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Biohythane production from palm oil mill effluent – a preliminary evaluation of a two-stage anaerobic digestion

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ABSTRACT

Aims: This research aims to investigate the potential of biohythane (biohydrogen and biomethane) production from palm oil mill effluent (POME) in a two-stage anaerobic digestion (AD) system.
Methodology and results: A two-stage AD system was configured with a thermophilic dark fermenter (TDF) for biohydrogen (H_2) production and a thermophilic anaerobic contact digester (TACD) for biomethane (CH_4) production. To adjust pH 5.5 for dark fermentation, the settled sludge was recirculated from TACD to TDF. The hydraulic retention time (HRT) applied in TDF and TACD was 3.75 and 6.25 day, respectively.
Conclusion, significance and impact of study: The sludge recirculation from TACD was able to adjust the pH in TDF to the optimum value of 5.5. The total COD and TSS degradation were 63.12 and 77.94 %, respectively. The H_2 production in TDF was 1.54 L H_2/L POME and the CH_4 production in TACD was 19.87 L CH_4/L POME. The H_2 and CH_4 yielded 0.085 L/g $COD_{removed}$ and 0.339 L/g $COD_{removed}$, respectively, with total energy recovery equivalent to 661.02 MJ/m^3 POME. Only 2.28 % of this energy was contributed by H_2 and the remaining was dominated by CH_4.

Keywords: biohydrogen, biomethane, two-stage, anaerobic digestion, palm oil mill effluent.

INTRODUCTION

Anaerobic digestion (AD) is a popular treatment technology for high organic strength wastewater such as palm oil mill effluent (POME). AD of POME can produce renewable energy gain in the form of biogas which can replace a fraction of the conventional biomass fuel in a boiler or even combust in biogas plant for renewable electricity production. The two-stage AD process has been reported as a practical biotechnology to produce biohythane (biohydrogen and biomethane) from a variety of organic materials (Schievano *et al.*, 2012). This advanced AD technology is gaining attention because biohydrogen (H_2) is a clean alternative energy source. It is environmentally friendly and has only an end product of water after combustion.

Dark fermentation process for H_2 production has been applied in various organic waste materials such as cassava starch processing wastewater (Khongkliang *et al.*, 2017), hydrolyzed wheat starch (Cakır *et al.*, 2010), cheese whey (Ghimire *et al.*, 2017), etc. Recent investigations indicated thermophilic dark fermentation (TDF) was more advantageous than mesophilic dark fermentation for H_2 production (Cakır *et al.*, 2010; Kargi *et al.*, 2012). On the other hand, thermophilic anaerobic contact digester (TACD) demonstrated higher efficiency in CH_4 production compared to mesophilic anaerobic contact digester (İnce, 2017). The anaerobic contact process able to reach steady state quickly due to mixing and short hydraulic retention times is usually sufficient to obtain high effluent quality (Senturk *et al.*, 2013). The discharge temperature of POME is usually 80–90 °C. Thus, a two-stage thermophilic anaerobic system can be a beneficial treatment technique because it eliminates the need for a conventional cooling pond.

Recently, investigation of a two-stage AD of POME has reported the use of different bioreactor combinations and operational conditions (Mamimin *et al.*, 2015; Krishnan *et al.*, 2016a; Krishnan *et al.*, 2016b; O-Thong *et al.*, 2016; Krishnan *et al.*, 2017). Recent investigations demonstrated varied treatment efficiency with promising biohythane production. These studies involved several practices to stimulate the two-stage AD of POME in laboratory scale study. For example, short hydraulic retention time (HRT) and high organic loading rate (OLR) was applied in the two-stage AD using diluted POME

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(Krishnan *et al.*, 2017). Chemicals were used to adjust proper operating pH and alkalinity for H_2 production (Krishnan *et al.*, 2016b). Furthermore, micronutrient was added and C:N:P of POME was adjusted to selected ration in a previous study (Krishnan *et al.*, 2016a). However, the practicality of such applications remains questionable to reflect the actual biohythane potential from POME. To our knowledge, these practices may be impractical in the industrial scale treatment system.

