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Accelerated Start-up of a Semi-commercial Digester Tank Treating Palm Oil Mill Effluent with Sludge Seeding for Methane Production

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Abstract: The modern closed digesters are becoming more popular for treating palm oil mill effluent (POME) and are currently being installed nationwide in Malaysia to replace the conventional open lagoons and tanks treatment system. This paper describes an accelerated start-up of the 500 m^3 semi-commercial anaerobic digester treating POME and methane gas recovery for clean development mechanism (CDM) project. Results showed that by direct seeding through the transfer of the sludge from either top or bottom of the open digester tank, the start-up period was significantly shortened. The bottom seed sludge transfer led to interesting results including a 24 day start-up period, stable pH condition (pH 6.8-7.2), high COD removal efficiency (>90%), satisfactory VFA to Alk ratio (<0.3), satisfactory biogas production of nearly 1.8 kg/m^3/d) and methane composition of 50 to 60%. The presence of high amount of methanogens in the seed sludge significantly reduced the need for a long acclimatization period and the digester could be fed with POME within less than a day after the seed sludge transfer process was completed. Close examination using scanning electron microscopy (SEM) and fluorescence *in situ* hybridization (*FISH*) revealed abundant amount of bacteria and methanogens, in particular *Methanosaeta* sp., in the seed sludge samples, which are very important for successful acidogenesis and methanogenesis processes.

Key words: Palm oil mill effluent (POME) · Anaerobic treatment · Digester start-up · Biogas · Methane

INTRODUCTION

Malaysia is currently the largest producer and exporter of palm oil in the world and the industry contributes significantly to the country's economy [1]. Despite huge benefits to the Malaysian economy, the palm oil industry also generates large amounts of wastes in the form of empty fruit bunch (EFB), oil palm frond, mesocarp fiber, palm kernel shell, palm oil mill effluent (POME) and sludge from ponds and anaerobic tanks [2-3]. In general, millions of tonnes of these wastes are available each year and ready to be exploited. In the case of POME, the most popular treatment method currently is using open ponds or tanks [4]. Therefore, its utilization for higher valued products such as methane in industrial scale is rather limited despite the fact that a considerable amount of literature has been published on methane production by using anaerobic digestion technology [4-13]. However, in the last few years there has been a great concern to utilize POME for methane production via clean development mechanism (CDM) project for certified emission reduction (CER) trading [14-20].

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In theory, POME could be used as a substrate for anaerobic digestion to produce methane gas because it contains high amount of organic substances with negligible inhibitory substances [4,10-13]. By utilizing the captured methane for electricity generation, high amount of CER could be generated which is very attractive to the industry for carbon trading. For any types of bioreactor design and organic wastes treatment systems, start-up operation remains the most critical stage of operation [21-31]. A study on anaerobic filter reactor (AFR), anaerobic fluidized bed reactor (AFBR) and upflow sludge blanket (UASB) reactors on high strength industrial wastes (pharmaceutical, glucose manufacturing, fruit processing and soft drink manufacturing), showed that between 50 and 75 days was required to start-up the reactors using stepped-loading of organic loading rate (OLR) technique [21]. UASB reactor showed shortest start-up period of 50 days in comparison to filter and fluidized bed reactors using similar start-up technique (i.e stepped-loading of OLR) technique. Using a 5.5 liters stirred reactor a study reported a longer start-up period of between 10-12 weeks was required by using stepwise addition of substrate with constant hydraulic retention time (HRT) for treating tomato-processing waste [22]. Stepwise seeding was proposed in a different study using anaerobic upflow biofilter (AUBFR) reactor for treating synthetic ethanol plant wastewater but long start-up time of 121 days was required [24]. A study using an anaerobic hybrid reactor (AHR) (combination of UASB and filter reactor) for treating coffee processing plant wastewater by varying the OLR, a fast start-up period of only 40 days was required [25]. However, they encountered a significant reduction of COD removal efficiency of down to 25.9% only just a week after the initial start-up.

In another study conducted using an upflow anaerobic filter reactor (UAFR) reactor, a shorter start-up period of 32 days was required for the treatment of synthetic wastewaters with limited and balanced nitrogen content by adopting stepped-loading of OLR [26]. On the other hand, by using the same technique however, longer start-up period of 100 days was reported for an anaerobic fixed-film reactor (AFFR) treating synthetic wastewater [27]. Similar long start-up period of between 122-140 days was also reported in a study using UASB for treating synthetic substrate by using the same technique [28].

For the case of POME, by using a conventional tank reactor for treating POME, a study showed that a start-up period of 3 months was required by means of stepped increment of POME loading rate [29]. Using an UASB for treating synthetic medium, a study claimed shorter start-up period of between 30-50 days only using stepped-loading of OLR with initial OLR of 3.8 g COD/L/d [30]. The simulated winery wastewater treatment was treated using inverse turbulence bed bioreactor (ITBR) and resulted in a shorter start-up period of only 45 days only by employing stepped- loading of OLR with low initial OLR of 0.5 g COD/L/d [31]. Based on the literatures, in general stepwise increment of OLR was a better technique for the start-up operation. However due to the longer time was required for cells acclimatization and growth, the length of the whole start-up operation could not be shortened. The present research was the continuation of our previous work carried out [29]. The objective of this study was to achieve an accelerated start-up process by reducing the acclimatization period of the microorganisms inside the digester. This was achieved by direct transfer of the matured POME sludge from an existing POME treatment facility. The digester stability was evaluated based on effluent pH, volatile fatty acid accumulation and COD removal efficiency whereas the digester performance was investigated based on biogas production rate and methane composition in biogas at different application of OLR.

