Volatile Fatty Acids Production from Fermentation of Secondary Sewage Sludge

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By

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Abstract

Sludge fermentation is used worldwide as an economical means to produce volatile fatty acids (VFA), which can be used as readily available carbon in biological nutrient removal (BNR) systems. In this research, secondary sludge was tested for its potential to generate VFA. Fermentation of secondary sludge was carried out in a lab-scale sequencing batch reactor (SBR). The SBR was fed with secondary sludge of 1% total solids and run with hydraulic retention time (HRT) of 48 hours and 28 hours in phase 1 (40 days) and phase 2 (12 days) respectively. The SBR produced net VFA (expressed as acetic acid) of 365 ±62.5 mg VFA_{HAc}/l which was equivalent to a VFA yield of 0.28 ±0.05 mg VFA_{HAc}/mg VSS_{feed} during phase 1. A change in operating HRT from 48 hours to 28 hours led to a reduction in solids retention time (SRT) from 2.65 days to 2 days in phase 2. The reduction in SRT during phase 2 led to poor hydrolysis and hence could not support the fermentation. Net VFA generation decreased during phase 2 and reached 0 mg/l. Acetic acid was the main acid produced comprising 45% of total VFA content during the run with 48 hours HRT.

The effect of total solids (TS) concentration on secondary sludge fermentation was tested using batch experiments. The batch with 2.8% TS secondary sludge showed a maximum net VFA production of 60 mg VFA_{HAc}/l, which appeared to be superior to the 1% TS secondary sludge batch fermentation where no net VFA production observed throughout the test period. Primary sludge (3% TS) exhibited 1200 mg VFA_{HAc}/l in a batch fermentation, which was superior to the net VFA produced during secondary sludge (2.8% TS) batch fermentation. The effects of sonication on fermentability of primary and secondary sludges were tested. A sonic power application of 0.0017 Watt/ml/min density increased soluble content of primary and secondary sludges. In batch fermentations, sonicated secondary sludge improved fermentation over unsonicated secondary sludge. A maximum net VFA production of 130 mg VFA_{HAc}/l was observed in the secondary sludge batch fermentation.

In this research work, an investigation into inhibiting VFA degradation in secondary sludge batch fermentations was also carried out. The effects of a methanogenic bacteria inhibitor (bromoethane sulfonic acid) and low pH (range of 4.02-6.07) were considered. The addition of 1 mM bromoethane sulfonic acid (BES) in secondary sludge (1% TS) batch fermentation successfully inhibited VFA degradation. pH values as low as 4.02 showed an inhibitory effect on secondary sludge (1% TS) batch fermentation which led to poor hydrolysis and hence no net VFA generated during the test period. However, low pH values reduced the VFA degradation rate in the batch fermentations.

Secondary sludge used in the present research showed the potential to generate VFA. The amount of VFA produced in the present work showed the potential to improve the performance of a BNR system. Moreover, in batch fermentations, VFA generation was improved using various pre-treatments like sonication and BES addition.

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1 Introduction

1.1 General:

Growing urbanization and limited water resources demand advances in technology to preserve water quality. Over the last few decades, the influx of excess nutrients into water bodies has brought water quality into question. Excess quantities of nitrogen and phosphorus can result in eutrophication (enrichment of nutrients like nitrogen and phosphorus) of receiving water bodies, mainly lakes and slow moving rivers (Sundblad et al., 1994). Nutrients can be treated by various chemical treatments. However, biological treatments are preferred over chemical treatments for economical and environmental reasons. Therefore, biological nutrient removal (BNR) systems are increasingly being incorporated in wastewater treatment plants.

In BNR systems, micro-organisms require readily available carbon as an energy source to remove nitrogen and phosphorus. Therefore, external carbon sources are supplemented to facilitate nutrients removal in BNR processes. However, external carbon sources incur high costs and increase organic load. McDonald (1990) reported that methanol costs were 70% of the total operating and maintenance expenditure of a municipal wastewater treatment facility. An internal carbon source (wastewater or sludge or a mixture of both) can be employed as an economical alternative to external carbon supplementation. However, quite often internal carbon sources are not inherently rich enough in readily available carbon to support BNR systems. As a result, fermentation of sludge or wastewater is carried out to produce volatile fatty acids (VFA) which can be used as a readily consumable substrate for bacteria in BNR systems.

1.2 Prior research:

Over the past few years, a lot of research has been conducted to optimise sludge-fermentation processes. The effects of various operational and sludge parameters like pH, hydraulic retention time (HRT), solids retention time (SRT), solids concentration and temperature on fermentation have been studied significantly. Also, a few studies have demonstrated the effect of different reactor configurations on VFA production. Furthermore, a myriad number of studies have dealt with various sludge pre-treatments to enhance solubility in order to boost fermentation.

Most of the previous studies of sludge fermentation were carried out on primary sludge. Secondary sludge fermentation has been given very little attention. It was believed that secondary sludge is hard to digest due to its characteristics, and hence its suitability to generate VFA via fermentation is lesser than primary sludge. Secondary sludge is reported as troublesome to stabilise because of difficulty in dewatering and digestion (Hogan et al., 2004; Mao et al., 2004). However, secondary sludge may contain high levels of organic matter and could be used to produce VFA, by which reduction and stabilization of organic wastes can also be achieved. Few full scale reactor and batch studies were attempted which focused on secondary sludge fermentation (Min et al., 2002; Yuan et al., 2006a, b; Chen et al., 2006). The findings of these studies are discussed later in the thesis.

1.3 Thesis aims and objectives:

The main objective of this research was to assess the potential of secondary sludge fermentation to facilitate VFA production.

Specifically, the objectives of this research were:

1. To quantify VFA production resulting from fermentation of secondary sludge.

2. To assess the effect of pH and solids concentration on secondary sludge fermentation.
3. To examine the effect of sonic power application on secondary sludge in terms of enhancement of solubility, and to compare VFA production resulting from the fermentation of sonicated and unsonicated secondary sludges.

Throughout the study, results will be put into context through comparisons with data pertaining to primary sludge fermentation.

2 Literature review

2.1 Importance of readily available carbon in BNR:

Biological nutrient removal (BNR) systems are widely used to eliminate nitrogen and phosphorus from wastewater in order to prevent eutrophication of receiving water-bodies. In BNR systems, nitrogen and phosphorus removal take place in the following ways:

1) Under anoxic conditions, nitrogen is removed by heterotrophic bacteria which reduce nitrate to nitrogen gas. Heterotrophic organisms use readily available carbon as the electron donor in the nitrogen removal process (Xu, 1996; Elefsinioitis et al., 2004; Purtschert and Gujer, 1999). Denitrification occurs as follows,

$$6NO_3^- + 5CH_3OH \longrightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$$

(Source: Metcalf & Eddy, 1991)

2) Enhanced biological phosphorus removal (EBPR) occurs in sequential aerobic and anaerobic periods. In the anaerobic phase, phosphate accumulating organisms (PAOs) take up carbon substrates, which are stored as polyhydroxyalkanoates (PHAs) in cells. Energy for their anaerobic activity is mainly derived through the utilisation and release of polyphosphate (Smolders et al., 1994; Pereira et al., 1996). In the subsequent aerobic phase, PAOs use stored PHAs as an energy source to take up phosphate and replenish their polyphosphate pool. The amount of phosphate uptake in the aerobic zone exceeds the released amount, thus a net phosphorus removal is achieved by wasting sludge from the end of the aerobic zone (Morse et al., 1998).

From the two above mentioned BNR processes it is clear that readily available carbon is crucial for optimum nutrient removal. This review investigates the

fermentation of sludge to acquire volatile fatty acids (VFA) (a readily available carbon source), in order to facilitate optimum and economical biological nutrient removal. The effect of various operational parameters like hydraulic retention time (HRT), solids retention time (SRT), solids concentration, and pH on fermentation of sludge are discussed. The review also outlines the effect of ultrasound pre-treatment on sludge to enhance its soluble content in order to accelerate the fermentation and explains the effect of bromoethane sulfonic acid (BES) to inhibit VFA degradation.

2.2 Carbon source for biological nutrient removal:

As mentioned in section 2.1, a readily available carbon source is of prime importance in BNR systems. It has been suggested that approximately 4.2 g COD / g N is required for total nitrogen removal (Henze et al., 1991), and a further, 6-9 g VFA are required to remove one gram of phosphorus (Pitman et al., 1992; Wentzel et al., 1988).

Organics within wastewater act as a carbon source. However, the quantity of internal carbon in wastewater is often not enough to support efficient nutrient removal, partly because pre-treatments like primary sedimentation and chemical unit processes reduce the organic load in the biological treatment sections in wastewater treatment facilities (McCue et al., 2003). Primary sedimentation, a preliminary stage in the further processing of wastewater, is used to remove readily settleable solids and floating material and in this way reduces suspended solids content. Ironically, an effectively designed and operated primary sedimentation unit can remove 25-40% BOD and 30-40% COD from wastewater (Ubay-Cokgor et al., 2005; Metcalf and Eddy, 2003). So, quite often wastewater has a low concentration of carbonaceous matter after primary treatments, resulting in the requirement for carbon supplementation for effective BNR. Various materials like methanol (Nyberg et al., 1996), ethanol (van Münch, 1998), sodium-acetate (Kargi and Uygur, 2003), glucose (Akin and Ugurlu, 2003) and

night soil (Choi et al., 1996) have been studied as readily available external carbon substrates when internal carbon for nutrient removal is limited.

Unfortunately, utilisation of external carbon incurs high costs (vanMünch, 1998; Moser-Engeler et al., 1998). Furthermore, carbon supplementation is not always beneficial. For example, glucose has been shown to be detrimental to EBPR (Appeldoorn et al., 1992; Cech et al., 1990), possibly because it stimulates activity of non-PAOs. Akin and Ugurulu (2001) showed that when glucose is used as a carbon source, no phosphorus removal occurs due to the competition between PAOs and glycogen accumulating organisms (GAOs). In the anaerobic phase, glucose was stored as the carbon source by GAOs without releasing polyphosphate energy, which caused the deterioration of EBPR in their study.

2.3 Sewage sludge:

Wastewater in the wastewater treatment plant passes through a series of physical, biological and chemical processes. A significant by-product of these processes is sewage sludge, which mainly results from the removal of solids present in the influent or from solids generated through biological activity during treatment. Sludge is classified in two categories as follows:

2.3.1 Primary sludge:

Primary sludge is the material that accumulates at the bottom of the primary sedimentation tank. A high proportion of primary sludge is organic matter - it includes faeces, vegetables, fruits, textiles, paper, etc (Yuan et al., 2006a).

2.3.2 Secondary sludge:

Secondary sludge is the material that accumulates in the secondary clarifier of the wastewater treatment plant after biological treatments; it consists mainly of

wasted biomass (Choi et al., 1997). It contains non-hydrolyzable particulate materials and biomass due to biological metabolism (Yuan, 2006).

In Europe alone, millions of tons of sewage sludge are produced annually. It is estimated that over 8.9 million tons of sludge was produced in Europe in 2000 (Onyeche, et al., 2002). Traditionally, sludge has been disposed of by landfilling and incineration.

2.4 Sludge as a carbon source:

Sewage sludge is rich in organic carbon and as such, fermentation of the waste can yield valuable products. Fermentation of sludge leads to the production of short-chain volatile fatty acids, which can be used as a substrate in nutrient removal processes. Fermentation of primary sludge has been widely studied (Elefsinioitis and Oldham, 1994a,b; Skalsky and Daigger, 1995; Banister and Pretorious, 1998; Bouzas et al., 2002 and Chen et al., 2004). It has been shown that maximum VFA production can be achieved with a wide range of solid retention times. For examples, various solids retention times from 6 days (Skalsky and Daigger, 1995) up to 20 days (Elefsinioitis and Oldham, 1994a,b) were studied.

Fermentation of biological waste (secondary sludge) is less common. Secondary sludge is difficult to digest as it mainly consists of aerobic bacteria. Choi et al. (1997) reported that when secondary sludge is supplied as a feedstock for anaerobic bacteria in the anaerobic digestion process, the microbial cell membrane (cell wall) of aerobic bacteria has an inhibitory effect on sludge digestibility. Still, several studies have shown that VFA production from secondary sludge is possible. Min et al. (2002) investigated the effects of pH, HRT and solids on VFA production in a mixed reactor and the amount of VFA produced was tested as a carbon source for denitrification. The authors compared VFA produced form secondary sludge fermentation with methanol (an

external carbon source) for denirification. They reported that specific denitrification rates of methanol and VFA were comparable, and so the latter has a potential to be used as an internal carbon source for denitrificaiton processes. Yuan et al. (2006a) tested secondary sludge under acidic and alkaline conditions to improve VFA production in a series of batch fermentations with pH values ranging from 4 to 11. The authors observed optimum VFA production of 256.2 mg VFACOD/g VSS at pH 10.

Based on these research studies, fermentation of secondary sludge could be considered a useful means of procuring VFA to support biological nutrient removal.

2.5 Fermentation process bio-chemistry:

The fermentation of raw wastewater or sludge in fermenters is similar to anaerobic digestion. In fact, fermentation can be described as a partial anaerobic digestion as it terminates after volatile fatty acid generation. Conversion of VFA into methane and carbon dioxide leads to a full anaerobic digestion. The whole anaerobic digestion process, as shown in
Figure 2-1 on the following page, involves four main phases:

2.5.1 Hydrolysis:

Hydrolysis is the solubilization of particulate matters. It is the rate-limiting step of fermentation. The process is catalysed by hydrolytic enzymes excreted by bacteria whereby complex substrates such as protein, lipids, amino acids and polysaccharides are broken down into soluble compounds (Eastman and Ferguson, 1981).

According to Elefsiniotis et al. (1996), hydrolysis depends upon pH, temperature, type of substrate, nature of biomass, size of particles and remaining

concentration of bio-degradable matters. First-order kinetics with respect to the remaining bio-degradable particulate substrate is the most widely used approach for describing the rate of hydrolysis. As a result of fermentation, the formation of VFA takes place, which in turn reduces the system pH. A reduction in pH can inhibit hydrolysis. Veeken et al. (2000) studied the effect of pH and VFA concentration on hydrolysis. The authors studied hydrolysis rates at a fixed VFA concentration and at a pH range between 5 and 7. They found that the hydrolysis rate constant is pH dependent and that it is not related with total or undissociated VFA concentrations. Yuan et al. (2006a) also studied the effect of pH on hydrolysis of secondary sludge fermentation. The authors tested a pH range of 4-11. They observed more of an increase in soluble protein and carbohydrate in the pH range of 7-11 than in the pH range of 4 to 7.

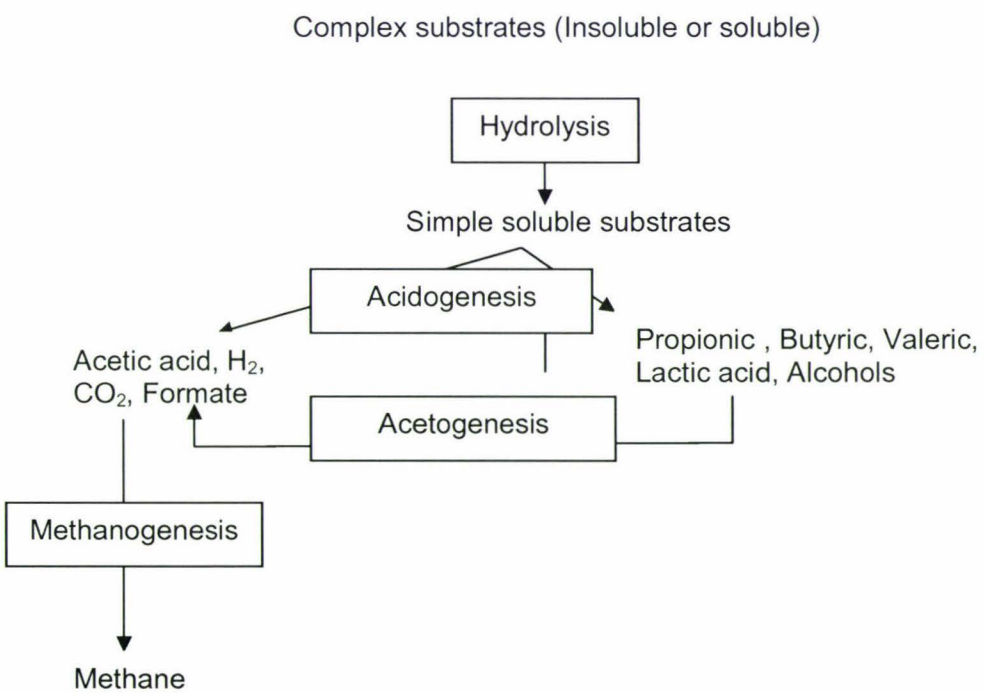


Figure 2-1 Anaerobic digestion process
(Source: van Münch, 1998)

Hydrolysis can be a concern because of solubilization of bound nitrogen and phosphorus. Solubilized nutrients can cause an additional load of nutrients for the biological nutrient removal system. Moser-Engler et al. (1988) observed nutrient release of 0.02-0.06 mg NH_4-N/mg COD and a maximum of 0.05 mg PO_4-P/ mg COD during hydrolysis.

2.5.2 Acidogenesis:

In this second sub-process of fermentation, volatile fatty acids generation takes place. VFA are generated from the soluble products that result from hydrolysis. Pyruvic acid is an intermediate product generated from glucose metabolism through carbohydrate hydrolysis. Depending upon the micro-organisms present, pyruvic acid is subsequently fermented into VFA (acetic, propionic and butyric acids), alcohols, ketones and aldehydes (Gottaschalk, 1986). According to Elsden and Hilton (1978), amino acids can also serve as energy and carbon sources for anaerobic bacteria to generate short-chain VFA (straight or branched). Among all VFA, acetic acid is the most common VFA produced, followed by propionic acid. Types of VFA and their characteristics are discussed in the following sections. The effect of various operational factors and substrate characteristics on VFA production is reviewed later in the literature review.

2.5.3 Acetogenesis:

As mentioned in section 2.5.2, VFA are intermediate products produced from soluble material. These intermediate products are oxidised by β-oxidation to hydrogen, carbon dioxide and acetate. The process is termed dehydrogenation and acetogenesis (McCarthy and Smith, 1986). Further in the anaerobic process, butyrate and propionate are degraded into acetate, hydrogen and bicarbonate, the main precursors of methanogenesis (Ahring and Westermann, 1987). Table 2-1 on the following page presents reactions and standard Gibbs free energy (pH 7, at 25°C, substrates and products are 1 mol/l concentrations) of degradation of propionate and into acetate.

Table 2-1 Standard free energy and equation of VFA degradation

Reaction	ΔG (kJ) at 25 °C
Propionate + H_2O ⟶ CH_3COO^- + HCO_3^- + H^+ + $3H_2$	+76.1
Butyrate + $2H_2O$ ⟶ $2CH_3COO^-$ + H^+ + $2H_2$	+48.1

(Source: Van Liër et al., 1993)

Wang et al. (1999a) carried out a study on degradation of VFA in anaerobic digestion. They observed that acetate was produced from propionate, butyrate, valerate and caproate. They also suggested that both, the concentration of acetate and H_2 partial pressure, should be maintained at a low level in order to achieve propionate and butyrate degradation. Kasper and Wuhrmann (1978) also suggested that hydrogen accumulation can inhibit propionate and butyrate turnover in anaerobic digestion.

2.5.4 Methanogenesis:

In this final step of anaerobic digestion, methane is generated from acetate, hydrogen and carbon dioxide. According to Jeris and McCarty (1965) 28% of methane generation takes place from reduction of H_2 with CO_2. However, acetate is the main precursor of methane. According to Kasper and Wurhmann (1978), 70% of methane is generated from acetate. System pH is one of the factors that affect methanogenesis; near neutral pH is generally considered optimal. Lay et al. (1997) suggested that most methanogenic bacteria function within a pH range of 6.7-7.4 and the rate of methanogenesis might decrease if the pH value is lower than 6.3 or higher than 7.8. Similarly, Neue (1993) and Floencio et al. (1996) suggested a pH range of 6-8 as optimal for methanogens. However, it should be noted that Taconi et al. (2006) showed that methane production can be increased by reducing pH from 7 to 4.5. Hydraulic retention time (HRT), solids retention time (SRT) and temperature are other factors which also affect methanogensis. The growth rate of methanogens is slower than that of acid-formers (Cohen et al., 1984). Ghosh and Klass (1978) reported that the maximum specific growth rate of acidogenic bacteria can be up to one order of magnitude higher than that of methane producing bacteria.