To overcome this issue, this study was conducted without chemicals or nutrients addition as well as a dilution to avoid the modification of physicochemical characteristics of POME. This research aims to investigate the potential of biohythane production from POME in a two-stage AD system using a thermophilic dark fermenter (TDF) and a thermophilic anaerobic contact digester (TACD).

MATERIALS AND METHODS

Substrate and inoculum preparation

POME was obtained from a palm oil mill located at Nibong Tebal, Pulau Pinang, Malaysia. POME was collected, then stored in a refrigerator at 4 °C to minimize microbial degradation. POME was preheated to 55 °C prior to the experiment. Table 1 shows the typical physicochemical characteristics of POME. Inocula for the two-stage AD was collected from an existing laboratory scale thermophilic anaerobic digester which had been operated for more than 12 months for treating POME.

Table 1: The physicochemical characteristics of POME used in this study.

Parameter	Range	Value
pH	4.4 – 4.7	4.5 ± 0.1
COD	70600 – 77560	74820 ± 2312
TSS	16000 – 21500	18688 ± 1953
VSS	15600 – 21000	17567 ± 2084
O & G	3950 – 5600	4683 ± 565

* All parameters are in mg/L except pH.

Equipment setup

Two water jacketed bioreactors were used as semi-continuous stirred tank reactor (SCSTR) for a two-stage AD of POME. One SCSTR with a capacity of 3.75 L was configured as thermophilic dark fermenter (TDF) while another SCSTR with a capacity of 6.25 L was configured as thermophilic anaerobic contact digester (TACD). A 2 L external settling tank was installed for liquid-solid separation. The operating temperature of TDF and TACD was maintained at 55 °C by a heating bath circulator. The daily withdrawal and feeding of the substrate were done by means of a peristaltic pump. Intermittent mixing at 120 rpm in 1 min for every 45 min was performed by an overhead stirrer with preprogrammed timers. A 40 L Tedlar gas sampling bag was connected to TDF and TACD for biogas collection.

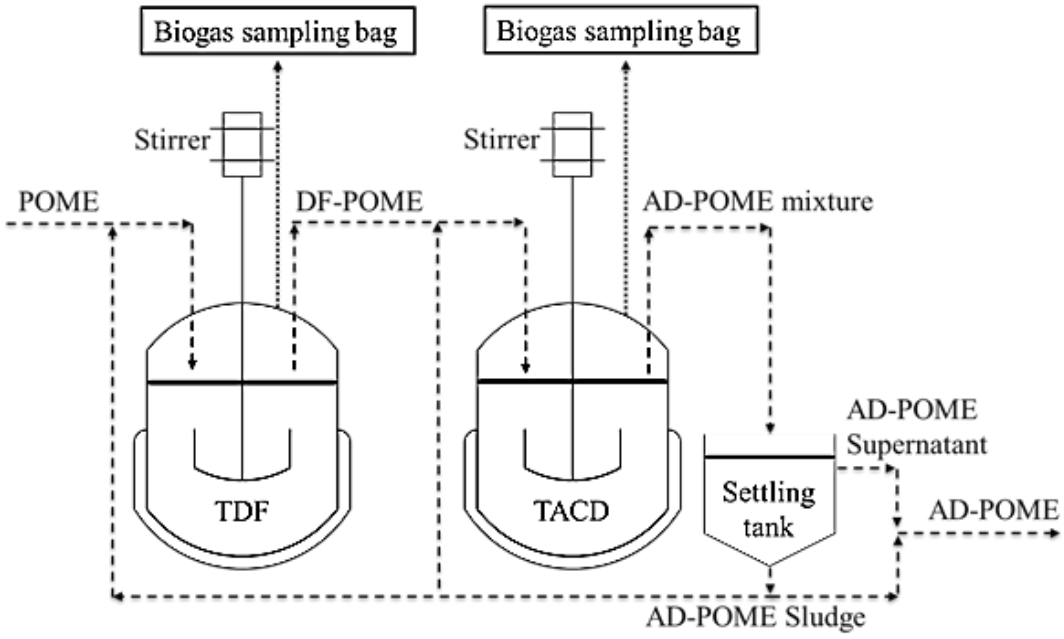

Figure 1: Schematic diagram of the two-stage AD of POME.