MATERIALS AND METHODS

Semi-Commercial Digester Set-Up: Figure 1 shows the set-up of the semi-commercial closed digester tank used in this study. The system consists of a 500 m^3 digester tank, a 30 m^3 settling tank and a 55 m^3 holding tank. The system was also equipped with effluent mixing system using centrifugal pump recirculation system. The parameters including pH, temperature, biogas flow-rate and POME flow-rate were measured on-line using CeraGel CPS 71, TST 266, Tmass AT70F and Proline Promag 50 (Endress+Hauser, Germany), respectively. The data were recorded on-line and stored using Eco-graph recorder (Endress+Hauser, Germany).

Source of Seed Sludge and POME: The seed sludge was obtained from a 3600 m^3 open digester tank for treating POME within the mill's compound. For this study, the seed sludge was obtained either from the bottom or from top of the tank through 6 inches pipeline connecting both digesters as shown in Figure 2. The direction of the seed sludge flow was controlled by opening and closing of valves V1 and V4 for bottom seed sludge and top seed sludge transfer, respectively. Throughout this experiment, the fresh POME was obtained directly from Serting Hilir Palm Oil Mill which was located in the vicinity of the

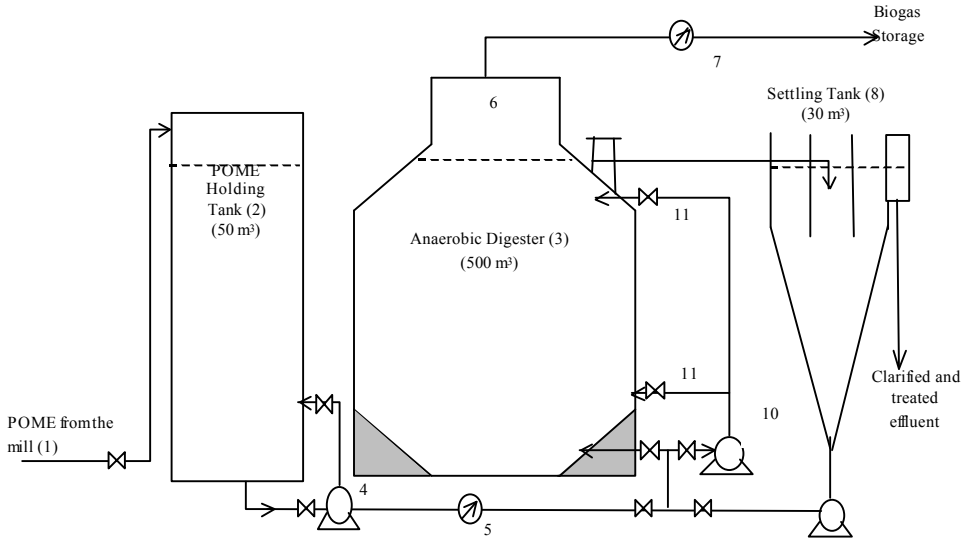

Fig. 1: Schematic diagram of the semi-commercial closed anaerobic digester complete with a holding tank and a sludge settling tank. 1- POME inlet from the mill; 2-Holding Tank; 3-500 m^3 anaerobic digester; 4-Centrifugal pump for feeding; 5-Endress+Hauser POME inlet mass flow meter; 6-Biogas chamber; 7-Endress+Hauser biogas mass flow meter; 8-Sludge settling tank; 9-Roto pump for sludge recycling; 10-Mixing pump; 11- Liquid recirculation line inlet.

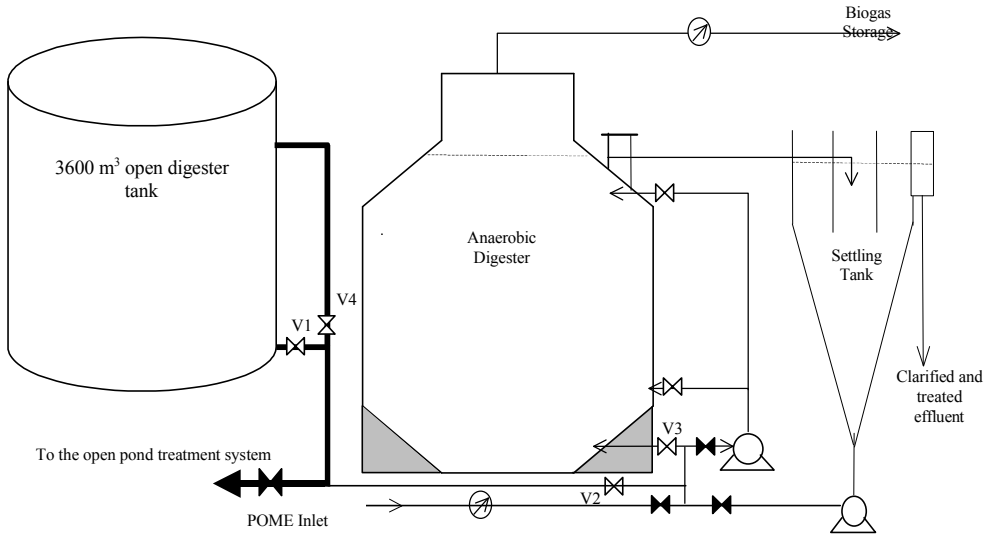

Fig. 2: Schematic diagram shows the pipeline connection between 3600 m^3 digester and 500 m^3 digester. To ensure less air penetration to the system valves V1, V2 and V3 are opened (⋈) and the rest are closed (⋈) during seeding process. The line (▬) represent 6 inches line.

reactor. The fresh POME used was directly pumped from the mill's mixing pond and stored in a 55 m^3 holding tank before being used. The POME samples were collected from this holding tank for analysis.