2.6 Characteristics of VFA produced during fermentation:

Fatty acids are organic acids, which consist of a number of carbon atoms arranged in a chain. The basic structure of VFA is CH_3-$(CH_2)_i$-COOH, where "i" represents the number of carbon atoms (van Münch, 1998). The term 'volatile fatty acids' represents the group of different species' of acids of which acetic acid, propionic acid, butyric acid and valeric acid are the most common forms (Buchauer; 1997). Table 2-2 describes the various types of VFA produced during fermentation. Branched forms of volatile fatty acids like iso-butyric, iso-valeric and iso-caproic acids can also be generated.

Table 2-2 Volatile fatty acids characterisation

Name	Chemical Formula	Molecular weight (g/mol)	COD equivalent (g COD/g acid)
Formic acid	HCOOH	46.00	0.348
Acetic acid	CH_3COOH	60.05	1.067
Propionic acid	CH_3CH_2COOH	74.08	1.514
n-Butyric or iso-butyric acid	$CH_3(CH_2)_2COOH$	88.11	1.818
n-Valeric or iso-valeric acid	$CH_3(CH_2)_3COOH$	102.13	2.039
n-Caproic or iso-caproic acid	$CH_3(CH_2)_4COOH$	116.16	2.207

(Sources: Rossle and Pritorious, 2001a; Moser-Engeler et al., 1998)

Acetic acid was reported to be the dominant species among all the VFA in several studies. Table 2-3 lists the composition of different volatile fatty acids observed in different studies.

Table 2-3 Composition of VFA distributions in various lab and full scale studies

Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Valeric acid (%)	Total (%)	Reference
63	25	12	0	100	Rodriguoz et al. (1998)
41	44	9	5	99	Skalsky and Daigger (1995)
38	36	16	10	90	Pitman et al.,1992
46	32	8	-	100	Elefsiniotis and Oldahm (1994a)
55	18	10	4.1	-	Yuan et al. (2006a)*

(Accounted as maximum of total VFA production at different retention times of the fermentation)*

According to Wang et al. (1999a), the presence of formate is very limited due to its rapid conversion to other compounds like carbon dioxide and methane. Also, the occurrence of VFA in anaerobic sludge decreases as the chain length increases (Moser – Engeler et al., 1998). The decomposition rates of branched forms are slower than those of the normal forms (straight-chain) (Wang et al., 1999a).

2.7 Suitability of VFA in BNR:

For BNR, most attention is given to two-carbon to five-carbon acids. *As* in section 2.6, the presence of formate (single-carbon acid) is very limited and moreover, according to Abu-Ghararah and Randal (1989), one-carbon formic acid does not support EBPR. Also, six-carbon VFA (Caproic acid) is present in negligible quantities during fermentation. Therefore, most attention is given to two-carbon to five-carbon acids in fermentation studies.

A number of studies have shown VFA to be a good carbon source for nutrient removal. Apart from dominance among all acids in terms of production, acetic acid is also reported, in a few research studies, as the appropriate substrate for the bacteria in BNR. Elefsiniotis et al. (2004) carried out denitrification on a mixture of digester sludge, synthetic nitrate solution and tap water in mixed batch reactors, using VFA from an acid-phase digester. The authors observed acetic acid as the most preferred carbon source for denitrification followed by butyric and propionic acids. Valeric acid was the least preferred by denitrifiers. The authors reported acetic acid as the most preferred substrate with compared to other acids for denitrification. A simple metabolic pathway of degradation and the highest constitution (53% of the total VFA) in the feed are the two reasons why acetic acid was found as the most preferred acid in their research study. A similar outcome was observed by Xu (1996) who used VFA for denitrificaiton of a mixture of synthetic nitrate, phosphate and carbon. The highest denitrification rate was observed when acetate was used as the carbon source.

Propionate was also reported as a preferable substrate for EBPR. Pijuan et al. (2004) used acetate and propionate as the sole carbon sources for enhanced biological phosphorus removal in a sequential batch reactor. The authors found slower phosphorus release and uptake rates when propionate was used as a carbon source. However, they found that phosphorus removal with propionate as a carbon source was similar, and sometimes even greater than acetate.

2.8 VFA production:

Various researchers have expressed VFA production in different manners. Following are some of the observed ways to express VFA production:

2.8.1 Net VFA production:

Net VFA production is expressed as mg of VFA (as acetic acid) per litre (mg VFA_{HAc}/l). The net value is obtained by subtracting the equivalent influent concentration from that in the reactor effluent (Elefsiniotis and Oldham, 1994a, b):

$$\text{Net VFA production (mg } VFA_{HAc}/l) = \frac{\text{mg } VFA_{HAc\text{-effluent}} - \text{mg } VFA_{HAc\text{-feed}}}{\text{Litre}}$$

2.8.2 VFA production yield:

The production yield is quantified in terms of mg of VFA_{HAc} produced per mg substrate consumed (Min et al., 2002):

$$\text{VFA production yield} = \frac{\text{mg } VFA_{HAc\text{-effluent}} - \text{mg } VFA_{HAc\text{-feed}}}{\text{mg } VSS_{feed}}$$

Volatile suspend solids (VSS) is a measure of organic matter present in sludge (APAHA, 1998), and therefore the degree of fermentation can be measured by calculating the amount of organic material converted into volatile fatty acids. Also, the production yield can be computed in terms of mg VFA_{COD}/mg VSS_{feed}, mg VFA_{HAc}/mg COD_{feed} and mg VFA_{HAc}/mg $SCOD_{feed}$.

2.8.3 Specific VFA production:

The specific VFA production rate is expressed in terms of mg VFA_{HAc} produced per unit time in the reactor (Elefsiniotis and Oldham, 1994a, b):

$$\text{Specific production rate} = \frac{\text{mg } VFA_{HAc\text{-effluent}} - \text{mg } VFA_{HAc\text{-feed}}}{\text{Unit time} * \text{mg } VSS_{effluent}}$$

2.8.4 Gross VFA yield:

Gross VFA yield is expressed as: mass of reactor effluent VFA per unit mass of volatile suspended solids of feed (Bouzas et al., 2002):

$$\text{Gross VFA yield} = \frac{\text{mg } VFA_{HAc\text{-effluent}}}{\text{mg } VSS_{feed}}$$

Table 2-4 gives the summary of VFA production values explored in different fermentation research studies conducted by various researchers.

Table 2-4 VFA Production outcome of various research works

VFA outcome	Expression	Associated details	Author(s)
300-500	mg VFA_{HAc}/l	Study on effect of hydraulic retention time (HRT) on VFA production	Elefsiniotis and Oldham (1994a)
0.145-0.176	mg VFA_{HAc}/mg VSS_{feed}	Study on effect of solids retention time (SRT) on wasted sludge fermentation	Min et al. (2002)
0.053-0.119	mg VFA_{HAc}/mg $VSS_{reactor}$/day	Study on effect of hydraulic retention time (HRT) on VFA production	Elefsiniotis and Oldham (1994b)
0.2-0.26	mg VFA_{HAC}/mg VS_{feed}	Study on effect of SRT on primary sludge fermentation	Skalsky and Daigger (1995)

2.9 Types of pre-fermenters (fermenters):

Pre-fermeters can be divided into two types.

1) In-line pre-fermenters
2) Side-stream pre-fermenters

In-line pre-fermenters treat the entire wastewater stream, while side-stream pre-fermenters treat the underflow from primary clarifiers (van Münch, 1998). An activated primary tank is an example of an in-line fermenter. It consists of a clarifier tank in which VFA is produced as the sludge settles under gravity. In addition, a recirculation system is installed to recycle the settled sludge with the feed stream for inoculation of incoming solids and to wash out VFA produced in the sludge blanket (Chanona et al., 2006).

The various kinds of pre-fermenters include upward anaerobic sludge bed (UASB), complete mixed, fixed film and sequencing batch reactor. Barjas et al. (2002) carried out fermentation of low VFA wastewater in a lab-scale activated primary tank. The authors observed remarkable solubilization and fermentation in the pre-fermenter with a VFA yield of 0.142 mg VFA_{COD}/mg VSS_{feed}. Other researcherds tested upward anaerobic sludge blanket (UASB), complete mixed and fixed film pre-fermenters to produce VFA. Mavnic et al. (2001) used a fixed film pre-fermenter to evaluate VFA production of primary effluent and screened wastewater. They found that short HRT (60 minutes) resulted in net VFA production of 11 mg VFA_{HAc}/l. Gonzalez-Barcelo and Gonzalez-Martinez (2006) studied anaerobic fermentation of wastewater in a sequencing batch reactor (SBR). They tested the effect of various organic loads (ranging from 0.27 kg COD/ Kg TSS/day) on VFA production. They observed that not less than 70% of total soluble COD was in the form of VFA. Moreover, for an organic load of 0.62 kg COD/Kg TSS/day, all soluble COD in effluent of the SBR was in the form of VFA. Also, the acidification of dissolved COD transformed to VFA increased from 50 to 63% when pH was reduced from 7.5 to 5.5.

2.10 Factors governing volatile fatty acid generation:

Various operational parameters influence volatile fatty acid production. The factors include solid retention time (SRT), hydraulic retention time (HRT) and solids concentration in the feed for pre-fermenter as well as other parameters like

ambient temperature, pH, mixing intensity and mass transfer, type and concentration of substrate, solids particle size, chemical inhibitors, oxidation-reduction potential, reactor configuration and trace metals (van Münch, 1998; Ghosh et al., 1975). In this review, only the main factors (HRT, SRT, solids concentration, and pH) are discussed.

2.10.1 Solids retention time:

Solids retention time (SRT) is defined as: mass of solids in the reactor divided by the mass of solids leaving the reactor per unit time. SRT affects the selection of micro-oganisms in according to their growth kinetics - acidogenic and methanogenic organisms differ to a great extent from each other (Elefsiniotis and Oldham, 1994b). The SRT also influences the reactor size and sludge handling facilities (Skalskya and Daigger, 1995). Insuffient SRT causes the wash out of microorganisms, process instability, diluted sludge and high sludge production. In contrast, prolonged SRT leads to growth of methanogenic organisms, which deplete the fermentation products (Chen et al., 2004; Rossle and Pretorious, 2001b). Various researchers, as described in Table 2-5, have reported different SRT for optimum VFA generation.

Table 2-5 Optimum SRT values for various studies

SRT(s) tested (days)	Optimum SRT (days)	VFA production	Author(s)
4-10	6	0.179 mg $VFA_{HAc\text{-}effluent}$/mg TVS_{feed}	Bouzase et al.(2002)
5 and 10	5	0.142 mg VFA_{HAc}/mg VSS_{feed}	Barjas et al.(2002)
2-6	2	0.2 mg $VFA_{HAc\text{-}effluent}$/mg VS_{feed}	Skalsky and Daigger (1996)
5-20	10	0.101 mg VFA_{HAc}/mg $VSS_{reactor}$/day	Elefsiniotis and Oldham (1994b)

It can be seen from the data summarised in Table 2-5 that various SRT values were found to be optimum. However, extended SRT can incur greater investment and increase methane production. Moreover, extended SRT can lessen the sludge thickening.

2.10.2 Hydraulic retention time:

Hydraulic retention time is the average duration for which liquid remains in the reactor. HRT is directly proportional to tank size and hence, the capital construction cost of a plant. Therefore, HRT is an important parameter in pre-fermenter design (Rossle and Pretorious, 2001b). HRT can be easily manipulated in the process and it controls the amount and types of substrates used by cells (Elefsiniotis and Oldham, 1994a). Table 2-6 provides a summary of the various HRT values used in different research studies. Table 2-6 covers the wide range of optimum HRTs observed in various fermentation studies.

Table 2-6 Optimum HRT values for various studies

HRT(s) tested	Optimum HRT	VFA production	Types of waste fermented	Author(s)
6,9,12,15 (hours)	12 hours	0.063 g VFA_{HAc}/g $VSS_{reactor/d}$	Primary sludge	Elefsiniotis and Oldham (1994a)
2.7,4.1,8.2 (days)	2.7 days	0.176 g VFA_{HAc}/g VS_{feed}	Wasted sludge	Min et al. (2002)
18,30,48,60 (hours)	30 hours	0.0453 g VFA_{HAc}/g $VSS_{reactor}$/d	Mixture of starch rich industirl wastewater and primary sludge	Maharaj and Elefsiniotis (2001)
1,2,3,4,5 (days)	3 days	0.27 g COD/g TVS_{feed}	Municiple solid waste	Bolzonella et al. (2005)
4,8,12,16,24 (hours)	16 hours	1200 mg VFA_{HAc}/l	Dairy wastewater	Fang and Yu (2000)

An HRT of approximately 12 hours has been shown to be optimal for VFA production from the fermentation of dairy wastes (Demirel and Yenigun, 2004; Fang and Yu, 2000). Fang and Yu (2000) tested HRTs ranging from 4 to 24 hours. Their results showed that under mesophilic temperature, the degree of acidification increased rapidly from 28.2% at HRT of 4 hours to 54.1% at HRT of 12 hours, but with a further increase of HRT from 16 hours to 24 hours, acidification increased only marginally from 56% to 59%. Elefsiniotis and Oldham (1994a) used primary sludge in USAB and complete mixed reactors at HRT ranging between 6-15 hours. They found that 12 hours is an optimal retention time for both reactors. Also, apart from optimum VFA production, they found good hydrolysis, as chemical oxygen demand also increased, when HRT was

increased up to 12 hours. Min et al. (2002) used HRT ranging from 2.7-8.4 days for the fermentation of secondary sludge. They found 2.7 days of HRT favourable and yielded maximum VFA generation. HRT of different values, have been found favourable for different types of substrate, under different experimental conditions.

It is difficult to select a particular range of optimum HRT values as characteristics of sludge/wastewater, experimental conditions and reactor configurations vary. But for wastes with high solids content the HRT may need to be longer than 12 hours to achieve optimum acidification. Bolzonella et al. (2005) fermented organic municipal solid waste in fermentation with total solids content of 2-3% (20000-30000 mg/l). Maximum net VFA production of about 1500 mg total VFA/l was observed up to HRTs as long as up to 3 days.

2.10.3 Solids concentration:

Along with HRT and SRT, solids concentration of sludge has been reported as a major influencing factor on fermentation. Solids management can be carried out easily in side-stream pre-fermenters because the two different tanks facilitate thickening and organic matter fermentation. In contrast, the use of an activated primary tank (APT) requires proper solids management strategies as the sewage solids concentration in an in-line pre-fermenter unit can not be controlled (Rossle and Pretorious, 2001b). There are quite a few studies that have been conducted on the effect of solids concentration on VFA yield and have resulted in contrary outcomes (see the following sections).

2.10.3.1 Effect of low solids on sludge fermentation:

Several studies indicated that net VFA yield improves with a decrease in solids concentration. Chen et al. (2004) found that diluted primary sludge with 1% total solids gave a better VFA yield than undiluted sludge with 2.7% total solids. The authors suggested that as long as a sufficient source of VSS is available, higher VFA production can be achieved at lower solids concentration. They also pointed

to the limitations of diluted fermentation which include the requirement of bigger reaction volumes and difficulty in temperature control in cold environments. Therefore, they suggested the optimum solids range of 1-1.5%. They also observed that the rate of pH reduction was directly proportional to sludge concentration. Therefore, Chen et al. (2004) indicate that inhibitory effects caused by fermentation products, like low pH and toxicity, can be lessened in a diluted environment, which is in agreement with the conclusions of Skalsky and Daigger (1995).

Skalsky and Daigger (1995) discovered that at a total solids concentration of 0.6%, higher VFA production was achieved than for fermentation of sludge with a solids content of 2.6%. They suggested that in a diluted environment better mixing takes place which enhances interaction between organisms and substrate; hence hydrolysis of particulate volatile solids is boosted. Similarly, Banister and Pretorious (1998) supported dilution of primary sludge and recommended a range of solids between 0.5-2% in order to achieve enhanced fermentation in a batch reaction. In all studies discussed in this section, the sludge used for fermentation was diluted with either primary or settled effluents. In another report, Ferreiro and Soto (2003) studied the influence of sludge concentration and temperature on anaerobic hydrolysis of primary sludge and also found that at increased solids concentration VFA production was decreased. According to Ferreiro and Soto (2003), specific VFA production primarily depends upon the sludge concentration, and to a lesser extent on the process temperature. They discovered 0.17-0.34 mg VFA_{COD}/mg VSS_{feed} concentration at volatile solids content of roughly 1000-13000 mg/l.

2.10.3.2 Effect of high solids on sludge fermentation:

Table 2-7 on the following page gives an overview of VFA yield found at an optimum total or volatile solid concentration in various research studies.

Table 2-7 Optimum solids concentration for various studies

Solids content tested (%)	Optimum solids content (%)	VFA yield	Author(s)
0.5-3.4 (TS)	0.5	0.062-0.074 mg VFA_{HAc}/mg VSS	Chen et al. (2004)
0.1-1.3 (VSS)	0.1	0.34 mg VFA_{COD}/mgVSS_{feed}	Ferreiro and Soto (2003)
0.12-0.36 (VSS)	0.36	0.176 mg VFA_{HAc}/mg VS_{feed}	Min et al. (2002)
0.6-2.8 (TVS)	2.8	0.110 mg VFA_{HAc}/mg TVS_{feed}	Bouzas et al. (2002)
0.43-2.6 (TS)	0.43	0.22 mg VFA_{HAc}/mg VS_{feed}	Skalsky and Daigger (1995)

Bouzas et al. (2004) observed an increment in VFA yield with an increase in volatile solids concentration. No inhibitory effects of fermentation products at high solids concentration were observed. Results of the study demonstrated that experiments conducted with diluted sludge showed a significant decrease in the VFA yields. Likewise, for secondary sludge fermentation, Min et al. (2002) found that an increase in volatile solids loading to the complete mixed reactor caused an increase in VFA yield and VFA production. Zeng et al. (2006) found that an increase in solids content not only enhanced the total VFA production but also boosted the propionic acid fraction.

Some studies showed that high solids level is preferable for sludge fermentation. However, some research works emphasised that dilution of sludge facilitates higher VFA production by lessening the inhibitory effect caused by products of fermentation. But it must be noted that dilution can reduce overall VSS content of the sludge and thus low VSS level might not lead to higher VFA generation. Also, very less VSS content may not support fermentation.

2.11 pH In the fermentation process:

Many studies have demonstrated that pH has a significant influence on VFA production/degradation (Min et al., 2002; Veeken et al., 2000; Ghosh 1975; Zeng et al., 2006; Gomec and Speece, 2003). It was discussed in section 2.5.1 that

hydrolysis is the most important and rate-limiting step of fermentation and that pH affects the rate of hydrolysis. Moreover, according to Gomec et al. (2002), pH affects enzymatic activity and a narrow pH range is suitable for each enzyme's activation. Therefore, in a fermentation process under an optimum pH range, maximum hydrolysis can be facilitated. Various researchers suggested different pH values as the optimum pH for enhanced hydrolysis and acid production. Skalsky and Daigger (1995) suggested that pH, in the range of 4.3-7.0, has little effect on acidogenesis of primary sludge. Veeken et al. (2000) observed an increase in hydrolysis rate in the pH range 5-7 and there was no enhancement in hydrolysis observed beyond neutral pH. Ghosh (1975) observed optimum hydrolysis and acidogenesis at pH 6. Min et al. (2002), observed that, for fermentation of secondary sludge, an increase in pH value from 6.6 to 7.5 decreased acids production by about 10%.

As fermentation progresses, acid production leads to a reduction in pH level (Chen et al., 2004; Barjas et al., 2002; Bolzonella et al., 2005). Furthermore, the rate of pH reduction is in direct proportion to the sludge concentration (Chen et al., 2002). Low pH values have been found to be inhibitory on acid production (Skalsky and Daigger, 1995; Veeken et al. 2000). Skalsky and Daigger (1995) pointed to the fact that the origin of fermentation products can decrease pH value and thus, inhibit fermentation. Therefore, the authors suggested fermentation in a diluted environment to be preferable in order to avoid product inhibition. However, Bouzos et al. (2004) did not observe any inhibition on fermentation of primary sludge due to a decrease in pH caused by VFA production.

