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Biomethanation of Palm Oil Mill Effluent (POME) with a thermophilic mixed culture cultivated using POME as a substrate

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ABSTRACT

Anaerobic systems operated under thermophilic condition often encounter longer start-up period and operating problems pertaining to temperature shift when mesophilic seed sludge were utilized as thermophilic sludge. Bioaugmentation products for thermophilic conditions were not readily available in the market. Hence, a thermophilic mixed culture has been cultivated specifically for Palm Oil Mill Effluent (POME) treatment at thermophilic conditions using a batch Continuous Stirred Tank Reactor (CSTR) where POME was utilized as a substrate for the growth of microbes. The thermophilic mixed culture managed to reduce at least 90% of Chemical Oxygen Demand (COD) in POME with a hydraulic retention time (HRT) of 6 days with a MLSS concentration of 14,000 mg/L. The biogas produced from the batch CSTR contained at least 64% of methane. The kinetic parameters for batch thermophilic POME treatment were obtained by fitting the Chen and Hashimoto's model to the experimental data. The maximum substrate utilization rate for this system was found to be 0.476 day^{-1}, which was higher than the systems operated under mesophilic range, and dimensionless kinetic parameters k and Q were −1.365 and 0.0007 respectively. The mixed culture had a methanogenic population which consisted of *Methanosaeta thermophila*, *Methanosarcina thermophila*, *Methanobacterium thermoautotrophicum*, *Methanobacterium thermoformicicum* and *Methanobacterium wolfei*.

1. Introduction

POME is a wastewater generated from palm oil milling activities and it is conventionally treated anaerobically using ponding systems or with open digesting tanks [1]. Nevertheless, the application of high-rate anaerobic bioreactors to replace conventional treatment methods for POME treatment has escalated due to the fact that these high-rate anaerobic bioreactors had smaller foot prints, producing better treated effluent quality and greater biogas volume with higher purity of methane which can be utilized for energy generation purposes. In addition, the introduction of Clean Development Mechanism (CDM) allows developing countries to earn Certified Emission Reduction (CER) credits which act as a source of revenue for companies working on methane capture from anaerobic digesters. This also further attracts palm oil millers to switch to high-rate anaerobic bioreactors for POME treatment.

The application of high-rate anaerobic bioreactors on the treatment of POME had distinct advantages. In addition, operation of anaerobic treatment of POME under thermophilic conditions was proven to produce effluent of better quality and also having higher biogas production rate [2]. Ibrahim et al. [3] managed more than 90% of BOD removal from POME treatment with anaerobic contact digester under thermophilic conditions, while Chin and Wong [4] and Cail and Barford [5] reduced more than 70% of COD in POME with batch and semi-continuous digesters respectively under thermophilic conditions. However, anaerobic thermophilic sludge or bioaugmentation products for thermophilic conditions are not readily available in the market. The start-up period for thermophilic systems requires a longer time to allow the mesophilic sludge to acclimatize with the substrate as well as to the temperature shift. In order to reduce the start-up period required by the anaerobic thermophilic POME treatment system and at the same time minimize the effect of temperature change to the mesophilic sludge (i.e., bioreactor upset), it is necessary to cultivate a thermophilic mixed culture tailored for anaerobic POME treatment at thermophilic condition.

Several studies showed that a cultivated mixed microbial consortium for the treatment of a targeted wastewater can be obtained by acclimatizing the existing seed sludge from any biological sludge basin with the targeted wastewater as the substrate at a specific set of operating conditions. Sreekanth et al. [6] obtained an inoculum specifically for pharmaceutical wastewater treatment by utilizing

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slaughterhouse wastewater sludge as the seed source and allowed the sludge to be acclimatized to the system by feeding pharmaceutical wastewater as the substrate. Similarly, Tan and Ji [7] also utilized sludge from oil-contaminated wastewater treatment as the seed source to obtain a carbazol-degrading microbial consortium by feeding carbazol-containing wastewater as the substrate. The carbazol-degrading mixed culture was able to show an increase of carbazol removal rate from 68% to 95% after two acclimatization stages of 24 days. This indicates that a cultivated anaerobic mixed culture suitable for anaerobic POME treatment at thermophilic condition is essential for a successful operation of the anaerobic thermophilic system.

Screening and identification of microbes in the mixed culture is essential to characterize the mixed culture and prove that the mixed culture had acclimatized to POME and at the same time identify the key methanogens which were responsible for the production of methane from POME. The identification of key methanogens responsible for methane conversion in the process of POME treatment will provide useful insights for further research to create a microbial cocktail specifically for anaerobic POME treatment at thermophilic conditions from pure cultures.

Conventionally, identification of microbes based on morphology, metabolic, biochemical and genetic assays were done through pure cultures that were obtained from a series of isolation [8]. Nevertheless, the conventional method does not reflect to the actual diversity of a microbial community, especially in wastewater treatment which involves a complex mixed culture where more than one species of microbes work to degrade the materials in the wastewater.

Molecular biology techniques were applied to aid the identification of the complex microbial communities in wastewater treatment sludge. These techniques have eliminated some of the problems encountered by the conventional identification method [9]. Among the most frequently used techniques are 16S rDNA [10–12], denaturing gradient gel electrophoresis (DGGE) [13,14], and fluorescence in-situ hybridization (FISH) [15,16].

Considering the complexity of the molecular biological techniques, a more convenient method following the Bergey's Manual of Determinative Bacteriology [17] was proposed for the identification of the methanogens in the mixed culture obtained in the present study. The methanogens present in the cultivated mixed culture can be identified through a series of screening processes based on the data of morphology, dimensions, Gram staining and biochemical properties and comparing the results with those listed in Bergey's Manual. This method allows the identification of microbial population to be conducted in the least amount of time while avoiding the isolation of pure cultures. In the present study, only methanogens were considered in the identification of microbes in the mixed culture owing to the fact that a lot of catabolic substrate tests will be involved if a larger community of microbes were to be identified.