Experimental set-up: The 500 m^3 digester was subjected to two experiments of a similar start-up method (i.e increasing the POME loading rate) with different seed sludge source. One source for obtaining seed sludge was the top of the existing 3600 m^3 open anaerobic digester tank and from the other was the bottom portion of the same tank. The transferring of the former portion took two days while one day was required to transfer the seed sludge from the latter portion of the tank. In both

experiments, the seed sludge was acclimatized only for less than a day and once the biogas production started, the digester start-up operation was initiated with minimal loading of 5 m^3/d of POME loading rate (PLR) on the following day.

Chemical and Microbial Analyses: Analysis on pH, chemical oxygen demand (COD), volatile fatty acids (VFA), alkalinity, total solid (TS) and volatile suspended solid (VSS) were carried out according to the American Public Health Association Standard Methods for the Examination of Water and Wastewater [32]. The methane composition was analyzed *in situ* using portable methane gas analyzer (XP-314A, Shin-Cosmos Electric Co. Ltd., Japan). For light microscope and scanning electron microspope (SEM) analyses, the sludge samples were collected from the bottom and top portions of the 3600 m^3 open digester tank and stored in 50 mL sterilized falcon tubes, placed in crushed ice and transported to the laboratory and stored at -20°C until use. The samples preparation and viewing was similar with our previous technique [33]. For fluorescent *in situ* hybridization (*FISH*) analysis and methanogenic cells counting, the technique applied was also similar with our previous work in this area [33]. 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).

RESULT AND DISCUSSION

Sludge Transfer Process and Acclimatization Period: The sludge samples from the bottom and top portions of the 3600 m^3 open digester tank were analyzed and characterized by lower total COD content as compared to untreated POME, neutral pH (7.2-7.5), high nitrogen content (300-350 mg/L of total TKN) and high alkalinity (3,500-4,000 mg/L $CaCO_3$). However the total solid (TS) and volatile suspended solid (VSS) was higher in the seed sludge taken from the bottom of the tank of up to 5.6-6.2% TS and 2.8-3.5% VSS, respectively. The carbon, nitrogen and phosphorous (C/N/P) contents in the sludge of both samples were in the range of 25.0-32.5 %, 3.1-3.9% and 1.5-2.1%, respectively. The higher percentage belonged to the sample obtained from the bottom of the tank which reflected the existence of higher amounts of microorganisms in the bottom seed sludge compared to the top seed sludge.

Microbial analysis using SEM of the seed sludge samples are shown in Fig. 3. The monogram revealed rod shape microorganisms which are believed to be important in the degradation process of organics in POME and bioconversion to methane. The amount of microorganisms was higher in the samples for the bottom seed sludge in comparison to the top seed sludge. Advanced molecular technique using *FISH* confirmed the presence of bacteria and methanogens in the seed sludge samples as shown in Fig. 4A, 4B and 4C. The light microscope picture (Fig. 4D) confirms the presence of cells in the sample. The picture obtained for the bottom seed sludge clearly shows the methanogen species to be *Methanosaeta concilii*. Using the technique previously described [33], the amount of *Methanosaeta concilii* in the bottom seed sludge sample was determined to be approximately 2.0 x 10^8 cells/ mL sludge whereas 1.9 x 10^6 cells/mL sludge was estimated for the top seed sludge sample.

The seed sludge was allowed to acclimatize for approximately less than a day in both experiments before the feeding started. Acclimatization period is time consuming as reported in several studies on anaerobic fixed-film reactor (AFFR) treating synthetic wastewater [27] and sequencing batch reactor (SBR) treating swine waste [34]. However in this study, the seed sludge obtained was obtained from the same type of waste treatment facility (i.e POME) and detected to contain high amount of bacteria and methanogens, thus long acclimatization period was unnecessary because the microorganisms was already acclimatized. Thus, the seed sludge was only let to stable for less than a day and this has helped to reduce the start-up period. For the case of bottom seed sludge experiment, a very low level of biogas production rate was measured of approximately 0.1 kg/hr during the initial stage of acclimatization period as shown in Figure 5. However, the biogas production rate was slightly increased to nearly 0.4 kg/hr just before POME feeding was initiated after 16 hours of acclimatization. Once loaded with 5 m^3/d of POME which corresponding to OLR of 0.68-0.77 kgCOD/m^3/d, the biogas production rate started to increase significantly as shown in Figure 6. A few hours after feeding, the biogas production rate increased from less than 5.0 kg/hr to nearly 20.0 kg/hr. Since only single feeding was applied at this stage, the biogas production rate started to reduce to only 10.0 kg/hr before feeding schedule on the following day. This is in agreement with previous studies where at low level of OLR applied, low level of biogas production rate was also observed during the start-up period [22, 28, 29, 35-38].

