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RESEARCH ARTICLE

Effect of temperature on the anaerobic digestion of palm oil mill effluent

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Abbreviations:
BOD: biochemical oxygen demand
COD: chemical oxygen demand
CSTRs: continuous stirred tank reactors
HRT: hydraulic retention time
OLRs: organic loading rates
POME: palm oil mill effluent
TKN: total Kjeldahl nitrogen
TS: total solid
TSS: total suspended solid
TVFA: total volatile fatty acid
UASFF: up-flow anaerobic sludge fixed film

Two continuous stirred tank reactors (CSTRs) each fed with palm oil mill effluent (POME), operated at 37ºC and 55ºC, respectively, were investigated for their performance under varies organic loading rates (OLRs). The 37ºC reactor operated successfully at a maximum OLR of 12.25 g[COD]/L/day and a hydraulic retention time (HRT) of 7 days. The 55ºC reactor operated successfully at the higher loading rate of 17.01 g[COD]/L/day and had a HRT of 5 days. The 37ºC reactor achieved a 71.10% reduction of chemical oxygen demand (COD), a biogas production rate of 3.73 L of gas/L[reactor]/day containing 71.04% methane, whereas the 55ºC reactor achieved a 70.32% reduction of COD, a biogas production rate of 4.66 L of gas/L[reactor]/day containing 69.53% methane. An OLR of 9.68 g[COD]/L/day, at a HRT of 7 days, was used to study the effects of changing the temperature by 3ºC increments. The reactor processes were reasonably stable during the increase from 37ºC to 43ºC and the decrease from 55ºC to 43ºC. When the temperature was increased from 37ºC to 46ºC, the total volatile fatty acid (TVFA) concentration and biogas production was 2,059 mg as acetic acid/L and 1.49 L of gas/L[reactor]/day at day 56, respectively. When the temperature was reduced from 55ºC to 40ºC, the TVFA concentration and biogas production was 2,368 mg as acetic acid/L and 2.01 L of gas/L[reactor]/day at day 102, respectively. By first reducing the OLR to 4.20 g[COD]/L/day then slowly increasing the OLR back to 9.68 g[COD]/L/day, both reactors were restored to stable conditions at 49ºC and 37ºC respectively. The initial 37ºC reactor became fully acclimatized at 55ºC with an efficiency similar to that when operated at the initial 37ºC whereas the 55ºC reactor also achieved stability at 37ºC but with a lower efficiency.

Anaerobic digestion is considered to be an effective treatment process for palm oil mill effluent (POME). This involves a consortium of microorganisms catalysing a complex series of biochemical reactions that mineralise organic matter producing methane and carbon dioxide. The key factors to successfully control the stability and efficiency of the process are reactor configurations,

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Table 1. Physico-chemical characteristics of the palm oil mill effluent.

Parameters	Values (mg/L)				Ahmad et al. (2003)	Najafpour et al. (2006)
	This study[c]					
	1st Sample	2nd Samplee	3rd Samplef	Ranges		
pH[a]	4.42	4.24	4.66	4.24-4.66	4.7	3.8-4.4
BOD	69,215	62,500	65,427	62,500-69,215	25,000	23,000-26,000
COD	112,023	95,465	100,600	95,465-112,023	50,000	42,500-55,700
TS	71,993	75,327	68,854	68,854-75,327	40,500	-
TSS	47,140	44,680	46,213	44,680-47,140	18,000	16,500-19,500
Oil and Grease	10,052	9,126	8,845	8,845-10,052	4,000	4,900-5,700
TVFA[b]	4,226	4,045	4,335	4,045-4,335	-	-
TKN	1,345	1,305	1,493	1,305-1,493	750	500-700[d]
NH_3-N	106	91	112	91-112	-	-

a: no unit.
b: mg as acetic acid/L.
c: values are means of three replicates.
d: total nitrogen.
e: for study effects of varying OLRs.
f: for study effect of temperature shifts.

hydraulic retention time (HRT), organic loading rates (OLR), pH, temperature, inhibitor concentrations, concentrations of total volatile fatty acid (TVFA) and substrate composition. In order to avoid a process failure and/or low efficiency, these parameters require an investigation so that they can be maintained at or near to optimum conditions.

