2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

Microbial and process performance in anaerobic digestion of palm oil mill effluent under normal and over loading in hybrid reactor

Kanlayanee Meesap[1], Somkiet Techkarnjanaruk[2] and Pawinee Chaiprasert[3*]

[1] The Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, Bangmod, Bangkok 10140, Thailand

[2] Excellent Center of Waste Utilization and Management (ECoWaste), National Center of Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Bangkhuntien,Bangkok 10150, Thailand

[3] Division of Biotechnology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand

[*]Corresponding author: pawinee.cha@kmutt.ac.th

Abstract. Influence of suspended solid (SS) and oil and grease (O&G) in palm oil mill effluent (POME) under normal and overload feeding on microbial characteristics and reactor performances was studied in anaerobic hybrid sludge bed-fixed film reactor (AHR). The normal condition was achieved in feeding operation of TCOD, SS and O&G concentration in the range of 10-20 g/l, 5.0-11.0 g/l and 1.0-2.3 g/l, respectively. The process stability and performance were in pH 7, TVA/Alk < 0.4, and methane yield 0.21 $lCH_4/gCOD_{removed}$. The shocked load operation was occurred in 12.5 gSS/l and reflected by pH < 6.0 with TVA/Alk 0.5-0.7 and dropping in methane yield. Under normal and shocked load not only showed differences in performance and stability but also microbial characteristics. The results in this study illustrated that microbial activity of non-methanogens and methanogens directly reflected in process stability and performance efficiency more than microbial population.

Keywords: Microbial characteristics, Normal and overload feeding, Palm oil mill effluent.

1. Introduction

Palm oil industry has become one of the rapidly growing sectors in Thailand. The rapid development has contributed to environmental pollution correspondingly to the large quantity of waste products produced from the oil extraction process. These waste products consist of fibrous materials, less fibrous material such as palm kernel cake and liquid discharge palm oil mill effluent (POME) [1]. POME is high strength wastewater with a high BOD load consisting of suspended solid (SS), oil and grease (O&G), colloidal and dissolved organic matters. It is also acidic and brown color. However, POME contains high organic substances which non-toxic and biodegradable [2]. Anaerobic treatment is one of the successful and powerful biological methods for POME treatment. However, the suspended and colloidal components are neither easily decomposed biologically, nor by other conventional means due to their floating on the surface of the wastewater having an impact on the microbial and reactor performance [3-5]. It is the major problem to cause failure of treatment system. Particularly, the major component in biological anaerobic digestion, microorganism plays an important role and core factor of the system to control reactor performance and stability. Nowadays, the anaerobic hybrid sludge bed and fixed film reactor (AHR) is applied to POME treatment for solving cell washout and material cost. It is interested to study the microbial characteristics in AHR for understanding the role of anaerobic microorganism in both normal and overload conditions. This

research intended to study the microbial characteristics through the molecular methods such as its quantities under both conditions for treatment and methane productions of POME. Activities of methanogens and non-methanogens had been monitored as well as process performance and stability. The process performance of AHR was investigated in term of SS, O&G and COD removal.

2. Materials and methods

2.1 Reactor system

The anaerobic hybrid sludge bed-fixed film reactor (AHR) was used in this study. AHR was made up of acrylic column with an internal diameter and height of 9.6 cm and 80 cm, respectively. Total volume of the reactor was 5,800 ml with working volume of 5,000 ml. Reactor working volume occupied by 50% of sludge zone and 50% of packed zone in the bottom and upper parts of reactor, respectively. In this packed zone fitted with nylon fibers as the supporting material with the specific surface area of 2 m^2/m^3. Seven sampling ports were located at several height level of sludge and packed zones in AHR by distribution of four and three points, respectively.