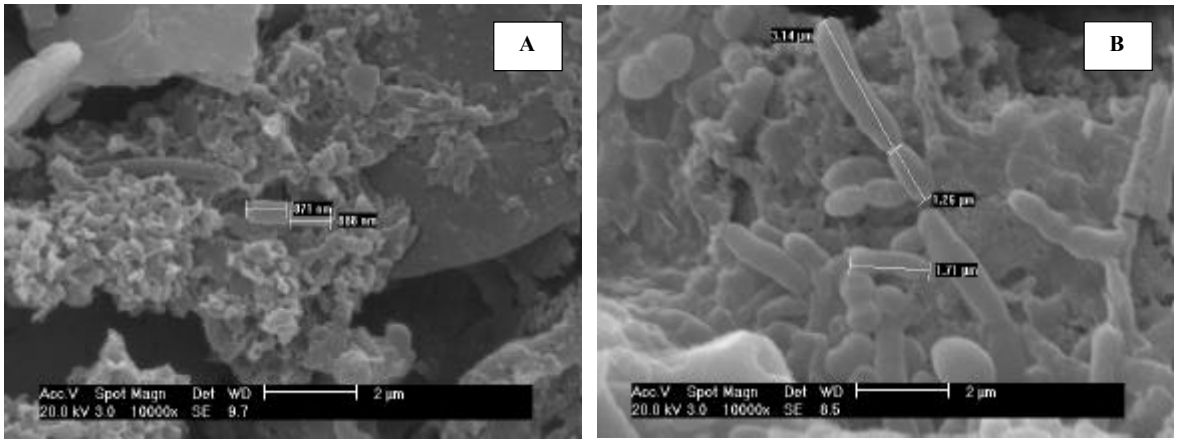

Fig. 3: The SEM monogram of the seed sludge sample (A-Top seed sludge sample; B-Bottom seed sludge sample from the 3600 m^3 open digester). The pictures reveal abundant amount of microorganisms in both samples with higher in bottom seed sludge sample.

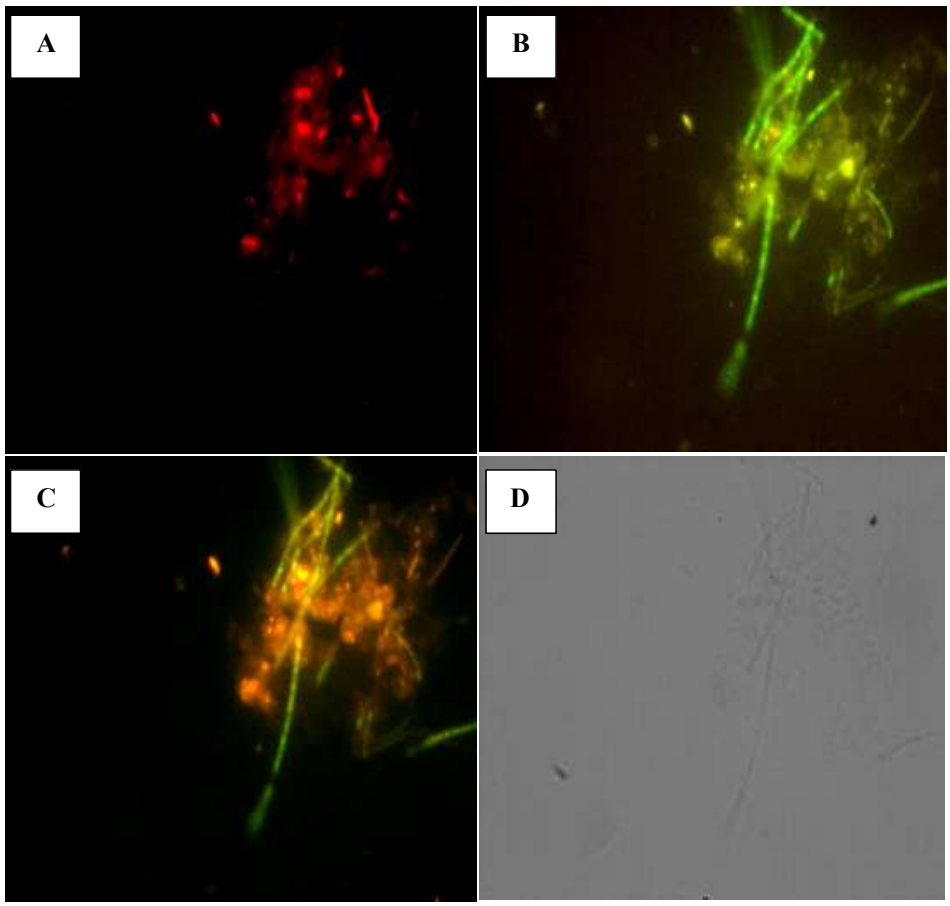

Fig. 4: *FISH* staining of the sludge sample used for seeding obtained from the bottonm portion of the 3600 m^3 open digester tank analyzed by confocal laser microscopy of fluorescent in situ-hybridized cells (A-Bacteria, B-*Methanosaeta Concilii* and C-Simultaneously hybridized with rhodamine-labeled bacterial-domain probe (EUB338) (red) and FITC-labeled methanogens probe (MSMX860) (green) showing the consortium between methanogens and bacteria) and D-Light microscopy picture.

