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The Effect of Mixing on Methane Production in a Semi-commercial Closed Digester Tank Treating Palm Oil Mill Effluent

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Abstract: The performance of a semi-commercial closed digester tank treating palm oil mill effluent (POME) was studied at four different mixing regimes i.e natural mixing (NM), minimal horizontal mixing (MHM), minimal horizontal and vertical mixing (MHVM) and vigorous mixing (VM). The chemical oxygen demand (COD) removal efficiency recorded satisfactory result at higher than 90% when subjected to the first three mixing regimes but reduced to the lowest of 85% when VM was applied. In the NM, MHM and MHVM experiments, the maximum total volatile fatty acids (VFA) concentration in the digester was recorded below the critical level of 1000 mg L^{-1}. The MHM gave the highest methane productivity at 1.4 m^3 m^{-3} d^{-1} in comparison to NM at 1.0 m^3 m^{-3} d^{-1} and MHVM at 1.1 m^3 m^{-3} d^{-1}. This indicates minimal mixing was required to provide good contact between substrate and microorganisms inside the digester and to release the entrapped biogas at the bottom of the digester. The VM on the other hand was discovered to inhibit the methane production process as methane was not produced at the end of the experiment and total VFA concentration was also recorded high at 3700 mg L^{-1}. The high total VFA concentration in the system may have disrupted the syntrophic relationship between acidogens and methanogens and inhibited the methanogenesis.

Key words: Palm Oil Mill Effluent, Anaerobic Treatment, Methane, Biogas, Mixing

INTRODUCTION

Palm oil industry is very important to the economy of Malaysia. Despite good economics return to the country, the industry also generates large amount of liquid waste known as palm oil mill effluent (POME). The most popular treatment method for this effluent is by the open pond or tank system. Recently with the introduction of Clean Development Mechanism (CDM), many of the Malaysian palm oil mills are converting the conventional open tanks or open ponds treatment system to the modern closed tanks or ponds treatment system in order to capture the methane gas as a potential source for renewable energy. In 2005, a semi-commercial scale 500 m^3 closed anaerobic digester tank was commissioned to study the anaerobic treatment of palm oil mill effluent (POME) and methane gas production for Clean Development Mechanism (CDM) project (Yacob *et al.,* 2006). Since then, many experiments have been conducted and recently the effects of mixing on the digester stability and performance were studied in order to improve the methane gas production. There have been many research reports on the effects of mixing on anaerobic treatment of various types of organic wastes but those were mainly at laboratory scales and furthermore did not utilize POME as the

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substrate (Stafford, 1982; Stroot *et al.,* 2001; Karim *et al.,* 2005a; Karim *et al.,* 2005b; Kaparaju *et al.,* 2007). Based on literature search this is the only large pilot scale (500 m^3) closed anaerobic digester tank dedicated for POME treatment and methane production research for CDM project in Malaysia (Faisal and Unno, 2001; Choorit and Wisarnwan, 2007; Najafpour *et al.,* 2006; Yejian *et al.,* 2008). As for the mixing study, the conclusions from various studies were consistent, that turbulent mixing is not suitable for high methane production (Stafford, 1982; Stroot *et al.,* 2001; Karim *et al.,* 2005a; Karim *et al.,* 2005b; Kaparaju *et al.,* 2007). In one study, vigorous mixing was found to reduce the average gas production rate for anaerobic treatment of sewage sludge due to shear force action on separating the hydrolytic bacteria from their substrate (Stafford, 1982). In addition to turbulent mixing, continuous mixing was also found to reduce the performance of the biogas production when organic fraction of municipal solid waste was co-digested with primary sludge and waste activated sludge (Stroot *et al.*, 2001). During anaerobic digestion of diluted animal waste effluent (5% of total solids), the unmixed and mixed digester performance were quite similar with biogas productivity ranging from 0.84-0.94 L L^{-1} d^{-1} (Karim *et al.,* 2005a; Karim *et al.*, 2005b). Based on both laboratory and pilot scale studies on manure, one study showed that in comparison with continuous mixing, minimal mixing strategy improved methane production by 12.5% and 7% respectively (Kaparaju *et al.,* 2007). Since there was no study conducted on the effects of mixing on anaerobic treatment of POME and methane gas production on a large pilot scale, this paper will discuss the digester performance in terms of COD removal efficiency and biogas productivity especially methane when the digester is subjected to natural mixing (NM), minimal horizontal mixing (MHM), minimal horizontal and vertical mixing (MHVM) and vigorous mixing (VM) regimes.