This paper elaborates on the cultivation, screening and identification of anaerobic thermophilic mixed culture for POME treatment which is able to achieve a shorter start-up period whilst lead to a better POME treatment performance. The performance of the cultivated anaerobic thermophilic mixed culture was assessed based on the treatment efficiency of POME under thermophilic condition. In addition, the kinetic parameters for anaerobic batch treatment of POME under thermophilic condition were also evaluated to characterize and assess the performance of the mixed culture.

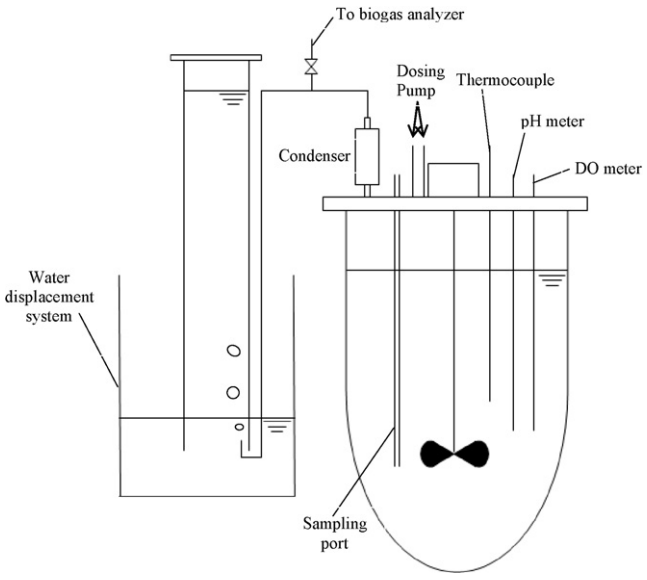

Fig. 2.1. Schematic diagram of batch CSTR setup for cultivation of microbes.

2. Material and methods

2.1. Batch Continuous Stirred Tank Reactor (CSTR) for bacteria cultivation

Minifors (Infors HT, Switzerland) batch CSTR was used for the cultivation of the mixed culture for POME treatment at thermophilic condition. Fig. 2.1 shows the schematic diagram of the batch fermentation system. The 2.5 L (total volume) CSTR was equipped with on-line temperature, pH and oxygen concentration measurement. The CSTR was also equipped with a stirrer of 2 impellers to provide even mixing in the CSTR whereby the rotational speed can be adjusted between 0 and 1250 rpm. A heating plate attached to the fermenting flask provides heating up to 60 °C for the system. pH adjustments in the system were done with two dosing pumps connected to acidic and alkaline buffers. To prevent loss of liquid from the CSTR through evaporation during operation at thermophilic condition, the biogas was cooled with a condenser attached at the gas stream outlet of the CSTR. The biogas volume was measured using a water displacement system, whereby, biogas produced in the CSTR will exit through the outlet of the condenser to an overturned measuring cylinder filled with water acidified to a pH $\leq$ 2 with nitric acid. The water has to be acidified in order to prevent carbon dioxide from dissolving in water, which in turn will affect the true biogas volume produced from POME treatment. The gas bubbled into the measuring cylinder will displace the water from the cylinder into the container.

2.2. Seed material

The seed sludge for the cultivation of anaerobic thermophilic mixed culture for POME treatment was taken from a mesophilic anaerobic wastewater treatment system of an oleo-chemical manufacturing plant (Pan Century Edible Oils Sdn. Bhd., Johor, Malaysia). The seed sludge obtained has a black colour appearance with VSS concentration of 13,390 mg/L. The specific methanogenic activity of the seed sludge was 0.11 g COD-CH_4/g VSS day.

2.3. Substrate for mixed culture cultivation

Raw POME was used as a substrate for the cultivation of mixed culture. POME was collected from Golconda Palm Oil Mill (Klang, Selangor) weekly and was preserved at 4 °C if not used immediately. The average characteristics of raw POME that was used during the cultivation of mixed culture for POME treatment at thermophilic condition is indicated in Table 2.1.

Table 2.1
Characteristics of raw POME used for the cultivation of thermophilic mixed culture.

Parameters	mg/L
COD	22,660–73,500
sCOD	12,500–40,100
BOD[1]	11,730–37,500
SS	7100–24,500
pH*	4.19–5.30
Oil & Grease	2500–16,100
Total Nitrogen	750–1020
Volatile Fatty Acid (VFA)	1215–2546

* no units for pH.
[1] BOD – biochemical oxygen demand.

2.4. CSTR start-up

The working volume of the CSTR for bacteria cultivation was 2 L. 0.5 L of seed sludge was inoculated into the bioreactor with 1.5 L of POME. POME was diluted by 10 times during the initial start-up and pH of the CSTR was adjusted to 6.8 with 1 M sodium hydroxide while sodium bicarbonate was further added into the mixture to provide alkalinity of at least 2000 mg/L to the system as well as to maintain the mixture at a pH of 7.0 throughout the entire start-up period. Nitrogen gas was used to purge oxygen from the CSTR. The oxygen concentration in the CSTR was kept below 5% throughout the cultivation. Stirring speed of the CSTR was fixed at 100 rpm to provide a complete mixing in the system.

In order to allow the adaptation of the mesophilic microbial seed sludge to the thermophilic condition, the initial temperature of the bioreactor was set at 35 °C for 4 h before a single step increase of temperature to 55 °C. This strategy was employed based on the study conducted by Bouškouvá et al. [18] which found that a one-step temperature increase, although caused a severe disturbance to the system, provided a shorter start-up period as compared to a stepwise increase approach.