2.2 Wastewater

Raw POME was collected directly from crude palm oil production plant, Thailand. Raw POME consists of total chemical oxygen demand (TCOD) 57-63 g/l, soluble chemical oxygen demand (SCOD) 40-44 g/l, suspended solid (SS) 11-13 g/l and oil and grease (O&G) 5.7-6.3 g/l. Before feeding into the reactor, POME was prepared by separating SS, O&G and supernatant through settling in water bath at 65°C for 6-8 h. Various SS concentration was used for the study in the range of 5-12.5 g/l. Characteristics of POME influents were shown in Table 1.

2.3 Reactor operation and monitoring

Reactor was operated under ambient temperature (~ 30-35°C). POME was continuous up-flow feeding from the bottom of sludge zone up to the top of packed zone. The inoculums seed for startup containing 14 kg/m^3 of volatile suspended solid (VSS) was used and taken from the anaerobic wastewater treatment system of POME. In order to allow the acclimatization of the mesophilic microbial seed sludge to the new environment, the reactor was semi-continuous fed once a day with the diluted POME (5–6 gCOD/l) and ran for 7 d at hydraulic retention time (HRT) of 10 d. Reactor was then fed with 5 gSS/l.d until the system reached to steady state. During steady state, performance, stability, microbial quality and quantity, and microbial communities were analyzed.

Table 1 Characteristics of POME influent

SS (g/l)	TCOD (g/l)	O&G (g/l)	OLR (gCOD/l.d)
5.0	10.0	1.0	1.2
7.0	18.0	1.5	1.8
10.0	20.0	1.9	2.0
11.0	24.0	2.3	2.2
12.5	30.0	3.6	2.7

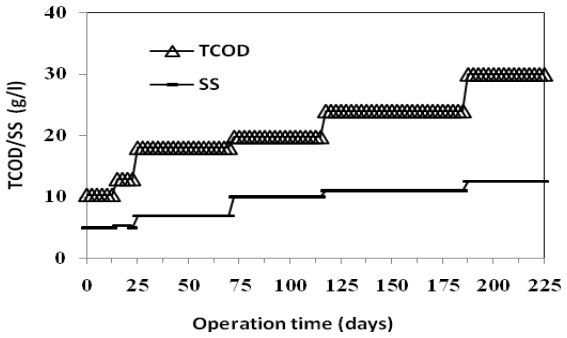


Fig.1 TCOD and SS concentrations of POME influent fed into AHR during operation

2.4 Determination of performance and microbial characteristics

2.4.1 Performance and stability

The performance of AHR was routinely monitored through the measurement of TCOD, SCOD, O&G and SS removal efficiency. Biogas production was daily measured with a wet gas flow counter. Methane and carbon dioxide composition in biogas were determined by gas chromatography (Shimadzu, Class-GC 14B, Japan), fitted with a thermal conductivity detector (TCD). The determination of volatile fatty acid (VFA) was

done using gas chromatograph equipped with a flame ionization detector (FID). In term of process stability, alkalinity (Alk), total volatile acid (TVA), and pH were determined according to APHA Standard Methods [6]. POME influents were stepwise increased to 7, 10, 11 and 12.5 gSS/l as showed in Fig. 1 by constant the HRT at 10 d.

2.4.2 Microbial characteristics

2.4.2.1 Determination of microbial activities

Microbial samples of sludge and packed zones of AHR at each steady state were collected. In sludge zone, suspended sludge was collected from wastewater in AHR. In packed zone, attached biofilm on supporting material was dispersed by ultra-sonication for 10 min. They were analyzed on the activities of methanogen and non-methanogen by spicing specific substrate with 1% (v/v) acetic acid and 1% (v/v) glucose, respectively [7].

2.4.2.2 Quantification of microbial population by 16S rDNA quantitative PCR

Copy numbers of 16S rDNA gene of eubacteria (EUB) and archaeal (ARC) were quantified by relative quantification real-time PCR (qPCR) using KAPA SYBR® Fast qPCR Kit (KAPA, Brazil) for real-time reactions. The qPCR assay was performed according to method of Kanlayanee et al. [8].