Generally, these anaerobic digestions are conducted at either mesophilic (30-37ºC) or thermophilic (50-60ºC) temperatures. In a palm oil mill processing system, the wastewater is discharged at relatively high temperatures (80-90ºC) (Najafpour et al. 2006), making it feasible to treat the POME at either mesophilic or thermophilic temperatures. With POME added at an OLR of 12.6 g[COD]/L/day and a HRT of 5.6 days under mesophilic temperature, Cail and Barford (1985) using a semi-continuous anaerobic reactor achieved a chemical oxygen demand (COD) removal of around 75%. Using a similar configuration, of a semi-continuous anaerobic reactor, but operating with thermophilic conditions and a maximum OLR of 15.1 g[COD]/L/day and a HRT of 4.3 days they achieved a COD removal of 85%, and a methane yield of 295 ml/g[COD] (Padilla and Banks, 1993). Using up flow reactors degrading synthetic wastewater of different OLRs, Yu et al. (2002), found that the operation at 55ºC achieved a higher substrate degradation rate, biogas production rate, and specific rate of aqueous product formation than when operated at 37ºC. de la Rubia et al. (2002) concluded that a reactor operating at a lower HRT and 55ºC produced more gas than at 35ºC with OLR's of up to 2.19 kg m^{-3} d^{-3} COD. The digestion of a distillery waste at anaerobic digestion temperatures ranging from 35-55ºC, gave a maximum total biogas and methane yield at a digester temperature of 50ºC (Banerjee and Biswas, 2004). According to these data, temperature is an important parameter that modifies the effectiveness of the anaerobic bacterial consortium to produce methane from organic matter.

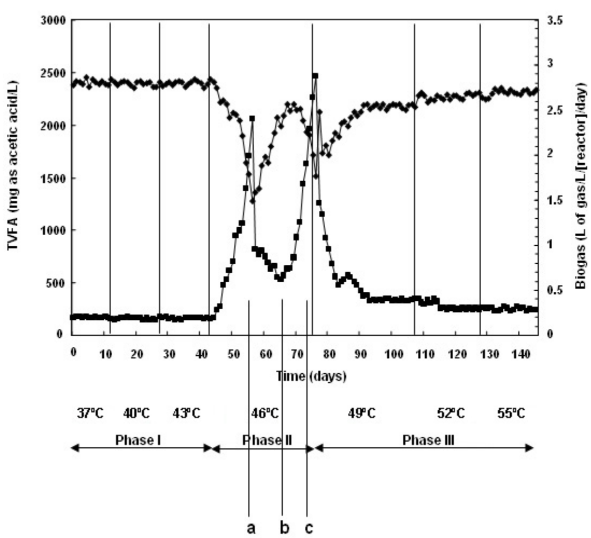

Figure 1. Variation of TVFA (■) and biogas production rate (♦) during operation of the mesophilic reactor at different temperatures. (a) decrease in OLR from 9.68 to 4.20 g[COD]/L/day; (b) increase in OLR from 4.20 to 9.68 g[COD]/L/day; (c) decrease in OLR from 9.68 to 4.20 g[COD]/L/day followed by a slow change from 4.20 to 9.68 g[COD]/L/day.

In practice, failure to control temperature increases can result in biomass washout with a resulting accumulation of TVFAs (Lau and Fang, 1997). Any sudden change in temperature caused a lowering of COD reduction, biogas production and coincided with an accumulation of TVFAs in both a mesophilic (35ºC) and a thermophilic (55ºC) up flow anaerobic filter, treating a simulated papermill wastewater (Ahn and Forster, 2002). Daily upward temperature fluctuations affected the maximum specific methanogenic activity more severely than did a daily imposed downward temperature fluctuation (El-Mashad et

Table 2. Performance of the mesophilic and thermophilic reactors at different OLRs under steady state conditions.

Parameters	Mesophilic reactor (37ºC)			Thermophilic reactor (55ºC)				
	HRT 7 days OLRs (g[COD]/L/day)			HRT 7 days OLRs (g[COD]/L/day)			HRT 5 days OLRs (g[COD]/L/day)	
	9.72	10.74	12.25	9.62	10.82	12.15	15.15	17.01
pH	7.54 ± 0.04[b]	7.44 ± 0.03[a]	7.42 ± 0.03[a]	7.80 ± 0.03[d]	7.72 ± 0.04[c]	7.72 ± 0.04[c]	7.72 ± 0.03[c]	7.71 ± 0.03[c]
TVFA (mg as acetic acid/L)	172.29 ± 8.94[a]	631.86 ± 10.15[e]	815.43 ± 12.82[g]	270.14 ± 21.93[b]	493.29 ± 11.39[c]	537.14 ± 11.00[d]	734.29 ± 19.25[f]	980.00 ± 15.43[h]
Alkalinity (mg/L)	3,000.00 ± 16.98[a]	3,156.57 ± 10.71[c]	3,157.14 ± 20.04[c]	3,126.79 ± 22.92[b]	3,144.64 ± 34.92[bc]	3,150.21 ± 32.27[c]	3,451.79 ± 21.29[e]	3,227.68 ± 26.93[d]
TVFA/alkalinity	0.06 ± 0.00[a]	0.20 ± 0.00[e]	0.26 ± 0.00[g]	0.09 ± 0.01[b]	0.16 ± 0.01[c]	0.17 ± 0.00[d]	0.21 ± 0.01[f]	0.30 ± 0.01[h]
Biogas production (L of gas/L[reactor]/day)	2.81 ± 0.04[b]	3.36 ± 0.03[d]	3.73 ± 0.05[e]	2.70 ± 0.05[a]	3.30 ± 0.02[c]	3.81 ± 0.04[f]	4.06 ± 0.05[g]	4.66 ± 0.03[h]
Gas yield (L/g[COD])	0.66 ± 0.01[b]	0.71 ± 0.01[e]	0.69 ± 0.01[c]	0.66 ± 0.01[b]	0.70 ± 0.01[d]	0.70 ± 0.01[d]	0.63 ± 0.0[a]	0.62 ± 0.01[a]
Methane production (L/L[reactor]/day)	1.96 ± 0.03[b]	2.39 ± 0.05[d]	2.65 ± 0.04[e]	1.91 ± 0.04[a]	2.31 ± 0.03[c]	2.68 ± 0.03[f]	2.82 ± 0.06[g]	3.24 ± 0.04[h]
Methane yield (L/g[COD])	0.46 ± 0.01[b]	0.51 ± 0.01[e]	0.49 ± 0.01[d]	0.47 ± 0.01[c]	0.49 ± 0.01[d]	0.49 ± 0.01[d]	0.44 ± 0.01[a]	0.44 ± 0.05[a]
COD reduction (%)	69.89 ± 0.65[c]	70.44 ± 0.33[d]	71.10 ± 0.31[e]	67.73 ± 0.63[a]	69.88 ± 0.15[c]	72.16 ± 0.33[f]	68.20 ± 0.24[b]	70.32 ± 0.30[d]