Raw POME fed into the batch CSTR was diluted to a COD concentration equivalent to 3476 mg/L. The dilution factor of POME fed into the batch CSTR was reduced each time the treated effluent was withdrawn from the system. The subsequent increase of organic loading rate (OLR) in the batch CSTR was shown in Fig. 2.2. The treated effluent was replaced when Mixed Liquor Suspended Solid (MLSS) concentration in the batch CSTR reduced as this indicates that the microbial population in the CSTR is depleting due to lack of substrate for utilization. Condition of the CSTR was constantly maintained at the desired MLSS concentration and pH. The performance of batch CSTR was also continuously monitored through the measurement of Chemical Oxygen Demand (COD), soluble Chemical Oxygen Demand (sCOD), suspended solids (SS), pH and MLSS according to APHA Standard Methods [19]. An adapted population of mixed culture is obtained when the effluent COD remained constant with less than 5% variation [20,21].

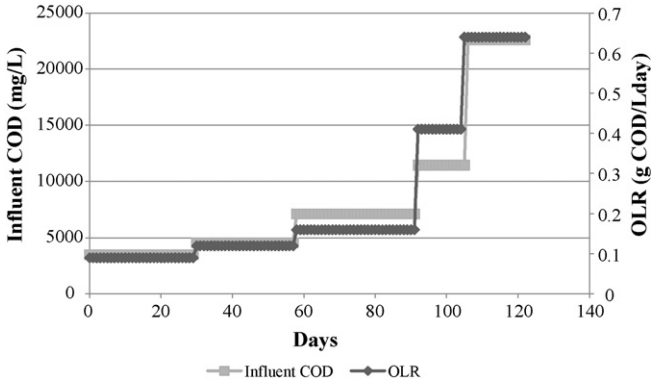

Fig. 2.2. Influent COD concentration and OLR profile during the start-up period.

2.5. Steady-state CSTR operation

After the batch CSTR reached steady-state, the treated POME effluent in the CSTR was withdrawn and replaced with new feed when at least 80% of COD is being degraded. This is to prevent POME from becoming a limiting substrate in the anaerobic system. It also enables a direct comparison between subsequent steady-state runs to evaluate the performance of the cultivated mixed culture. The COD, sCOD and SS concentrations as well as pH in the batch CSTR were measured daily. Biogas volume and composition produced were also recorded daily to evaluate the performance of the thermophilic mixed culture on POME treatment using the batch CSTR.

The MLSS concentration in the CSTR was increased for each run by reducing the volume of POME fed into the CSTR and topping up with microbial seed sludge in order to investigate the effect of MLSS concentration to the performance of POME treatment at thermophilic conditions with the cultivated mixed culture.

For the analysis of COD, sCOD and SS of the treated effluent, 25 ml of sample was extracted from the CSTR and was allowed to settle for 15 min. The supernatant was then extracted for analysis and the remainder of the sample was returned to the bioreactor to reduce the loss of biomass in the system. Biogas volume was measured with a water displacement system while the biogas composition was measured with Gas Data GFM 416 series biogas analyzer.

2.6. Microbial screening and enumeration

2.6.1. Microscopy examination and Gram staining

Examinations were conducted using the microscope for the supernatant and granules from samples withdrawn from the batch CSTR, raw POME samples and seed sludge to determine the morphology and the dimensions of microbes. Microscopy examinations on the supernatant and granule samples were conducted after the batch treatment of POME at thermophilic condition has reached its steady-state. Gram stains were applied on all the samples in order to differentiate the different types (Gram positive and Gram negative) of microbes under the light microscope. All the information gathered from the microscopy examinations were used to identify the associated species of methanogens with Bergey's Manual of Determinative Bacteriology [17].

All samples for microscopy examination were fixed with heat before the application of Gram staining reagents. Microscopy examination on the Gram stained slides were conducted under the Nikon Eclipse 80i microscope with either 400x or 1000x magnifications. Microscopic images were captured with the built in digital imaging device of the microscope. The measurements of the length and radius of microbes were done with the aid of digital imaging software (NIS-Elements, Nikon).

2.6.2. Identification of methanogens

The identification of thermophilic methanogens was conducted in accordance with Bergey's Manual of Determinative Bacteriology [17] which aids to identify bacteria or archaea according to its morphology, dimension, Gram staining and parameters optimum for culture growth. Only thermophilic methanogens were being considered in the determination process although mesophilic methanogens could be present in the cultivated thermophilic mixed culture. This is because the operating temperature of the batch CSTR (55 °C) is not optimal for the growth of mesophilic microbes.

2.6.3. Most Probable Number (MPN) enumeration

MPN enumeration was conducted on raw POME, seed sludge and samples of supernatant and granules from the batch CSTR to obtain the distribution of the microbial population. Trypticase Soy Broth (TSB) was chosen as the medium for the enumeration of both total anaerobes and methanogens present in the thermophilic mixed culture cultivated from the batch POME treatment system. More specific selective medium was not selected for the enumeration study on methanogens as TSB supports the growth of fastidious microorganisms and a wide variety of anaerobic bacteria. The use of selective medium might have inhibited the growth of certain microbes that are actually desired and the MPN enumeration would not reflect the real microbial population in the thermophilic mixed culture. TSB has been used in the MPN enumeration of fermentative anaerobes and methanogens by Horn et al. [22]. This media does not inhibit the growth of any anaerobic bacteria and thus the data on MPN enumeration can be representative.