MATERIALS AND METHODS

Experimental Set-up:

Fig. 1 shows the set-up of the 500 m^3 semi-commercial closed digester tank equipted with horizontal and vertical mixing systems. In this study three different pumps were used; a centrifugal pump for mixing (11 kW power and 125 m^3 hr^{-1} capacity), a centrifugal pump for feeding (7.5 kW power and approximately 30 m^3 hr^{-1} capacity) and a roto pump for sludge recycling (1.1 kW power and 3 m^3 hr^{-1} capacity). During the experiment period, there was no major pump's leaking observed. For the experiment, the digester was subjected to four different mixing regimes namely natural mixing (NM), minimal horizontal mixing (MHM), minimal horizontal and vertical mixing (MHVM) and vigorous mixing (VM) and the details are in Table 1. The sludge from the settling tank was recycled for approximately 6 m^3 d^{-1} in all the experiments.

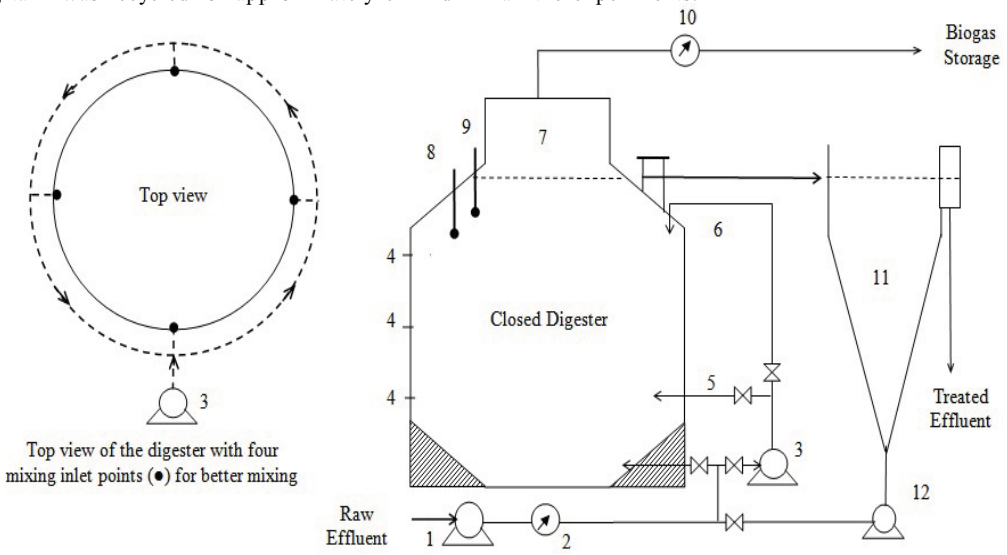

Fig. 1: Process flow diagram of the 500 m^3 semi-commercial closed digester; 1-POME feed pump; 2-POME mass flow meter; 3- Mixing pump; 4-Three different sampling port; 5-Horizontal mixing inlet; 6-Vertical mixing inlet; 7-Gas collection chamber; 8- pH probe; 9- Temperature probe; 10-Biogas mass flow meter; 11-Settling tank; 12- Sludge recycling pump.

Table 1: The explanation of different mixing regimes

Mixing regimes applied	Details
Natural mixing (NM)	The mixing was due to the biogas rising in the digester, raw POME feeding and sludge recycling mechanisms. The mixing pump was not used in this experiment.
Minimal horizontal mixing (MHM)	The horizontal mixing action was created by the mixing pump for 30 minutes, intermittently every 6hours through the horizontal inlet only.
Minimal horizontal and vertical mixing (MHVM)	The combined vertical and horizontal mixing action was created by the mixing pump for 30 minutes, intermittently every 6 hours through two inlets i.e horizontal and vertical.
Vigorous mixing (VM)	The vigorous mixing action was created by the mixing pump for 30 minutes, intermittently every 2 hours through two inlets i.e horizontal and vertical.