Surprisingly, high pH has in some cases been shown to be preferable for VFA production. Yuan et al. (2006a), when fermenting secondary sludge, showed that total VFA production at pH 9 and 10 was much greater than acidic or neutral pH. The maximum yield of 0.256 mg VFA_{COD}/ mg VSS was achieved at pH 10, which was 3 times higher than at pH 5. There was less consumption of acids observed at pH 10 and hydrolysis was improved in terms of higher soluble COD production.

From prior research studies, it seems that pH influences fermentation significantly by enhancing hydrolysis or volatile fatty acids production. It can also be seen that pH near neutral value favours methanogenic activity and hence, acids degradation through methane production. However, pH values of 9 or 10 have been found to be inhibitory for gas production and have increased VFA production. Also, excessively acidic pH values like 4 could not support fermentation.

2.12 Pre-treatment of sludge to enhance fermentation:

It is discussed in section 2.5.1 that hydrolysis, the conversion of complex matter into soluble products, is a rate-limiting step in the fermentation process. Therefore, to lessen the impact of rate-limitation and to enhance hydrolysis, many research studies on the pre-treatment of sludge have been conducted. Pre-treatment strategies include mechanical integration like the application of ultrasound (Tiehm et al., 2001; Wang et al., 1999b; Wang et al., 2005), ɣ-irradiation (Lafitte-Torque and Forster, 2002), high pressure homogenisation (Choi et al., 1997; Nah et al., 2000), ball mill and colloid mill (Harrison, 1991), thermal treatment like high temperature (Sawayama et al., 1996) and chemical treatments such as treating the sludge with NaOH, $Ca(OH)_2$ (Rajan et al., 1989; Ray et al., 1990; Tanaka et al., 1997; Lin et al., 1997), KOH and $Mg(OH)_2$ (Penaud et al., 1999; Kim et. al., 2003). Although the principles of all methods were different, the aim of each is to release the soluble substances from inside and outside of the cells in order to increase the soluble chemical oxygen demand (SCOD). Sonication treatment is discussed in the following section.

2.12.1 Sludge pre-treatment by sonication:

Among all pre-treatment methods, ultrasonic exposure of the sludge is best known. This method demonstrates a great potential as it is not noxious to the environment and is economically competitive (Bien and Wolny, 1997; Tiehm et.

al., 1997). It is also the most powerful method to disintegrate sludge cells and at higher power levels 100% cell disruption can be achieved (Weemaes and Verstraete, 1998). However, it is an energy intensive technique; thus, cost-effectiveness of sonication is a concern for the wastewater treatment industry.

The basic principle of sonication is the propagation of ultrasound into the sludge which induces a vast amount of energy dissipation due to cavitation bubbles (Weemaes and Verstraete, 1998; Mao et al., 2004). Tiehm et al. (1997) noted that hydromechanical shear forces produced by ultrasonic cavitation were responsible for sludge disintegration. Firstly, sludge flocs would be effectively broken (Tiehm et al., 1997) and then some portion of the insoluble particulate matter might be converted into a soluble state due to sonication of sludges (Tatsuo et al., 1993; Chiu et al., 1997; Jorand et. al., 1995). Moreover, it was noted that excess sonic power can markedly raise the temperature after a long-duration operation (Chiu et al., 1997; Chu et al., 2001). Wang et al. (2006) stated that secondary sludge contains extracellular polymeric substances (EPS) such as protein, carbohydrates and a small quantity of DNA, which increase the stability of the sludge. Intercellular substances contain proteins, carbohydrates, lipids, inorganic salts and water. Sonic exposure can release substances from EPS and cells into the aqueous phase, causing an increase in the soluble fraction of the sludge.

Wide ranges of frequency were tested for sonic reaction. Sonication can take place in a range of frequencies from 20 KHz to 1 MHz with the highest efficiency of sonication being observed at a frequency of more than 100 KHz (Hua and Hoffmann, 1997; Petrier and Francony, 1997). Wang et al. (2006) carried out a study in which they sonicated secondary sludge at a frequency of 20 KHz. They observed an increase in soluble chemical oxygen demand (SCOD) from 0 mg/l to about 10000 mg/l in a disintegration time of 30 minutes. They observed that the predominant compound of the liquid released was protein. They also observed an increase of Ca^{+2} and Mg^{+2} captions in the sludge following sonication, which

can be linked with the fact that EPS were released into the sludge because according to Keilding and Nielsen (1997), divalent cations like Mg^{2+} and Ca^{2+} play an important role in binding the EPS.

Tiehm et al. (1997) showed that ultrasound application in the sludge of 3.6 KW and 31 KHz can increase SCOD content from 630 to 2270 mg/l. A couple of studies have demonstrated a boost in solubility due to the application of ultrasound to secondary sludge. Tiehm et al. (2001) studied secondary sludge sonication with frequencies varying within a range of 41 to 3217 KHz. They found that sludge disintegration was significant at low frequencies. Mao et al. (2004) compared the sonication of primary and secondary sludge. They found a decrease in solids concentration and an increase in the soluble content of the sludges. Secondary sludge showed more notable improvements than primary sludge. Furthermore, they found that solids concentrations of sludge can influence the disintegration efficiency as they observed attenuation in sonication when the solids content of primary sludge and secondary sludge were increased from 0.98 % to 3.6% and from 1% to 3.75% respectively. In contrast, Wang et al. (2005) observed that efficient sonication of secondary sludge was achieved for total solids of 1% over the sludge with total solids content of 0.5%.

2.13 Inhibition of methanogens to prevail acidogenic condition:

VFA produced during acidogenesis can be consumed by methogenic bacteria under anaerobic conditions. As discussed in section 2.10, by controlling factors like HRT, SRT and pH, methanogenic activity can be minimised or VFA accumulation can be prolonged. However, some studies have proven that several chemicals can completely suppress methanogens. Bromoethane sulfonic acid (BES or BESA) (Balch and Wolfe, 1979), iodopropane (Aiello-Mazzarri et al., 2006), trichloroacetic acid and chloroform (Hickey et al., 1987), were tested as methanogenic inhibitors in various studies.

BES has shown promising impact as a methanogenic inhibitor compound. Zinder et al. (1984) observed methanogen inhibition when 1 µmol of BES per ml was added in thermophilic anaerobic digestion. They found that acetate, ethanol and hydrogen were accumulated due to methanogenic inhibition. In another study, Ruel et al. (2002) added 1 mM BES into the wastewater fermentation reactors. The authors observed that gas yield dropped from 10 mg COD/l CH_4 (without BES wastewater fermentation) to less than 0.1 mg COD/l CH_4 (BES added wastewater fermentation). Zhu et al. (2006) observed that 0.01 mM BES addition in digester sludge inhibited methanogens.

2.14 Summary:

Following are the conclusions that can be drawn from the literature review,

1. Fermentation of volatile fatty acids from sludge is a very important process as it significantly affects the biological nutrients removal system. Sludge acidification can prove to be a very economical process as it substitutes various external carbon sources. Moreover, sludge being the waste product, incurs disposal problems. Thus, utilisation of sludge via fermentation can reduce sludge management costs.

2. Acetic acid seems to be the dominant species among all short chain volatile fatty acids. It also plays a major role in enhanced nutrients removal processes.

3. Various kinds of fermenters are used in practise. However, they should be selected based upon the appropriate process design required for local conditions.

4. Various control parameters like hydraulic retention time (HRT), solids retention time (SRT), and solids concentration have been identified as the

important factors that govern fermentation processes. SRT and HRT values should be selected in such a way that optimum and economical VFA production can be achieved, because these values directly affect the capital cost of the fermenters. Also, the effect of solids concentration on sludge fermentation demonstrated contrasting outcomes for different studies. Therefore, various ranges of solids should be assessed in order to achieve enhanced VFA production.

5. pH is considered as an important factor for hydrolysis, but pH is not generally controlled in practise. However, various studies have shown that the proliferation of methanogens due to neutral or near neutral pH values leads to acids degradation. However, alkaline pH values such as 9 and 10 boosted VFA generation due to improved hydrolysis. Also, acidic pH values like 4 and 5 did not support the fermentation process. However, acids degradation can be lowered at low pH levels. Therefore, an optimum pH range should be determined for the respective fermentation conditions.

6. Among various sludge disintegration techniques, sonication has proved to be efficient, as well as cost-effective if operated at economical power and frequency levels. Ultrasound propagation into the sludge breaks the sludge flocs which in turn releases EPS and other soluble products into the liquid. Therefore, the soluble content of COD can be boosted which lowers the impact of rate-limiting step of hydrolysis and hence, better VFA generation can be achieved through the use of sonication.

7. BES has the potential to act as a methanogen inhibitor to facilitate acidogenic condition. 1 mM addition to sludge can significantly affect the methanogenic activity, despite some VFA degradation might take place.

2.15 Opportunity of research:

Increasing commissioning of biological nutrients removal systems in wastewater treatment plants has demanded an economical means of procuring readily available carbon sources in order to support efficient nutrient removal. Extensive research on primary sludge fermentation has been carried out in the past few years.

A number of laboratory and bench scale reactor as well as batch fermentation studies demonstrated the suitability of primary sludge to produce VFA. Operational parameters like HRT, SRT, solids concentration and pH have been tested for their effects on primary sludge fermentation. However, few studies have tested the production of VFA through secondary sludge fermentation. Similar to primary sludge, secondary sludge is produced in large quantities in waste treatment facilities and raises the need for a useful way of disposal. After receiving biological treatment, secondary sludge might not have a sufficient amount of organic content to be fermented for the generation of VFA. However, very few studies have confirmed that secondary sludge has the potential to produce VFA via fermentation.

2.15.1 Based on different characteristics, does secondary sludge have potential to support fermentation?

In chapter 4, various characteristics of primary and secondary sludge used in the present study will be discussed and compared with each other and with those highlighted in other literature.

2.15.2 Does secondary sludge have the ability to support fermentation in a reactor study?

Chapter 5 will detail secondary sludge fermentation using a sequential batch reactor. Any possible VFA generation will be discussed by analysing the net VFA

generation. VFA yield will be measured to assess the degree of fermentation. Other parameters like pH, solids and COD will be measured to establish their link with fermentation. The amount of VFA generated will be compared with prior fermentation research.

2.15.3 Do secondary sludge characteristics have the effect on fermentation?

Chapter 6 will investigate the effect of sludge characteristics in batch fermentations. Various issues will be covered in the following manner:

2.15.3.1 Effects of solids concentration on secondary sludge fermentation:

Diluted secondary sludge (1% total solids) will be compared with undiluted secondary sludge (2.8% total solids) for VFA production in the batch fermentation.

2.15.3.2 Comparison between primary and secondary sludges fermentation:

Primary sludge (3% total solids) and secondary sludge (2.8% total solids) will be compared for net VFA production and VFA yield in the batch fermentation.

2.15.3.3 Effects of pre-treatments like sonication and bromoethane sulfonic acid (BES) addition on secondary sludge fermentation:

Effects of sonic power application on secondary sludge will be tested by measuring the enhancement in soluble content. Later, sonically treated secondary sludge will be compared with unsonicated secondary sludge for VFA production in the batch fermentation. Also, the effects of bromoethane sulfonic acid (BES) addition will be tested by comparing the net VFA production of BES

added secondary sludge with that of non-BES added secondary sludge fermentation in bath fermentations.

2.15.3.4 Effects of pH on secondary sludge fermentation:

In the first batch study, the effects of pH on the fermentation of secondary sludge will be studied. Over the duration of study, VFA and pH will be measured for the batch fermentations. In the second batch study, the pH values of three batch reactors will be lowered to 4, 5 and 6 through the addition of 0.1 N hydrochloric acid (HCl). In a series of batch fermentations, VFA production in low pH set reactors will be compared with the production in unchanged pH reactors.

3 Materials and methods

3.1 Sludge sources:

Primary and secondary sludges were employed for various studies in this research work. Both sludges were refrigerated at 4°C in 15 L plastic containers. The source and characteristics of both types are discussed individually in the following sections.

3.1.1 Primary sludge:

Primary sludge was obtained from Palmerston North wastewater treatment plant. The plant treats the wastewater of a population of around 76,000 people. Figure 3-1 represents the unit processes of the treatment plant.

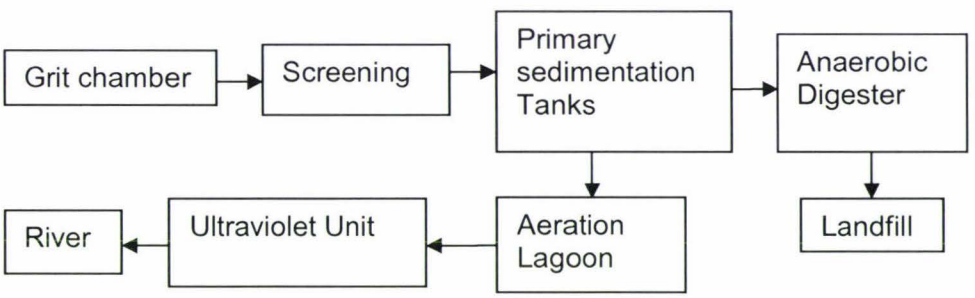


Figure 3-1 Unit operations of Palmerston North wastewater treatment plant

Fresh primary sludge for this study was drawn from the underflow at the bottom of primary sedimentation tanks (PSTs). PSTs were operated at hydraulic retention time of approximately 4 hours.

3.1.2 Secondary sludge:

Secondary sludge was procured from Fielding sewage treatment plant. This plant treats the wastewater for a population of around 28,000 people. The secondary

sludge was obtained from a settling tank which was receiving effluent from an aeration pond. The different unit operations of the sewage plant are shown in Figure 3-2.

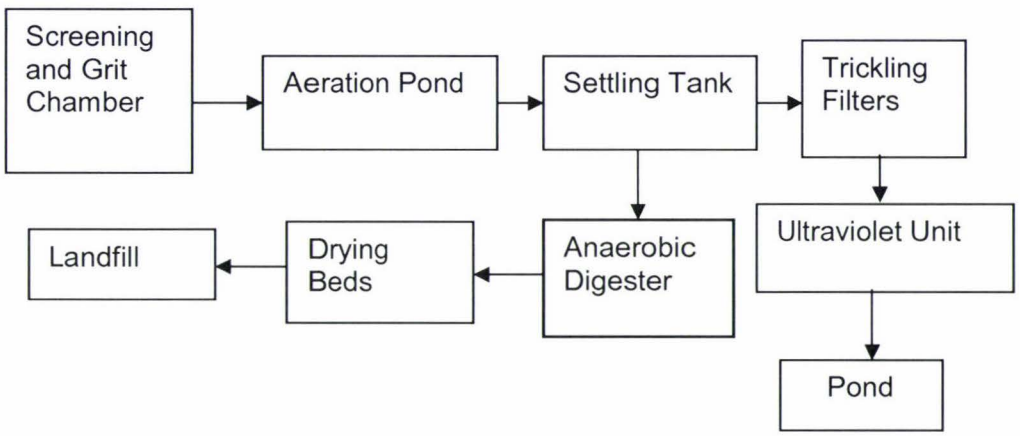


Figure 3-2 Unit operations of Fielding sewage treatment plant

3.2 Reactor study:

3.2.1 Sequencing batch reactor:

A sequencing batch reactor (SBR) was used as a fermenter in this research. The volume of the reactor was 6 L. Figure 3-3 shows the reactor's configuration.

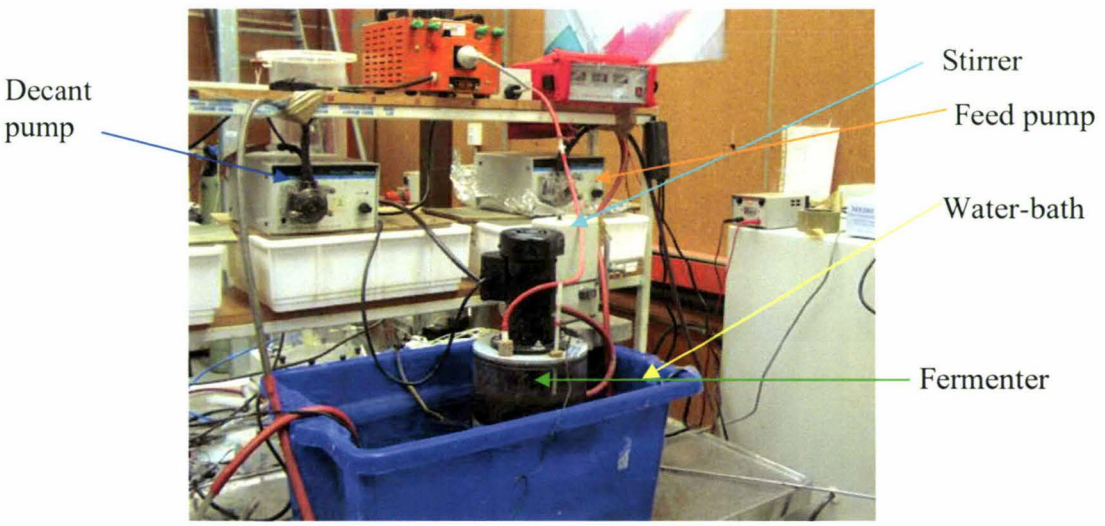


Figure 3-3 Sequencing batch reactor

The reactor was intermittently mixed. An electric stirrer was used to keep the reactor under suspension. The reactor's pH was not controlled. The reactor was run at temperature between 20-22°C. A water-bath was used to maintain constant temperature.

3.2.2 Feed:

Secondary sludge from Fielding sewage treatment plant was fed to the reactor. Prior to being fed to the reactor, the sludge was diluted using tap water to keep total solids content around 1% throughout the study. The feed was kept under suspension in a refrigerator at temperature range of 5-7°C. Feed was prepared at the interval of 3-4 days.

3.2.3 Reactor operation:

The reactor was run for three cycles (each of 8 hours) per day. Each cycle was characterised by three stages: feed, react and decant. The reactor was mixed for 45 minutes during the feed stage. At all other times the reactor remained unmixed. Hydraulic retention time for the SBR was calculated as follows:

$$\text{HRT in hours} = \frac{\text{Volume of the reactor in L}}{(\text{Volume fed in L/cycle} * 1 \text{ cycle}/8 \text{ hours})}$$

The reactor was run for a total duration of 52 days. The total duration of 52 days was divided in two phases. The reactor was tested for hydraulic retention time (HRT) of 48 hours during phase 1 and of 28 hours during phase 2. Solids retention time (SRT) of the reactor was found by calculating the average amount of solids retained in the reactor during each cycle.

$$\text{SRT} = \frac{\text{Solids concentration of the reactor in mg/L} * \text{Volume of the reactor in L}}{\text{Solids leaving reactor in mg/L} * \text{Volume of liquor leaving per day}}$$

Total suspended solids (TSS), Volatile suspended solids (VSS) and pH of the reactor were measured once daily. Volatile fatty acids (VFA) of decant and feed of the reactor were measured from the 25th day of the total duration of reactor operation. The soluble chemical oxygen demand of decant and feed of the reactor was measured from the 29th day of the total duration of reactor operation. Table 3-1 describes the various operational parameters for both phases.

Table 3-1 Duration of different phases of the SBR

parameters	**Phase 1**	**Phase 2**
Feed time (min)	7	12
React time (min)	458	458
Decant time (min)	15	10
Total time per cycle (hours)	8	8
Volume fed to the SBR/cycle (ml)	1000	1425
HRT (hours)	48	28
SRT (days)	2.65	2.0
Duration of phase (days)	40	12

3.3 Sonication of sludge:

Primary and secondary sludges were conditioned by ultrasound application to study the enhancements in soluble content. Sonication was carried out using MSE sonication unit. Figure 3-4 shows the sonication set-up used in this research.