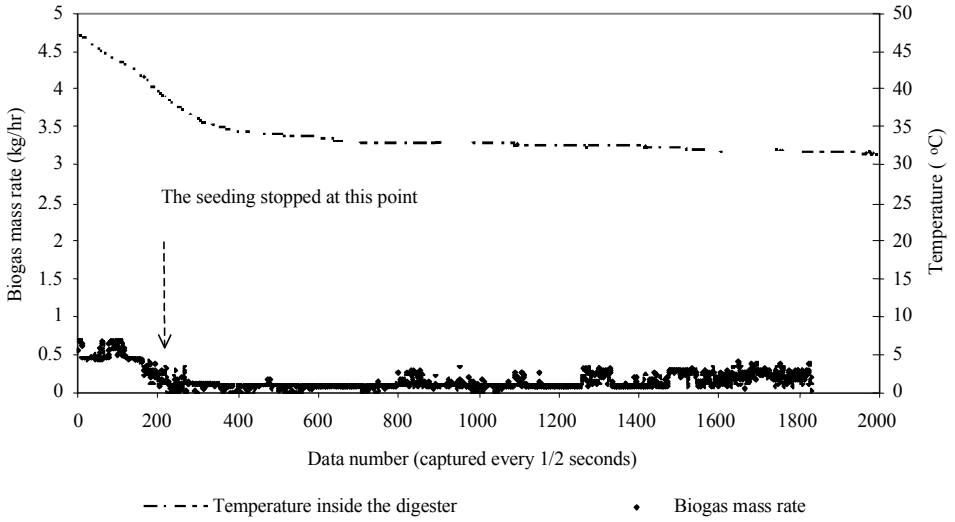

Fig. 5: The graphs showing the biogas mass rate (kg/h) and temperature drop inside the digester during the acclimatization period

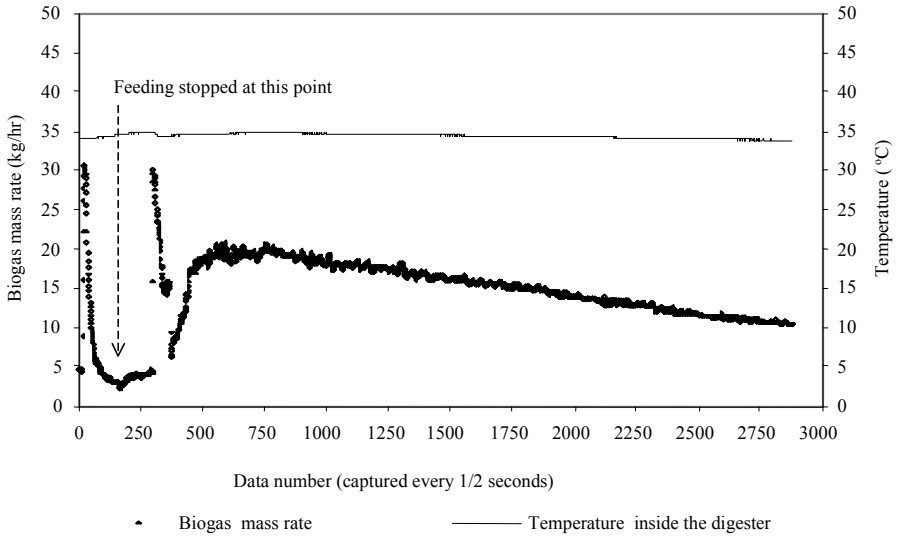

Fig. 6: The graphs showing the biogas mass rate (kg/hr) and the temperature inside the digester on the first POME feeding

Start-up Strategy: In this study, the start-up strategy adopted was stepwise increase of POME loading rate (PLR) using COD removal efficiency and biogas production as indicators for increasing the daily feeding rate or OLR. These indicators were easier and faster to be determined on-site in comparison to methane yield as proposed in other study [27]. The stepwise increase of PLR with time during the start-up period is given in Table 2. For the bottom seed sludge experiment, soon after completing the transfer process from the existing 3600 m^3 open digester tank, the seed sludge was allowed to stablilise for less than a day before loading with 5.0 m^3/d or OLR of 0.68-0.77 kgCOD/m^3/d of fresh POME from the mill until day 7th. From days 8-12, PLR was increased slightly to 10 m^3/d, which correspond to OLR of 1.23-1.68 kgCOD/m^3/d. A slightly higher PLR of 20 m^3/d (OLR of 2.12-3.56 kgCOD/m^3/d was applied from days 13-17. Finally, from days 18-25 the digester was loaded with PLR of 30 m^3/d (OLR of 3.90-4.74 kgCOD/m^3/d) and continued with normal operation of OLR fixed at 5.0 kgCOD/m^3/d from days 26 onwards. In Table 2, the OLR was slowly increased from low values (between 0.68 and

Table 1: Start-up period for different types of reactor design and organic wastes treated

Reactor	Waste type	Start-up method	Period	Ref.
AFR	Pharmaceutical waste containing N-propanol	Stepped-loading of OLR	69 days	[21]
AFR	Pharmaceutical waste containing dimethylformamide	Stepped-loading of OLR	75 days	[21]
UASB	Pharmaceutical waste containing N-propanol	Stepped-loading of OLR	50 days	[21]
UASB	Pharmaceutical waste containing dimethylformamide	Stepped-loading of OLR	50 days	[21]
AFBR	Pharmaceutical waste containing N-propanol	Stepped-loading of OLR	69 days	[21]
AFBR	Pharmaceutical waste containing dimethylformamide	Stepped-loading of OLR	75 days	[21]
-	Tomato-processing waste	Stepwise addition substrate fraction with constant HRT 10-12 weeks	[22]	
AUBFR	Synthetic substrate to simulate alcohol production plant	Stepwise seeding of inoculum with constant OLR at 0.26 kg COD/m^3/d	121 days	[24]
AHR (UASB/Filter)	Wastewater from coffee processing plant	Fluctuate OLR starting with 1.06 kgCOD/m^3/d.	40 days	[25]
UAFR	Synthetic wastewaters (nitrogen limited-NL and nitrogen balanced-NB)	Stepped-loading of OLR	32 days	[26]
AFFR	Synthetic wastewater	Increasing OLR from 1.1 gCOD/L/d	100 days	[27]
UASB	Synthetic substrate	Stepwise-loading of OLR	122-140 days	[28]
TR	Palm oil mill effluent	Stepped increment of substrate loading rate	3 months	[29]
UASB	Synthetic medium	Stepped–loading of OLR from 3.8 g/L/d	30-50 days	[30]
ITBR	Simulated winery wastewater	Stepped-loading of OLR from 0.5 g/ L/d	45 days	[31]

