

*Full Length Research Paper*

# The Effect of C:N:P ratio, volatile fatty acids and Na<sup>+</sup> levels on the performance of an anaerobic treatment of fresh leachate from municipal solid waste transfer station

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Anaerobic digestion was carried out in this study to treat fresh leachate from municipal solid waste transfer station in a 10 L stirred tank reactor (STR). The treatment process was performed in batch and semi-continuous process. Palm oil mill effluent (POME) sludge was used as an inoculum. A high BOD reduction was achieved in 3 different treatment conditions in this study. A BOD removal of 85, 77 and 90% for the batch (Experiment 1), semi-continuous process without pH adjustment (Experiment 2) and semi-continuous process with pH adjustment (Experiment 3), respectively were recorded. It was observed that there was no significant deficiency in required nutrients for Experiment 1, 2 and 3 in this work. High concentration of volatile fatty acids (VFAs) was detected in Experiment 3, which indicated the instability of bioreactor in which lower methanogenic activity was observed. The levels of acetic acid (HAc) and propionic acid (HPr) appeared to be the VFA species that accumulated and started to cause an imbalance in the reactor. It was found that the use of large amount of sodium hydroxide (NaOH) to adjust the bioreactor pH had caused an inhibition of the metabolic activity of methanogenesis bacteria that involved in the methane production.

**Key words:** Anaerobic treatment, batch, semi-continuous, fresh leachate, BOD removal, C:N:P ratio, volatile fatty acid.

## INTRODUCTION

Leachates from municipal solid waste (MSW) are often defined as a hazardous and heavily polluted wastewater. The discharge of landfill leachate can lead to serious environmental problems, since leachate contains a large amount of organic matter (both biodegradable and non-biodegradable carbon), ammonia-nitrogen, heavy metals, chlorinated organic and inorganic salts. Although some of these pollutants can be degraded by microorganisms, the limitation of common biological processes (degradation is

only part of the COD and limited removal of bio-refractory organic pollutants) has made it difficult to meet the correlative discharge standard (Wang et al., 2002).

Meanwhile, the characteristics of the leachate varied with regards to its composition and biodegradable matter content (Andreottola and Cannas, 1992; Chu et al., 1994). All these factors make leachate treatment difficult and, thus, have to be seriously considered before an appropriate treatment processes is decided. Therefore, pollution of groundwater and surface water with leachate has become one of the major problems associated with sanitary landfill waste disposal.

There are many factors affecting the characteristic of such leachates, that is, age, precipitation, seasonal

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weather variation, waste type and composition. In particular, the composition of landfill leachates varies greatly depending on the age of the landfill (Baig et al., 1999). In young landfills that contain large amounts of biodegradable organic matter, a rapid anaerobic fermentation takes place and hence the volatile fatty acids (VFA) is the main fermentation products (Welander et al., 1997). Acid production is enhanced if the moisture or water content of the solid waste is high (Wang et al., 2003). This early phase of a landfill's lifetime is called the acidogenic phase, and leads to the release of large quantities of free VFAs into the treated leachate, and organic content as high as 95% has been reported in the literature (Harsem, 1983).

In a mature landfill, the methanogenic microorganisms are accumulated in the leachate, and the VFAs in mature landfill are converted to biogas such as methane and carbon dioxide. At this stage the organic fraction in the leachate becomes dominated by refractory (non-biodegradable) compounds such as humic substances (Chian and DeWalle, 1976). The characteristics of the landfill leachate can usually be characterised by the parameters such as COD, BOD, the ratio BOD/COD, pH, suspended solids (SS), ammonia nitrogen and heavy metals.

It is well known that the composition of landfill leachate is complex, due to the conditions within a landfill such as chemical and biological activities, moisture content and the degree of stabilization. In spite of many attempts to treat landfill leachate, it appears that no general recommendations for leachate treatment can be made at the present time. The treatment of leachate involves both biological and physicochemical methods. Initially, the biological treatment of leachate takes place in anaerobic or aerated lagoons with elongated mean period of retention (Blakey et al., 1992; Robinson, 1992; Robinson and Grantham, 1988).

It is common knowledge that high efficiency of leachate treatment is achieved in multistage biological processes. The leachate composition varies with time and depends on atmospheric conditions, hence, the appropriate selection of reactors and their operational conditions are of key importance (Klimiuk and Kulikowska, 2006). Therefore, the main objective of this study was to investigate the effect C:N:P ratio, VFAs and  $\text{Na}^+$  levels on the performance of an anaerobic treatment of fresh leachate. Three types of treatment; batch with no pH adjustment (Experiment 1), semi-continuous process with no pH adjustment (Experiment 2) and semi-continuous process with pH adjustment (Experiment 3) were conducted and their performance were compared and discussed.