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Operation of two-stage AD

First, 3.75 L and 6.25 L of anaerobic inocula were loaded into TDF and TACD, respectively. Later, the preheated POME was fed into TDF for H_2 production whereas the dark-fermented POME (DF-POME) was fed into TACD for CH_4 production. The anaerobically digested (AD-POME) mixture was allowed to settle for 1 h. The sludge recirculation ratio (return sludge to POME flow) was fixed to 1 where the feeding rate of POME equal to the sludge recirculation flow rate. In this study, 45 % v/v of settled sludge was returned to TDF and 55 % was returned to TACD. Excess sludge combined with supernatant to become AD-POME. Figure 1 shows the schematic flow of the two-stage AD of POME. The TDF operated at organic loading rate (OLR) of 19.95 ± 0.39 g COD/L.d which is equivalent to hydraulic retention time (HRT) of 3.75 days. The HRT of TACD was 6.25 days. The two-stage AD of POME achieved steady-state conditions after 30 days of operation where the H_2 and CH_4 production were stable (< 15 % variation). Then, six consecutive results (from day 30 to day 45) of selected parameters were determined once every three days.

Analysis of samples

The measurement of pH, total alkalinity (TA), chemical oxygen demand (COD), total suspended solids (TSS), mixed liquor volatile suspended solids (MLVSS) were according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Free fatty acid (FFA) was analyzed using GC system (Shimadzu GC-2010 Plus) with a flame ionization detector (FID) and equipped with a BP 21, 25 m × 0.22 mm ID × 0.25 µm (SGE) column. Biogas volumes were recorded using a graduated gas-tight syringe. Biogas compositions were analyzed using Clarus 500 Gas Chromatography (Perkin Elmer).

RESULTS AND DISCUSSION

The sludge recycled back into TDF was sufficient to provide a high pH buffering capacity of the biological treatment system and maintained the initial operating pH to approximately 5.5 to inhibit the H_2 consuming homoacetogenic activity (Ghimire *et al.*, 2015). Both hydrolytic and non-hydrolytic acidogens were more active at pH 5.5 to 6.0 (Lv *et al.*, 2010). The final pH dropped to 5.2 (Table 2) although an average TA buffering capacity of 2103 g $CaCO_3$/L was achieved by the sludge recirculation. The corresponding observation possibly due to the accumulation of fatty acids during fermentation of POME in TDF. The sludge recirculation effectively stabilized TACD at pH value of 8.1 and TA of 7170 mg $CaCO_3$/L along the experimental period.

The MLVSS concentration in TDF was 18342 mg/L. This unexpected high value was due to the enclosure of MLVSS from recycled sludge as well as high VSS concentration in POME (Table 2). Consequently, high TSS concentration of 19942 mg/L was observed. On the other hand, the MLVSS concentration in TACD was only 12262 mg/L because it received 55% (volume) of the recycled sludge. The TSS concentration of AD-POME was 6800 mg/L, which indicated the total TSS degradation was 63.12%. The average COD concentration of AD-POME from TACD was 16620 mg/L, equivalent to COD degradation of 77.94 % in the two-stage AD of POME.

Table 2: The operating conditions and process performance at steady state conditions of the two-stage AD of POME.