Values are averages ± SD of three determinations taken over fourteen days during steady state conditions.
Averages followed by the different letters in the same row are statistically different at 95% level by Duncan's multiple Range test.

al. 2004). Because of this information, together with the high temperature of POME and the variation of the POME wastewater volumes during high and low seasons, we have investigated the performance of a continuous stirred tank reactor (CSTR) operating in a steady state at both 37ºC and 55ºC and the effects of variations of OLRs and temperatures shifts on the performance of reactors operating at a relatively low OLR level.

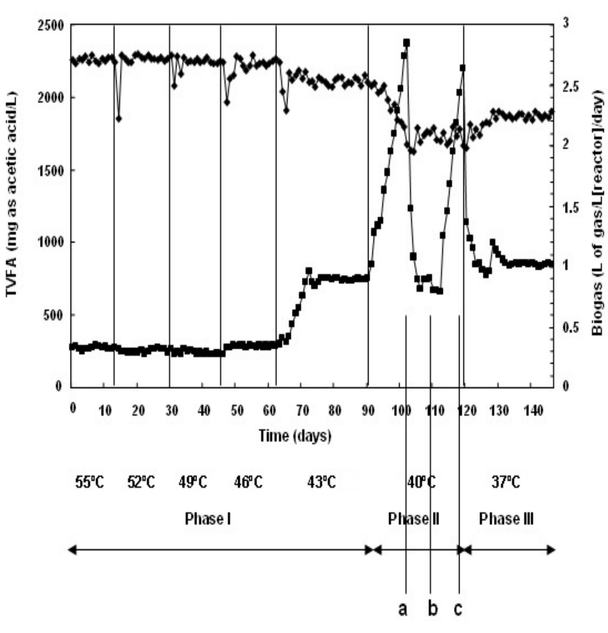

Figure 2. Variation of TVFA (■) and biogas production rate (♦) during operation of the thermophilic reactor at different temperatures. (a)decrease in OLR from 9.68 to 4.20 g[COD]/L/day; (b) increase in OLR from 4.20 to 9.68 g[COD]/L/day; (c) decrease in OLR from 9.68 to 4.20 g[COD]/L/day followed by a slow change from 4.20 to 9.68 g[COD]/L/day.

MATERIALS AND METHODS

POME characterization

Fresh POME was collected monthly from a conventional palm oil mill factory located in Surat-thani province, Thailand. After the determination of its physico-chemical properties, the wastewater was stored in a sealed container and kept in a cold room at 4ºC until used.

Equipment

The CSTR reactors used have a 12 cm internal diameter with a height of 27 cm. The reactors were maintained at the constant desired temperature using hot water circulation around the reactors. Feed was pumped semi-continuously through the feeding hole, (5 mm in diameter), near the bottom by means of a peristaltic pump. Samples were withdrawn from sampling holes (5 mm in diameter) located 1, 8 and 15 cm from the bottom. Mixing was achieved by stirring the medium at 70 rpm with a magnetic bar (0.8 x 5 cm).

Inoculum

A conventional POME treatment pond with an area of 110 x 191 m^2, and 4 m in depth was fed with an OLR of 0.5-1.5 kg[COD]/L/day with a residence time of 20-30 days. The inoculum sludge for seeding the reactors was brought from this site and adapted with diluted POME (POME:tap water = 1:4 v/v) for 7-10 days at the desired temperature, and then

Table 3. Division of the process performance based on the effect of temperature shifts during operation of the reactors.

Reactor	Temperature shifts (ºC)		
	Phase I	Phase II	Phase III
Mesophilic	37-43	46	49-55
Thermophilic	55-43	40	37

inoculated into the digesters with an initial total solid (TS) and volatile suspended solid (VSS) of around 35-37 and 15-16 g/L, respectively.