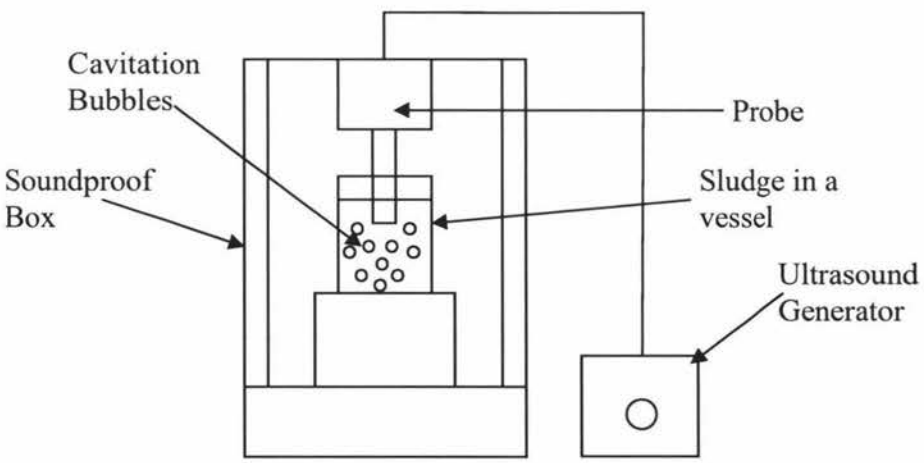


Figure 3-4 Sonication set-up

As shown in Figure 3-4, the probe was immersed 1 cm into the sludge during disintegration. Sludge samples were kept in 50 ml vessels for disintegration. Sonically treated sludges were measured for COD, BOD_7 and carbohydrates after and prior to sonication. Frequency for the set-up was 20 KHz. The same power was applied for both types of sludges in order to produce comparable results. Sonic power was measured using following equation:

$$E = M C_p dT/t$$

Where,

M = mass of sludge sample in kg

C_p = Specific heat capacity of the sludge (assumed to be the same as water)

dT = change in temperature over the sonication period

t = Time in minutes

Sludge samples were sonicated for up to 30 minutes. COD and soluble carbohydrates content of the samples were measured at 0, 2, 4, 5, 8, 10, 12, 15, 20, 25 and 30 minutes of sonication. Also, BOD_7 of sludge samples that had been sonicated for 30 minutes, were measured and compared with those of unsonicated samples.

3.4 Batch study 1:

In the first batch fermentations study, the relationship between system pH and VFA production/degradation was studied. In each case, 500 ml of biomass was mixed with 500 ml diluted secondary sludge. Table 3-2 on the following page shows the initial conditions of the batch fermentation. Biomass was taken from the SBR reactor. The resulting volume was placed in an orbital shaker at 23°C. All samples were analysed for pH, SCOD and VFA for up to 50 hours.

Hydrochloric acid (HCl) of 0.1 N concentration was used to set the pH values of the batch experiments.

Table 3-2 Initial conditions for batch fermentations 1

Exp	Initial pH	Description of 'feed'
1	6.26 (unchanged)	Secondary sludge: 1% solids; SCOD≈5000 mg/l
2	6.07	
3	5.54	
4	4.02	
5	6.46 (unchanged)	Glucose solution: ≈0% solids, SCOD=5000 mg/l

The fermentation of glucose (a known readily fermentable substrate) was included for reference purposes in batch fermentations.

3.5 Batch study 2:

In the second batch study, 250 ml of biomass (taken from the SBR during phase 1) was mixed with 250 ml of feed. The resulting volume was placed in an orbital shaker at 23°C for up to 84 hours. pH and VFA were analyzed for this batch study. Table 3-3 shows the initial conditions of the batch fermentations.

Table 3-3 Initial conditions for batch fermentations 2

Exp	Feed (250 ml)	'Biomass'
1	Diluted secondary sludge (1% solids)	Biomass from the SBR (250 ml)
2	Secondary sludge (2.8% solids)	
3	Primary sludge (4.8% solids)	
4	Sonicated diluted secondary sludge (1% solids)	
5	BES (1mM) added diluted secondary sludge (1% solids)	
6	Glucose solution: ≈0% solids, SCOD≈5000mg/L	

The fermentation of glucose (a known readily fermentable substrate) was included for reference purposes in batch fermentations.

For experiment no 4 as shown in Table 3-3, secondary sludge was sonicated for 30 minutes duration at power of 0.52 Watt/ml before mixing with the biomass. Moreover, for experiment no 5, powder of bromoethane sulfonic acid was dissolved in distil water to make 1 mM concentration and mixed with diluted secondary sludge before placing in an orbital shaker. The main objectives of this set of batch fermentations were:

1. To established the relationship between VFA production and different solids concentration of secondary sludge.
2. To compare primary and secondary sludges for VFA production.
3. To compare diluted sonicated and unsonicated secondary sludges for VFA production.
4. To compare diluted secondary sludge and 1mM BES added diluted secondary sludge for VFA production and to check any possible methanogens inhibition.

3.6 Characteristics of sludges:

All sludge samples were tested for different parameters. Most of the parameters were measured in accordance with the standard methods (APHA, 1998). However, some of the characteristics were estimated using other analytical methods which are referenced below.

3.6.1 Solids:

All types of solids (total solids, total suspended solids and volatile suspended solids) were measured in accordance with standard methods (APHA, 1998). Sludge samples were filtered under vacuum through a glass-fibre filter (Whatman, GF/C, 0.45 micron). Filters with residues of solids were dried in an oven at a constant temperature between 103-105°C. Then, filters were cooled in desiccators and total suspended solids were measured based upon the volume of sludge filtered. Filters were dried further in a furnace at temperature of 550°C. Followed by cooling the filters in desiccators, volatile suspended solids were measured as the difference between the remaining fixed solids and the total suspended solids.

Total solids were measured by drying sludge samples in evaporating dishes, of known weight, in an oven at temperatures between 103-105°C. After cooling the

residues, the percentage total solids of the sludge samples were estimated based upon the amount of solids evaporated in the furnace, the measured weight of the dishes and residues.

3.6.2 Chemical oxygen demand:

Chemical oxygen demand (COD) of sludge samples were measured using the closed reflux, colorimetric method (APHA, 1998). For soluble COD, samples were filtered using 0.45 micron syringe membrane filters. Unfiltered samples were used for total COD analysis. Samples were digested using dichromate and sulphuric acid reagents and the change of chromium from the hexavalent to the trivalent state was measured by photospectrometer. Firstly, a standard curve was prepared using Potassium Hydrogen Phthalate (KHP) solution. Dichromate solution ($K_2Cr_2O_7$) (1.5 ml) and sulphuric acid reagents (3.5 ml) were mixed in clean culture tubes with 2.5 ml of standard KHP solution known COD concentrations. The mixture was digested at 150°C for 2 hours in a HACH COD reactor. After cooling, absorption of the samples was measured using a photospectrometer at a wavelength of 600 nM and the standard curve was prepared. Instead of KHP solutions, sludge samples were analysed using the same procedure and COD values were found from the standard curve. A blank sample was prepared with every batch of samples.

3.6.3 pH:

Sludge samples were tested for pH using a portable Orion pH meter (Model 230A). The pH electrode was calibrated using KOH and $NaHCO_3$ standard solutions of known pH values of 4 and 7 respectively.

3.6.4 Biochemical oxygen demand:

Biochemical oxygen demand (BOD) test was carried out in accordance with the HACH BODTrak test manual. A BODTrak reactor was used to measure BOD of

samples. Diluted samples were kept under suspension in sealed BODTrak sample bottles. The volume of samples was selected to ensure a BOD range of 0-700 mg BOD/l. Lithium Hydroxide was placed in the seal cap to avoid possible occurrence of carbon dioxide (CO_2) during the test duration. Then BODTrak bottles were attached with the tubes of the reactor. The reactor was placed in an incubator for 5 days at 20^{O}C. BOD results for each bottle were displayed on a control panel of the reactor for the duration of 7 days.

3.6.5 Ammonia:

Ammonia was estimated using a titrimetric method (4500 – NH_3 C) from standard methods (APHA, 1998). Firstly, ammonia in sludge samples was distilled, in a solution of 4% Boric acid, using a BUCHi distillation unit. Subsequently, ammonia concentrations in the distilled samples were determined by titration using an electronic titrator (Mettiler DL 25). HCl of 0.1 M was used as a titratant.

3.6.6 Total Kjedahl nitrogen:

Total Kjedahl nitrogen (TKN) was measured using TKN apparatus. The method was in accordance with the Macro-Kjeldahl Method of Standard Methods (4500-N_{org} B) (APHA, 1998). Sludge samples were mixed with 13ml sulphuric acid and Kjedahl catalyst tablets in TKN glass tubes. The mixtures were digested in TKN apparatus until the colour of samples changed to pale green. Then, the same procedures of distillation and titration, as described in section 3.6.5, were followed to measure TKN content of the sludge samples.

3.6.7 Volatile fatty acids (VFA):

VFA concentrations of sludge samples were measured using Ion-Chromatography. The Ion-Chromatograph (Dionex ICS-2000) with an Ion Pac AS11-HC column was used to measure volatile fatty acids content in samples via

conductivity selection. Acetic, propionic, butyric and valeric acids were the type of acids detected using Ion-Chromatography.

Ion-Chromatograph (IC) consisted of an eluent generator, a high-pressure pump, a sample injector, a separator column, a suppressor column, a conductivity cell and a data collection system. The IC was calibrated using standard acids solutions. Diluted sludge samples were filtered through 0.45 micron Millipore syringe filters in 10ml polyvinyl vials to analyse for VFA concentrations. Sludge samples were analysed in bulk to measure VFA content using an AS 50 auto-sampler.

3.6.8 Alkalinity:

Alkalinity was measured using an end-point pH titration method which was in accordance with method 2320 B-Alkalinity in standard method (APHA, 1998). HCl of 0.1N was used as a titratant and pH value of 4.5 was selected as the end-point value. Samples were titrated to the end-point pH value without reading intermediate values. Alkalinity was measured using amount of titratant used to reach the end-point pH.

3.6.9 Carbohydrates:

Phenol-sulphuric acid method was used to measure carbohydrates in sludge samples (Dubois, et al., 1956). Firstly, a standard curve was prepared using glucose of known concentrations. Known concentration of glucose samples of 1 ml volume were mixed with 1 ml of phenol (80% solution in water) and 5 ml of sulphuric acid (grade 95.5%) in clean culture tubes. The mixtures were left at room temperature for 10 minutes after vortexing the tubes. Then, absorption values of the samples were measured at a wavelength of 490 nM and the standard curve was prepared. Carbohydrates concentrations of sludge samples were measured from the standard curve using the same procedure.

4 Sludge characteristics

Table 4-1 shows the various characteristics of primary and secondary sludges.

Table 4-1 Characteristics of primary and secondary sludge

Parameters	Primary sludge	Secondary sludge
Total solids (TS)	4.8% ±0.5	2.3% ±0.7
Total suspended solids (TSS)	46000 ±4591	23840 ±6522
Volatile suspended solids (VSS)	35130 ±4316	4300 ±1256
VSS/TSS	76%	18%
Chemical oxygen demand (COD)	68170 ±14878	39260 ±19564
Soluble chemical oxygen demand (SCOD)	6540 ±1583	5200 ±1100
Volatile fatty acids (VFA):		
• Acetate (HAc)	776 ±207	453 ±192
• Propionate (HPr)	762 ±269	274 ±106
• Butyrate (HBr)	220 ±93	24 ±12
Biological oxygen demand (BOD_7)	10400	9300
pH	5.7 ±0.46	6.43 ±0.30
Alkalinity	700 ±222	560 ±152
Ammonia (NH_3)	130 ±35	80 ±22
Total Kjedahl nitrogen (TKN)	880 ±28	1200 ±61
Organic nitrogen	760	1120

(All parameters are expressed in mg/l except for pH and total solids)

4.1 Solids:

It appears from Table 4-1 that the solids content was a distinguishable parameter for the sludges studied. Average total solids value for the primary sludge was almost two times more than the value of secondary sludge. The value of VSS/TSS gives an indication of the organic fraction of sludge (APHA, 1998). From Table 4-1, VSS/TSS was higher in primary sludge than secondary sludge. Therefore it appears that the organic content of the primary sludge was much higher than that of the secondary sludge. VSS/TSS content of primary sludge was 75% in the study carried out by Elefsiniotis and Oldham (1994a), which is quite similar to the ratio observed in this research. However, the VSS/TSS

content for activated (secondary) sludge was 78% in the sludge fermentation study carried out by Chen et al. (2004), which is significantly higher than the VSS/TSS value of the secondary sludge used in this research.

4.2 Carbonaceous content:

Carbonaceous content of both sludges was measured in terms of COD, SCOD, VFA and BOD_7. For primary sludge the soluble COD was around 9.5% of total COD, while for secondary sludge it was around 13% of total COD. The SCOD/COD fraction of the primary sludge used in this work seems similar to the fractions reported in previous fermentation studies carried out by Elefsiniotis et al. (2004) and Bouzas et al. (2002). However, for the secondary sludge it is high compared to the fractions reported in previous works carried out by Yuan et al. (2006a) and Min et al. (2004). BOD values give a quite good rough estimate of the amount of bio-degradable matters present in the sludge. From Table 4-1 it is clear that unlike the COD contents, there was not a vast difference between BOD_7 values for both sludges. The volatile fatty acids contents of the sludges were quite different. The amount of VFA present in the primary sludge was significantly higher than that present in the secondary sludge. A higher degree of fermentation was observed in primary sludge with 36% VFA/SCOD, against 18% in secondary sludge. Since an average pH value was 5.7 for the primary sludge, it was likely that the primary sludge was getting fermented in the pipe network of the Palmerston North wastewater treatment plant. Propionate content was similar to acetate for the primary sludge, while for the secondary sludge, acetate was considerable higher than propionate.

4.3 pH:

From Table 4-1, it appears that the primary sludge was more acidic than the secondary sludge. Bouzas et al. (2002) and Chen et al. (2004) also reported primary sludge pH values in their studies as 5.88 and 5.8 respectively which are

similar to the pH value of primary sludge used in this research. Min et al. (2002) reported secondary sludge pH value as 6.7 in their sludge fermentation study. Therefore it appears that the pH values for both sludges are suitable for VFA production in this study.

4.4 Nitrogen content:

The TKN value of secondary sludge is higher than that of primary sludge. As discussed in section 2.3.2, secondary sludge is a mixture of wasted biomass, therefore it contains a considerably higher amount of cells than does primary sludge. Microbial cells represent a considerable fraction of organic nitrogen. Therefore, it appears that the organic nitrogen content of secondary sludge is higher than that of primary sludge.

4.5 Summary:

It appears from the various parameters observed in the study that both sludges are suitable for generating VFA through fermentation. Primary sludge contains a significantly higher organic fraction than secondary sludge. Furthermore, pH of primary sludge is more acidic than that of secondary sludge. Therefore, fermentation of primary sludge might be more advantageous than secondary sludge.

5 VFA production in a laboratory scale SBR

The intermittently-mixed sequential batch reactor (SBR) was run for a period of 52 days. The various parameters observed for the SBR were total suspended solids (TSS), volatile suspended solids (VSS), short chain volatile fatty acids (VFA) and pH. All parameters are discussed in the following sections.

5.1.1 Solids:

Figure 5-1 shows the TSS and VSS profiles of the reactor over the 52 day period. The profile of the SBR was divided into two phases (Phase 1 and Phase 2) as shown in Figure 5-1. Phase 1 includes the start-up of the reactor. A drastic fluctuation in the TSS level was observed during the first phase of the reactor's operation. While in phase 2, at 28 hours of HRT, a drop in both solids levels were observed.

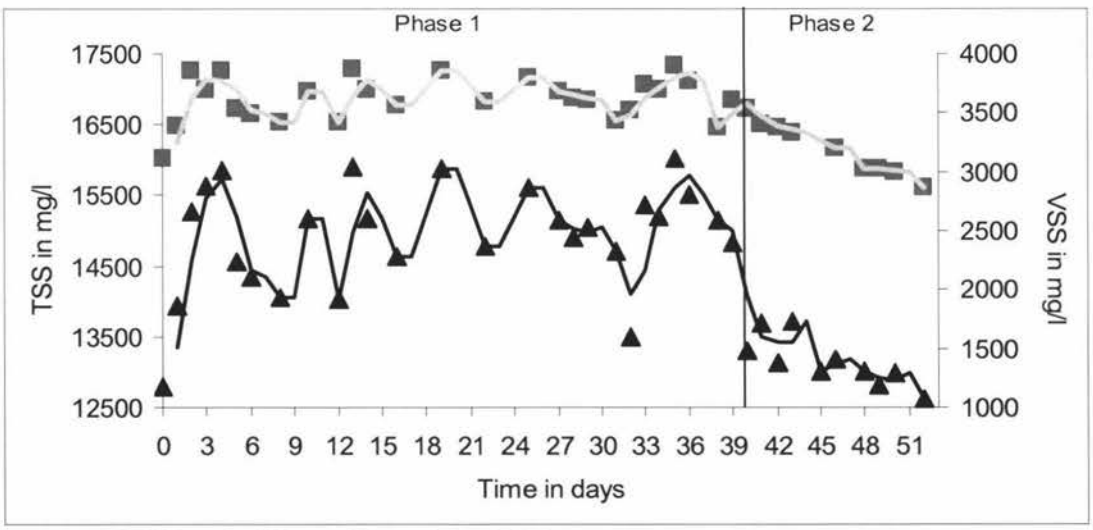


Figure 5-1 TSS and VSS profiles of the SBR
(■ VSS of the reactor, ▲TSS of the reactor)

The reactor configuration was similar to that of a 'static pre-fermenter' which is also defined as a single-stage pre-fermenter (van Münch, 1998). However, 'static pre-fermenters' are totally non-mixed while the reactor in this study was

intermittently mixed. Solids retention time in a static pre-fermenter is controlled by keeping the sludge blanket height constant by wasting sludge through a waste line provided at the bottom of the pre-fermenter. In contrast, in this reactor SRT was not independently controlled because of the poor settling capacity of the secondary sludge. Even after remaining unmixed for almost 7 hours per cycle during both phases, solids in the reactor had not settled well. On average, a total suspended solids level of 12,700 mg/l in decant (not shown in the graph) was observed throughout the study. Contrary to this research study, Gonzalez-Barcelo and Gonzalez-Martinez (2006) observed good settling in an SBR study. They used an SBR both, as a primary settler and a fermenter, using raw wastewater for different organic loads. Cycle time of their reactor was 8 hours (similar to the present study). However, the mixing condition was different from the present study's SBR. Even though the SBR in their study was mixed throughout the anaerobic phase, a good settling of solids with maximum 55% TSS removal in their reactor was observed. Although the reactor in the present study was unmixed during the anaerobic phase, solids were observed in decant, which strongly indicates that the liquid in the reactor had very poor settling capacity.

As discussed earlier in this section, the liquid in the reactor has poor settling ability even at 48 hours HRT. Furthermore, at HRT of 28 hours, solids had less time to settle compared to at HRT of 48 hours. Solids retention time was approximately 2.65 and 2 days for phase 1 and 2 respectively. The most likely reason for solids retention time to decline in phase 2 is due to a change in hydraulic retention time. It is suggested in section 2.10.3 that too short SRT can cause solids wash out in the reactor. Hence, it seems that SRT of 2 days was insufficient and eventually solids washed out in the decant.

Figure 5-2 on the following page shows the TSS and VSS concentration of feed throughout the study. The average concentration of TSS in the feed was 9,750 mg/l (approximately 1% solids). The VSS concentration was also quite constant

at 1,380 mg/l (on average). It is clear from the graph that the volatile fraction of total solids is very low and the ratio of VSS to TSS was around 14%. Min et al. (2004) observed that an influent sludge has VSS/TSS of 82%. Also, Yuan et al. (2006a) observed VSS/TSS ratio of 78% for secondary sludge in their study.