Parameter	TDF	TACD
HRT, days	3.75	6.25
OLR, g COD/L.d		
Value	20.11 ± 0.39	11.80 ± 0.68
Range	19.60 – 20.68	11.16 – 12.97
pH		
Value	5.2 ± 0.1	8.1 ± 0.1
Range	5.1 – 5.3	8.0 – 8.2
TA, $CaCO_3$ mg/L		
Value	2103 ± 70	7170 ± 153
Range	2030 – 2220	7000 – 7350
MLVSS, mg/L		
Value	18342 ± 793	12262 ± 525
Range	17950 – 19950	11600 – 13100
TSS, mg/L		
Value	19942 ± 1060	6800 ± 252
Range	19100 – 22000	6550 – 7160
COD, mg/L		
Value	56522 ± 3247	16620 ± 1911
Range	53450 – 62100	15000 – 19760

The decline of pH in TDF was due to remarkable FFA production, as shown in Table 3. Accumulation of FFA in DF-POME was observed, especially for acetic acid (9450 mg/L), propionic acid (825 mg/L) and butyric acid (4520 mg/L). The higher concentration of acetic acid than butyric acid implied the major H_2 produced in TDF may follow the acetic acid pathway. However, the propionic acid inhibition for H_2 production shall not be neglected although its concentrations were less than 1000 mg/L. The butyric acid and acetic acid ratio (B/A) in TDF was 0.326, which is relatively close to the experimental results from previous literature (O-Thong *et al.*, 2016). Other research on thermophilic fermentation process of POME for H_2 production had recorded the B/A ratio ranged from 0.30 to 1.20 (Prasertsan *et al.*, 2009). The concentration of palmitic acid and oleic acid in DF-POME was 1510 and 1033 mg/L, respectively. However, its concentrations were

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slightly lower compared to raw POME possibly due to the incomplete hydrolysis of residue oil. Other fatty acids with a low concentration were observed, including valeric acid (70 mg/L), caproic acid (435 mg/L), stearic acid (120 mg/L), and linolenic acid (145 mg/L). Although the high concentration of FFA either in dissociated or undissociated form may inhibit H_2 production (Bundhoo and Mohee, 2016). A trace amount of FFA remained in AD-POME which is palmitic acid (190 mg/L), stearic acid (48 mg/L) and oleic acid (162 mg/L). This indicates there is no inhibition in biogas production because most of the FFA were rapidly degraded and utilized by anaerobic microorganisms.

Table 3: FFA of POME, DF-POME and AD-POME at steady state conditions of the two-stage AD of POME.

FFA, mg/L	POME	DF-POME	AD-POME
Acetic acid	2546 ± 250	9450 ± 851	< MQL
Propionic acid	162 ± 46	825 ± 61	< MQL
Butyric acid	287 ± 77	4520 ± 910	< MQL
Valeric acid	79 ± 12	70 ± 10	< MQL
Caproic acid	129 ± 11	435 ± 25	< MQL
Enanthic acid	126 ± 12	< MQL	< MQL
Myristic acid	47 ± 16	< MQL	< MQL
Palmitic acid	1790 ± 151	1510 ± 120	190 ± 44
Stearic acid	131 ± 21	120 ± 15	48 ± 11
Oleic acid	1381 ± 156	1033 ± 75	162 ± 57
Linoleic acid	141 ± 19	145 ± 21	< MQL

MQL = Method detection limit

As shown in Figure 2, the H_2 production detected at the early stage of the experiment indicated the rapid growth of thermophilic H_2 producing bacteria. The enrichment of bacteria, as well as pretreatment of sludge that practiced in published report (Prasertsan *et al.*, 2009; Krishnan *et al.*, 2017), are not necessary to initiate hydrogen production. However, the production was inconsistent and a steady H_2 production was observed only after 30 days. At steady state conditions, the biogas in TDF contained 26.60% of H_2 with a production rate of 1.54 L/L POME (Table 4). On average, 25.01% of COD was removed for H_2 production to give a H_2 yield of 0.085 L H_2/g $COD_{removed}$. In the TDF, methanogenic activity was inhibited because CH_4 was not detected. The acidic pH suppressed methanogen but allowed acidogens to grow (Krishnan *et al.*, 2017). Rapid CH_4 production was detected at the early stage of the experiment where the production trend seems less sensitive to the instability in TDF (Figure 3). At steady state conditions, the biogas in TACD contained 67.09% of CH_4 with a production rate of 19.87 L/L POME. On average, 70.59% of COD was removed for CH_4 production to give a CH_4 yield of 0.339 L CH_4/g $COD_{removed}$. This study shows that a two-stage AD of POME was able to produce biogas of 34.833 L/L POME containing 4.00% of H_2, 57.05% of CH_4 and 38.95% of CO_2. The energy recoveries were calculated based on the density of H_2 and CH_4 of 0.08988 and 0.717 kg/m^3, respectively, at standard temperature and pressure (STP) and the lower heating value of H_2 and CH_4 of 119.96 and 50 MJ/kg, respectively. The total energy of approximately 661.02 MJ/m^3 POME can be recovered from it. The experimental results indicated there was room for improvement because the renewable energy was dominated by CH_4 whereas H_2 only contributed to 2.28% of the total energy.