## MATERIALS AND METHODS

### Leachate and POME sludge inoculum

Fresh leachate was taken directly from Taman Beringin Transfer Station (TBTS), Kuala Lumpur, Malaysia and POME sludge was obtained from anaerobic treatment pond at the Seri Ulu Langa Palm

Oil Mill Sdn.Bhd, Dengkil, Malaysia. The POME sludge were collected and sieved using a 1.5 mm mesh to remove solid particles such as shell, fiber and small stone and stored at 4°C before it was used as an inoculum for anaerobic treatment process.

### Process configuration and system design

The Stirrer tank reactor (STR) used for this study was a 13 L BIOSTAT<sup>®</sup>B bioreactor (10 L working volume). It was filled with 10 L POME sludge as inoculum and was operated at 28±2°C. The fresh leachate with a flow rate of 1 L/day in semi-continuous mode was being added into bioreactor and mixed with POME sludge as the sources of microbial complex. The anaerobic treatment process was operated in three different mode; (1) batch process (Experiment 1) pH was not controlled ; (2) semi-continuous process (Experiment 2) pH was not controlled and (3) semi-continuous process (Experiment 3) pH was adjusted to a range of 6.8-7.5 using sodium hydroxide (NaOH). The treatment was conducted in a period of two and three months for semi-continuous and batch processes, respectively. The organic loading rate (OLR) of 6 kg COD/m<sup>3</sup>.day and the hydraulic retention time (HRT) of 10 days was used for semi-continuous process.

### Analytical methods

Standard Method for the Examination of Water and Wastewater (APHA, 2000) was used for measurement of COD, BOD<sub>5</sub>, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, VFA, and pH. Ammonia nitrogen concentration ( $\text{NH}_3\text{-N}$ ) was detected by the Nessler method (380 HACH DR/2500 spectrophotometer) and phosphorous by the React PV method (490 HACH DR/2500 spectrophotometer). The experimental conditions for these 3 treatment process are summarized in Table 1.

## RESULTS AND DISCUSSION

### Biodegradation of BOD and COD in batch and semi-continuous anaerobic treatment of fresh leachate

The characteristics of fresh and treated leachate in batch and semi-continuous anaerobic treatment from transfer station are tabulated in Table 2. The whole duration of treatment for Experiment 1, 2 and 3 took 91, 27 and 30 days, respectively. Fresh leachate is difficult to treat due to its high concentration of COD, BOD and ammonia nitrogen. BOD/COD ratio is used to determine the proportion of biodegradable organic carbon in the leachate. The BOD/COD ratio indicates the changes in the amount of biodegradable compounds in the leachate. The biodegradation of organic content of leachate during the treatment would lead to the decrease of BOD/COD ratio. This decrease indicated that the organic wastes were degraded via the microbial metabolic activity.

Initial BOD/COD ratio for both batch and semi-continuous process were in the range of 0.5 to 0.55. After the anaerobic treatment process, the BOD/COD ratio of treated leachate was reduced to 0.19, 0.14 and 0.11 for Experiments 1, 2 and 3, respectively. The lowest BOD/COD ratio, 0.11, was obtained in semi-continuous system (Experiment 3) which showed that a great proportion of

**Table 1.** Conditions of fresh leachate treatment process.

Experiment	Batch Experiment 1	Semi-continuous Experiment 2	Semi-continuous Experiment 3
Biomass sedimentation	No	No	No
Duration (day)	91	27	30
Initial OLR ( kg COD / m <sup>3</sup> .day)	---	6	6
Temperature	28±2 °C	28±2 °C	28±2 °C
pH adjustment	No	No	(6.8-7.5)

OLR = Organic loading rate, --- = not applicable.