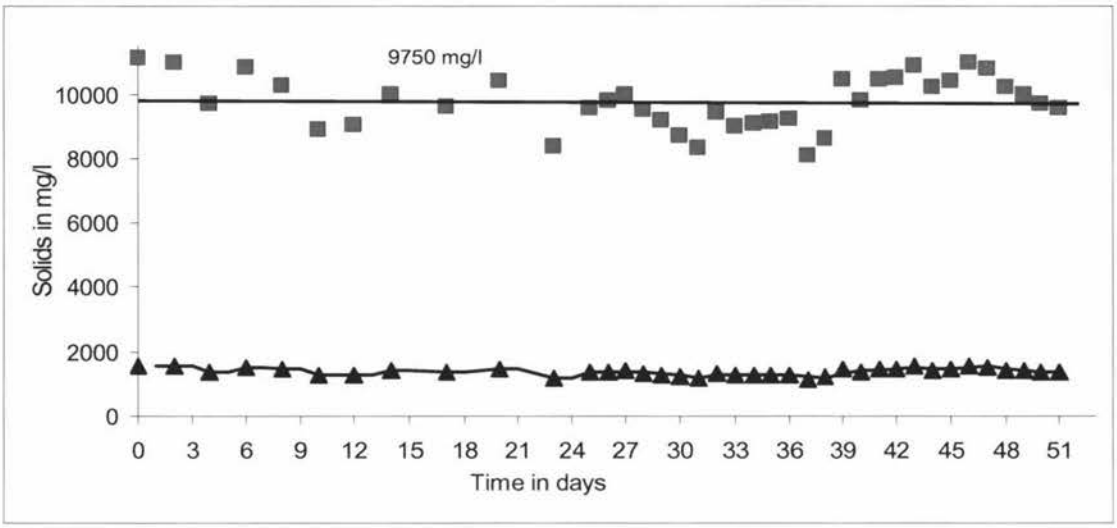


Figure 5-2 TSS and VSS profile of feed
(■ TSS of the feed, ▲ VSS of the feed)

It seems that the secondary sludge used for the feed in the present study had low organic content compared with the secondary sludge used in previous studies.

5.1.2 VFA Production:

Figure 5-3 on the following page shows the overall profile of VFA in the effluent and influent along with net production (mg VFA_{HAc}/l) over the final 28 days of the study. It is clear from the graph that the amount of VFA produced was almost constant until day 40, after which the amount started to decrease. During phase 2, VFA production had declined (eventually to zero mg/l). It is widely known that HRT governs the contact time between organisms and substrate in the feed (Elefsiniotis and Oldham, 1994a) and that SRT affects the mix of organisms as it tends to select organisms in accordance with their generation times (Elefsiniotis and Oldham, 1994a). As mentioned in section 5.1.1, hydraulic retention time (HRT) was reduced from 48 hours to 28 hours in phase 2, which led SRT to

reduce from 2.65 days to 2 days. It is possible that at 2 days SRT during phase 2, the growth of organisms which are responsible for hydrolysis, was not promoted.

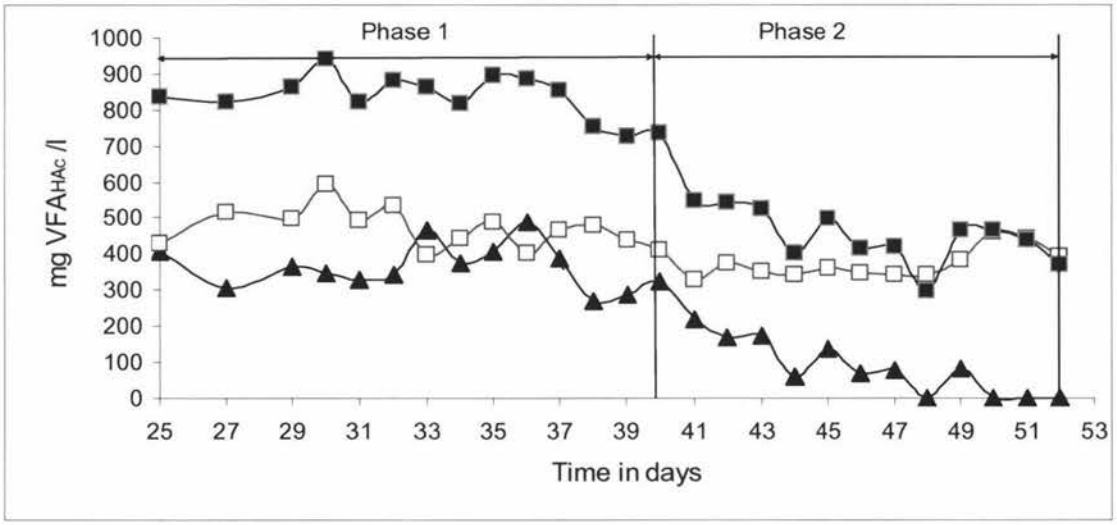


Figure 5-3 VFA profile of the SBR
(■ VFA out, □ VFA in, ▲ VFA produced)

Therefore, no net VFA production was observed due to poor hydrolysis and consequently complex matters were not degraded during phase 2. Hydrolysis is the rate limiting step which results in the formation of soluble products, known as solubilization. Later in the thesis, solubilization is discussed for the present study in section 5.1.3. An average net VFA production (VFA concentration in effluent – VFA concentration in influent) during both phases is shown in Table 5-1. On average, net VFA production of 365 ±62.5 mg VFA_{HAc}/l was observed for 48 hours HRT during phase 1.

Table 5-1 Net VFA production of the SBR

VFA production	Phase 1	Phase 2
Net VFA production mg VFA_{HAc} /l	365 ±62.5	76.4 ±85.4
Net VFA production mgVFA_{COD}/l	389 ±66.6	81 ±91.2

This amount is lower than the amount observed by Min et al. (2004) who used secondary sludge for fermentation in a complete mixed reactor at temperature of

20°C. The authors found a maximum net VFA production of 538 mg VFA/l at HRT of 64.8 hours. However, the secondary sludge that those authors used was from a fibre industry, while the sludge used in this study was from a sewage treatment plant.

The amount of Maximum net VFA in this study is significantly lower than the domestic primary sludge fermentation observed in other reactor research studies. For instance, Ubay-Cokgor (2005) observed a maximum VFA production of 2,740 mg/l in a reactor study. Maharaj and Elefsinioitis (2001) reported net VFA production of around 780 mg/l (as acetic acid) for HRT of 30 hours and temperature of 25°C in a mixed reactor. But, Elefsinioitis and Oldham (1994a) reported optimum VFA generation of about 580 mg/l (as acetic acid) for primary sludge fermentation at HRT of 12 hours in a UASB reactor, which is similar to the amount produced in the present study. Table 5-2 summarises the net VFA production compared with HRT using either primary sludge or secondary sludge as a substrate in previous reactor studies.

Table 5-2 Optimum VFA production for various studies

Substrate	Net VFA (mg/l)	HRT (hours)	Author(s)
Secondary sludge	538	64.8	Min et al. (2004)
Primary sludge	580	12	Elefsiniotis and Oldham (1994a)
Primary sludge and industrial wastewater	780	30	Maharaj and Elefsiniotis (2001)

From Table 5-2, it can be seen that Elefsiniotis and Oldham (1994a) and Maharaja and Elefsiniotis (2001) observed optimum net VFA production for primary sludge fermentations at HRT values lower than that used in the present study. The net VFA degradation took place when the authors used HRT values higher than those mentioned in Table 5-2 in their research. The amount of VFA generated at HRT of 48 hours in the present study is not comparable with findings for primary sludge fermentation in previous studies. Also, the net VFA produced in the present work is less than the amount achieved by Min et al. (2004) in their reactor study at 64.8 hours HRT. Therefore, it is clear that

fermentation of secondary sludge in this work yielded low VFA production in comparison with prior studies.

On the whole, it was confirmed that the intermittently mixed sequencing batch reactor in this study has the potential to produce VFA from secondary sludge at 48 hours HRT in phase 1. However, a drop in net VFA production was noted when HRT was decreased to 28 hours during phase 2. Poor hydrolysis due to a drop in SRT during phase 2 is most likely the factor that affected net VFA production.

5.1.3 Solubilization:

Soluble COD in the influent and effluent of the SBR was measured throughout the study. The difference between soluble COD found in the influent and soluble COD that found in the effluent was expressed as SCOD accumulation or production. Monitoring SCOD data gives an idea of the degree of hydrolysis. In Figure 5-4, accumulated SCOD during the study is shown. From Figure 5-4, it appears that during phase 1, soluble COD accumulation remained almost constant and, on average, net SCOD of 655 ±89.1 mg/l was observed.

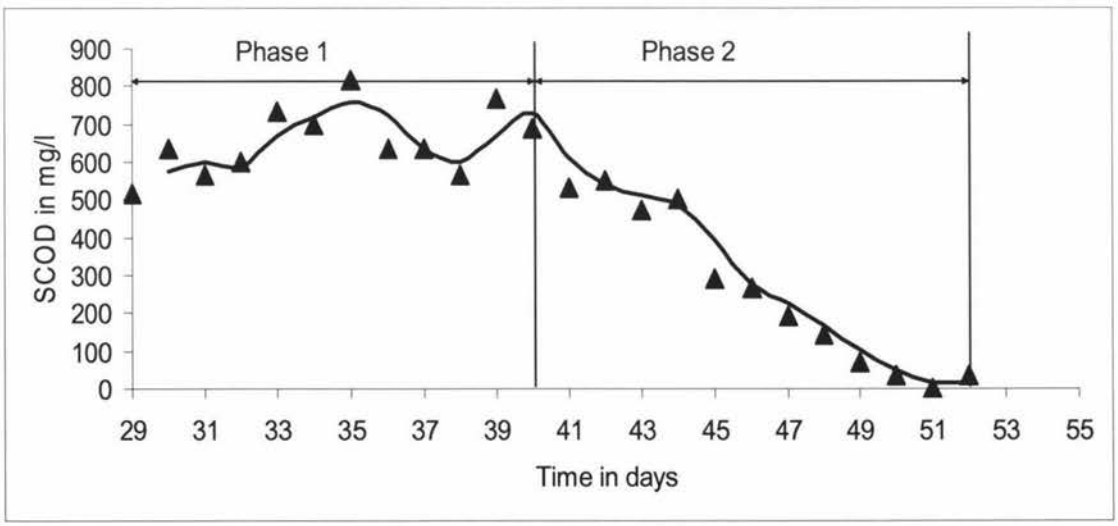


Figure 5-4 Profile of Soluble COD accumulation of the SBR
(▲ SCOD accumulated)

During phase 2, at HRT of 28 hours and SRT of 2 days, SCOD accumulation declined, which gives an indication of poor hydrolysis. As discussed in section 5.1.2, SRT of 2 days might not have encouraged the growth of the bacteria responsible for hydrolysis, causing net VFA production to decline during phase 2. Poor solubilization observed in phase 2 is also in accordance with declined net VFA production in that phase. Therefore, it appears that hydrolysis is a function of SRT and that a drop in SRT in phase 2, causing acidogenesis to decline in the SBR during that phase.

During phase 1, the net VFA fraction of accumulated SCOD was 60%. While, during phase 2, the fraction of VFA of total SCOD declined to 32%. The ratio of soluble COD to total COD during phase 1 was 12%, which is considerably lower than the ratio reported by Ghosh et al. (1975) and Eastman and Ferguson (1981) for primary sludge acidogenesis. In the SBR study carried out by Gonzalez-Barcelo and Gonzalez-Martinez (2006), maximum SCOD accumulated was 100% in the form of VFA for raw wastewater. However, the maximum accumulated SCOD of 34 mg/l in their study is significantly lower than the average SCOD accumulation of 655 ±89.1 mg/l in this study. Therefore, the degree of solubilization occurred in the present work was lower than the amount reported in previous studies.

5.1.4 Specific VFA production rate and VFA yield:

The degree of fermentation should be measured in terms of the amount of organic matter converted into VFA. It is possible that high VFA production can be observed, but a large fraction of organics in the feed is not converted to VFA. Therefore, net VFA production gives an indication of whether fermentation has taken place or not, but not the extent of fermentation. Specific volatile fatty acids production per day in this study is defined as mg of net VFA produced per mg of volatile suspended solids in the reactor per day.

Figure 5-5 depicts the overall picture of specific VFA production rate. VFA production rate is defined as mg VFA produced per mg VSS of the biomass per day. Similar to the net VFA production, specific VFA production was consistent in phase 1 and declined during phase 2 due to the change in HRT from 48 to 28 hours. The specific production rate in phase 2 was 76% less than that observed during Phase 1.

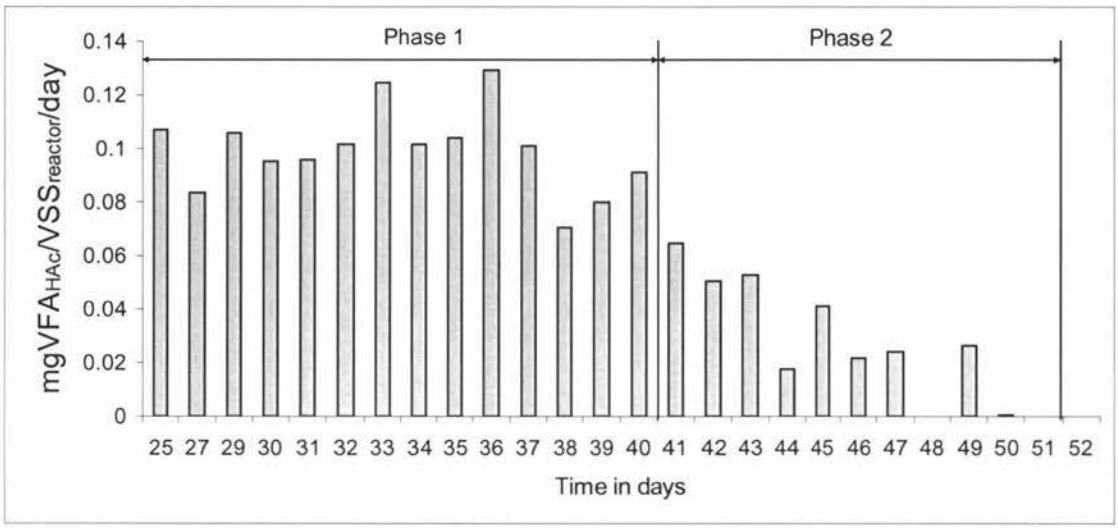


Figure 5-5 Specific VFA production rate of the SBR

Table 5-3 shows the specific production rates, net VFA yields and gross VFA yields of the SBR observed for both phases. In accordance with net VFA production mentioned in section 5.1.2, all yields and production rates were significantly lower in phase 2 compared to phase 1.

Table 5-3 VFA yield and specific production rate for the SBR

VFA outcome	Unit	Phase 1	Phase 2
VFA specific production rate	(mg VFA_{COD}/mg $VSS_{reactor}$/day)	0.10 ±0.02	0.024 ±0.03
	(mg VFA_{HAc}/mg $VSS_{reactor}$/day)	0.10 ±0.02	0.024 ±0.02
VFA Yield	(mg VFA_{HAc}/mg VSS_{feed})	0.28 ±0.05	0.056 ±0.06
	(mg VFA_{COD}/mg VSS_{feed})	0.30 ±0.05	0.060 ±0.06
	(mg VFA_{COD}/mg COD_{feed}	0.012 ±0.002	0.003 ±0.003

Various VFA yields have been cited: Ubay-Cokgor et al. (2005) and Banister and Pretorious (1998) observed a net VFA value of 0.13 mg VFA_{COD}/mg $COD_{influent}$ for primary sludge fermentation in their research studies, which is substantially higher than the 0.012 ±0.002 mg VFA_{COD}/mg COD_{feed} obtained in this study. However, the net VFA yield in this study 0.28 ±0.05 mg VFA_{HAc}/mg VSS_{feed} is higher than the range of 0.034-0.081 mg VFA_{HAc}/mg VSS in the primary sludge batch fermentations observed by Chen et al. (2004). Also, it is higher than the range (0.145-0.176) mg VFA_{HAc}/mg VS_{feed} reported by Min et al. (2004) for the secondary sludge fermentation. However, the authors used volatile solids concentration in calculation of the yield. Elefsiniotis and Oldham (1994a) reported that for their studies the highest VFA production rate for primary sludge fermentation was 0.10 mg VFA_{HAc}/mg $VSS_{reactor}$/day which is similar to 0.10 ±0.02 mg VFA_{HAc}/mg $VSS_{reactor}$/day achieved in this study. The production rate for the present study is significantly higher than the maximum amounts of 0.029 and 0.036 mg VFA_{HAc}/mg $VSS_{reactor}$/day reported by Banerjee et al., (1999) and Maharaj and Elefsiniotis (2001) who used primary sludge as a substrate in their reactor studies.

It is clear from the results that although the net VFA production was lesser for secondary sludge fermentation in the present study, the specific VFA production rate was higher than previous fermentation studies using primary sludge. Also, VFA yield for the present research was higher than the values reported in some of the prior research studies.

5.1.5 VFA speciation:

Acetate, propionate, butyrate (single and branched) and valerate (single and branched) are the types of VFA measured during the study. Figure 5-6 on the following page shows the fraction of each type of VFA in the total amount of VFA produced. As discussed in section 2.6 (see Table 2-3), acetic acid was the most common of all VFA occurred during fermentation

in previous sludge fermentation studies. Similarly, in the present study it can be seen from Figure 5-6 that acetate was the most common and along with propionate made 82% of the total VFA content during the study. Among other acids, both butyrate/iso-butyrate and valerate/iso-valerate compositions comprised 9% of the total VFA content.

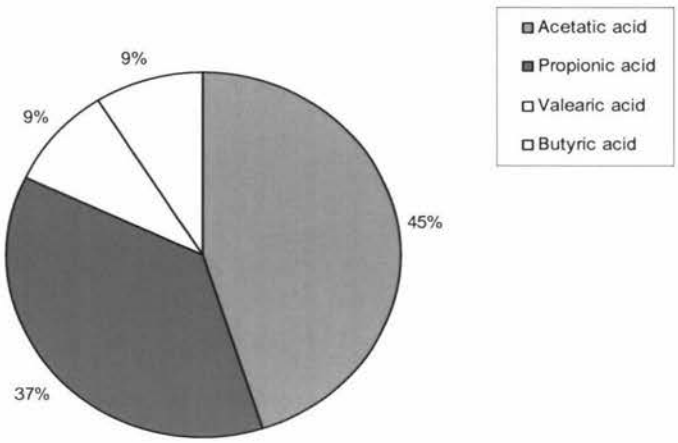


Figure 5-6 Fractions of VFA produced during fermentation

Similar to the present study, Elefsiniotis and Oldham (1994a) reported the sum of acetic acid and propionic acid as 78-82% in the study of primary sludge fermentation. Also, Ubay-Cokgor et al. (2005) observed acetate and propionate as constituting 80% of total VFA content. However, Min et al. (2002) reported 30-31% of acetic acid and 24-32% propionic acid of total VFA content in experiments on fermentation of secondary sludge, which is different from findings in the present study.

The characteristics and source of influent, and also the reactor's configuration, might affect the type of acids generated through fermentation. Acetic acid, as expected, was observed as the most frequent acid produced during fermentation in the present research.

5.1.6 pH:

Figure 5-7 shows the pH profile of the SBR and the feed throughout the study.The SBR was started with an initial pH of 6.8. Following the start-up, pH of the reactor decreased and stayed around 6.25.

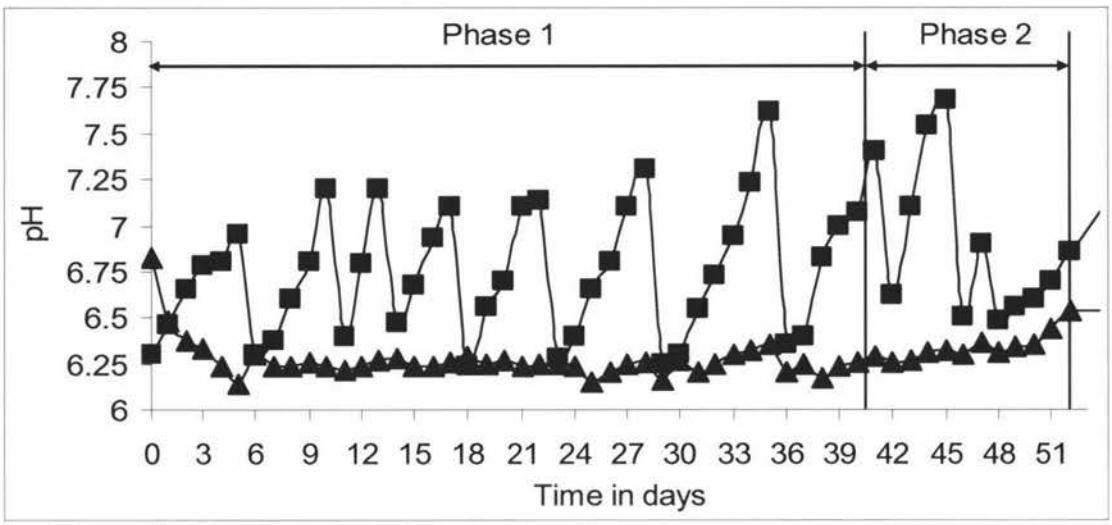


Figure 5-7 pH profile of the SBR set-up
(■ pH of the feed, ▲ pH of the reactor)

Another interesting phenomenon was the fluctuation in pH of feed over the study. During the study, the pH of feed, on average, was 6.8. A wide variation in feed-pH was observed which could be seen from Figure 5-7. Feed was kept under suspension in a refrigerator at 6°C. Throughout the study, once feed was made, within four to five days the pH value increased at a steep rate and reached around 7.25. It seems that a wide variation in pH value was due to the storage of feed for long periods in the refrigerator.