The Feeding Profiles:

The raw POME was daily pumped from the mill and stored in the holding tank prior to feeding. The feeding was done every 6 hours by using the centrifugal pump. Table 2 shows the profiles of the COD, pH, OLR and feeding rate of the raw POME utilized at different experiment periods. The COD concentration of the raw POME varied daily (30-70 kg m^{-3}) and resulted in variation of OLR applied. In this study, after the initial start-up period the raw POME volumetric feeding rate was fixed at 50 m^3 d^{-1} in all the experiments. The pH value of the raw POME was recorded between 4.5 and 5.0 and this is comparable to the literatures (Faisal and Unno, 2001; Choorit and Wisarnwan, 2007; Najafpour *et al.,* 2006; Yejian *et al.,* 2008). In between different experiments the process was let to stable to low VFA and substrate levels in order for the experiment to start on the same initial condition.

Table 2: The value of COD, Feeding rate, pH, Feeding rate, HRT and OLR on raw POME utilized at different mixing regime experiments.

Period	Days of operation	COD mg L^{-1}	pH value	Feeding rate m^3 d^{-1}	HRT days	OLR kg COD m^{-3} d^{-1}
Start-up	47	20,300-71,400	4.8-4.9	10.0-40.0	12.5-50.0	0.6-5.3
Natural mixing (NM)	32	39,400-77,500	4.3-4.9	50.0	10	3.9-7.8
Minimal horizontal mixing (MHM)	29	24,800-77,500	4.3-4.7	50.0	10	2.5-7.8
Minimal horizontal and vertical mixing (MHVM)	25	31,000-74,700	4.5-4.8	50.0	10	3.1-7.5
Vigorous mixing (VM)	20	40,000-84,500	4.4-4.8	50.0	10	4.0-8.5

Chemical Analyses:

Chemical oxygen demand (COD), total volatile fatty acids (VFA) and total solid (TS) were performed according to the APHA standard methods (APHA, 1985). The raw POME fed was measured by the electromagnetic flow measuring system (*PROline* promag 50, Endress+Hauser, Germany) and the biogas produced was measured by the thermal mass flow meter (T-Mass AT70, Endress+Hauser, Germany). The methane concentration was determined using a calibrated portable methane gas analyzer (XP-314A, Shin-Cosmos Electric Co. Ltd, Japan). The pH and oxidation redox potential (ORP) were measured using the HANNA pH/ORP/Temperature meter (HI 991002, HANNA Instrument, Romania).

Fluoroscent In-Situ Hybridization (FISH) Technique:

The probe MSMX860, complementary to the 16S rRNA of some methanogens including *Methanosarcina* spp., *Methanococcoides* spp., *Methanolobus* spp., *Methanohalophilus* spp. and *Methanosaeta* spp. was used to directly analyze the methanogenic population (Crocetti *et al*., 2006). To determine the sludge bacteria, the 16S rRNA probe EUB338 for the bacteria domain was used as suggested in the literature (Amann *et al.,* 1990). Oligonucleotides and their fluorescent derivatives (5′-labelled with either FITC or rhodamine) were purchased from First Base (Malaysia) Sdn. Bhd. Cells were fixed and hybridized using the protocol suggested (Amman *et al.,* 1995) with some modifications (Sakai, *et al.,* 2004). Fluorescence was observed using an epifluorescence microscope (Axiolab, Carl Zeiss, München-Hallbergmoos, Germany) and the pictures were taken using a color camera (AxioCam, Carl Zeiss, München-Hallbergmoss, Germany).