OLR–organic loading rate; HRT-hydraulic retention time; AFR- Anaerobic filter reactor; UASB-Upflow anaerobic sludge blanket; AFBR-Anaerobic fluidized bed reactor; AFFR-Anaerobic fixed-film reactor; AUBFR-Anaerobic upflow biofilter reactor; AHR(UASB/Filter)-Anaerobic hybrid reactor (UASB/Filter); UAFR-upflow anaerobic filter reactor; TR-Tank reactor; ITBR-Inverse turbulent bed bioreactor.

Table 2: The start-up profiles in terms of PLR, OLR, HRT, pH, COD concentration, TS and VSS for the bottom POME sludge transfer experiment

	Palm oil mill effluent (POME) parameters					Organic loading rate	Hydraulic retention time
Days	PLR (m^3/d)	pH	COD g/L	TS g/L	VSS g/L	kgCOD/ m^3/d	d
1-7	5.0	4.12-4.42	68.2-76.7	32.0-40.0	13.2-21.5	0.68-0.77	100
8-12	10.0	4.38-5.30	61.7-84.1	30.0-34.0	15.2-25.6	1.23-1.68	50
13-17	20.0	4.36-4.56	52.9-88.9	28.0-36.0	13.1-18.9	2.12-3.56	25
18-25	30.0	4.22-4.69	65.0-79.0	20.0-38.0	17.8-21.3	3.90-4.74	16.7
26-51*	22.0-40.0	4.25-4.52	63.2-110.8	30.0-44.0	17.1-44.0	5.00	12.5-22.7

*Start-up operation completed on day 24th. From day 25th onwards the digester was operated at OLR of 5.0 kgCOD/m^3/d.

Table 3: The start-up profiles in terms of PLR, OLR, HRT, pH, COD concentration, TS and VSS for the top POME sludge transfer experiment

	Palm oil mill effluent (POME) parameters					Organic loading rate	Hydraulic retention time
Days	PLR (m^3/d)	pH	COD g/L	TS g/L	VSS g/L	kgCOD m^3/d	d
1	5.0	4.27	67.7	42	25.2	0.68	100
2-8	10.0	4.19-4.38	67.9-86.5	36-48	22.1-30.1	1.36-1.73	50
9-14	20.0	4.25-4.31	66.6-80.2	28-58	22.0-33.9	2.66-3.21	25
15-23	30.0	4.23-4.51	68.7-96.0	34-46	14.8-26.7	4.12-5.76	16.7
24-29	40.0	4.27-4.43	69.5-88.5	36-50	19.2-35-3	5.56-7.08	12.5
30-40	20.0	4.36-5.07	68.2-92.0	34-56	17.4-33.4	2.86-3.68	16.7
41-78*	21.0-49.0	4.18-4.88	47.9-117.9	18-42	20.0-36.7	5.00	10.2-23.8

*Start-up operation completed on day 40th. From day 41st onwards the digester was operated at OLR of 5.0 kgCOD/m^3/d

Table 4: The stability in terms of VFA:Alk, pH and COD removal efficiency for the bottom and top seed sludge transfer experiments

Bottom sludge transfer				Top sludge transfer			
PLR (m^3/d)	pH value	VFA:Alk	COD removal efficiency	PLR (m^3/d)	pH value	VFA:Alk	COD removal efficiency
5.0	6.9-7.1	0.05-0.1	96.3-97.6	5.0	7.1	0.12	95.9
10.0	7.0-7.1	0.06-0.09	95.9-97.3	10.0	7.0-7.2	0.05-0.13	95.5-97.0
20.0	6.9-7.0	0.05-0.14	95.1-97.4	20.0	6.9-7.1	0.09-0.15	95.6-96.2
30.0	6.9-7.1	0.07-0.23	96.1-97.0	30.0	7.0-7.2	0.14-0.39	93.6-96.2
40.0	6.8-7.0	0.08-0.23	94.9-97.9	40.0	7.1-7.3	0.20-0.48	94.0-95.4
-	-	-	-	20.0	7.0-7.2	0.14-0.50	92.8-96.4

0.77 kgCOD/m^3/d) to higher values (between 3.9 and 4.74 kgCOD/m^3/d) because of increasing the PLR strategy and this has proved to be a good technique to avoid shock loading to the system. This is in agreement with previous study where low level of OLR has been applied at the beginning of start-up operation in order to avoid shock loading to the system regardless of the system design, substrate used and environmental condition [21, 24-29, 36, 38]. Although the acclimatization was significantly shortened, the start-up operation was able to proceed because the seed sludge was already acclimatized in the previous operation and need not to be further acclimatized. Furthermore, the bottom seed sludge contained higher amount of solids, which provided higher number of microorganism to the system and eventually make the start-up process more stable against the increasing organic loading.