In the present study during phase 1, at pH 6.25, specific VFA production rate and VFA yield were observed as 0.10 ±0.02 mg VSS_{HAc} /mg $VSS_{reactor}$/day and 0.28 ±0.05 mg VFA_{HAc}/mg VSS_{feed} respectively which are higher than some of the previous sludge fermentation studies. Therefore it appears that pH value of 6.25 for the current study is suitable for VFA production.

5.2 Implications for nutrient removal:

The SBR study has shown net VFA production of almost 370 mg VFA_{HAc}/l for HRT of 48 hours. Elefsiniotis et al. (1996) assumed that a typical primary clarifier underflow rate represents 10% of the plant influent flow. Similarly, it is assumed that a secondary clarifier might also correspond to 10% of the plant influent flow; therefore around 400 mg/l net VFA production in the SBR in the present study, is equivalent to 37 mg of VFA per litre of influent wastewater entering into a BNR system. According to Wentzel, et al. (1988) and Pitman et al. (1992) a VFA range of 6-9 mg per litre (approximately 7 mg/l) is required to remove 1 mg of phosphorus. The supplement of 40 mg VFA/l into a BNR facility can reduce phosphorus in the effluent of the process by about 5 mg/l (37/7=5.5 mg/l). The soluble phosphorus concentration in American municipal wastewater is in the range of 5-8 mg/l (Metcalf & Eddy, 2001). Therefore it appears that the amount of VFA generated in the SBR study can improve a BNR treatment facility.

5.3 Brief summary of the SBR:

Overall, after relating all parameters, it was seen that the reactor experienced two phases of different retention times. By linking all the measured parameters, phase 1 was fruitful in terms of VFA production. Following an initial start-up period, almost a constant net VFA production of 365 ±62.5 mg VFA_{HAc}/l was achieved for HRT of 48 hours. In phase 2, it appears that reduction of HRT from 48 to 28 hours decreased VFA generation. One reason could be the fact that the drop in HRT also led to the reduction in SRT from 2.65 days to 2 days in phase 2. SRT of 2 days did not promote the growth of bacteria responsible for hydrolysis and so a decrease in SCOD accumulation was observed during phase 2. Therefore, net VFA production declined to zero due to shorter SRT. Throughout the study, a poor solids settling was observed for the SBR. At SRT of 2 days, solids in the reactor remained suspended and finally washed out in the decant.

In the present study, specific VFA production rate and VFA yield were observed as 0.10 ±0.02 mg VSS_{HAc}/mg $VSS_{reactor}$/day and 0.28 ±0.05 mg VFA_{HAc}/mg VSS_{feed} respectively. These values are higher than those found in other reactor sludge fermentation studies using primary and secondary sludges. The results also indicate that a large fraction of VSS was converted into VFA. However, it also appears that the amount of net VFA produced in the present study is low compared to that produced in other sludge fermentation studies. VFA production at 48 HRT observed in the present SBR study has a potential to improve a BNR system's operation.

6 Effects of feed sludge characteristics on VFA production

The sequencing batch reactor study confirmed that volatile fatty acids (VFA) can be produced during the fermentation of secondary sludge. However, prolonged net VFA production was not attained, likely due to poor hydrolysis of secondary sludge. In this chapter of the thesis, the contribution of the characteristics of the feed sludge to achieve net VFA production is considered. A set of batch fermentations was carried out to assess the potential for VFA production from the fermentation of secondary sludge (as used for the SBR study). VFA production from fermentation of secondary sludge was compared with that from fermentation of primary sludge. Glucose was used as a reference substrate. The characteristics of the sludges were varied: the effects of preconditioning (by sonication), solids concentrations, and low pH values were all tested. The effect of bromoethane sulfonic acid (BES) was also tested when it was suspected that VFA degradation during batch fermentations might have occurred due to the presence of methanogens.

6.1 *Solids concentration:*

Figure 6-1 on the next page shows the net VFA production profiles of secondary sludge batch fermentations containing total solids (TS) of 2.8% and 1%. It is clear from the graph that secondary sludge containing 1% total solids could not support fermentation. Throughout the study, zero net VFA production was observed for 1% TS secondary sludge batch fermentation. On the other hand, secondary sludge with 2.8% TS showed a maximum net VFA production of 60 mg VFA_{HAc}/l (64 mg VFA_{COD}/l) at a retention time of 12 hours, and from this point on, net production dropped to zero throughout the rest of the study. As discussed in sections 4.1 and 5.1.1, secondary sludge used in the present work contained a relatively low amount of organic matter (as per its low VSS/TSS ratio). Therefore, it is possible that, upon dilution, the amount of VSS of 1% TS

secondary sludge batch could have dropped. Therefore, a low amount of organic matter might not have been enough to sustain fermentation in this batch study.

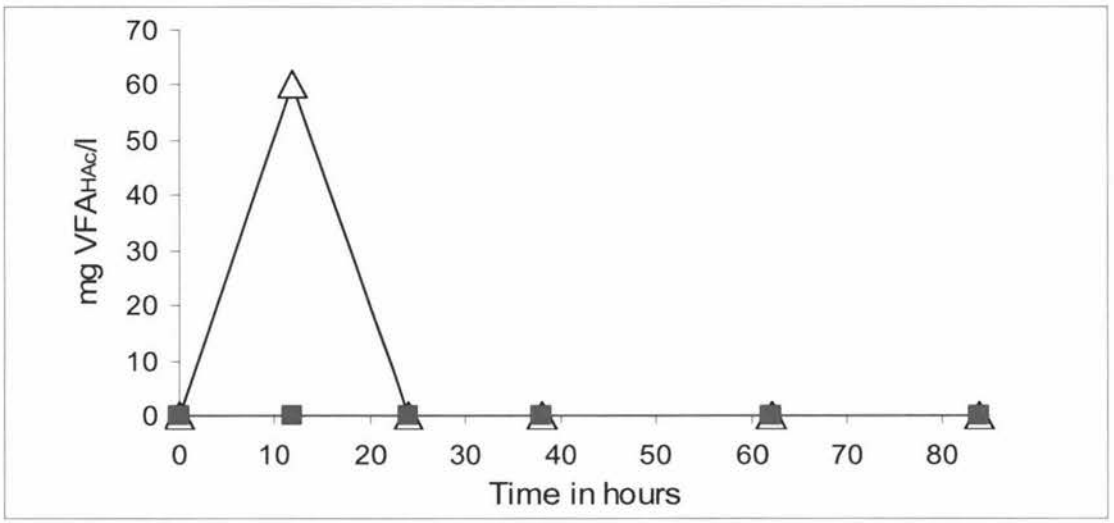


Figure 6-1 Net VFA production profile for secondary sludges batch fermentation
Δ Secondary sludge (2.8 % solids), ■ Secondary sludge (1% solids)

Solids concentration of sludge is believed to be a driving factor because in diluted environments, better interaction between biomass and substrate takes place. Moreover, a diluted environment might also reduce any possible inhibitory effect of the products generated during fermentation (Skalsky and Daigger, 1995). The authors also suggested that a diluted environment provides better mixing; hence better hydrolysis of solids into the soluble products.They observed better VFA production at total solids of 0.43% than at total solids of 2.6% in their research, which is contrary to the results obtained in the present study. Similarly, Banister and Pretorious, (1998) supported dilution of primary sludge and recommended the range of solids to be between 0.5-2%, in order to achieve enhanced fermentation in a batch reaction.

As mentioned in the previous paragraph, a few previous research studies suggested that dilution of sludge could be beneficial for fermentation. However, in the present study, dilution of secondary sludge did not improve fermentation. Dilution of secondary sludge cause a drop in the VSS level per volume of sludge;

hence a very low amount of organic matter would be available, which might not be able to support fermentation. However, for the present study, one responsive data point is not conclusive and further study is required in order to make a better judgement about the effects of dilution on secondary sludge fermentation.

6.2 Primary v secondary sludges fermentation:

It was confirmed in section 6.1 that net VFA production of secondary sludge with TS of 2.8% was better than that of secondary sludge with TS of 1%. However, net VFA produced in secondary sludge was very low compared to the amount generated in primary sludge fermentation in the present study. The profile for net VFA production for primary sludge is shown in Figure 6-2.

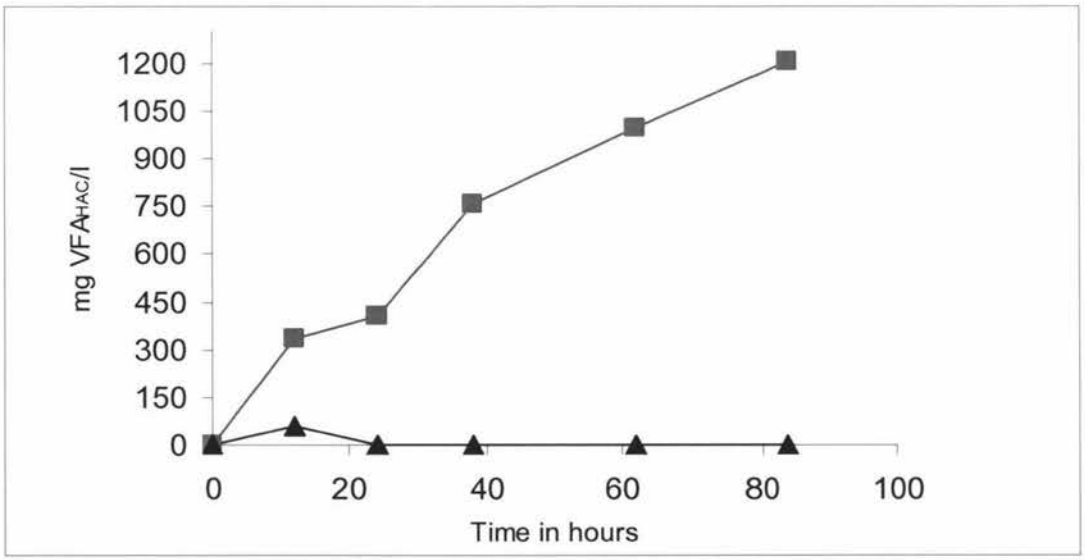


Figure 6-2 Net VFA production profile for primary and secondary sludges
■ Primary sludge (3% total solids), ▲Secondary sludge (2.8% total solids)

The highest amount of net VFA produced was 1200 mg VFA_{HAc}/l which is significantly higher than the highest amount of net VFA (60 mg VFA_{HAc}/l) generated during secondary sludge (total solids of 2.8%) fermentation. Also, in contrast with observations on secondary sludge fermentation, no reduction in net VFA production was observed for primary sludge over the study period.

Figure 6-3 shows the VFA yield in terms of mg VFA_{HAc}/mg VSS_{feed} for primary and secondary sludge batch fermentations. It is clear that the net VFA yield achieved for primary sludge during fermentation was much higher than that achieved for secondary sludge. As shown in Figure 6-3, net yields at 12 hours retention time were 0.019 and 0.017 mg VFA_{HAc}/mg VSS_{feed} for primary and secondary sludge respectively.

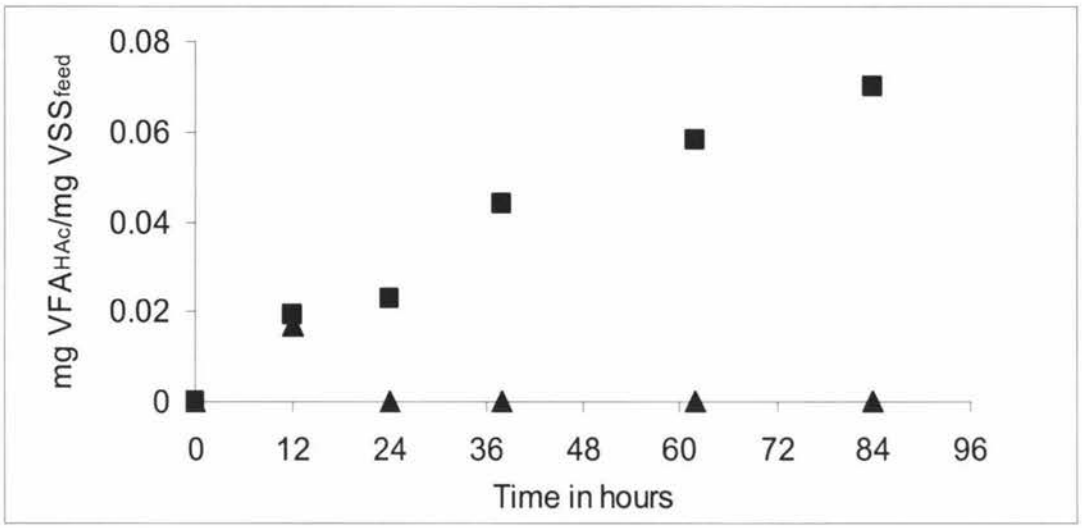


Figure 6-3 Net VFA yield for primary and secondary sludges batch fermentation
■ Primary sludge (3% total solids), ▲Secondary sludge (2.8% total solids)

Even though the net VFA production (shown in Figure 6-2) for primary sludge at retention time of 12 hours was much higher than the net production for secondary sludge, there was very little difference between the net yields for both. Therefore, secondary sludge fermentation can be considered as comparable to primary sludge fermentation in terms of net VFA yield at 12 hours of retention time for the present study.

6.3 Preconditioning of sludge by sonication:

Sonication, the application of ultrasound through a medium, has exhibited a significant enhancement in the fermentability of sludge. Various researchers have observed that sonication causes an increase in the soluble fraction of chemical oxygen demand (SCOD), protein and carbohydrates (Chu et al., 2001; Burus et

al., 1992; Urban et al., 1993; Wang et al., 2005; Wang et al., 2006). In this research, primary sludge and secondary sludges were treated with ultrasonic waves over a 30 minute period. Soluble chemical oxygen demand (SCOD), soluble carbohydrates and biological oxygen demand (BOD_7) were all recorded.

6.3.1 Characteristics of sonicated sludge:

6.3.1.1 Soluble chemical oxygen demand (SCOD):

Figure 6-4 shows the amount of SCOD released for primary and secondary sludges under sonic exposure over 30 minutes. It is apparent from the graph that the amount of SCOD increased for both sludges at 0.0017 Watt/ml/min of sonic exposure. There was a sharp increase from 0 to over 2500 mg/l for primary sludge, and from 0 to over 1600 mg/l for secondary sludge, which is equivalent to 70% (3600 mg/l to 6100 mg/l) and 40% (4066 mg/l to 5666 mg/l) increases respectively, in the first five minutes of disintegration time.

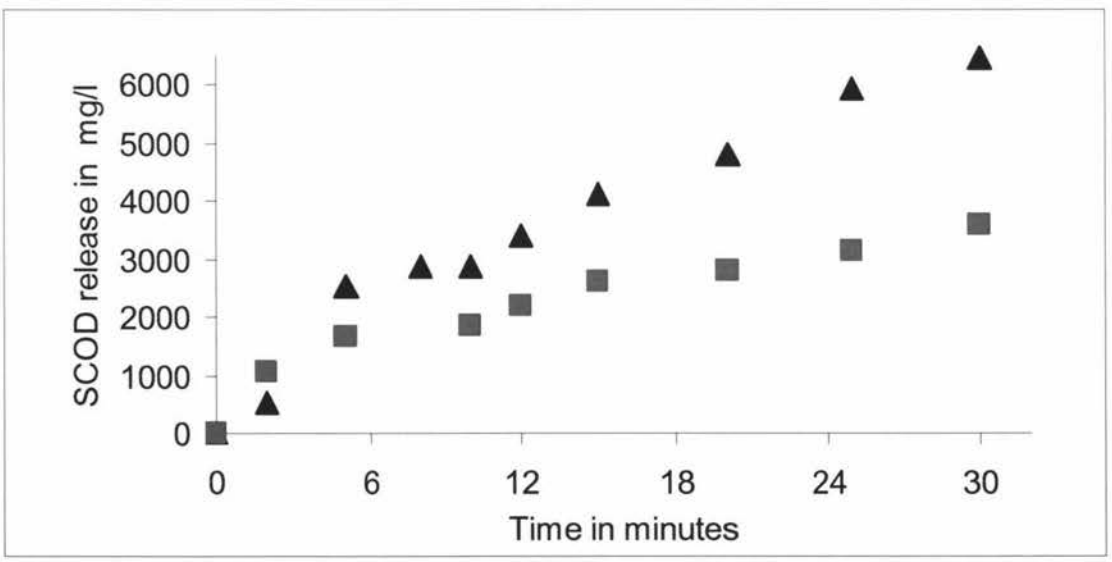


Figure 6-4 SCOD release over sonication
■ Secondary sludge (2.5% solids), ▲ Primary sludge (3% total solids)

Similarly, Wang et al. (2006) observed an increment in SCOD level for wasted sludge from 2581 mg/l to 8912 mg/l for sonication time from 5 to 10 minutes. In

the present sonication study, the increase in the release of SCOD for secondary sludge started to slow down after 15 minutes which is in accordance with research by Wang et al. (2006). However, the amount of sonic power (0.0256 Watt/ml/min) used in their study was much higher than that of used in this research. In the present work, higher SCOD release was observed for primary sludge compared with secondary sludge, which is in contradiction of the study carried out by Mao et al. (2004). The authors observed an increase in SCOD from 1020 mg/l to 3980 mg/l (by 4 times) for primary sludge and 670 mg/l to 5260 mg/l (by 7.7 times) for secondary sludge.

Total solids content might have an impact on the difference in the rate of solubility increase for primary and secondary sludges. Total solids content for primary sludge was 3% which was higher than secondary sludge with 2.5% total solids. It is possible that the higher total solids content in primary sludge attributed the higher solubility release than secondary sludge. Wang et al. (2005) observed a similar phenomenon in their work. In their study, the levels of SCOD detected were 9019 mg/l and 3966 mg/l for sludges with total solids content of 1% and 0.5% respectively. However, Wang et al. (2005) used the same sludge with different total solids contents to test the effect of total solids on sonication, while both sludges used in the present work had different sources and characteristics.

6.3.1.2 Soluble carbohydrates:

Carbohydrates and proteins contribute to the microbial cell's constitution. They are also defined as extracellular polymeric substances (EPS) outside the cells in the floc structure of sludge. They represent a fraction of the total chemical oxygen demand of sludge. Disintegration of sludge causes these compounds to be released into the aqueous phase which elevates its soluble concentration. Figure 6-5 on the following page shows the increase in soluble concentration of carbohydrates via sonication for primary and secondary sludges. Similar to

findings for SCOD outlined in section 6.3.1.1, soluble concentration increased for both of the sludges over the period of 30 minutes sonication. However, the elevation in soluble carbohydrates concentration was higher for secondary sludge than for primary sludge. Primary sludge is mostly untreated solids. On the other hand, secondary sludge is a wasted material and could consist of mostly microbial cells. Furthermore, carbohydrates are one of the constituents of microbial cells composition. Therefore, it is likely that sonication can cause higher release of carbohydrates for secondary sludge than for primary sludge.