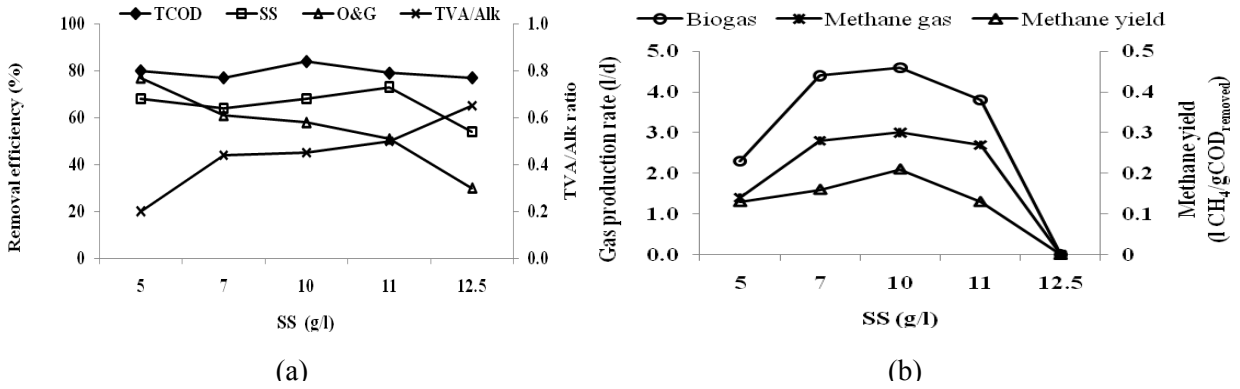
3. Results and discussions

3.1 Reactor performance and stability

The overall performance efficiency of AHR at each load (Fig. 2a) was shown in removal of SS, O&G and TCOD which were in the range of 60-80, 30-70 and 80-85 %, respectively. According to the results found that high performance of TCOD and SS removal (70-80%) were achieved at TCOD and SS concentration 10-24 g/l and 5-11 g/l, respectively. TCOD removal efficiency was in the same range of previous work which study solid waste treatment by laboratory-scale completely stirred tank reactor at mesophilic temperature (35 ◦C). TCOD removal efficiency was 88.4% at OLR 12.02 gCOD/l.d [9-10]. It indicated that high reactor performance was achieved under these SS and O&G concentrations.

Moreover, the system ran in the normal condition with system pH was in the range of 6.2-7.3 and TVA/Alk ratio of 0.22-0.64. After increasing to 30 gTCOD/l with 12.5 g SS/l, the deteriorated performance was found due to the overload feeding and reflected the decrease of TCOD and SS removal to 77% and 54%, respectively. The accumulation of SS was settled in the bottom part of reactor. O&G removal was continuously decreased along with increasing of O&G and SS concentrations in POME due to its hardly degradable and need more time to degrade by anaerobic microorganisms. Generally, O&G was hydrolyzed by bacterial enzymes under anaerobic condition which sensitive to pH and inhibitory substances. At normal condition O&G could remove to 50-80% depending on the initial concentration of SS and O&G feeding. Dramatically drop in 30% O&G removal was found under overloading condition. It can be found in two phases that either suspending in the supernatant or floating on the upper layer of the suspension. Under this operation condition, pH and ratio of TVA/Alk were acidic (5.7) and lower buffer capacity (>0.4) that effected to inhibit the lipase producer bacterial activity. This enzyme activity can work well in neutral pH [11, 12].

Process stability (pH, TVA, Alk and TVA/Alk) will reflect process performance of biogas and methane production. As general anaerobic digestion, a variety of primary producers (acidogens) break down the raw wastes into simpler fatty acids in the first stage. In the second stage, a different group of organisms (methanogens) consumes the organic acids produced by the acidogens, generating biogas as a metabolic byproduct [13]. This relation could be seen in Figs. 2a and 2b. At normal conditions of process stability (5-11 gSS/l), biogas and methane gas production rate were also high as in the range of 2.3-4.6 l/d and 1.4-3.0 l/d, respectively. These values suddenly decreased and finally stopped when SS concentration overloaded to12.5 gSS/l. Moreover, methane yield became lower < 0.13 $lCH_4/gCOD_{removed}$ as showed in Fig. 2 (b).