Tenfold serial dilutions were prepared for all the samples for total anaerobe enumeration and the dilutions were inoculated into TSB tubes in triplicates aseptically. All the culture tubes were incubated in the GasPak system (BD Diagnostics, USA) at 55 °C for 8 days. For the enumeration of methanogens, the culture tubes with TSB broth were incubated in a water bath shaker at 55 °C for 8 days with a shaking speed of 100 rpm to ensure equal mixing. An incubation period of 8 days was selected based on the study conducted by Siebert and Hattingh [23] which showed that this time frame yielded a more consistent MPN enumeration result.

For total anaerobes, tubes with visible turbidity were considered positive and negative for otherwise. Culture tubes for the enumeration of methanogens were considered positive when methane gas reading was detected and negative for otherwise. The amount of total anaerobes and methanogens were determined using the MPN table [24].

3. Results and discussion

3.1. Performance of thermophilic mixed culture on anaerobic POME treatment

3.1.1. CSTR start-up

During the single step temperature increase from 35 °C to 55 °C, the only severe disturbance observed on the operation of the CSTR was a drop in pH of the system which was quickly rectified through the dosing of sodium bicarbonate with the dosing pump. Fig. 3.1 shows the profiles for pH, COD, SS removal efficiency in the batch CSTR during the start-up period. During the initial start-up (Run 1), the COD removal efficiency of the system was highly dependent on the pH of the system. During the first 6 days of start-up, the removal of COD from the system increased steadily to 44% until the pH of the system reduced from 7.68 to 7.0 where the COD removal efficiency was found to decline thereafter. $NaHCO_3$ was dosed into the batch CSTR when pH of the CSTR dropped to less than 7 to maintain it at a pH of 7. A COD removal efficiency of 54% was achieved when pH in the batch CSTR was restored to 7 and above. Similar observation was obtained for Run 2. In subsequent runs (3, 4, 5), pH of the CSTR was maintained within the optimum operating pH range (6.8–7.2) without the dosing of alkaline buffer.

The batch CSTR required a period of 120 days to reach steady-state. A COD removal efficiency of 84% was achieved with the batch CSTR with an OLR of 0.64 g COD/L day when the steady-state was achieved. No methane was detected for the first two runs during start-up possibly due to the fact that the POME fed to the batch CSTR was too diluted and insufficient for significant methane production. The highest methane concentration detected from batch CSTR was 72% while the average methane concentration recorded during start-up period was 64.5%. This methane concentration was similar to the methane concentration recorded from the study conducted by Ibrahim et al. [3], where the average methane concentration detected from the thermophilic anaerobic contact digestion system for POME treatment was only 65%.

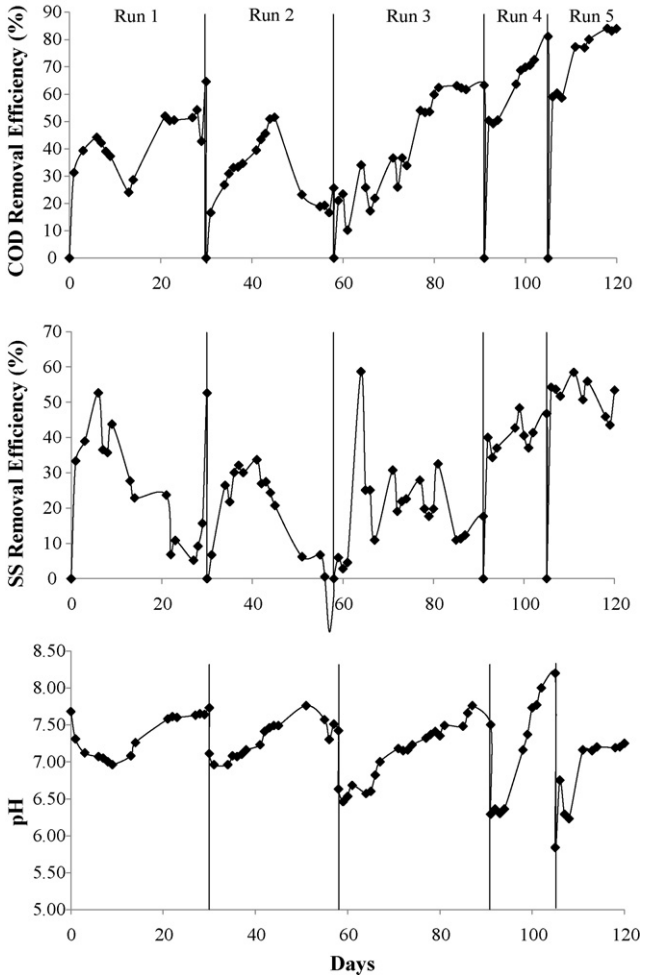

Fig. 3.1. Profiles of COD, SS removal efficiencies and pH of the batch fermenter for POME treatment at thermophilic condition.

When the COD removal efficiency of the batch CSTR has maintained constant around 84 ± 0.5% for 3 consecutive days, the batch CSTR was fed with undiluted raw POME to evaluate the performance of the mixed culture on the treatment of POME under thermophilic conditions.

3.1.2. Mixed culture performance under steady-state conditions

No pH adjustments were required during the steady-state runs as the pH in the batch CSTR increased steadily to reach the optimum pH range for methanogenesis (6.8–7.2) for each run. The pH rise in the system indicates that the methanogens have adapted to the system [25]. Table 3.1 lists the feed conditions, operating conditions as well as the quality of the treated effluent.

The average methane concentration produced from anaerobic digestion of POME at mesophilic condition from an anaerobic pond and open digesting tank are 54.4% and 36.0% respectively [26,27] while the average methane concentration produced from mesophilic high-rate bioreactor for anaerobic POME treatment ranged between 62% and 84% [28,29]. The average methane concentration in the biogas produced from the batch CSTR for POME treatment at thermophilic condition was found to be 66%, which was higher than conventional treatment methods and falls within

Table 3.1
Feed and operating conditions and treated effluent quality.