Effects of varying OLRs on the performance of the reactors

Two CSTRs each with a 1.6-L working volume were fed with acclimatized POME. One reactor was controlled at a temperature of 37ºC (mesophilic reactor) and the other at 55ºC (thermophilic reactor). The COD of the POME was adjusted to the desired value with tap water. The various OLRs were achieved at a HRT of 7 days for the 37ºC reactor and 5 days for the 55ºC reactor. The physico-chemical characteristics of the effluent used in this section are shown in Table 1 (2nd sample).

Effect of temperature shifts on the performance of the reactors

Temperatures were changed in both reactors while being operated at a HRT of 7 days (OLR 9.68 g[COD]/L/day). The temperature of the 37ºC reactor was increased up to 55ºC gradually by 3ºC at a time, while the temperature of the 55ºC reactor was decreased until it reached 37ºC again by 3ºC at a time. After each temperature change the reactor was left at the new temperature until a steady state was achieved. This took at least 2 weeks and sometimes longer before the next temperature shift. The 37ºC reactor was operated at 37, 40, 43, 46, 49, 52 and 55ºC for 14, 15, 15, 33, 31, 22 and 16 days, respectively. The 55ºC reactor was operated at 55, 52, 49, 46, 43, 40 and 37ºC for 14, 17, 16, 17, 27, 29 and 27 days, respectively. After the 37ºC and 55ºC reactors were operated at 46ºC and 40ºC, respectively a major loss of steady state as indicated from the increase in TVFA and the decreasing biogas production. The OLR was reduced to try to stabilize the system, then, gradually increased to the normal working OLR to achieve a new steady state. Physico-chemical characteristics of the effluent used in this section are shown in Table 1 (3rd sample).

It was considered that a steady-state had been achieved when the levels of TVFA, COD removal, biogas production rate and composition varied by less than 3% on three consecutive days (Borja et al. 1996). The values shown in Table 2, Table 4 and Table 5 were the average values obtained after measuring the given parameters for a 2 week period of that steady-state.

Chemical analyses

Gas volume was measured by using a displacement of acidified water (pH 2-3) and methane by KOH solution displacement in a serum bottle, as described previously (Ergüder et al. 2001). Alkalinity was measured by the direct titration method (Jenkins et al. 1983). Biochemical oxygen demand (BOD), COD, TVFA, pH, TS, total suspended solid (TSS), total Kjeldahl nitrogen (TKN), NH_3-N and oil and grease were determined in triplicate according to standard methods (Clescerl et al. 1998).

Data analysis

Means ± SD of pH, TVFA, alkalinity, TVFA/alkalinity, biogas and methane productions, biogas and methane yields and COD removal were calculated from data to be collected from the reactors operated under steady state (14 days) under variation of OLRs and temperature shifts. These data were subjected to statistical analyses using SPSS program version 10. Completely randomized design was employed for analysis of variance (ANOVA). The difference between means was evaluated by using Duncan's multiple range test. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Characteristics of POME

The physico-chemical parameters of POME used in this study (Table 1) were very different from those previously reported (Ahmad et al. 2003; Najafpour et al. 2006). This is due to a change in the mill operation. For example, a much smaller water volume was used to remove the majority of the suspended material from the lipids. This allowed for a considerable reduction in the amount of wastewater generated in the process, and consequently a higher content of organic and inorganic matter. Since the COD/BOD ratio of POME is about 1.56 a good possibility exists that the organic matter is biodegradable (Raj and Anjaneyulu, 2005). The main recalcitrant organic material found in POME was lignocellulose (Oswal et al. 2002). The large amounts of identified biodegradable components were oil and grease, which can be hydrolyzed by microorganisms to fatty acids. Some of these fatty acids are potential substrates for methane production which does allow a favourable economic outcome (Angelidaki et al. 1990). In contrast, the lipid-rich waste contains long chain fatty acids,

Table 4. Performance of the mesophilic reactor at different operating temperatures under steady-state conditions.