Table 4: H_2 and CH_4 production at steady state conditions of the two-stage AD of POME.

Parameter	TDF	TACD
H_2 production, L/d		
Value	1.54 ± 0.06	-
Range	1.45 – 1.62	-
H_2 content, %		
Value	26.60 ± 1.17	-
Range	25.00 – 27.90	-
H_2 yield, L H_2/g $COD_{removed}$		
Value	0.085 ± 0.020	-
Range	0.064 – 0.121	-
Energy from H_2, MJ/m^3 POME		
Value	15.04 ± 0.59	-
Range	14.19 – 15.81	-
CH_4 production, L/d		
Value	-	19.87 ± 0.59
Range	-	19.11 – 20.48
CH_4 content, %		
Value	-	67.09 ± 0.62
Range	-	66.03 – 67.73
CH_4 yield, L CH_4/g $COD_{removed}$		
Value	-	0.339 ± 0.021
Range	-	0.308 – 0.369
Energy from CH_4, MJ/m^3 POME		
Value	-	645.98 ± 19.19
Range	-	621.34 – 665.97
COD removal, %		
Value	25.01 ± 4.76	70.59 ± 2.99
Range	17.42 – 31.09	65.98 – 73.63

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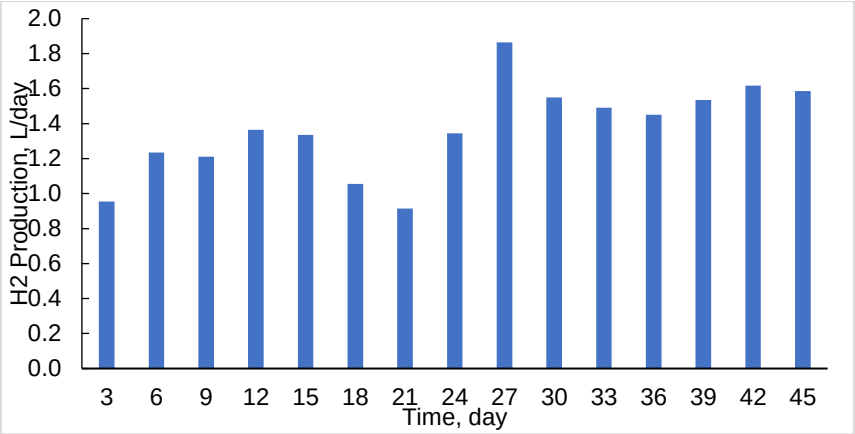

Figure 2: Profiles of continuous H_2 production in TDF of the two-stage AD of POME.

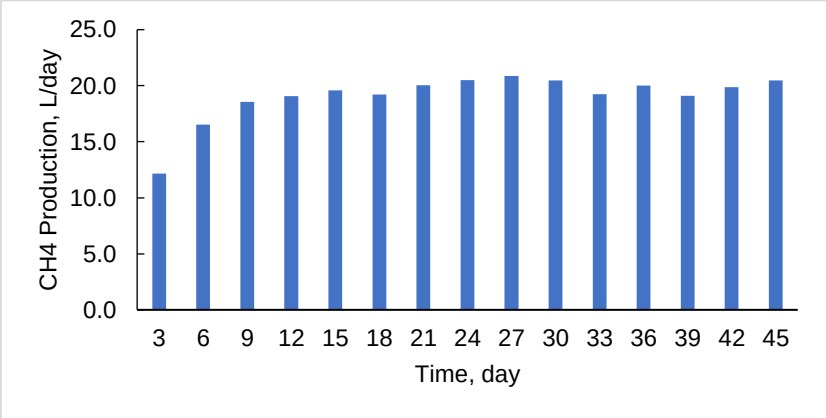

Figure 3: Profiles of continuous CH_4 production in TACD of the two-stage AD of POME.