**Table 2.** Leachate characteristic in batch and semi-continuous treatment process.

Parameter	Initial fresh Leachate (batch)	Experiment 1 (Day 91)	Initial fresh leachate (semi- continuous)	Experiment 2 (Day 27)	Experiment 3 (Day 30)
pH	5	6.5	4.8	4.5	7.6
Temperature	28±2 °C	28±2 °C	28±2 °C	28±2 °C	28±2 °C
COD (mg/L)	55000	30720	60000	34560	25780
COD reduction (%)	---	44	---	37	52.7
BOD (mg/L)	30250	4500	30000	6912	2882
BOD/COD	0.55	0.14	0.5	0.2	0.11
BOD reduction (%)	---	85.12	---	76.96	90.39
TS (mg/L)	39190	17200	26970	27780	21130
VS (mg/L)	n.d	n.d	21060	16270	11580
TSS (mg/L)	4700	3300	4200	4800	3012.5
VSS (mg/L)	3925	2820	3350	3988	2262.5
VSS/TSS	0.83	0.85	79.76	0.83	0.75
NH <sub>3</sub> -N (mg/L)	492	680	268.5	450	663
PO <sub>4</sub> <sup>-3</sup> (mg/L)	300	173.5	312	321	138
Alkalinity (as mg CaCO <sub>3</sub> /L)	1940	3120	n.d	n.d	5450

n.d = Not determined, --- = not applicable.

the organic matter in the leachate was refractory to the biological degradation. The highest BOD/COD was recorded in the batch process suggested that accumulation of the metabolite as a result of the COD biodegradation may cause a feed back inhibition to some of those microbial biodegradation pathways.

A high BOD removal was achieved in the present study, in which 85, 77 and 90 % was obtained for Experiments 1, 2 and 3, respectively. This has led to a COD reduction of 44, 37 and 52.7% for batch and Experiments 2 and 3 of semi-continuous process, respectively. The VSS/TSS ratios were in good condition during the anaerobic treatment. Ammonia nitrogen is produced as a by-product of anaerobic digestion, principally from the mineralization of organic nitrogen during the deamination of proteins and amino acids. NH<sub>3</sub>-N increased during the experiments as pH increased. Ammonia toxicity can be avoided if the reactor pH is maintained within the optimum range of 6.8 to 7.5 and the ammonia nitrogen concentration does not reach the

range of 1500 to 3000 mg/L (Gerardi, 2003). Highest value for COD and BOD<sub>5</sub> removal was obtained in Experiment 3 of semi-continuous system at pH 7.6 and the lowest was obtained from the semi-continuous system in Experiment 2 at pH 4.5.

This result suggested that pH value may affect the COD removal. Indeed, semi-continuous anaerobic treatment with pH adjustment shows a better reduction in COD and BOD<sub>5</sub> compared to the batch process and semi-continuous (Experiment 2) where pH was not adjusted. The COD and BOD removal efficiency achieved in the present study are comparable to other stabilized landfill leachate treatment (Horan et al., 1997; Henderson and Atwater, 1995; Hosomi et al., 1989; Roger et al., 2005) except that from Diamadopoulos (1994), in which they achieved a very high COD removal from the stable leachate treatment. The comparisons of the performance of the fresh leachate treatment in the present study to other reported leachate treatment using stabilize landfill leachate are depicted in Table 3. Generally, fresh

**Table 3.** Biological treatment of landfill leachate and transfer station leachate.

Methods	Past literature	COD removal (%)	BOD removal (%)
Recirculation (stabilized leachate)	Diamadopoulos (1994)	90	98
Granular activated carbon-biological fluidized bed (stabilized leachate)	Horan et al. (1997)	55	---
Rotating biological contactors (RBC) (stabilized leachate)	Henderson and Atwater (1995)	49	92
Sequencing batch reactors (SBR) (stabilized leachate)	Hosomi et al. (1989)	50	---
Physico-chemical treatment (stabilized leachate)	Roger et al. (2005)	47	---
Batch anaerobic (leachate from transfer station)	This study	44	85
Semi-continuous anaerobic (leachate from transfer station)	This study	52.7	90

--- = Not available.

**Table 4.** Chemical composition of the methanogenic microorganism (Lettinga et al., 1996).

Macronutrients		Micronutrients	
Element	Concentration (g /kg TSS)	Element	Concentration (mg /kg TSS)
Nitrogen	65	Iron	1,800
Phosphorous	15	Nickel	100
Potassium	10	Cobalt	75
Sulfur	10	Molybdenum	60
Calcium	4	Zinc	60
Magnesium	3	Manganese	20
		Copper	10

leachate is characterized with high pollution such as COD, BOD compared to stabilize landfill leachate which has lower COD and BOD.

### C:N:P ratio

Fermentable carbohydrate, nitrogen, phosphorous and other nutrients such as sodium, potassium, calcium, magnesium, iron and sulphur are needed for normal growth of bacteria involved in anaerobic wastewater treatment (Isa, 1991). Therefore, it is important to ensure that during the anaerobic treatment nutrients required for biomass to grow are sufficient. For biological treatment to be successful, the inorganic nutrient necessary for the growth of microorganisms should be supplied in sufficient amounts; if the ideal concentration of nutrient is not supplied, there should be some form of compensation, either by applying smaller nutrient loads to the treatment system, or by allowing a reduced efficiency of the system (Sperling and Chernicharo, 2005).