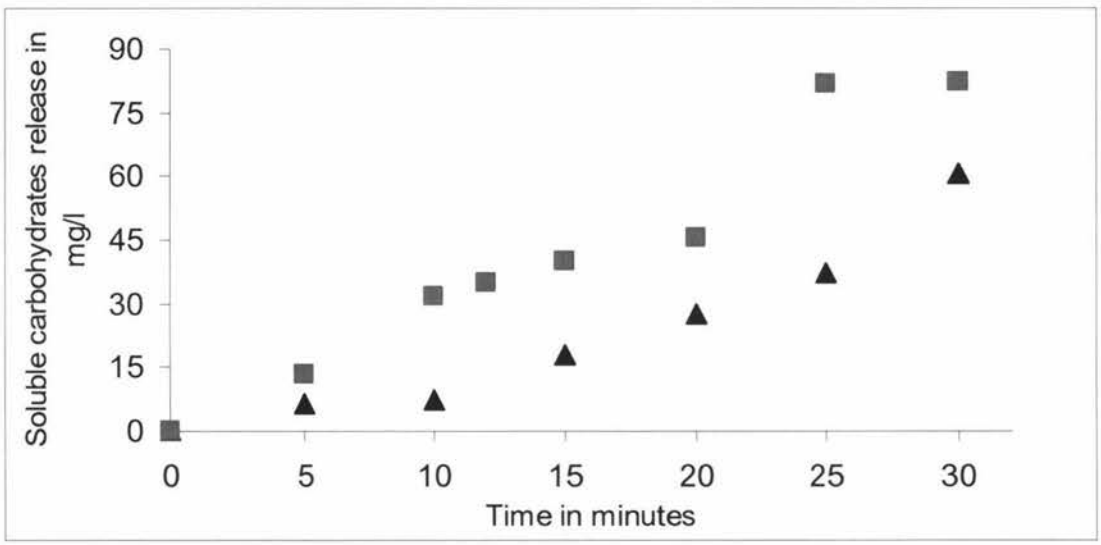


Figure 6-5 Soluble carbohydrates release over sonication
■ Secondary sludge (2.5% solids), ▲ Primary sludge (3% total solids)

As shown in Figure 6-5, for the first 20 and 25 minutes, the soluble carbohydrate level increased from 0 to 45 mg/l and 27 mg/l for secondary and primary sludge respectively. Soon after that, a sudden increase was observed for both. According to Chiu et al. (1997) and Keiding and Neilsen (1997) some of the divalent cations, such as Ca^{2+} and Mg^{2+} play a major role in binding EPS in sludges. Wang et al. (2006) also found the linkage between those cations and biopolymers like proteins and carbohydrates in sludge in their study. Similarly, for the present study, it is possible that a greater release of cations might have occurred during sonication, resulting in a higher release of soluble carbohydrates for both sludges.

6.3.1.3 Biochemical oxygen demand (BOD):

Figure 6-6 and Figure 6-7 show the oxygen consumption over the test period of 7 days for sonicated and unsonicated primary and secondary sludges. Similar to the SCOD test in section 6.3.1.1, an increase in BOD was observed in both sonically treated sludge types.

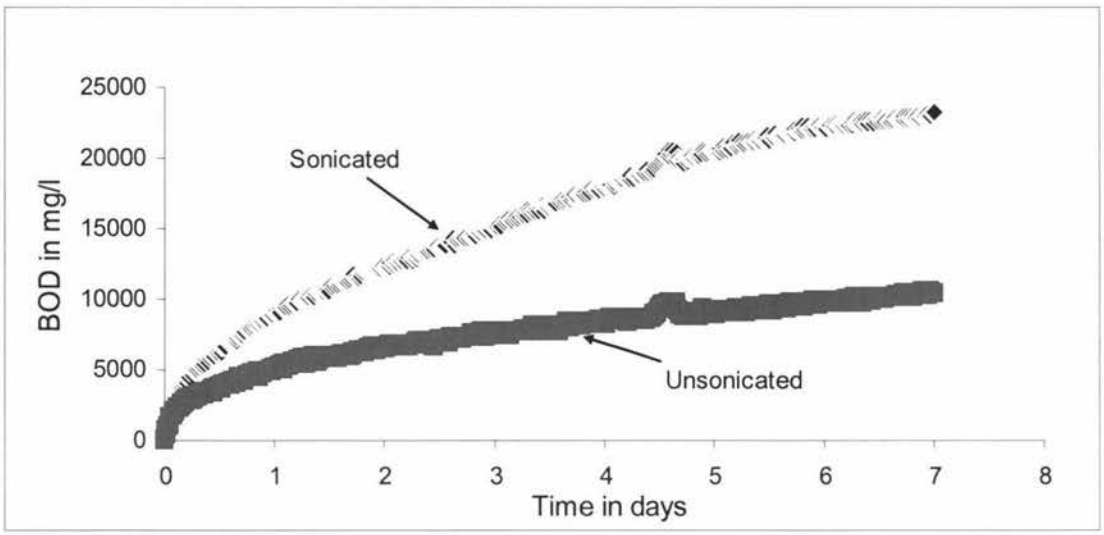


Figure 6-6 BOD profile of sonicated and unsonicated primary sludges

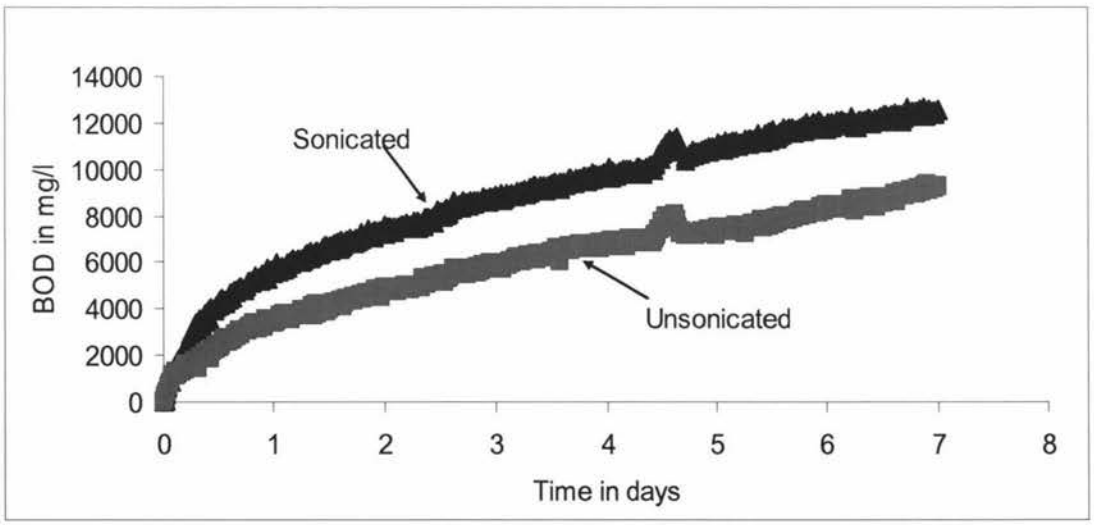


Figure 6-7 BOD profile of sonicated and unsonicated secondary sludges

At the end of a 7 day measurement period, 55 % (from10360 mg/l to 23200 mg/l) and 28 % (from 9280 mg/l to 12980 mg/l) increases in BOD levels were observed

for primary and secondary sludges respectively. It is clear from Figure 6-6 and Figure 6-7 that ultrasonic sound released higher degradable matter in primary sludge than in secondary sludge.

6.4 Effect of pretreatments to improve fermentation:

6.4.1 Effect of sonication on secondary sludges fermentation:

As mentioned above, secondary sludge with 1% solids did not show any sign of VFA production. Therefore, conditions of fermentation were altered by treating the sludge sonically. Total solids content was kept at 1% and all other conditions were maintained constant with those of the former set of secondary sludge batch fermentations. Figure 6-8 shows the results of net production for sonicated and unsonicated secondary sludges.

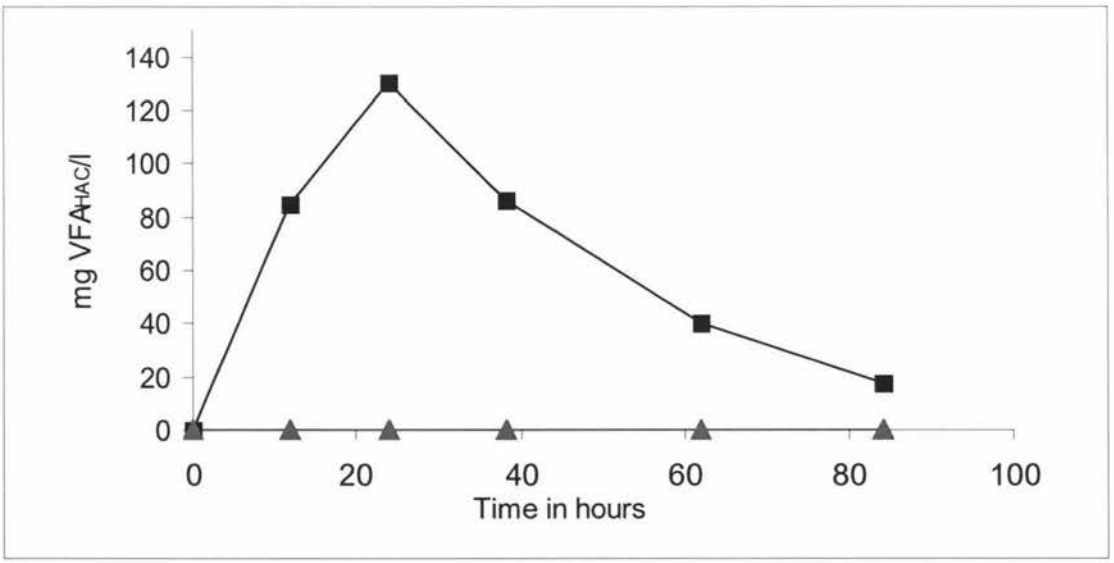


Figure 6-8 Net total VFA profile for sonicated and unsonicated sludges
■ sonicated sludge, ▲ unsonicated sludge

Overall, sonically treated sludge exhibited net VFA production with a maximum of 130 mg VFA_{HAc}/l at a retention time of 24 hours. Subsequently, the rate of production retarded and at the termination of the study, a very minimal net production (20 mg/l) was observed. The highest net VFA yield of 0.094 mg

VFA_{HAc}/mg VSS_{feed} was observed for sonicated sludge at a retention time of 24 hours. It appears from Figure 6-8 that sonication has improved the secondary sludge fermentation compared with unsonicated sludge. However, similar to undiluted 2.8% TS secondary sludge batch fermentation outlined in section 4.1, net VFA produced in sonicated sludge batch fermentation also declined during fermentation. Various studies have exhibited that sonic exposure elevates the soluble content of sludge (Wang et al., 2006; Chu et al., 2001). It was also proven in the study undertaken in this research (section 6.3) that the soluble organic matter in terms of SCOD and soluble carbohydrates of secondary sludge can be increased through sonic exposure. Hence, more readily biodegradable soluble content is available to produce VFA through fermentation. Therefore, more net VFA producion was achieved in the case of sonically treated sludge than with unsonicated sludge in the present study.

6.4.2 Effect of methanogenic inhibitor on fermentation:

Figure 6-9 shows the net VFA production for batch fermentations of with and without BES added 2.8% TS secondary sludges. The net VFA amount for BES

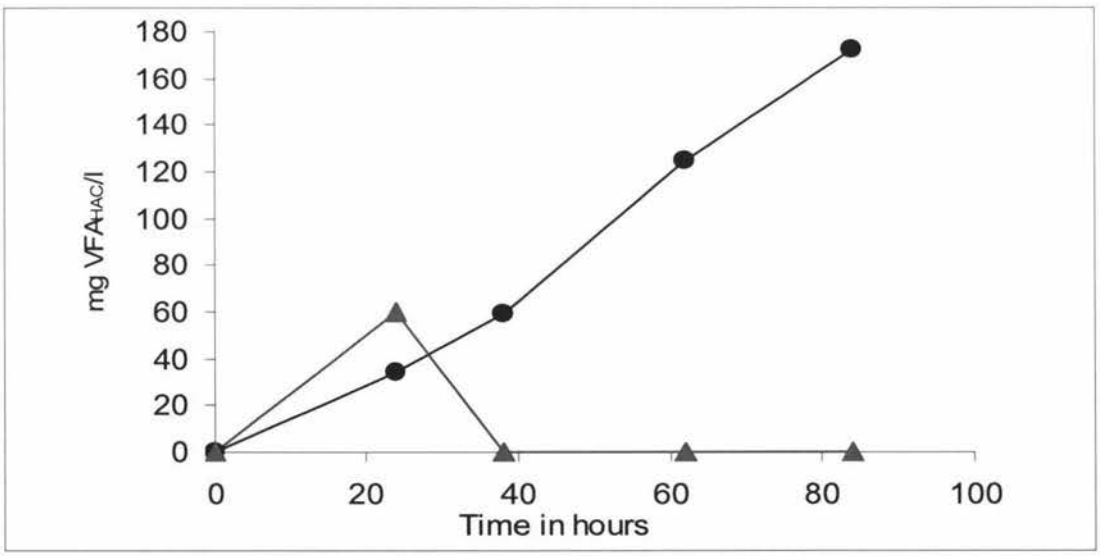


Figure 6-9 Net VFA production for with and without BES added sludge fermentation
● BES added sludge, ▲ non-BES added sludge

added sludge increased at every sampling time until the end of the fermentation. The highest net VFA production of 170 mg VFA_{COD}/l was recorded for BES added secondary sludge. Figure 6-9 also shows that for the same experimental conditions, a maximum of 64 mg VFA_{COD}/l net VFA production was found for non-BES added secondary sludge fermentation. Therefore, it is likely that BES inhibited the methanogens in the batch study, and hence the net VFA produced during fermentation in BES added batch were not degraded.

Acetate degradation is the main precursor of methanogenic activity. According to Kasper and Wurhmann (1978), 70% of methane is generated from acetate. Therefore it is possible that a drop in acetic acid level indicates that the methanogens are degrading acetic acid to produce methane gas. Figure 6-10 shows the individual VFA profile for the BES added sludge fermentation. It appears from Figure 6-10 that no acetic acid degradation was observed over the study, which indicates that an increase in the amount of acetate contributed to the total net VFA accumulation. Similarly, Zinder et al. (1984) observed that acetate, along with ethanol and hydrogen, accumulated due to methanogenic bacteria inhibition when 1 µmol BES was added to an anaerobic digestion study.

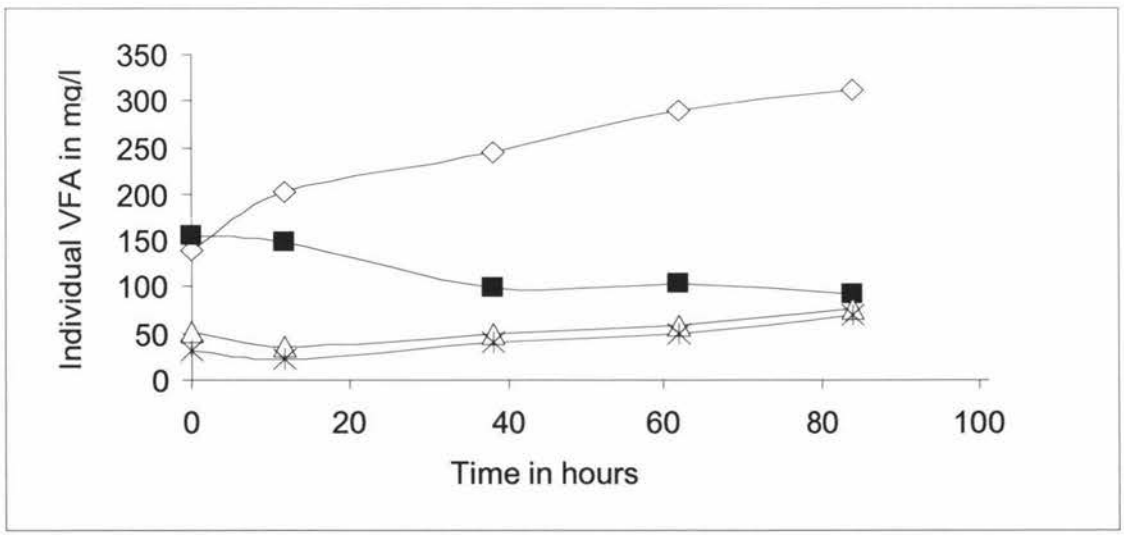
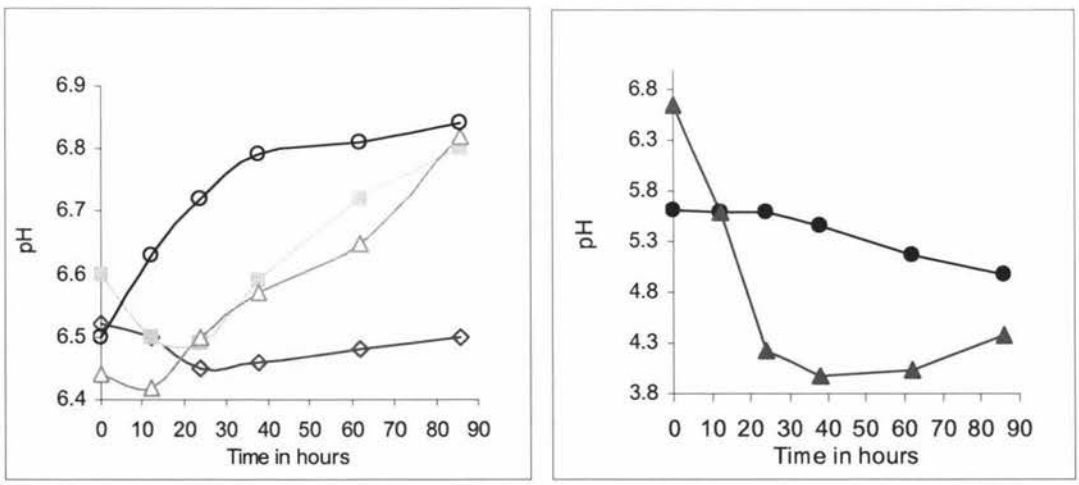


Figure 6-10 Individual VFA profile for BES added sludge fermentation
◊ Acetate, ■ Propionate, Δ Butyrate, ж Valerate

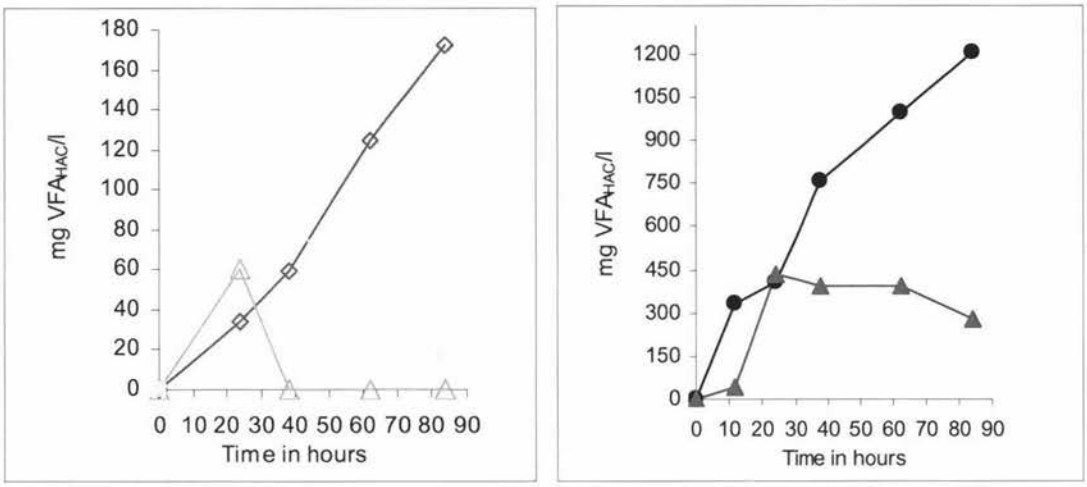
In the present study, propionate was degraded over the time period which can be linked with its conversion into acetate. Similarly, butyrate and valerate degradation up 12 hours retention time can be linked with their conversion into acetate. After the retention time of 12 hours, butyrate and valerate accumulated. Elefsiniotis and Oldham (1994a) reported slower degradation rates for proteins compared to carbohydrates and lipids. Also, Yuan et al. (2006a) linked accumulation of iso-valeric and n-valeric acid with protein degradation. Therefore, it is likely that butyrate and valerate accumulation in the present study can be due to a late hydrolysis of some complex organic matters. Overall, BES addition inhibited VFA degradation, most likely by suppressing the growth of methanogens. A significant acetate accumulation in BES added secondary sludge batch fermentation contributed to net VFA production for the present study.