Run	HRT (days)	pH	COD (mg/L)	sCOD (mg/L)	SS (mg/L)
Feed conditions					
6	7	5.28	24,600	15,350	8550
7	6	4.29	29,800	16,600	11,750
8	6	4.33	37,800	19,900	13,550
9	7	4.20	40,850	19,900	12,950

Run	Temperature (°C)	OLR (kg COD/m^{3}day)	Feed volume (mL)	Seed sludge volume (mL)	MLVSS concentration (mg/L)
Operating conditions					
6	55	1.41	800	700	3803
7	55	1.74	700	800	4244
8	55	1.89	600	900	7870
9	55	0.94	900	900	8527

Run	pH	COD removal efficiency (%)	sCOD removal efficiency (%)	SS removal efficiency (%)	Methane (%)	Carbon dioxide (%)	Hydrogen sulphide (ppm)
Treated effluent conditions							
6	7.44	82.9	79.2	70.2	70.1	20.9	668
7	7.19	85.1	82.5	75.2	64.9	23.0	280
8	7.15	90.4	83.2	69.0	69.5	26.3	655
9	7.25	82.9	81.2	53.3	66.7	23.4	555

the range of methane concentration produced from high-rate anaerobic bioreactors. In addition to that, the methane concentration was also comparable with the 65% quoted in a study by Ibrabim et al. [3] on thermophilic anaerobic contact digestion of POME.

The effect of MLSS concentration on POME treatment under thermophilic condition was also evaluated simultaneously during the steady-state runs (Runs 6–9). In order to increase the MLSS concentration, seed sludge was added into the system and the feed volume of POME was reduced. The amount of volume of seed sludge and POME in the batch CSTR for each steady-state runs were indicated in Table 3.1.

Fig. 3.2 indicates the COD removal efficiency profile during steady-state runs (Runs 6–9) in the batch CSTR. The COD removal efficiency in the batch CSTR improved over each run. The HRT required to reduce more than 80% of COD of POME was reduced from 16 days (Run 5) during start-up period to 6 days (Runs 7, 8, 9) during steady-state period. This suggests that a mixed culture consisting of thermophilic microbes has been successfully cultivated as the culture has shown great improvements in terms of COD removal efficiency and methane composition in the biogas which is comparable to the value from thermophilic high-rate bioreactor.

Fig. 3.3 was plotted to show the relationship between the concentration of MLSS in the batch CSTR and the COD removal efficiency attainable with HRT of 6 days. It was found that COD removal efficiency can be significantly improved with the increase in MLSS concentration in the batch CSTR. The COD removal efficiency improved from an 80% to 90% when the MLSS concentration of the CSTR increased from 5600 mg/L to 14,000 mg/L. Nevertheless, no significant increase in the COD removal efficiency was observed when the MLSS concentration was increased to 15,000 mg/L. This indicated that the MLSS concentration should be maintained at 14,000 mg/L in the batch CSTR for at least 90% COD removal.

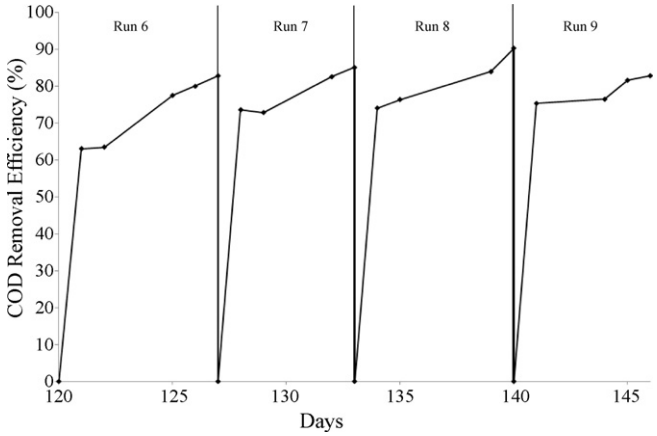

Fig. 3.2. COD removal efficiency profile for steady-state runs.

The removal of suspended solid in this system showed better performance as compared to a similar study conducted by Chin and Wong [4] whereby more than 50% of suspended solids were removed due to hydrolysis and mixing [30] after 1 day retention time while 5 days retention time was required in Chin and Wong's study to remove 46% of suspended solids at thermophilic condition. Referring to Fig. 3.3, a COD reduction of at least 73% was attainable for 6 days HRT with the mixed culture. The HRT required to achieve at least 80% of COD removal from POME was significantly shortened from 14 (Run 5 of start-up) to 6 days. Within 6 days of treatment, the COD of POME was reduced to 90% with an operating OLR of 1.89 g COD/L day. This indicated that the mixed culture had acclimatized to the operating conditions and the fed substrate.

3.1.3. Kinetic evaluation of the thermophilic mixed culture in the batch CSTR

The evaluation of kinetic parameters is important to obtain design parameters for bioreactor design. Furthermore, the performance of the thermophilic mixed culture on thermophilic POME treatment can be assessed through the evaluation of kinetic parameters. Kinetic model developed by Contois suggested that the bacterial specific growth rate (μ) was a function of cell mass concentration (M) and the limiting substrate concentration (S) [31]. Chen and Hashimoto's kinetic model was applied to evaluate the kinetic parameters of the thermophilic mixed culture as the model accounted for the dependence of effluent substrate concentration on the influent substrate concentration [32]. The mass balance for a completely mixed batch system without recycle in terms of substrate concentration change is expressed as:

$$\frac{dS}{dt} = -F \quad (1)$$

where S is the effluent substrate concentration; F is the volumetric substrate utilization rate and t represents time.