Nitrogen and phosphate requirements were calculated and tabulated for batch and semi-continuous anaerobic treatment of fresh leachate based on empirical chemical composition of the microbial cell that shown in Table 4 and Equation 1 (Lettinga et al., 1996).

$$Nr = S_o \cdot Y \cdot N_{abc} \cdot TSS/VSS \quad (1)$$

Where  $Nr$  = nutrient requirement (g/L),  $S_o$  = concentration of influent COD (g/L),  $Y$  = yield coefficient (g VSS / g COD),  $N_{abc}$  = concentration of nutrient in the bacterial cell (g/g VSS), and  $TSS/VSS$  = total solids/volatile solids ratio for the bacterial cell (usually 1.14).

It is clearly shown in Table 5 that the availability of nitrogen and phosphate is sufficient for the growth of the microorganisms in batch and semi-continuous anaerobic treatment of leachate except ammonia nitrogen in Experiment 2. Hence the yield coefficient,  $Y$ , achieved in the semi-continuous (0.09-0.1) and batch process (0.06) can be classified as medium and low yield coefficient, respectively, as compared to that reported by Lettinga et al. (1996).

An appropriate carbon-to-nitrogen ratio (C:N) is a prerequisite for the continued successful functioning of a digester (Gerardi, 2003). Lettinga et al. (1996) reported that carbon to nitrogen ratio is 26 and 66 for biomass with high and low yield coefficient, respectively. In the present study, the C:N ratio calculated for Experiments 1, 2 and 3 was 80, 46 and 42, respectively. High C:N ratio in Experiment 1 is due to the large amount of nitrogen and phosphate produced in the bioreactor under batch condition. However, according to McCarty (1964) a nitrogen concentration of less than 1000 mg/L has no adverse effect on anaerobic process. An excessively high C:N ratio cause the increase in acid formation which retards methanogenesis activity and, thus, methane production is

**Table 5.** Leachate characteristics for Experiment 1, 2 and 3.

Experiment	COD influent (mg/L) ( $S_o$ )	g VSS/g COD (Y)	TSS/VSS	Average $NH_3-N$ (mg/L)	Required $NH_3-N$ (mg/L)	Average $PO_4^{3-}-P$ (mg/L)	Required $PO_4^{3-}-P$ (mg/L)	COD:N:P (C:N:P)
Exp. 1	55000	0.06	1.14	610	245	205	56.43	975/4.3/1 (340:4.3:1)
Exp. 2	60000	0.09	1.30	428	456	286	105	600/4.5/1 (210:4.5:1)
Exp. 3	55000	0.1	1.24	500	443	208	102	550/4.5/1 (190:4.5:1)
Biomass with low yield coefficient (Lettinga et al., 1996)	---	0.05	1.14	---	---	---	---	1000:5:1 (330:5:1)
Biomass with high yield coefficient (Lettinga et al., 1996)	---	0.15	1.14	---	---	---	---	350:5:1 (130:5:1)

--- = Not available.

**Table 6.** Average percentages of VFAs to TVFA production for each Experiment.

Experiment	Acetic (%)	Propionic (%)	I-Butyric (%)	N-Butyric (%)	I-Valeric (%)	N-Valeric (%)	TVFA (mg/L)	COD Removal (%)
Exp. 1	18.9	7.7	9.6	39.3	11.6	12.9	8483	44
Exp. 2	30.0	24.4	2.0	24.9	2.9	15.2	7735	37
Exp. 3	47.0	25.3	1.8	13.9	1.9	9.9	9868	53

inhibited. On the other hand, when the C:N ratio is too low, nitrogen is converted to ammonium-N at a faster rate than it can be assimilated by the methanogens. For readily degradable substrates, the optimum C:N ratio is on the order of 20:1 to 25:1. However, for materials that are resistant to microbial degradation, the C:N ratio can be as high as 40:1 (UNEP IETC, 2005).