6.5 Effect of pH:

Figure 6-11 shows the pH and VFA profiles of the various batch fermentations discussed in sections 6.1, 0 and 6.4. It seems Figure 6-11 that net VFA production/degradation during batch fermentations has affected the system pH.



(A) (B)



(C) (D)

Figure 6-11 (A) and (B) pH profile (C) and (D) VFA profile
▲ Glucose, ○ Secondary sludge (1% solids), ● Primary sludge (4.8% solids), ◊ BES added secondary sludge, Δ Secondary sludge (2.8 % solids), ■ Sonicated secondary sludge (1% solids)
(Net VFA production for diluted secondary sludge (1%TS) is not shown as it was zero throughout this bath study)

The general picture is that there is a correlation between VFA generation and reduction in pH. For secondary sludge with dilution (1% total solids), pH increased throughout the study as no VFA generation was achieved. For secondary sludge (2.8% total solids), pH decreased up to 12 hours of retention time which coincided with VFA generation. The same scenario was observed for sonicated sludge, primary sludge and BES added set-ups where VFA accumulation tended to correlate with a drop in pH values and acids consumption led to an increase in pH values.

The pH of the reference experiment (glucose fermentation) was not observed to rise. It is suspected that this is because the significant drop in pH (from net acid production and the lack of alkalinity) resulted in conditions that inhibited the subsequent action of methanogens, which are generally considered to be neutrophilic (pH 6-8) organisms (Neue, 1993). It is hypothesized that fermentation of glucose is significantly more rapid than the fermentation of the secondary sludge. Similarly for primary sludge, where fermentation started with

initial pH of 5.9 played an important role to subdue any possible acids degradation due to any possible methanogenic activity.

From Figure 6-11 (D), it seems that primary sludge fermentation boosted and pH of that batch had gone down below 5.5 after 24 hours retention time. It appears that a low pH value of 5.5 might have subdued methanogens successfully in primary sludge fermentation. In the case of secondary sludge where VFA production was little or nil, higher pH after 24 hours might have encouraged methanogens, which would have attributed to the zero VFA yield observed. Lay et al. (1997) reported a pH range of 6.7-7.4 as suitable for the functionality of most methanogenic bacteria and that this functionality decreases if the pH goes below 6.3 or above 7.8. Min et al. (2002) also observed acids degradation when pH was increased from 6.6 to 7.5.

It is hypothesized that pH could be a governing factor for the present study and that higher pH (around 6.5) might have supported methanogenic activity by which produced acids were converted into gaseous products. Moreover, the VFA degradation rates were higher than the rates of their production.

6.6 Effect of low pHs:

As discussed in section 6.5, higher pH might be a governing factor for net acids consumption; low pH might be the inhibitory factor for acids degradation. Consequently, another set of fermentations was carried out with pH of 6.07, 5.54 and 4.02 along with unchanged pH for secondary sludge (1% total solids). Glucose was used as a reference solution. Figure 6-12 shows the VFA profile over the time period of 50 hours.

It was expected that some VFA accumulation would have occurred in each of the fermentations, and that the extent of VFA accumulation would be greatest in the systems with lowest pH. However, as can be seen in Figure 6-12, significant VFA

accumulation was generally not observed. It is assumed that the slight VFA accumulation observed at the beginning of each of the experiments might be due to both a lag in the activity of methanogens and the production of VFA from any readily biodegradable organics in the feed.

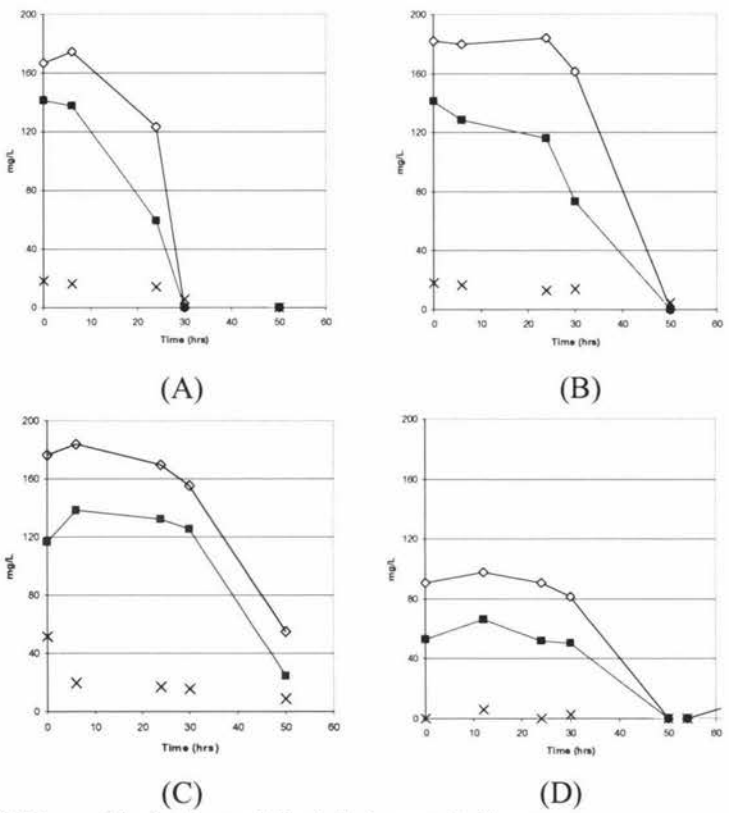


Figure 6-12 VFA profile for low pH batch fermentations
[A] initial pH 6.26, [B] initial pH 6.07, [C] initial pH 5.54, [D] initial pH 4.02.
(◊ Acetate, ■ Propionate, X Butyrate)

It is not surprising that the fraction of readily biodegradable material of the feed is low, as the feed material, secondary sludge, is a by-product of a process in which readily biodegradable material is removed. Also, Gomec and Speece (2003) showed that although secondary sludge can be solubilized, solubilization was not observed as VFA production. Significant VFA consumption, likely caused by the production of methane, was noted. This was surprising for the systems with low pH. Jain and Mattiasson (1998) showed that shock pH reduction to below 5.5 prevented gas production. But, Burmmeler et al. (1985) suggested that

methanogens can grow at pH as low as 5.5. Taconi et al. (2006) reported that methanogens can be acclimatized at pH 4.5. The authors reported that biogas production increased by 30% when initial pH was decreased from 7 to 4.5 in the batch digestion of synthetic acetic acid wastewater.

Figure 6-13 shows VFA for a system fed with glucose. It can be seen that significant VFA accumulation (and the degradation of propionic acid to acetic acid) occurred. It can be seen in Figure 6-13 that for the range of VFA concentrations observed, the rate of degradation of propionic acid was not sensitive to the high acetic acid concentration. This is contrary to the findings of Maswson et al. (1991) who showed that elevated acetic acid concentrations inhibited the degradation of propionic acid. Poor hydrolysis is another factor that can lead to poor VFA production.

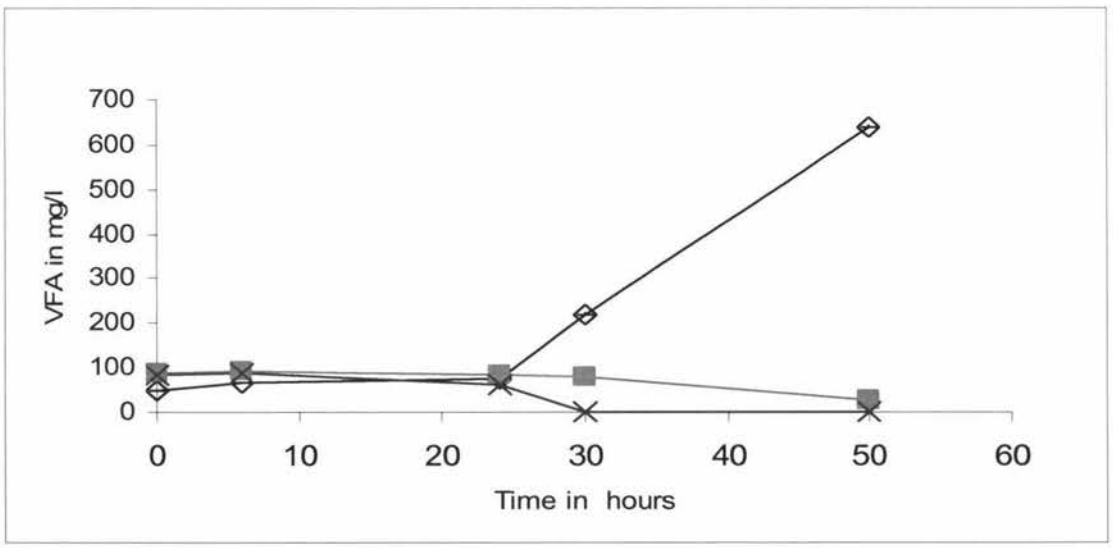


Figure 6-13 VFA profile of batch with glucose as substrate.
(◊ Acetate, ■ Propionate, X Butyrate)

It was expected that at low pH values in batch fermentations, net VFA degradation could be stopped. However, as shown in Figure 6-12, net VFA degradation was observed for the low pH batch study. SCOD of low pH fermentations were measured to observe solubilization. Figure 6-14 shows the SCOD profile for the batch fermentations of pH value 4.02 and 5.54. It is clear

from Figure 6-14 that no soluble COD accumulation took place for either of the fermentations. A 17% increase in the amount of SCOD was observed for the batch with pH of 5.54 at 6 hours retention time, after which the SCOD level decreased.

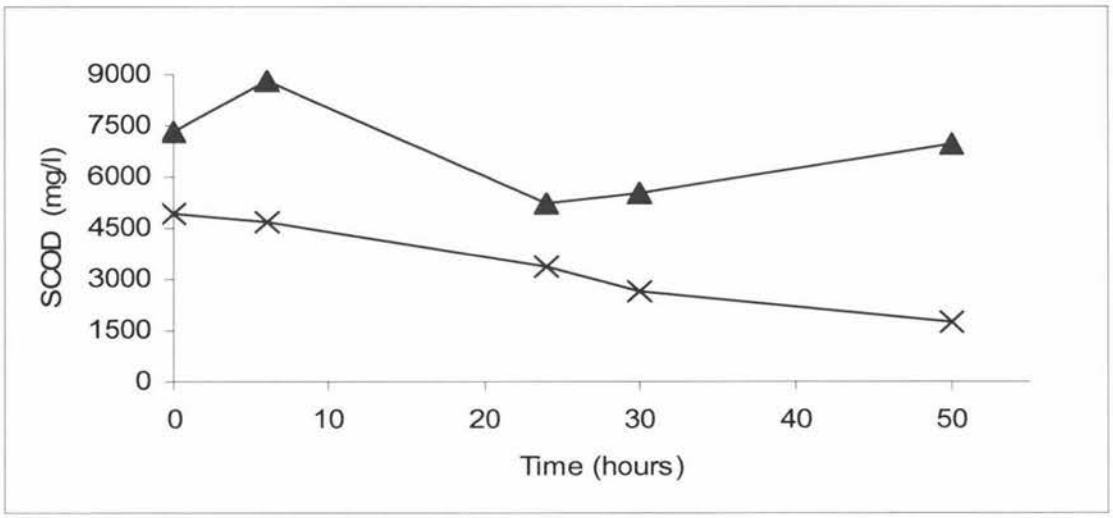


Figure 6-14 SCOD profile for low pH batch fermentations
X pH 4.02, ▲ pH 5.54

Hydrolysis, a rate-limiting step, is pH dependent (Elefsiniotis et al., 1996; Veeken et al., 2000). Yuan et al. (2006b) studied the effect of pH range (4-11) on hydrolysis and VFA production in batch fermentations. They observed a little hydrolysis at pH ranging from 4 to 7. Furthermore, no VFA or methane production took place at pH 4 in their study which is in accordance with the present study. Therefore, it seems that a pH value as low as 4.02 might have been toxic for acidogenic bacteria in their study. Similarly, for the present study, there is a possibility that a pH value as low as 4.02 might be toxic for organisms since poor hydrolysis and almost no overall VFA production were observed in batch fermentations.

6.6.1 pH profile for low set pH study:

The pH profiles for the batch experiments are shown in Figure 6-15. The production of VFA (without subsequent VFA consumption) results in a decrease in system pH. For the cases in which the substrate is secondary sludge, there is an initial tendency for pH to remain at the starting level.

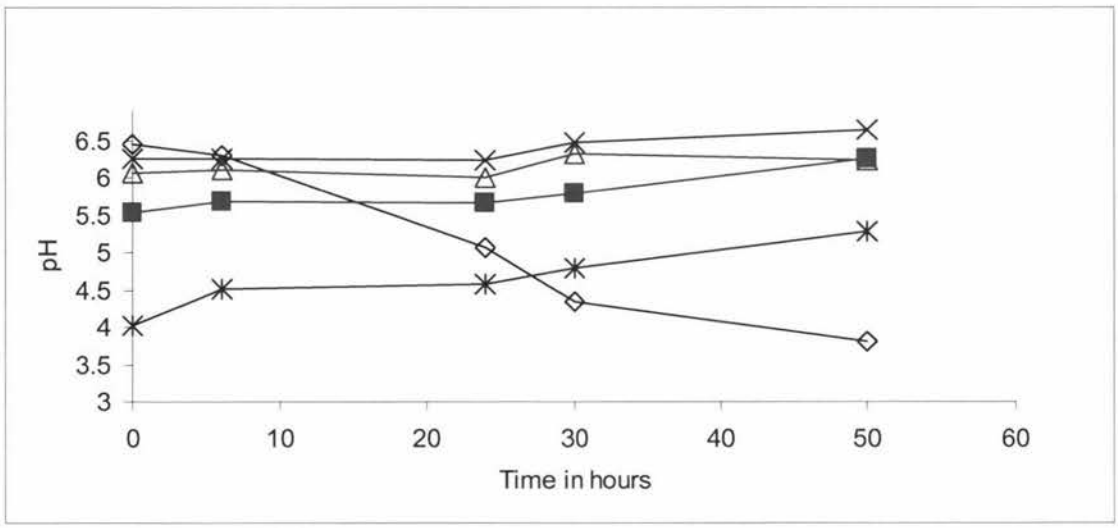


Figure 6-15 pH profile for low pH batch fermentations
(◊ Glucose, ■ pH 5.54, Δ pH 6.07, ж pH 4.02, × pH 6.26)

However, from approximately 25 hours a rise in pH can clearly be seen. It is hypothesized that the degradation of VFA led to the rise in pH values for secondary sludge fermentation. Similar to the effects of pH examined in section 6.5, glucose showed the same trend in that the pH of the batch containing glucose did not rise.

6.7 Summary of the batch fermentations:

It is suggested, based on the results of the batch fermentations, that solids concentration could be a governing factor for secondary sludge fermentation; secondary sludge with 2.8% total solids was superior in the production of VFA compared with secondary sludge with 1% total solids. Primary sludge fermentation yielded 1200 mg VFA_{HAc}/l over retention time of 84 hours, which is

significantly higher than the net production from secondary sludge fermentation. However, there was not much difference between VFA yields of primary and secondary sludges fermentation at retention time of 12 hours.

Pre-treatments like sonication and BES addition improved secondary sludge fermentation. Sonication at 20 KHz frequency for 30 minutes application time improved the solubility of primary and secondary sludge. Sonicated sludge with 1% total solids showed net VFA production; VFA production from unsonicated sludge with 1% solids was not observed. Therefore, it was clear that sonic power application improved secondary sludge fermentation. The pre-treatment of secondary sludge by the addition of 1 mM BES also improved fermentation. VFA were accumulated in BES added secondary sludge batch fermentation over the study period of 84 hours. Accumulation of acetate might indicate the inhibition of methanogens. Pre-treatment of secondary sludge (1% solids) by lowering pH value to as low as 4.02 did not improve fermentation in a batch study. No SCOD accumulation took place for pH 4.02 fermentation which suggests that such a low pH value can lead to poor hydrolysis.

7 Conclusions and recommendations

The SBR study showed that secondary sludge used in the present research work has a potential to generate VFA during fermentation. At HRT of 48 hours during phase 1, the reactor produced net VFA of 365 ±62.5 mg VFA_{HAc}/l. Net VFA yield and specific production rate found during the SBR study revealed that the fermentation of secondary sludge used is not only comparable to primary sludge fermentation studied in prior research, but in fact could be more beneficial to generate VFA. Also, the amount of VFA produced in this work showed the potential to improve the performance of a BNR system.

HRT reduced from 48 hours to 28 hours during phase 2 could not support fermentation. A drop in HRT during phase 2 caused the SRT of the reactor to drop from 2.65 days to 2 days. It appears from the reactor study that hydrolysis is a function of SRT. A drop in SRT during phase 2 led to poor solubilization which suggests that poor hydrolysis occurred in the reactor. SRT affects the selection of micro-oganisms according to their generation times (Elefsiniotis and Oldham, 1994b). Similarly, it appears that shorter SRT was not sufficient for organisms to settle in the reactor as, at SRT of 2 days during phase 2, biomass of the reactor was washed out and hence net VFA production was halted. Therefore, it is concluded that SRT is an important factor which governs the degree of fermentation. It is recommended that HRT values more than 48 hours should be studied in order to gain a broad perspective about the potential of fermentation for secondary sludge used in the present study. However, it should be noted that HRT governs the reactor size and thus the capital cost of the treatment facility. Therefore, longer HRT can incur higher operational costs at a commercial level.

In batch fermentations, diluted secondary sludge (1% total solids) did not show any VFA production while the batch containing undiluted sludge (2.8% total solids) produced 60 mg VFA_{HAc}/l. However, it is clear that the secondary sludge used in the present study had very low VSS/TSS ratio which indicates low organic level.

Therefore, it is likely that dilution of the sludge may not sustain fermentation due to low VSS level. It is recommended to test various dilution ranges in the batch fermentation which would give a better understanding of the effect of solids on sludge fermentation.

Sonic power of 0.0017 Watt/ml/min for 30 minutes application time induced an increase in solubility of primary and secondary sludge. Significant increases in SCOD, BOD_7 and soluble carbohydrates levels were observed in sonicated primary and secondary sludges. It is clear that sonication brings about solubilization in sludge which improves the degree of fermentation. Sonicated sludge in the batch fermentation, exhibited a maximum of 130 mg VFA_{HAc}/l net VFA production which was superior to unsonicated sludge which did not show any net VFA production under the same experimental conditions.

It is likely that the addition of BES in sludge fermentation can inhibit methanogens. Secondary sludge (2.8% total solids) demonstrated net VFA production of 60 mg VFA_{HAc}/l at a retention time of 12 hours in the batch fermentation. Afterward, net VFA production declined and dropped to 0 mg/l throughout the fermentation period. It was possible that methanogens produced at longer retention times consumed net VFA produced during fermentation. Under the same batch fermentation conditions, when 1 mM bromoethane sulfonic acid (BES) was added with secondary sludge (total solids 2.8%); no VFA degradation was observed over a fermentation period of 86 hours. Therefore, it appears that BES has a potential to be used as a methanogenic bacteria inhibitor. It is recommended that various BES concentrations should be tested in batch fermentations. Also, methane gas measurements should be carried out to a get better idea about the inhibitory effects of BES on methanogenic organisms in sludge fermentation.

A correlation between fermentation and pH was noted as pH values tended to drop when VFA were produced during batch fermentations. Reduction in pH did

not improve fermentation as the batch fermentations with pH values set at 4.02, 5.54 and 6.07 did not show any significant VFA production. Furthermore, in the batch fermentation with a pH value of 4.02, poor solubilization was observed. Therefore, it is likely that low pH values can lead to poor hydrolysis and thus fermentation is inhibited. It is recommended that sludge fermentation under alkaline conditions should be studied by measuring the degree of hydrolysis and VFA production.

8 References

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