RESULTS AND DISCUSSION

The Performance of the Closed Digester:

The digester's performance in terms of COD removal efficiency and productivities of biogas and methane is shown in Table 3. During the start-up period, the COD removal efficiency was consistently above 95% which indicates good substrate utilization by the microorganisms. The digester was ready for the experiments after 47 days of initial start-up. The fast start-up achieved was partly due to the suitable seed sludge used from

the existing 3600 m^3 open digesting tank for POME treatment available at the site. In addition, the small increment of the volumetric feeding rate applied (from 10 m^3 d^{-1} to 20 m^3 d^{-1}, 30 m^3 d^{-1} and finally to 40 m^3 d^{-1}) managed to avoid the possibility of loading shock to the system. The trend of high COD removal efficiency of higher than 95% continued even after the raw POME volumetric feeding rate was increased to 50 m^3 d^{-1} in the NM experiment. Throughout this study, the productivity of the biogas produced was calculated based on the volume of biogas produced at standard temperature and pressure per day per digester's volume (i.e m^3 m^{-3} d^{-1}). In the NM experiment, the biogas and methane productivity was found to fluctuate in the range of 0.35-2.14 m^3 m^{-3} d^{-1} and 0.21-1.18 m^3 m^{-3} d^{-1}, respectively. This was higher than what previously reported in the other mixing study at only in the range 0.84-0.94 L L^{-1} d^{-1} (Karim *et al.,* 2005a; Karim *et al.,* 2005b) probably due to different kind of liquid waste utilized as the substrate. In this experiment, the digester's pH was neutral (7.0±0.2) and the temperature was maintained in the mesophilic range (36 ^oC±2). Nevertheless, towards the end of the natural mixing, the COD removal efficiency slightly reduced to 90% which was caused by the increased of total VFA of approximately 1000 mg L^{-1} inside the digester. In many studies, it was reported that high total VFA concentration had caused digester failure by reducing the pH in the system and inhibited the methanogenesis process (Poh and Chong, 2009). In this experiment it can be concluded that with NM alone the VFA was not homogenously distributed inside the digester and its utilization by the methanogens was limited and this might have caused total VFA accumulation inside the digester. Before continuing with the MHM experiment, the digester was allowed to stabilize to lower VFA concentration inside the digester as shown in Fig. 2. In the MHM experiment, the COD removal efficiency was also maintained above 95% and both productivities were recorded in the range of 1.6-3.1 m^3 m^{-3} d^{-1} and 0.8-1.8 m^3 m^{-3} d^{-1} for biogas and methane, respectively. This biogas productivity is also higher than what previously reported at only 1.14 L L^{-1} day^{-1} (Karim *et al.,* 2005a; Karim *et al.,* 2005b) in spite of the higher power input used in this experiment. In fact it was also higher than NM experiment. In this experiment, the mixing pump wattage per unit volume of the digester was calculated to be 22 W m^{-3} which was higher than what was recommended by United States Environmental Protection Agency (USEPA) at only 5.26-7.91 W m^{-3} (Karim *et al.,* 2005b). The minimal mixing was performed intermittently every 6 hours for 30 minutes mixing time and this was sufficient to release the entrapped biogas at the bottom of the digester without disturbing the activity of microorganisms. The digester's temperature was slightly higher (approximately 40 ^oC) than NM and the pH value was neutral (approximately 7.0) which reflected high microorganisms' activity inside the digester. For the MHVM, high COD removal efficiency of higher than 95% was recorded and productivity for biogas and methane ranged from 1.9-2.2 m^3 m^{-3} d^{-1} and 0.9-1.2 m^3 m^{-3} d^{-1}, respectively. In this experiment the productivity of methane produced was less than the MHM experiment probably due higher mixing disturbance resulted by both vertical and horizontal mixing inside the digester. In the last experiment which was VM experiment, the digester was continuously subjected to vigorous mixing. The digester's performance in terms of COD removal efficiency, productivity of biogas and methane productivity reduced significantly in just 13 days and finally the process was stopped for recovery because no methane gas was produced. The VM has created continuous turbulence flow with high shear stress at the mixing pump impeller. Consequently, the spatial juxtaposition of syntrophic bacteria and their methanogenic partners might have been disrupted and affected the anaerobic process as reported by McMohan *et al., (2001)* and Stroot *el al*., (2001). This was further supported by the high VFA accumulation inside the digester of 3700 mg L^{-1} on the day 13 as shown in Fig. 2. As a result the pH value was also recorded low at only pH 6.5 and was reported not conducive for methanogenesis (Stroot *et al*., 2001). This pH value was also lower than what was recorded in the NM, MHM and MHVM experiments.