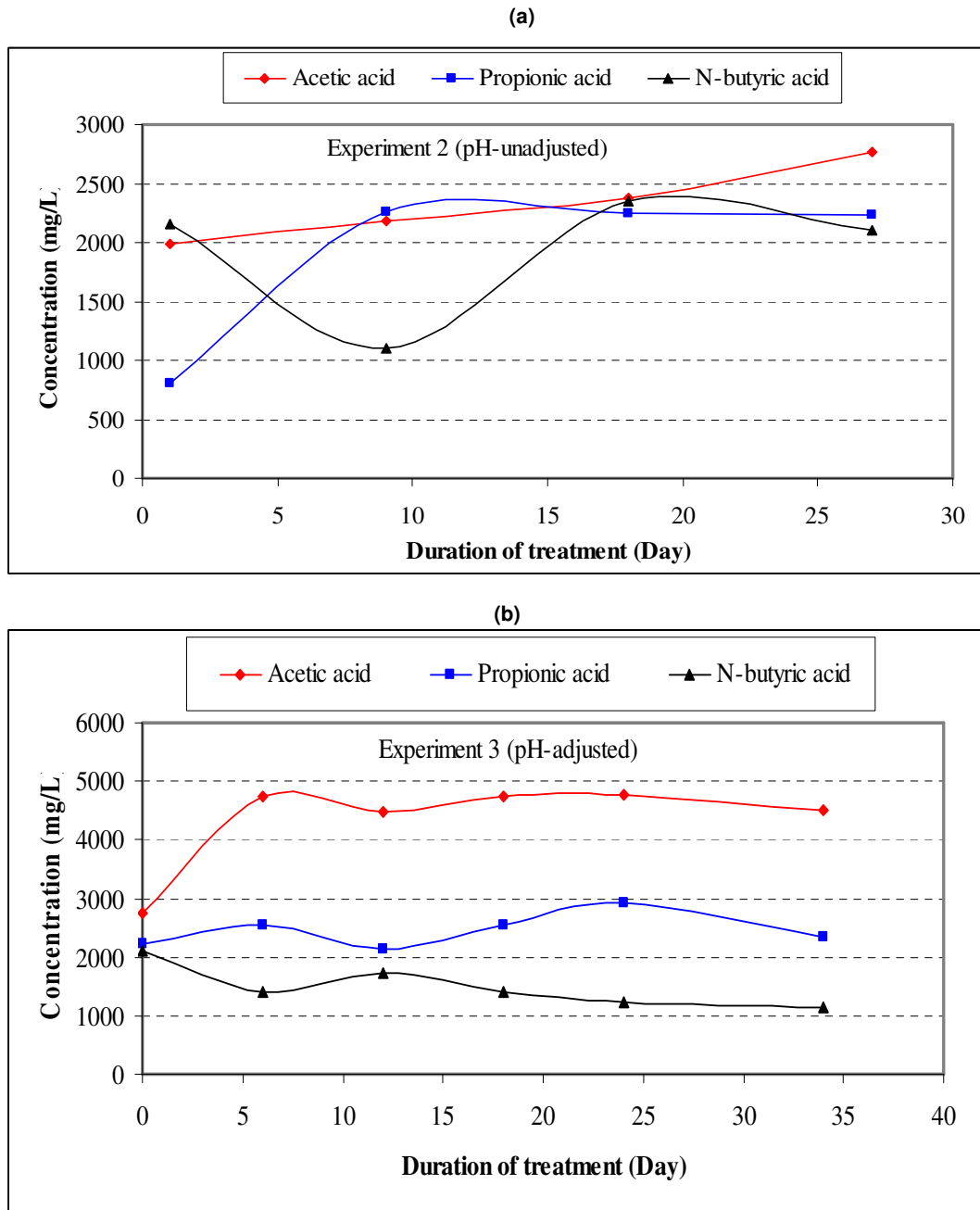
#### VFAs production during anaerobic treatment of leachate

Knowledge on the type and quantity of VFAs formed provides useful information to the dominating species of acidogens within a reactor. In this study, major intermediate products of anaerobic digestion of fresh leachate, were acetic (HAc), propionate (HPr), isobutyrate (i-HBu), n-butyrate (n-HBu), isovalerate (i-HVa) and n-valerate (n-HVa) acids. Table 6 shows the average percentages of VFAs to TVFA production in batch (Experiment 1) and semi-continuous anaerobic treatment of fresh leachate (Experiments 2 and 3).

The percentage for HAc, HPr, i-HBu, n-HBu, i-HVa and n-HVa acids in Experiment 1 was found to be 18.9, 7.7, 9.6, 39.3, 11.6 and 12.9, respectively. HAc and HPr

were the dominant organic acids found in the reactor in Experiment 2 and 3 of semi-continuous process in which they represent 54 and 72% of TVFA, respectively. Indeed Elefsiniotis and Oldham (1994a) had reported that in a complete mixed system of anaerobic continuous process treatment fed with primary sludge, acetic and propionic acid were the dominating VFAs found in the reactor.