(a) (b)

Fig. 2 Removal efficiency of organic compounds with ratio of TVA/Alk (a) and gas production (b) at the different SS concentration operation

3.2 Microbial characteristics

AHR consists of sludge zone in the bottom part and packed zone with supporting media as nylon fiber in upper part. Microbial characteristics of both sludge and packed zones in AHR at each operation were identified. According to results of overall reactor performances, it can be categorized into two conditions as normal condition (5-11 gSS/l) and shocked load condition (12.5 gSS/l). In term of microbial characteristics found that microbial activity (Table 2) seem be clearly indicator of microbial dynamic in anaerobic digest POME system. Under normal condition, non-methanogen or EUB activity in sludge zone showed high performance than packed zone, whereas methanogen or ARC activity in packed zone was higher than sludge zone.

EUB and ARC population did not show the difference between sludge and packed zones it was in the range of 10^6-10^8 and 10^3-10^5 copies 16S DNA/gVSS, respectively. The number of non-methanogen and methanogen was observed by Shigematsu et al (2006) in chemostat cultivation and found that there were mesophilic methanogenic consortium that can degrade long-chain fatty acid 1.14 x 10^8 and 1.54 x 10^7 copies 16S rDNA/50 ng-DNA, respectively. It can be seen non-methanogen normally higher than methanogen [14]. Under shocked load condition, non-methanogenic activity in both sludge and packed zone has been high (1.70 gCOD/gVSS.d) which indicated that the native bacteria could growth and resist to high concentration of organic substances. Whereas methanogenic activity decreasing was found, especially sludge zone decreased to 0.09 gCOD-CH_4/gVSS.d due to the suspended microorganism character compared to biofilm in packed zone.

Table 2 Microbial characteristics in sludge and packed zones of AHR on POME treatment

Parameter	Non-methanogen		Methanogen	
	Sludge zone	Packed zone	Sludge zone	Packed zone
Normal load				
Activity[a]	1.08 - 1.65	0.43 - 1.11	0.10 - 0.16	0.18 - 0.26
Population[b]	3.5 x 10^6 - 1.1 x 10^8	4.0 x 10^6 - 4.2 x 10^8	1.4 x 10^3 - 1.3 x 10^5	1.2 x 10^3 - 7.3 x 10^5
Shock load				
Activity[a]	1.50 - 1.90	1.32 - 1.72	0.07 - 0.12	0.02 - 0.042
Population[b]	3.7 x 10^6 -4.2 x 10^6	6.3 x 10^6 -6.7 x 10^6	0.9 x 10^3-1.3 x 10^3	1.2 x 10^4-1.6 x 10^4

[a] Unit of non-methanogenic and methanogenic activities is gCOD/gVSS.d, and gCOD-CH_4/gVSS.d, respectively
[b] Unit of microbial population is copies 16S DNA/gVSS

4. Conclusions

The results of this study demonstrated the influence of POME with 10-30 gTCOD/l composed of 5-12.5 gSS/l and 1.2-2.7 gO&G/l at 10 d of HRT on AHR stability and performance including microbial characteristics. The studied condition can categorize into two conditions namely normal and overload condition operated at 5-11 and 12.5 gSS/l, respectively. At normal conditions, overall performance via TCOD, SS and O&G removal efficiencies were high in 80%, 70%, and > 50%, respectively. Biogas and methane production rate in normal condition were in the rage of 2.3-4.6 and 1.4-3.0 l/d, respectively. The methane yield was 0.13-0.21 $lCH_4/gCOD_{removed}$. This AHR system had loading capacity at SS concentration to 11 g/l. The shock loading was occurred when increasing SS concentration to 12.5 g/l. The process stability in pH and ratio of TVA/Alk was < 6 and > 0.4. This was the signal warning of reactor failure as seen by the reactor performance dropped in biogas and methane production. In this study microbial activities of EUB and ARC clearly represented the relationship between process performance and microbial character more than microbial population. The action zone of AHR system in sludge and packed areas seem to act as hydrolytic/fermentative and methane producing zones, respectively.