Table 3: The digester performances and stability in terms of COD removal efficiency and productivities of biogas and methane determined at different mixing regime experiments.

Period of study	Days of operation	COD removal efficiency (%)	Productivity (m^3 m^{-3} day^{-1})			
		Range	Biogas Range	Mean±SD	Range	Methane Mean±SD
Start-up	47	94-98	0.35-2.14	1.09±0.45	0.21-1.18	0.62±0.24
Natural mixing (NM)	32	91-97	1.5-2.7	2.1±0.3	0.7-1.3	1.0±0.1
Minimal horizontal mixing (MHM)	29	92-97	1.6-3.1	2.5±0.4	0.8-1.8	1.4±0.3
Minimal horizontal and vertical mixing (MHVM)	25	92-96	1.9-2.2	2.1±0.1	0.9-1.2	1.1±0.1
Vigorous mixing (VM)	20	85-95	0.1-1.4	0.9±0.4	0-0.6	0.4±0.2

SD is the standard deviation

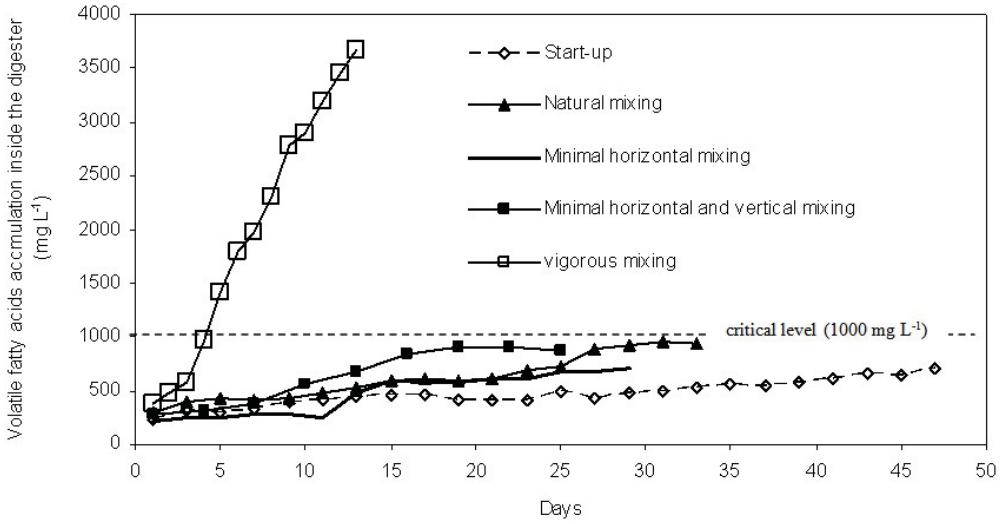

Fig. 2: VFA accumulation measured inside the digester at different mixing regime experiments. The graphs also show that each experiment was started at approximately same initial total VFA concentration.

The Stability of the Closed Digester:

The digester's stability in terms of total VFA concentration and oxidation redox potential (ORP) in the digester is shown in Fig. 3 and Table 4, respectively. In the first three experiments (NM,MHM and MHVM), the VFA concentration was below the critical level of 1000 mg L^{-1}, however, in VM experiment, the concentration of total VFA critical level exceeded in just 5 days after starting the experiment. The maximum total VFA concentration in the digester was recorded at approximately 3700 mg L^{-1} at the end of 13 days which signify the negative effect of VM on VFA utilization by the methanogens. This is in agreement with the findings by Stroot *et al.,* (2001) who argue that continuous mixing is more likely to severely affect the biogas production owing to increasing levels of volatile fatty acids than the minimal mixing. In a stable anaerobic digester with low total VFA concentration, Stroot *el al.,* (2001), Kaparaju *et al.* (2007) and McMohan *et al*.(2001) reported the microorganisms exist in syntrophic relationship. The FISH picture taken from the sludge sample from a stable digester tank is shown in Fig 3. In the picture the existence of bacteria and methanogens (*Methanosarcina* spp. and *Methanosaeta concilii*) were clearly observed. However the FISH picture could not be taken from the sludge sample from the digester operated under VM regime. Both bacteria and methanogens were unable to be observed probably due to non-conducive environment at high total VFA concentration inside the digester. At the end of the experiment the methane production rate was negatively affected as at this stage methanogenesis was inhibited by the high VFA concentration distributed in the system. The ORP was also measured in all experiments to understand the degree of anaerobic condition inside the digester at different mixing conditions. In the first three experiments (NM. MHM and MHVM) showed quite similar degree of anaerobic condition inside the digester (between -255 and -305 mV), however, not in the vigorous mixing condition. The ORP was measured highest at the end of the vigorous mixing experiment (+20 mV) which reflected the non-conducive environment for methanogenesis. Methane producing bacteria are obligate anaerobes and their metabolic functions are performed in a highly reduced environment having a highly negative potential (Fannin, 1987). Thus at +20 mV of ORP recorded, the methanogenesis was badly affected and as a result methane gas was not produced. Thus ORP with positive value could also be used as an indicator for poor methogenesis in the anaerobic treatment of POME.

Table 4: The range of the oxidation reduction potential (ORP) recorded at different mixing regimes

Mixing regimes applied	Range of ORP recorded
Natural mixing (NM)	-298 to -305 mV
Minimal horizontal mixing (MHM)	-286 to -310 mV
Minimal horizontal and vertical mixing (MHVM)	-255 to -286 mV
Vigorous mixing (VM)	-278 to +20 mV[a]

[a]The +20 mV value was measured at the end of VM experiment

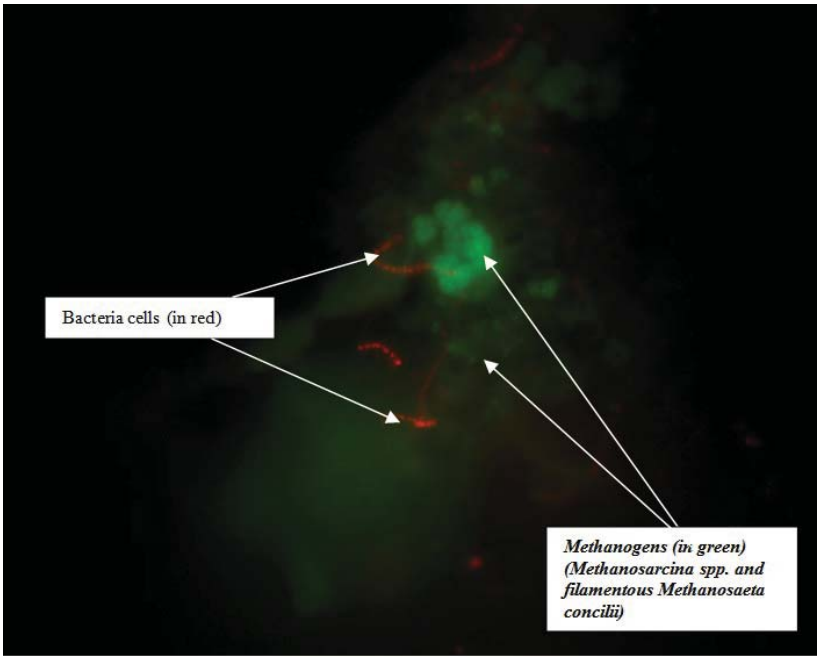

Fig. 3: Fluorescent in situ Hybridization (FISH) picture for the samples taken from a, minimal mixing (1000X magnifications) showing the distribution of methanogens (*Methanosaeta concilii* and *Methanosarcina spp.*) and bacteria in the system.

Conclusions:

The COD removal efficiency showed satisfactory results of higher than 90% when subjected to NM, MHM and MHVM regimes but reduced to the lowest of 85% in the VM regime. The MHM gave the highest methane productivity of 1.4 m^3 m^{-3} d^{-1} in comparison to NM of 1.0 m^3 m^{-3} d^{-1} and MHVM of 1.1 m^3 m^{-3} d^{-1} .This showed that minimal mixing was sufficient to provide good contact between the substrate and microorganisms and to release the entrapped biogas at the bottom of the digester. On the other hand, the VM regime inhibited the methane production process by disrupting the syntrophic relationship between acidogens and methanogens.