Figure 1 shows the trend of three major VFAs produced in Experiment 2 (Figure 1a) and 3 (Figure 1b) of semi-continuous process. Accumulation of VFAs resulted in a decrease in pH from 6.5 to 4.5 for Experiment 2. The distribution of residual VFA in the reactor shows HAc, HPr and n-HBu to accumulate in the ratio of about 1.2:1:1 and 3.4:2:1 on a COD basis for Experiments 2 and 3, respectively. It was observed that HPr concentration in some period of time was higher than HAc and n-HBu concentration in Experiment 2 (Figure 1a). This may indicate that the conversion from propionic acid to acetic acid might become the rate controlling step of biodegradation process. In Experiment 3, attempts were made to increase the pH from acidic zone to the slight alkaline pH that favoured for the growth of methanogens bacteria (6.8-7.5) in order to achieve a better COD biodegradation efficiency. There were remarkable signs of accumulation of acetic acid since beginning of Experiment 3 until end of



**Figure 1.** Variation on VFAs vs. duration of treatment (Day) in (a) Experiment 2 and (b) Experiment 3.

treatment process. At the end of treatment of Experiment 3, 39% increment in HAc was obtained while n-HBu acid was reduced by 46%. However, negligible amount of methane was obtained in Experiment 3. This indicates that further conversion of acetate to methane was not occurred in Experiment 3. It has been reported that even when process pH is optimal, the accumulation of VFAs can contribute to a reduced rate of hydrolysis of the solid organic substrate (Banks and Wang, 1999), or inhibit the biodegradation if it is present at an extremely high levels (>10 g/L) (Palmisano and Barlaz, 1971).

The concentration of VFAs produced in Experiments 1, 2 and 3 of anaerobic treatment of fresh leachate is illustrated in Figure 2. Process imbalance in an anaerobic reactor will normally lead to accumulation of VFAs and then resulting in a decrease in pH. The increase in acid concentration may not register as a drop in pH immediately if the buffer capacity of the material in the acids and bases is high. The organic acid accumulation, therefore, has to reach a level before it causes a drop in pH. At that point the accumulation of VFAs may have already significantly reached high levels and caused the

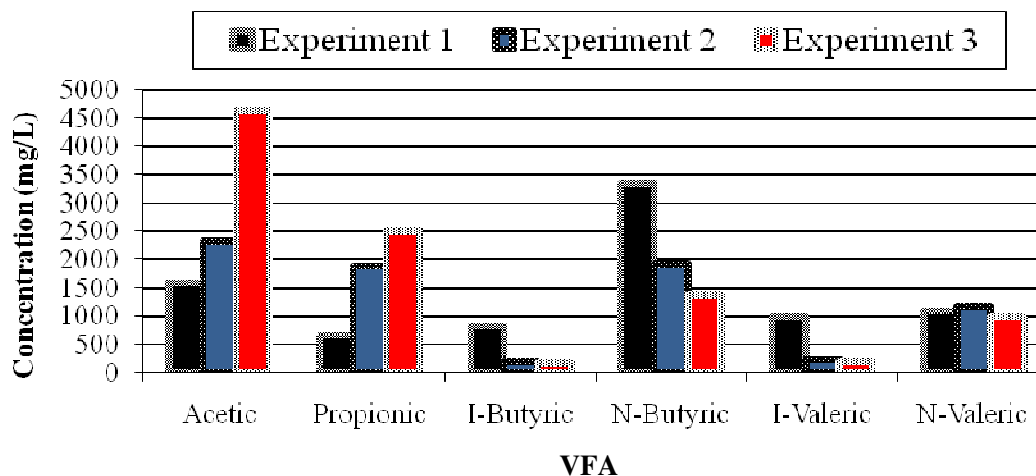


Figure 2. Average VFAs concentration for batch and semi-continuous process.

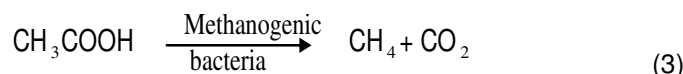
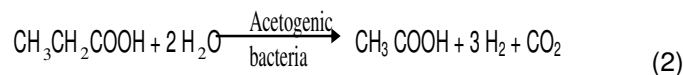
inhibition of the biodegradation (Poulsen, 2003).