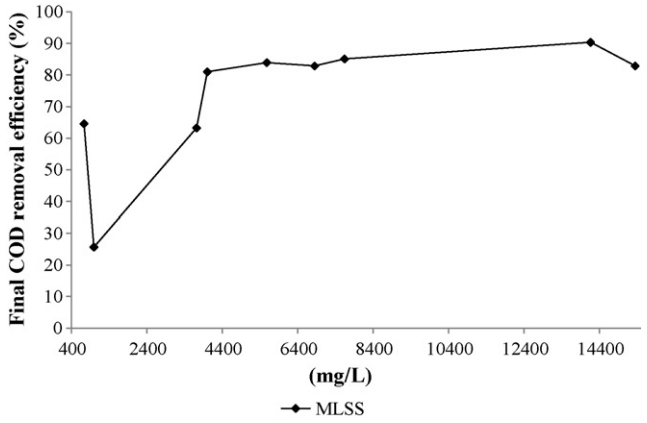

Fig. 3.3. MLSS concentration against the attainable COD removal efficiency with a HRT of 6 days.

In a completely mixed system without solids recycle, the hydraulic retention time will be equivalent to the solids retention time. The relationship between F and specific growth rate of microorganisms, μ is given by:

$$\mu = \frac{Y}{M} \times F \tag{2}$$

where Y is the growth yield coefficient. μ can also be expressed in terms of Contois' kinetic model, whereby:

$$\mu = \frac{\mu_m S}{BM + S} \tag{3}$$

μ_m is regarded as the maximum specific growth rate [31] of the microbes and B is the kinetic coefficient of Contois' kinetic model. Rearranging Eq. (2) and substituting Eqs. (3) and (4) into Eq. (1) to obtain Eq. (5),

$$F = \frac{\mu M}{Y} \tag{4}$$

$$\frac{dS}{dt} = \frac{-\mu_m S}{k + S(Y/M)} \tag{5}$$

where k is a dimensionless kinetic parameter, which is a product of B and Y [32]. Let Q, another dimensionless kinetic parameter be the ratio of growth yield coefficient and cell mass concentration, thus Eq. (5) can be rewritten as:

$$\frac{dS}{dt} = \frac{-\mu_m S}{k + SQ} \tag{6}$$

The kinetic parameters of μ_m, k and Q were evaluated by fitting Eq. (6) with the experimental data using non-linear regression with SigmaPlot v11.0. The values of the kinetic constants for thermophilic POME treatment are listed in Table 3.2 while the plots of experimental and simulated values were presented in Fig. 3.4.

The experimental data fitted to the model showed acceptable R^2 values between 0.82 and 0.97. μ_m was found to increase with COD influent concentration. This implies that the maximum specific growth rate of microbes in the anaerobic system is affected by the concentration of the feed where higher concentration of substrate would be required to increase the growth of the microbes.

Table 3.2
Kinetic constants for thermophilic POME treatment with batch fermenter.

Influent COD concentration (mg/L)	μ_m (day^{-1})	k	Q	R^2 value
73,100	0.4755	−1.3650	0.0007	0.9395
65,450	0.4533	−1.3024	0.0008	0.8233
22,600	0.3999	−1.0059	0.0010	0.9226
11,400	0.3228	−0.9300	0.0013	0.9720

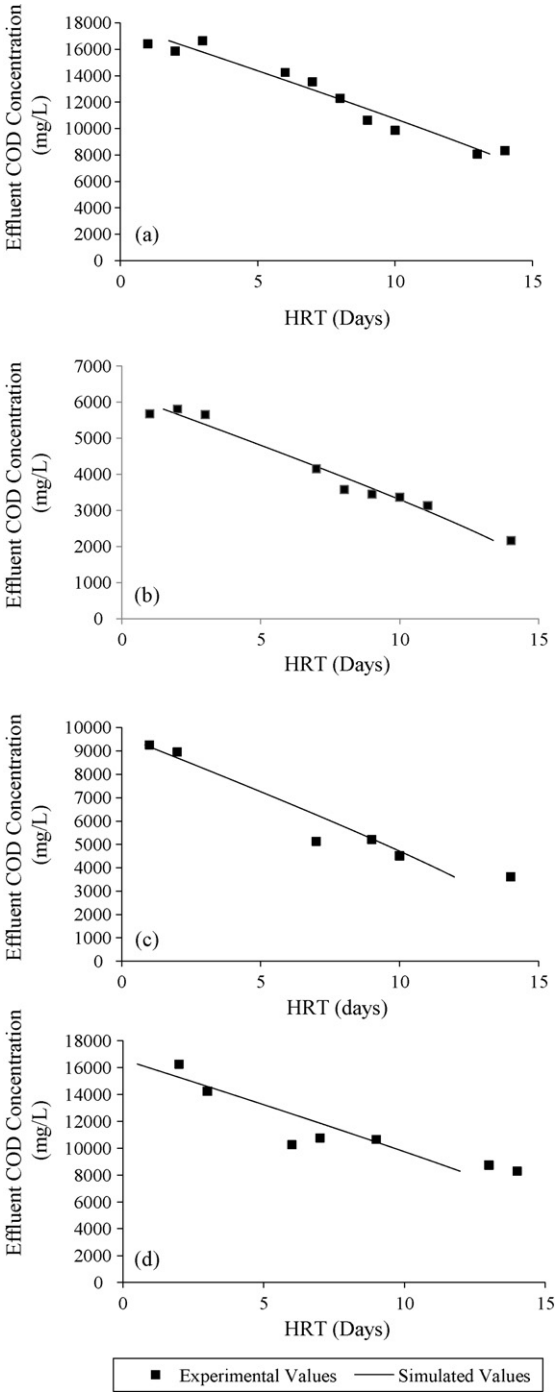

Fig. 3.4. Plot of experimental value fitted to the Contois' kinetic model with influent COD values: (a) 73,100 mg/L; (b) 11,400 mg/L; (c) 22,600 mg/L; and (d) 65,450 mg/L.