5. Acknowledgements

I would like to express the sincerely gratitude to Joint Graduate School of Energy and Environment (JGSEE) for the Ph.D. scholarship to Ms. Kanlayanee Meesap and Excellent Center of Waste Utilization and Management (ECoWaste) for facility and experimental support.

6. References

[1] S. Cheah. Understanding the causes of oil palm tissue culture abnormalities on the road to commercialization. Proceedings of the PIPOC 2003 International Palm Oil Congress. *AgricultureConference*, 2003, 121 – 128. Malaysia.

[2] N.A. Nik Norulaini, Ahmad Zuhairi, A.l, Muhamad Hakimi, and Mohd Omar, A.K. Chemicalcoagulation of setteable solid-free palm oil mill effluent (POME) for organic loading ratereduction . *J. Ind. Tech*, 2001, **10**(1): 55-72.

[3] J.M. Megat and Mohd Noor, A. J. a. A. H. G. POME treatment utilization high rate hybrid anaerobic reactor. *J. Islamic Acad. Sci*.1989,**2**(1): 13-16.

[4] U.Stoll and H. Gupta. Managemetn strategies for oil and grease residues. *Was.Manage. Res.* 1997, **15**: 23-32.

[5] A.Torkian, A. Eqbali and S.J. Hashemian. The effect of organic loading rate on the performance of UASB reactor treating slaughterhouse effluent. *Resource Conv Recycling*. 2003, (40):1-11.

[6] American Public Health Association, 2005. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association, American Public Association (APHA).

[7] R. Amann, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev.* 1995, **59**:143-169.

[8] Kanlayanee Meesap, Somkiet Techkarnjanaruk and Pawinee Chaiprasert. Microbial performance characteristics of Anaerobic Hybrid Sludge Bed Fixed-Film Reactor in Palm Oil Mill Effluent Treatment: *International Conference for a Sustainable Greater Mekong Subregion (GMSTEC 2010)*, 26-27 August 2010, Bangkok, Thailand.

[9] R. Borja, B. Rinc´on, F. Raposo, J. Alba, A. Mart´ın, A study of anaerobic digestibility of two-phases olive mill solid waste (OMSW) at mesophilic temperature, *Process Biochem.* 2002, **38**:733–742.

[10] R. Borja, A. Mart´ın, B. Rinc´on, F. Raposo, Kinetics for substrate utilization and methane production during the mesophilic anaerobic digestion of two-phases olive pomace (TPOP), J. Agric. *Food Chem.* 2003, **51**(11): 3390–3395.

[11] G.Tchobanoglous, F.L.,Burton and Stensel, *H.D.Wastewater Engineering: Treatment and Reuse, Fourth Edition*. New York: McGraw Hill Companies, Inc. 2003.

[12] Lee Chee Siang. Biodegradation of oil and grease in upflow anaerobic sludge blanket reactor for pail oil mill effluent treatment, Thesis *of Faculty of Civil Engineering, University of Technology Malaysia*. 2007.

[13] A, Demirbas, Pehlivan E, Altun T. Potential evolution of Turkish agricultural residues as bio-gas, bio-char and bio-oil sources. *Int J Hydrogen Energy*. 2006, **31**:613–620.

[14] Shigematsu Toru, Yueqin Tang, Yuko Mizuno, Hiromi Kawaguchi, Shigeru Morimura,1 and Kenji Kida. Microbial diversity of mesophilic methanogenic consortium that can degrade long-chain fatty acids in chemostat cultivation. J. Biosci Bioen. 2006, **102**(6): 535-544.