Therefore, pH is not a good indicator at high buffer capacity medium. However, pH is an effective indicator in system with low buffer capacity since the pH will response quickly to the accumulation of VFA. It is noteworthy to mention that the concentration of longer straight chain acids (n-HBu and n-HVa acids) were dominant products compared to their iso-forms (i-HBu and i-HVa acids) in this study as shown in Figure 2. It was also found that almost the achieved values for n-butyric, n-valeric acid and their iso-forms in Experiment 1 were higher than that in Experiments 2 and 3 of semi-continuous process; however, the concentration of HAc and HPr acids produced in Experiment 1 was lower compared to that in Experiments 2 and 3 as shown in Figure 2.

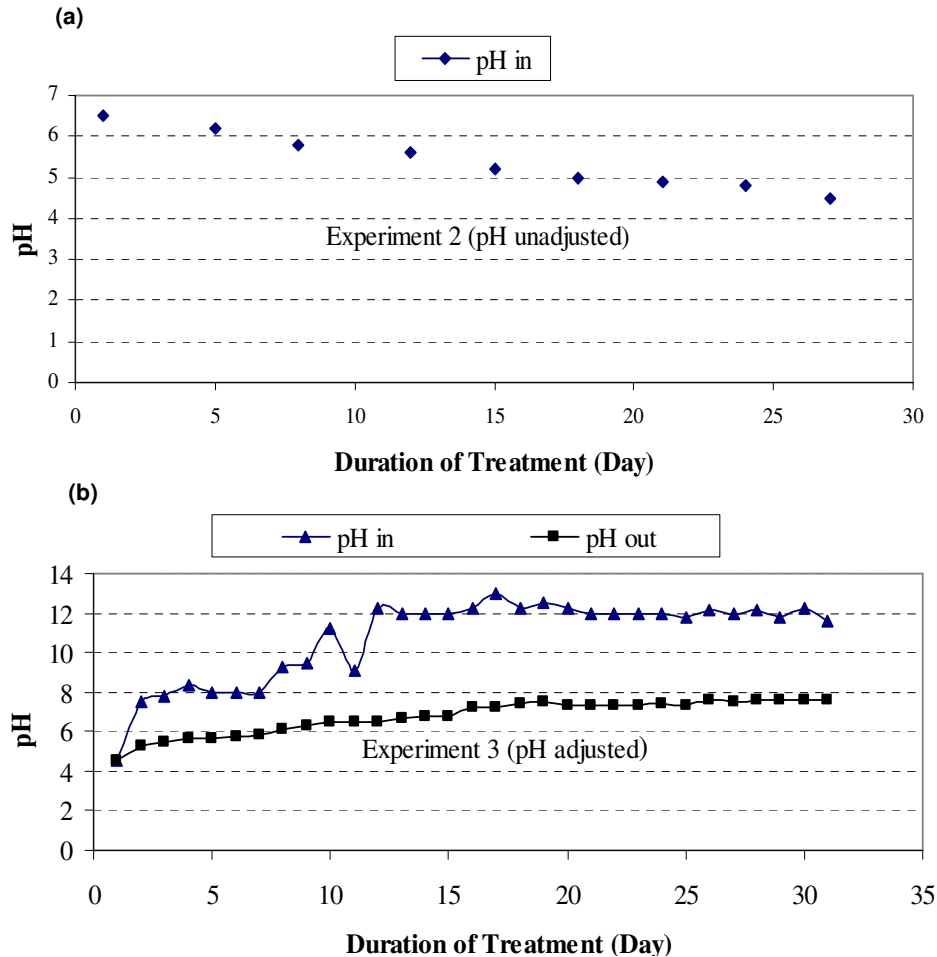
The high production of n-HBu acid in this work is mainly attributed to the larger amount of carbohydrate present in the substrate especially in Experiment 1 which had high C:N ratio (Elefsiniotis and Oldham, 1994b; Banerjee et al., 1999). The high molecular weight VFAs, including i-HBu, n-HVa and i-HVa acids, were present at low concentration in Experiments 2 and 3.

HAc, HPr and n-HBu were the major VFAs produced during anaerobic digestion in Experiments 2 and 3. Their concentration provides a useful measure of bioreactor performance. Low acid value indicates stable operation while high acid concentration is constantly associated with low biogas production (Pohland and Bloodgood, 1963; Hill et al., 1987). It has been reported by many researchers that HPr acid appears particularly sensitive to changes in the bioreactor environment although rapid accumulation of both HAc and HPr acids during stressed operation and prior to failure has also been frequently noted (Pohland and Bloodgood, 1963; Andrews and Pearson, 1965; Hobson et al., 1974; San Marzano et al., 1981; Speece, 1983). Degradation of these accumulated acids is essential to the recovery and control of the digestion process (Mawson et al., 1991).