The feeding profiles for the stepwise increase of PLR with time for the start-up experiment of the top seed sludge transfer is given in Table 3. Similar with the previous experiment, soon after the seed sludge, the seed was also allowed to stable for less than a day before started to feed with PLR of 5.0 m^3/d (OLR of 0.68 kgCOD/m^3/d) on the following day. From days 2-8, PLR was increased to 10 m^3/d (OLR of 1.36-1.73 kgCOD/m^3/d). Then slightly higher PLR of 20 m^3/d (OLR of 2.66-3.21 kgCOD/m^3/d) was applied from days 9-14. From days 15-23, the digester was loaded with PLR of 30 m^3/d (OLR of 4.12-5.76 kgCOD/m^3/d) and increased further to 40 m^3/d (5.56-7.08 kgCOD/m^3/d) from days 24-29. However, the digester became unstable with high VFA level and thus PLR was decreased to 20 m^3/d (OLR of 2.86-3.68 kgCOD/m^3/d) from days 30-40 to stabilize the system. Once the system became stable, the OLR was fixed at 5.0 kgCOD/m^3/d from day 41 onwards. Generally, the OLR was slowly increased from low value of 0.68 kgCOD/m^3/d to higher values (between 3.9 and 4.74 kgCOD/m^3/d). However the system became slightly unstable at PLR of 40 m^3/d (OLR of 5.56-7.08 kgCOD/m^3/d), but quickly recovered once PLR was reduced down to 20 m^3/d (OLR of 2.86-3.68 kgCOD/m^3/d). This phenomenon could be explained due low amount of microorganisms available in the seed sludge to encounter high PLR applied (40 m^3/d) which then led to shock loading. Problem associated with shock loading was also observed in another study using AHR (UASB/Filter) for treatment of wastewater from coffee processing plant where treatment performance significantly dropped to 22.4% only after two weeks of start-up at OLR of only 2.59 kgCOD/m^3/d [25].

Stability of the Digester: A stable digester operation for various types of digester designs, substrates used and environmental conditions during the start-up period has been widely reported in many studies [21, 26-29, 36, 38]. In these studies, the parameters used were volatile fatty acids, alkalinity, pH, biogas production, biochemical oxygen demand and chemical oxygen demand removal efficiency, methane yield and composition in biogas, specific methanogenic acivity (SMA), mixed liquor volatile suspended solid concentration, sludge-to-volume index (SVI) to indicate the stability of the digester. The analysis of some of these parameters were time consuming, thus in this study the stability was assessed based on volatile fatty acids-to-alkalinity ratio (VFA:Alk), pH of the treated effluent and chemical oxygen demand (COD) removal efficiency. These indicators were faster to be determined and thus are important on-site parameters to understand the stability of the digester. The values of these parameters are shown in Table 4 for the bottom seed sludge transfer and top seed sludge transfer experiments, respectively.

Generally, the stability of the digester seeded with the bottom sludge was better in comparison to the top sludge. This can be seen from the values of VFA:Alk ratio, where throughout the experiment the former recorded values less than 0.23 whereas the latter recorded higher values of nearly 0.5 when the PLR was increased to 40 m^3/d (OLR of 5.56-7.08 kgCOD/m^3/d).

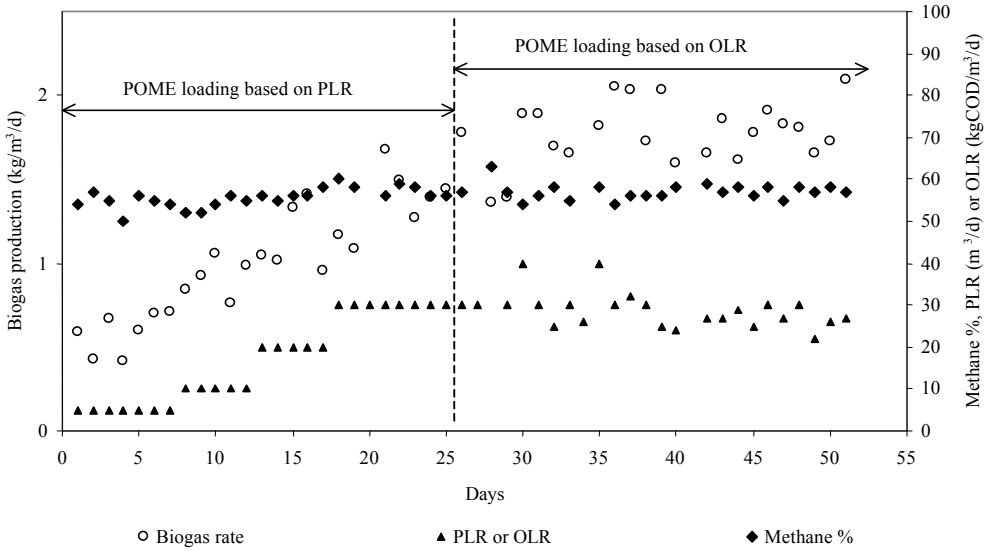

Fig. 7: Graphs showing the profiles for the biogas production (kg/m^3/d) and methane composition (%v/v) at different PLR (m^3/d) and OLR (kgCOD/m^3/d) applied during the start-up operation for the bottom seed sludge transfer experiment