The values of μ_m obtained fall into the range of literature values for anaerobic digestion of POME as shown in Table 3.3. The μ_m from this study was lower as compared to the value obtained by Yeoh et al. [33] indicates that anaerobic contact digestion system is more efficient in terms of COD removal from POME as compared to a batch CSTR. However, the value of 0.476 day^{-1} was higher than those operating under mesophilic range which shows that the mixed culture which was seeded with mesophilic sludge had adapted to the thermophilic operating conditions.

Based on the performance study and kinetic evaluation, the cultivation of thermophilic mixed culture has been considered successful as the mixed culture has the ability to match the POME

Table 3.3
μ_m values for anaerobic POME treatment.

Temperature range	μ_m (day^{-1})	Type of reactor	Reference
Thermophilic	0.476	Batch CSTR	Present study
	0.570	Anaerobic contact	Yeoh et al. [33]
Mesophilic	0.304	MABR	Faisal and Unno[34]
	0.207	UASFF	Zinatizadeh et al. [35]

MABR – modified anaerobic baffled reactor; UASFF – upflow anaerobic sludge-fixed film.

treatment performance under thermophilic condition of high-rate anaerobic bioreactors in the treatment of POME with a batch CSTR. Furthermore, the high value of μ_m indicated the better efficiency of this mixed culture for the treatment of POME under thermophilic condition.

3.2. Identification and enumeration of microbes

Microscopic observations were conducted on raw POME samples, seed sludge, and granules extracted from the batch CSTR after the CSTR had reached its steady-state where Gram staining was applied to all samples for microscopic observation except for raw POME samples. Microscopic observations were conducted in order to identify the key microbes forming the thermophilic mixed culture using Bergey's Manual of Determinative Bacteriology [17].

Figs. 3.5 and 3.6 are microscopic images of seed sludge and granule of the thermophilic mixed culture. All images were captured at a magnification of 1000 times. Rod and coccus-type microbes were observed in both samples from mesophilic seed sludge and the thermophilic mixed culture, as shown in Figs. 3.5 and 3.6. From the microscopic observations conducted on all samples, it was found that long sheathed rods were found mostly in the samples from the thermophilic mixed culture. Referring to the morphology obtained from the microscopic images and the growth condition of the mixed culture, it can be deduced from Bergey's Manual of Determinative Bacteriology that the long sheathed rods present in the samples are *Methanosaeta thermophila*.

Fig. 3.6 shows the microscopic image from the sample of a granule extracted from the thermophilic mixed culture which has been evenly spread on the microscopic slide. Comparing Figs. 3.5 and 3.6, the microbial population contained in the granules was denser than the population suspended in liquid. This indicated that it is important to retain granules in an anaerobic wastewater treatment system in order to maintain suitable MLSS concentration for efficient POME treatment as most microbes were densely packed in the granular form.

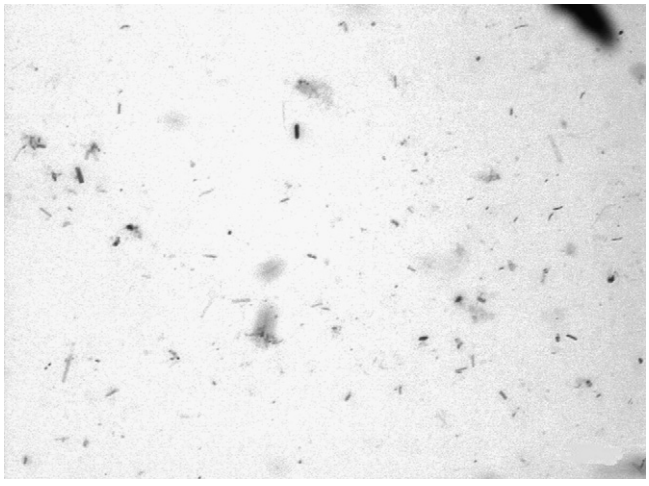

Fig. 3.5. Population of microbes from the mesophilic seed sludge (Pan Century Edible Oils Sdn. Bhd) at 1000× magnification (Gram stain).

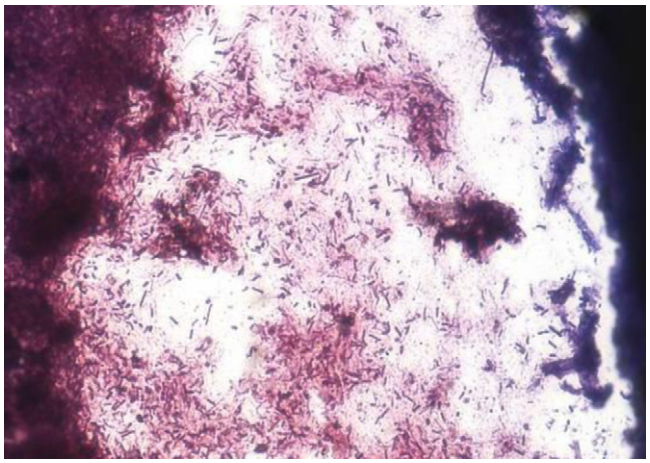

Fig. 3.6. Microbial population of a granule taken from thermophilic batch POME treatment at 1000× magnification (Gram stain).

Most microbes that were observed from the seed sludge and thermophilic mixed culture were short rods, sheathed rods, curved rods, single cocci, diplococcus or coccus in aggregates (sarcina). To enable the identifications of the species of methanogens present in the anaerobic sludge samples, dimensions of the microbes were measured to match with the descriptions in the Bergey's Manual of Determinative Bacteriology. Table 3.4 lists the range of dimensions of the types of microbes detected from samples both from the seed sludge and thermophilic mixed culture.