Parameters	Temperature (ºC)					
	Phase I			Phase III		
	37	40	43	49	52	55
pH	7.55 ± 0.05[a]	7.57 ± 0.03[a]	7.61 ± 0.02[b]	7.68 ± 0.07[c]	7.73 ± 0.04[d]	7.80 ± 0.03[e]
TVFA (mg as acetic acid/L)	166.64 ± 7.44[a]	162.43 ± 8.31[a]	160.71 ± 6.73[a]	338.71 ± 14.87[c]	253.07 ± 7.32[b]	245.07 ± 15.32[b]
Alkalinity (mg/L)	3,051.00 ± 33.70[b]	3,053.79 ± 32.74[b]	3,061.71 ± 31.58[b]	2,981.43 ± 31.31[a]	2,974.36 ± 23.82[a]	2,984.14 ± 28.34[a]
TVFA/Alkalinity	0.06 ± 0.00[b]	0.05 ± 0.00[a]	0.05 ± 0.00[a]	0.11 ± 0.01[d]	0.08 ± 0.00[c]	0.08 ± 0.00[c]
Biogas production (L of gas/L[reactor]/day)	2.81 ± 0.03[d]	2.79 ± 0.02[d]	2.80 ± 0.03[d]	2.54 ± 0.02[a]	2.65 ± 0.03[b]	2.70 ± 0.02[c]
Gas yield (L/g[COD])	0.66 ± 0.01[d]	0.65 ± 0.01[c]	0.66 ± 0.01[d]	0.61 ± 0.01[a]	0.64 ± 0.01[b]	0.66 ± 0.01[d]
Methane production (L/L[reactor]/day)	1.97 ± 0.02[c]	1.97 ± 0.02[c]	1.98 ± 0.02[c]	1.72 ± 0.16[a]	1.86 ± 0.02[b]	1.91 ± 0.03[b]
Methane yield (L/g[COD])	0.46 ± 0.01[c]	0.46 ± 0.01[c]	0.46 ± 0.1[c]	0.42 ± 0.01[a]	0.45 ± 0.00[b]	0.47 ± 0.01[d]
COD reduction (%)	70.34 ± 0.39[d]	70.76 ± 0.02[e]	70.85 ± 0.45[e]	69.08 ± 0.92[c]	68.31 ± 0.36[b]	67.62 ± 0.40[a]

Values are averages ± SD of three determinations taken over fourteen days during steady state conditions.
Averages followed by the different letters in the same row are statistically different at 95% level by Duncan's multiple Range test.

especially palmitate (higher than 50 $mg/g_{dry\ weight}$) and oleate (higher than 200 mg/L), that were hydrolysis products of fat & oil and these have been reported to inhibit bacterial growth and methane formation (Cirne et al. 2007). The high amounts of TS and TSS in the POME comes from insoluble organic substances being washed out during the production process. It has to be emphasized that the up-flow anaerobic sludge blanket process appears to be particularly sensitive to the loading of solids. Thus Borja et al. (1996) used a two-stage up-flow anaerobic sludge blanket for treating POME. As soon as the suspended solids concentration of POME in the acidogenic reactor was increased to 10.8 g/L, an accumulation of organic solids in the reactor was observed.

Effects of varying OLRs on the performance of the reactors

Reactor performance is usually evaluated in terms of stability and efficiency of the process estimated through the measurement of pH, TVFA and alkalinity, COD removal, gas production and methane production (Table 2). For the 37ºC reactor, as the OLR was increased from 9.72 to 12.25 g[COD]/L/day, the pH was significantly [$p < 0.05$] reduced from 7.54 to 7.42 with a significant [$p < 0.05$] increase of TVFA from 172.29 to 815.43 mg acetic acid/L. At an OLR of 12.25 g[COD]/L/day, the ratio of TVFA/alkalinity was 0.26. Zinatizadeh et al. (2006) demonstrated that treating POME in an up-flow anaerobic sludge fixed film (UASFF) reactor at 38ºC, with OLRs of 14.49, 21.31, 26.21 and 34.73 g[COD]/L/day with a HRT of 1 day, the TVFA concentration increased to 93.5, 165.1, 365.2 and 843.2 mg/L respectively. This implied an increasing unbalance between acid formation and methane production in the system. However under the conditions of this experiment, the pH of the effluent (7.42) was in the optimal range (6.9-7.9) for anaerobic digestion, far from a pH of 5.3, known to decrease methane concentration by about 59% (Björnsson et al. 2000). Also Song et al. (2004) reported that the buffering capacity was sufficient when the TVFA/alkalinity was maintained below 0.4.

The pH values in the 55ºC reactor were significantly [$p < 0.05$] higher than those in the 37ºC reactor at all OLRs tested (Table 2). However, the ranges of pH values were all within the optimal pH values for methane production (Wheatley, 1990). At an HRT of 7 days and an OLR increasing from 9.62 to 12.15 g[COD]/L/day, the levels of TVFAs in the 55ºC reactor increased from 270.14 to 537.14 mg acetic acid/L, with the TVFA/alkalinity ratio changing only between 0.09-0.17 compared to a change from 0.06 to 0.26 at 37ºC using the same loadings and HRT. These results imply that at the same level of OLR, the process in the 55ºC reactor was more stable than in the 37ºC reactor. Increasing the OLR in the 55ºC reactor to 17.01 g[COD]/L/day with an HRT of 5 days, caused a significant [$p < 0.05$] increase of the TVFA/alkalinity ratio to 0.30 and therefore this system was under severe stress.

The efficiency of COD reduction was between 69.89-71.10% at OLRs from 9.72-12.25 g[COD]/L/day for the 37ºC reactor with a methane yield of 0.46-0.51 L/g[COD] and methane production was 1.96-2.65 L/L[reactor]/day. The 55ºC reactor gave a COD reduction of between 67.73-72.16%, a methane yield of 0.44-0.49 L/g[COD] and methane production was 1.91-3.24 L/L[reactor]/day. The biogas and methane productions of both reactors significantly [$p < 0.05$] increased with an increasing OLR (Table 2).