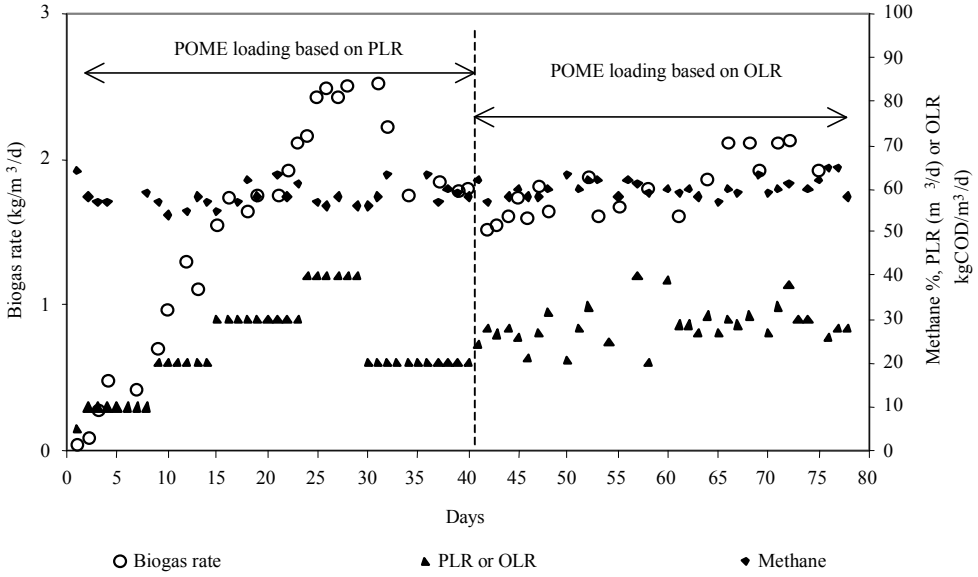

Fig. 8: Graphs showing the profiles for the biogas production (kg/m^3/d) and methane composition (%v/v) at different PLR (m^3/d) and OLR (kgCOD/m^3/d) applied during the start-up operation for the top seed sludge transfer experiment.

Despite the difference in VFA:Alk ratio values, both experiments recorded a stable operation with pH value of the treated effluent between 6.8 and 7.3 and high COD removal efficiency of more than 90% removal. This indicates suitability of both seed sludge used and stepwise increase of POME loading rate method for the start-up operation.

Methane Production: In previous studies, both biogas and methane production and yield were used to monitor the performance of the anaerobic digester during the start-up period [22, 24, 27-29, 31, 36-38]. However in this study, only biogas production rate and methane percentage in biogas were shown to be sufficient as additional on-site parameters to monitor the performance of the digester.

The values of the biogas productivity expressed in mass rate of biogas over the digester volume (kg/m^3/d) are shown in Figure 7 and Figure 8 for the bottom and top seed sludge transfer experiments, respectively.

At low range of PLR applied of 5.0-10.0 m^3/d, the biogas production was observed to be slightly higher in the digester seeded with bottom sludge with value recorded nearly 0.6 kg/m^3/d. However, once the PLR was increased to between 20.0 and 30.0 m^3/d, the biogas production was recorded higher in the digester seeded with top sludge of nearly 1.8 kg/m^3/d. This could be explained in terms of OLR applied, where at this stage the OLR applied was higher in the digester seeded with top sludge in comparison to bottom sludge. Furthermore, both systems were stable and microorganisms were able to efficiently utilize the organics available in the system. Consequently, this resulted in higher biogas production as well. This is in agreement with other studies, where biogas production increases with OLR applied [24, 27-29, 36, 38]. For the latter case, once the OLR was fixed at 5.0 kgCOD/m^3/d, the biogas production was observed to steadily increase to nearly 1.8 kg/m^3/d. As for the methane composition of biogas, high values between 50-60 % were recorded in both experiments throughout the thsi study even when the HRT was steadily climbing down from 100 days (at the beginning of the experiment when loading rate was only 5 m^3 of POME/d) to 16.7 days (at the end of start-up period before OLR was fixed at 5 kgCOD/m^3/d). This was ascribed to the availability of high amounts of active microorganisms (especially methanogens) in the seed sludge obtained from the existing POME treatment facility, which were able to convert POME to methane gas efficiently. Although the biogas production was slightly deteriorated from days 30-40 in the top seed sludge experiment due to high VFA accumulation in the system but the process was quickly recovered. This was accomplished by reducing the OLR applied and therefore, the methane composition did not change and remained high at nearly 60%. This finding further strengthened our previous conclusion on the availability of high amounts of active methanogens in the system, which helped to improve the recovery process.

For the digester seeded with top sludge, once the PLR was increased to 40 m^3/d (OLR of 5.56-7.08 kgCOD/m^3/d), the digester became slightly unstable due to acidification with VFA:Alk ratio measured as high as 0.48. Despite this, the system still maintained neutral pH (7.1-7.3), high COD removal efficiency (>90%) and satisfactory biogas production (1.8 kg/m^3/d). To avoid further acidification on the system, the PLR was reduced to 20 m^3/d (OLR of 2.86-3.68 kgCOD/m^3/d) in order to stabilize the system. As a result the biogas production slightly reduced to approximately 1.4 kg/m^3/d. Once the digester became stable, the operation of the digester was fixed at OLR of 5.0 kgCOD/m^3/d. At this stage, the biogas production rate started to increase to more than 2.0 kg/m^3/d and the methane composition in the biogas was maintained in the range 50.0-60.0%.

CONCLUSIONS

This study successfully demonstrated the use of seed sludge from the bottom and top of an existing 3600 m^3 open tank digester to accelerate the start-up process in stable operation. The start-up periods were accelerated to 24 days and 40 days for bottom and top seed sludge, respectively. However, the bottom seed sludge transfer exhibited more stable operation with VFA:Alk ratio less than 0.25 and COD removal higher than 90%. The biogas production and methane composition in biogas were also satisfactory with more than 1.8 kg/m^3/d and 50-60%, respectively once the digester was operated and fixed at OLR of 5.0 kgCOD/m^3/d.

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