Based on the operating parameters, microscopy observation and data obtained from the sizing of the microbes, the methanogenic population present in the cultivated mixed culture was deduced from Bergey's Manual. The presence of *M. thermophila* in the cultivated thermophilic mixed culture does not coincide with other findings on methanogenic population analysis of thermophilic anaerobic digestions. Studies conducted by Sasaki et al. [36], Chackhiani et al. [37] and Ueno and Tatara [38] did not find *M. thermophila* in their community of microbes. However, these studies [36–38] indicated the presence of *Methanosarcina thermophila*, *Methanobacterium thermoautotrophicum* and *Methanobacterium wolfei* in their population of microbes which coincided with the population of methanogens present in the thermophilic mixed culture.

The differences of methanogenic species between the mixed culture cultivated with raw POME and literature studies were attributed by the difference in substrate and culture conditions as the distribution of methanogens are completely dependent on the physical conditions and the ability to adapt to the conditions during culture [39]. Another interpretation of this result might be due to the fact that granular sludge was used for cultivation. van Lier et al. [40] reported that Methanosaeta sp. were dominant in the granular anaerobic sludge both in mesophilic and thermophilic operation, out-competing microbes of Methanosarcina genus. Methanosaeta

Table 3.4
Range of dimensions of microbes detected from seed sludge and mixed culture sludge samples.

Microbe shape	Range of dimensions ($L \times W$) or diameter (μm)
Short rods	0.7 × 0.2–3.0 × 0.6
Sheathed rods	4.1 × 0.8–22.01 × 1.3
Cocci (including diplococcus)	0.4–1.0
Sarcina	1.0–1.8

Table 3.5
MPN values of total anaerobes and methanogens.

Total anaerobes (ml^{-1})	Methanogens (ml^{-1})	Source	Temperature of system (°C)	Reference
MPN enumeration				
1.1×10^9	1.8×10^7	POME treatment	55	Present study
2.0×10^8	<3	Raw POME	60	Present study
1.5×10^8	1.5×10^5	Oleo-chemical wastewater treatment	38	Present study
–	4.6×10^5	Aeration tank	–	Wu et al. [41]
–	1.5×10^6	Sedimentation tank	–	Wu et al. [41]
1.1×10^8	2.2×10^6	POME treatment	55	Yeoh et al. [33]

sp. generally out-competes Methanosarcina sp. when the concentration of acetate in the anaerobic system is low [9]. The presence of Methanosaeta sp. in the cultivated mixed culture thus indicates that the performance of the batch CSTR for thermophilic POME treatment had been of satisfactory condition.

MPN enumeration tests on the bacteria population were conducted for raw POME samples, seed sludge samples and thermophilic mixed culture to evaluate the performance of the cultivated thermophilic mixed culture. The results of MPN enumeration were presented in Table 3.5 together with other literature values. Anaerobes were present and no methanogens were detected in raw POME samples. Carbon dioxide was detected in most of the MPN samples from raw POME. This suggested that fermentative bacteria were active in raw POME samples as carbon dioxide is a product formed through fermentation process. This is advantageous for anaerobic treatment as POME can be degraded easily into substrates suitable for the consumption of methanogens (i.e., carbon dioxide).

Comparing with the MPN values published by Yeoh et al. [33], the number of total anaerobes and methanogens were found to be greater in this study although the microbes were grown on the same type of substrate. This might be attributed by the fact that the batch CSTR has the ability to retain more biomass as compared to a continuous system and is therefore useful for the cultivation of mixed culture for inoculation into an anaerobic bioreactor.

The methanogen MPN value of the cultivated thermophilic mixed culture for POME treatment was significantly higher than the rest of the seed sludge listed in Table 3.5. This indicates that the mixed culture has been successfully cultivated and it is an advantage to cultivate mixed culture for specific purposes as the cultivation produces a seed source with higher density of microbial population whereby shorter adaption period is required for the anaerobic system.

MPN enumeration of total anaerobes and methanogens were also carried out for a single granule of the mesophilic seed sludge and granule of the mixed culture throughout the cultivation phase. This is done to monitor the population of microbes in a granule throughout the cultivation. It was found that methanogens constitutes between 1.6% and 2.3% of the total anaerobe population in the granules. The increase in the number of total anaerobes and methanogens indicated that microbial growth occurs in the granules. The population of microbes in a granule maintained at a MPN value of 9.3×10^5 ml^{-1} for anaerobes and 15,000 ml^{-1} for methanogens after 134 days of cultivation. The constant MPN values of both anaerobes and methanogens but an increase in total microbial population in the system was reflected in the addition of seed sludge into the batch CSTR.

Based on the results from the MPN enumeration of total anaerobes and methanogens, a significant increase in the MPN values of both total anaerobes and methanogens from the original seed sludge to the cultivated thermophilic mixed culture showed that a thermophilic mixed culture had been successfully cultivated from the mesophilic seed sludge and was showing significant growth in the batch CSTR.

4. Conclusion

A mixed culture specifically for the treatment of POME under thermophilic condition was successfully cultivated. The thermophilic mixed culture managed to reduce at least 90% of Chemical Oxygen Demand (COD) in POME with a hydraulic retention time (HRT) of 6 day from POME treatment with a MLSS concentration of 14,000 mg/L in the batch CSTR. The biogas produced from the batch CSTR for POME treatment contained at least 64% of methane. The μ_m for this system was found to be 0.476 day^{-1} which was higher than systems operated under mesophilic range. The performance study and kinetic evaluation showed that the mixed culture was adapted to the thermophilic condition. The mixed culture had a methanogenic population which consisted of *M. thermophila*, *M. thermophila*, *M. thermoautotrophicum* and *M. wolfei*.

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