It has been shown that high HAc content and high CO<sub>2</sub> partial pressure inhibit HPr degradation by acidogenic bacteria (Kaspar and Wuhrmann, 1978; Hansson and Molin, 1981). Lin et al. (1986) has reported that high CO<sub>2</sub> contents (> 40%) in the reactor with feed substrate concentration higher than 50,000 mg COD/L might affect the HPr degradation. The splitting of HPr to HAc (Equation 2) and conversion of HAc to methane (Equation 3) are reported to be rate-limiting steps of HPr and HBu degradation in methanogenic digestion, respectively (Chang et al., 1983).



The above-mentioned information is taken into account to be two of the reasons causing accumulation of these acids throughout the experiment. Concentration of HAc, HPr and n-HBu acids recorded at day 33 were 4730, 2535 and 1399 mg/L, respectively, for Experiment 3. Furthermore, at feed substrate concentration of 55000 mg COD/L, the CO<sub>2</sub> percentage of 37.53% was obtained as measured by gas chromatography. These results were fully in accordance with above information expressed from other researchers which might indicate the lack of propionate degradation by the dominated presence of acidogenic microorganisms in the bioreactor. In addition, the effect of volatile intermediates on methanogenesis has been extensively studied and it is generally accepted that propionic acid has a stronger inhibitory effect on methanogenesis than acetic or butyric acid (Siegert and Banks, 2005). Since there is a consistent link between methane production and COD removal in anaerobic treatment and taking into consideration the obtained results in terms of COD removal and negligible biogas



**Figure 3.** Variation of pH outlet in (a) Experiment 2 and (b) Experiment 3. The pH was adjusted in Experiment 3.

production in this work, it is concluded that accumulation of VFAs has caused inhibition in Experiment 3 of semi-continuous process of fresh leachate. However, recovery of a reactor from a period of retarded operation will necessarily require adequate time for the accumulated acids to be degraded at the lower rate.

Figure 3 depicts the variation of pH during the duration of treatment for Experiments 2 (Figure 3a) and 3 (Figure 3b) of semi-continuous process. The pH of the Experiment 3 was controlled by the addition of NaOH. Although, high levels of biodegradation of COD to VFAs was achieved in Experiment 3, however, further conversion of acetate to methane was inhibited. One of the most important factors that contributed to this inhibition could be the toxicity caused by excessive addition of  $\text{Na}^+$  during pH adjustment which increased the alkalinity from zero to 5450 mg  $\text{CaCO}_3/\text{L}$  in Experiment 3 of semi-continuous process as presented in Table 2.

At low concentrations, sodium is essential for methanogens, probably because of its role in the formation of ATP or in the oxidation of NADH (Dimroth and Thomer, 1989). At high concentrations, sodium could readily affect

the activity of microorganisms and interfere with their metabolism. McCarty (1964) has reported that sodium concentration ranging from 100-200 mg/L to be beneficial for the growth of mesophilic anaerobes; 3500 to 5500 mg/L to be moderately; and 8000 mg/L to be strongly inhibitory to methanogens at mesophilic temperatures (Chen et al., 2008). However, the concentration of  $\text{Na}^+$  was not measured in this study, but the large amount of NaOH used to adjust the pH in Experiment 3 might have affected negatively on semi-continuous anaerobic treatment process of fresh leachate.

## Conclusion

In this study, biological treatment of fresh leachate in anaerobic stirred tank reactor were conducted through series of experiments (Experiments 1, 2 and 3). In general, anaerobic digestion using POME seed sludge and pH adjustment in semi-continuous process (Experiment 3) showed better efficiency in COD reduction and especially in  $\text{BOD}_5$  compared to the batch and unadjusted pH in semi-continuous process (Experiment 1 and 2).



However, further conversion of acetate to methane was inhibited in this treatment. Analysis showed that shortage of nutrient (nitrogen and phosphorous) was not occurred during these processes. Accumulation of HAc and HPr acid was recorded by 50 and 23% in Experiment 3 compared to that in Experiment 2, respectively. High accumulation of HAc and HPr concentration was found to be major factors to the bioreactor inhibition in Experiment 3 which led to low biogas production. It is believed that extra use of Na<sup>+</sup> to adjust the pH in Experiment 3 could have contributed to the inhibition of methane conversion in anaerobic treatment process of fresh leachate.

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