Table 5: Current research of two-stage AD of POME for biohythane production.

Bioreactor (T, °C)	HRT, d	OLR, g COD/L.d	Biogas content, %	Biogas production rate, L/L.d	Biogas yield, L/g $COD_{removed}$	Ref.
ASBR (55)	2	60	55, H_2	1.8	0.210	(Mamimin *et al.*, 2015)
UASB (28-34)	15	6	73, CH_4	2.6	0.315	
UASB (55)	2	75	55, H_2	1.92	0.215	(Krishnan *et al.*, 2016a)
CSTR (37)	5	-	70–80, CH_4	3.2	0.320	
CSTR (55)	2	14.3[a]	55, H_2	1.31	0.180	(O-Thong *et al.*, 2016)
UASB (37)	15	1.58[a]	73, CH_4	1.18	0.271	
UASB (55)	0.375	75	35, H_2	2.1[b]	0.075[b]	(Krishnan *et al.*, 2016b)
CSTR (55)	12	12	65, CH_4	13[b]	0.156[b]	
UASB (55)	0.5	50	45.08, H_2	2.5[b]	0.033	(Krishnan *et al.*, 2017)
CSTR (60)	5	13.1	67.74, CH_4,	10.58[b]	0.11	
TDF (55)	3.75	20.11	26.60, H_2	0.41	0.085	This study
TACD (55)	6.25	11.80	67.09, CH_4	3.18	0.339	

[a] Self-estimated; [b] Unit in L/d.

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The performance of current research of two-stage AD of POME for biohythane production is summarized in Table 5. These studies demonstrated promising biohythane production using different bioreactor combinations and operating conditions. The investigation of a two-stage AD of POME reported the high H_2 content of 35-55% and CH_4 content of 65-80%. However, the corresponding H_2 and CH_4 yields were relatively lower than the results observed in this study. It should be noted that current research involved the use of diluted POME, enriched inoculum, adjusting pH and alkalinity using chemicals, adding micronutrients as well as balancing the C:N:P to a selected ratio (Mamimin *et al.*, 2015; Krishnan *et al.*, 2016a; Krishnan *et al.*, 2016b; O-Thong *et al.*, 2016; Krishnan *et al.*, 2017). These practices may stimulate biohythane production but its practicality and economic feasibility remain questionable in industrial scale application. Instead of focus in treatability study, future research should consider the operational conditions in laboratory scale investigation that is able to mimic the performance of an industrial scale application. The H_2 yield in this study was lower than the current research which implied there's room for improvement in TDF. Also, the recorded H_2 production rate was much lower than current research. On the other hand, the CH_4 yield in this study was the highest among the published reports. The experimental results indicated TDF was operated in a suboptimal condition which may be linked to the comparatively long HRT in TDF that caused accumulation of FFA. Operational control such as HRT and sludge recirculation ratio could be modified to optimize biohythane production as well as treatment efficiency.

CONCLUSION

Biohythane production using two-stage AD of POME was demonstrated in this study. The total energy of 661.02 MJ/m^3 POME could be recovered in biogas which contains 4.00% of H_2, 57.05% of CH_4 and 38.95% of CO_2. The total degradation of COD and TSS in the two-stage AD of POME was 77.94 and 63.12%, respectively. The sludge recirculation from TACD is able to adjust the pH in TDF to the optimum value of 5.5. Low H_2 production in TDF was observed which may be due to its suboptimal operating conditions.

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