As far as the performance of the process is concerned, the 37ºC reactor ran successfully at the maximum OLR tested (12.25 g[COD]/L/day) and an HRT of 7 days. At an HRT of 5 days, the 55ºC reactor also ran successfully at the maximum OLR tested (17.01 g[COD]/L/day). In both these cases, although the TVFA levels were significantly [$p < 0.05$] raised to 815.43 and 980.00 mg acetic acid/L, respectively; the reduction of % COD was still high and a significant increase in the production of biogas and methane occurred. This work showed that the capital cost of the anaerobic digester could be lowered by operating the reactor at a thermophilic temperature. Borja and Banks (1995) reported that changing the type of reactor also affected OLRs; for example, using an anaerobic filter or a fluidized-bed reactor or an UASFF reactor, each had its own characteristics (Zinatizadeh et al. 2006). However, these reactor types did not work well with wastewater of high solid content (Björnsson et al. 1997). Thermophilic digestion is now becoming of great interest for sewage sludge treatment due to its potential for a better reduction of potential pathogens compared to that using mesophilic digestion (Boušková et al. 2005). Since POME has an initial temperature of 80-90ºC (Najafpour et al. 2006), operating the reactor under thermophilic conditions would be more economical than under mesophilic conditions in terms of the ability to use a smaller digester and obtaining a better methane production rate (Table 2).

Effect of temperature shifts on the performance of the reactors

The responses of the performance of the processes to changes in temperature were investigated in the 37 and 55ºC reactors at an HRT of 7 days and an OLR of 9.68 g[COD]/L/day. The performance of both reactors was divided into three phases (Table 3).

Phase I. After changing the temperature, only minor changes in the operating processes were observed in phase I with either reactor. The performance of the mesophilic reactor is shown in Figure 1 and the thermophilic reactor in Figure 2. The results illustrate that the performanceof the 37ºC reactor changed insignificantly in terms of any of the measured parameters as the temperature was raised to 40ºC and 43ºC. These results were confirmed by statistic tests which showed that the levels of TVFA, alkalinity, biogas and methane productions and methane yield did not significantly [$p < 0.05$] change (Table 4). For example, the TVFA levels ranged from 160.71-166.64 mg as acetic acid/L and biogas production was 2.79-2.81 L of gas/L[reactor]/day (Table 4). The 55ºC reactor, operated over the reducing temperature range of 52ºC, 49ºC and 46ºC also produced only minor changes in efficiency. The biogas production varied from 2.67-2.72 L of gas/L[reactor]/day and methane production ranged from 1.86-1.89 L/L[reactor]/day. However when the temperature was reduced from 46 to 43ºC the efficiency of the process became lower with a significant [$p < 0.05$] drop in methane (1.77 L/L[reactor]/day) and biogas productions (2.53 L of gas/L[reactor]/day) (Table 5). The process became unstable with a significant increase in TVFA levels (746.14 mg as acetic acid/L). Moreover, at each 3ºC temperature shift from 55ºC to 52ºC, 49ºC, 46ºC and 43ºC there was a rapid initial drop in biogas production rate that was quickly reversed over a few days (Figure 2). These results indicated that the 55ºC reactor was quite sensitive to the temperature disturbances, probably due to induction of a temporary unbalance of the microorganisms in the reactor. Speece (1996) reported that methanogens are more sensitive to

Table 5. Performance of the thermophilic reactor at different operating temperatures under steady-state conditions.

Parameters	Temperature (ºC)					
	Phase I					Phase III
	55	52	49	46	43	37
pH	7.78 ± 0.04^{c}	7.78 ± 0.04^{c}	7.76 ± 0.04^{c}	7.60 ± 0.13^{a}	7.69 ± 0.13^{b}	7.57 ± 0.04^{a}
TVFA (mg as acetic acid/L)	274.86 ± 8.99^{c}	256.43 ± 14.64^{b}	239.71 ± 12.44^{a}	284.29 ± 6.30^{d}	746.14 ± 6.05^{e}	851.29 ± 7.96^{f}
Alkalinity (mg/L)	3,122.32 ± 23.6^{bc}	3,114.29 ± 16.16^{bc}	3,117.86 ± 34.22^{bc}	3,134.82 ± 25.09^{c}	3,058.93 ± 27.49^{a}	3,106.79 ± 21.15^{b}
TVFA/Alkalinity	0.09 ± 0.00^{c}	0.08 ± 0.00b	0.07 ± 0.00^{a}	0.09 ± 0.00^{c}	0.24 ± 0.00^{d}	0.27 ± 0.00e
Biogas production (L of gas/L[reactor]/day)	2.71 ± 0.02^{d}e	2.72 ± 0.02^{e}	2.69 ± 0.03^{cd}	2.67 ± 0.06^{c}	2.53 ± 0.03^{b}	2.24 ± 0.02^{a}
Gas yield (L/g[COD])	0.65 ± 0.01^{c}	0.66 ± 0.01^{d}	0.65 ± 0.01^{c}	0.65 ± 0.01^{c}	0.62 ± 0.01^{b}	0.56 ± 0.01^{a}
Methane production (L/L[reactor]/day)	1.86 ± 0.02^{c}	1.89 ± 0.02^{d}	1.89 ± 0.02^{d}	1.89 ± 0.04^{d}	1.77 ± 0.02^{b}	1.55 ± 0.02^{a}
Methane yield (L/g[COD])	0.44 ± 0.01^{c}	0.46 ± 0.01^{d}	0.46 ± 0.01d	0.46 ± 0.01^{d}	0.43 ± 0.01^{b}	0.39 ± 0.00^{a}
COD reduction (%)	69.27 ± 0.06^{d}	68.26 ± 0.48^{c}	68.54 ± 0.45^{c}	67.80 ± 0.43^{b}	67.65 ± 0.69^{b}	65.71 ± 0.32^{a}