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REFERENCES

Amann, R.I., 1995. In Situ Identification of Micro-Organisms by Whole Cell Hybridization with rRNA-Targeted Nucleic Acid Probes, 3.3.6. In Molecular Microbial Ecology Manual, Eds., Akkermans, A.D.L., J.D. Elsas and F.J. Bruijn, Kluwer Academic Publisher, Dordrecht, pp: 1-15.

Amann, R.I., L. Krumholz and D.A. Stahl, 1990. Fluorescent oligonucleotide probing of whole cells for determinative phylogenetic, and environmental studies in microbiology. J. Bacteriol., 172: 762-770.

APHA, 1985. Standard Methods for the Examination of Water and Wastewater. New York: American Public Health Association.

Choorit, W. and P. Wisarnwan, 2007. Effect of temperature on the anaerobic digestion of palm oil mill effluent. Electronic Journal of Biotechnology, 10(3): 376-385.

Fannin, K.F., 1987. Start-up, Operation, Stability and Control. In Anaerobic Digestion of Biomass, Eds., Chynoweth, D.P. and R. Isaacson. Elsevier Applied Science Publishers Ltd, pp: 171-196.

Crocetti, G., M. Murto and L. Björnsson, 2006. An update and optimisation of oligonucleotide probes targeting methanogenic Archaea for use in fluorescence in situ hybridisation (FISH). J. Microbiol. Methods, 65: 194-201.

Faisal, M. and H. Unno, 2001. Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. Biochem Eng., 9: 25-31.

Kaparaju, P., I. Buendia, L. Ellegaard and I. Angelidakia, 2007. Effects of mixing on methane production during thermophilic anaerobic digestion of manure: Lab scale and pilot scale studies, Bioresour Technol., 99: 4919–4928.

Karim, K., R. Hoffmann, K.T. Klasson and M.H. Al-Dahhan, 2005a. Anaerobic digestion of animal waste: Waste strength versus impact of mixing. Bioresour Technol., 96: 1771–1781.

Karim, K., R. Hoffmann, K.T. Klasson and M.H. Al-Dahhan, 2005b. Anaerobic digestion of animal waste: Effect of mode of mixing. Water Res., 39: 3597-3606.

McMahon, K.D., P.G. Stroot, R.I. Mackie, L. Raskin, 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions-II: Microbial population dynamics. Water Res., 35: 1817-1827.

Najafpour, G.D., A.A.L. Zinatizadeh, A.R. Mohamed, M.H. Isa and H. Nasrollahzadeh, 2006. High rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. Process Biochem., 41: 370-379.

Poh, P.E. and M.F. Chong, 2009. Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Bioresour. Technol., 100: 1-9.

Sakai, K., M. Mori, A. Fujii, Y. Iwami, E. Chukeatirote and Y. Shirai, 2004. Fluorescent in situ hybridization analysis of open lactic acid fermentation of kitchen refuse using rrna-targeted oligonucleotide probes. J. Biosci. Bioeng., 98: 48-56.

Stafford, D.A., 1982. The effects of mixing and volatile fatty acid concentrations on anaerobic digester performance. Biomass, 2: 43-55.

Stroot, P.G., K.D. McMahon, R.I. Mackie and L. Raskin, 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions-I.Digester performance. Water Res., 35: 1804–1816.

Yacob, S., Y. Shirai, M.A. Hassan, M. Wakisaka and S. Subash, 2006. Start-up operation of semi-commercial closed anaerobic digester for palm oil mill effluent treatment. Process Biochem., 41: 962-964.

Yejian Z., Y. Li, C. Lina, L Xiuhua, M. Zhijian and Z. Zhenjia, 2008. Startup and operation of anaerobic EGSB reactor treating palm oil mill effluent. Journal of Environmental Sciences, 20: 658-663.