD reduction achieved in this study, it is likely that the anaerobic digestion system could tolerate the concomitantly-produced VFAs. Further observation revealed that the VFAs under the thermophilic and mesophilic conditions subsequently declined, indicating the proliferation of the methanogen and its consumption of the VFAs. The decline can be seen on 12th day and 16th day onwards. The COD removal also can be noted to increase at the same time. Compared to the study by [6], this study has discussed the effect of propionic acid in anaerobic digestion. [17] suggested that propionic acid above 951 mg/l would not be viable for anaerobic digestion. In this study, the concentrations of propionic acid were respectively 446±260 mg/l and 174±180 mg/l for mesophilic and thermophilic, thus not affecting the system.
3.7 The total alkalinity variation upon start-up operation

Total alkalinity and VFA are effective parameters for monitoring the progress of anaerobic digestion. The production of VFA must be balanced by controlling the level of total alkalinity such that the anaerobic digestion remains stable. Besides, total alkalinity acts as a pH buffer as it prevents rapid drastic changes in pH. Based on the result from Figure 6, the thermophilic condition exhibited a total alkalinity interval of 11617.81±4246 mg CaCO₃/l, whereas the mesophilic condition exhibited a much lower one of 9813.75±2550 mg CaCO₃/l. The high total alkalinity at the end of the start-up corresponded to the low VFA in the same period. It is believed that the higher temperature could lead to a higher breakdown of organic nitrogen into ammonium bicarbonate, which could eventually produce a higher total alkalinity and also as a result of successful conversion of the VFA by the methanogen into methane and the prevention of the accumulation of VFA [18]. High total alkalinity has resulted in superior performance of biogas production and COD removal as the VFA is favourably converted to biogas.

4. Conclusion

This study has proven the importance of monitoring the conditions and behavior of acclimatization in the anaerobic digestion of methane. Total alkalinity should be balanced with the higher production of VFA to avoid anaerobic digestion failure. In this study, the high concentrations of VFA of mesophilic and thermophilic conditions, which are 4581.37 and 2765.06 mg CH₃COOH/l, respectively, were well balanced with the total alkalinity system. The selection of thermophilic in anaerobic digestion has resulted in better performance in biogas production and COD removal as well as requires a shorter time to complete acclimatization compared to the mesophilic condition.

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