Values are averages ± SD of three determinations taken over fourteen days during steady state conditions.
Averages followed by the different letters in the same row are statistically different at 95% level by Duncan's multiple Range test.

temperature changes than acidogens. It maybe that the rate at which the methanogens converted the fatty acids to methane was initially reduced far more than the rate at which the acidogens produced acids.

As shown in Table 5, the pH in the 55ºC reactor (operating in the steady state at 55, 52, 49, 46 and with slightly changed parameters at 43ºC) were in the range of 7.60-7.78. The higher pH levels (7.78-7.76) occurring in the 55-49ºC reactor were in agreement with results from a previous study (de la Rubia et al. 2002). The alkalinity levels of the 55ºC reactor were also higher than those of the 37ºC reactor (Table 4), thus an increase of TVFA levels in the 55ºC reactor was compensated by an increased alkalinity. This allowed neutralization of the TVFA and prevented a pH drop (Borja et al. 1995). In addition, the process is considered to be operating effectively as TVFA/alkalinity ratios between 0.05-0.06 (Table 4) and 0.08-0.09 (Table 5), are still some way from the failure limit of 0.3-0.4 (Rittmann and McCarty, 2001).

In addition, temperature shifts of the 55ºC reactor to 52, 49 and 46ºC and for the 37ºC to 40 and 43ºC had no detrimental effect on reactor performance with the COD removal efficiencies remaining about 68% in both reactors. Also the methane production and yields did not vary significantly at these different temperatures. It seems therefore that temperature shifts do not directly affect the gas composition (Table 4 and Table 5). From these results, we can conclude that the microorganisms present in these reactors must have a tolerance for a fairly wide range of temperatures. This may be attributed to the presence of thermotolerant organisms that can quickly adapt to any newly imposed temperature change. Chen (1983) reported that the development of a bacterial community involved in the degradative system could be related to the percentage of mesophilic and thermophilic bacteria in the initial sludge. Iranpour et al. (2002) have also suggested that an upward temperature shift may lead to the development of a culture dominated by thermotolerant mesophilic organism rather than true thermophiles.

Moreover, both reactors could be operated successfully at 43ºC, which is considered to be the optimal change-over temperature from mesophiles to thermophiles.

Phase II. In phase II a temperature shift of 3ºC did cause a loss of stability and a change in the performance of the reactors. This was clearly observed when the 37ºC reactor temperature was raised from 43ºC to 46ºC and for the 55ºC reactor, the temperature was lowered from 43ºC to 40ºC. Process instability was observed as TVFA concentrations rapidly increased from 156 to 2,059 over the first 13 days after the change from 43ºC to 46ºC and from 750 to 2,368 over the first 12 days from 43ºC to 40ºC. This indicated a significant change in the balance among the microbial groups involved in the system. It is unlikely that such temperature changes occur in the normal operating environment of the methanogenic sludge. During these periods of operation the performance of the processes were poor with the biogas production rates dropping to a minimum value (1.49 L of gas/L[reactor]/day for the 37ºC reactor and 2.01 L of gas/L[reactor]/day for the 55ºC reactor). Perhaps a consortium adapted to operate at 37ºC ceases to function effectively at 46ºC while a consortium adapted to operate at 55ºC ceases to operate effectively at 40ºC. Griffin et al. (1998) reported that the methanogenic bacteria are the limiting microbial group during the period of adaptation to thermophilic conditions. Boušková et al. (2005) also observed a strong disturbance when the reactor temperature was adjusted from 42ºC to 47ºC. In particular, the optimal growth rate of any particular bacterial strain occurs over a limited temperature range. Once this temperature range is exceeded, growth rate drops off rapidly due to denaturation of key proteins (Rittmann and McCarty, 2001). The loss of function of any one of the microbes involved in the degradative system will alter the overall process.

Since, the unstable conditions of both reactors were most clearly shown in the changes in TVFA levels, the OLR of the reactors was lowered in an attempt to restore an effective process. Decreasing the OLR from 9.68 to 4.20 g[COD]/L/day during the transition period resulted in a significant drop in the TVFA levels in both reactors to 532 mg as acetic acid/L (37ºC reactor) and 665 mg as acetic acid/L (55ºC reactor) and the process stabilized. However, when the OLRs were again increased to 9.68 g[COD]/L/day between days 67-76 (37ºC reactor) and days 111-119 (55ºC reactor) the performance was again reduced as shown by a marked continued rise in TVFA to 2,459 for the 37ºC and 2,198 for the 55ºC reactor and a concomitant decline in biogas production.

The responses of pH, alkalinity, reduction of COD, and methane content were somewhat delayed after the instability developed (data not shown). However, the increased TVFA was not accompanied by a corresponding increase in alkalinity so the pH fell during this period.

Phase III. In an attempt to recover the reactor performance after the onset of the unstable transient conditions in phase II, both reactors were fed with a decreased OLR (4.20) followed by a gradual increase (to 9.68) over a period of time to allow for any adaptation of the microbial populations. This readjustment occurred during days 77-93 for the 37ºC reactor and from 120-132 for the 55ºC reactor and during this time the temperature was altered again to 49ºC and 37ºC for the 37ºC and 55ºC reactors, respectively. The 37ºC reactor became stabilized again at 49ºC after about day 93 with a substantial drop in TVFA levels to 338.71 mg (as acetic acid/L). The 55ºC became stabilized at 37ºC at about day 130 with a TVFA level of 851.29 mg as acetic acid/L. Both these new TVFA levels were significantly [$p < 0.05$] higher than those of the steady state in phase I, especially with the 55ºC reactor (Table 4 and Table 5). This data revealed that the bacterial consortium and in particular the methanogens, in both digesters were able to adapt to new conditions of temperature and OLR and achieve a new steady-state. Once methanogenesis had recovered, a relatively stable environmental condition could be maintained in the system.

The adaptation of the mesophilic population operating at 37ºC to thermophilic conditions at 55ºC led to a stable process that is significantly different from that previously found at 37ºC (Table 4) and was different from the performance of the initial 55ºC reactor (Table 5). This new process allows for a conversion of organic matter into the final end-product without accumulation of intermediates. This could be attributed to the rapid development of thermophilic methanogens, that were originally present in the mesophilic sludge, to become dominant under the new thermophilic conditions (Chachkhiani et al. 2004). In contrast when the 55ºC reactor was shifted to a temperature of 37ºC and the new set of stable conditions were established from days 132 onwards, these properties were different from those of either the initial properties of the 37ºC or 55ºC reactors (Table 5). A reduced amount of biogas production coincided with a significantly [$p < 0.05$] higher TVFA level (851.29 mg as acetic acid/L) even at day 146, when the experiment was terminated. This indicates that the new microbial consortium is probably different from that operating initially in the 37ºC reactor and results in a population that is less efficient in terms of % COD removal, biogas and methane productions. This could be related to the poor development of mesophiles that should become dominant under the new mesophilic conditions. The results indicate that the microbial population present in the 55ºC reactor found it more difficult to recover from temperature changes than did the population of the 37ºC reactor. This indicates that temperature regulation here is complex and may depend on the composition of the initial sludge in the system. Lau and Fang (1997) found that a temperature shock had a less adverse effect on the acetotrophic methanogens than on other methanogens. According to Cabirol et al. (2003), who studied the adaptation of a mesophilic anaerobic sludge to thermophilic conditions, this also showed a rapid adaptation with an increase in the proportion of hydrogenotrophic methanogens.

We have shown that the adverse effect of a temperature shift can be alleviated by an initial lowering of the OLR. When this is followed by a slow increase back to the initial OLR value the system can return to a steady state that in some cases is not too different from the original but at a different temperature. We strongly recommend the use of this procedure in order to help microbial populations adapt to any temperature shifts. Moreover, a continuous feed should be used to eliminate problems that may arise from transient growth conditions and to permit flexibility in adjustment of the time course of temperature shifts. It also can be used to select a culture that will grow under conditions of stress.

CONCLUDING REMARKS

Based on these results, the operation of a 37ºC reactor at an OLR of 10.74 g[COD]/L/day and the 55ºC reactor at an OLR of 12.15 g[COD]/L/day equivalent to an HRT of 7 days is likely to achieve satisfactory results.

The 37ºC reactor could tolerate temperature variations in the range of 37-43ºC without significant changes in an index for process stability (TVFA and TVFA/Alkalinity) whereas, the 55ºC reactor could tolerate temperature variations in the range of 55-43ºC. However, minor instabilities of the processes in terms of TVFA and TVFA/alkalinity were observed when the temperature was changed.

The first indication of a loss of stability of the processes, due to a temperature shift, in both reactors was an accumulation of TVFAs. This occurred at a temperature of 46ºC for the 55ºC reactor and at 40ºC for the 37ºC reactor.

The instability could be overcome by lowering the OLR. After the stability was regained the OLR could be gradually increased to that of the initial value, without major changes to the stability.

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