Inhibitory effects of ammonium, lipids, and salt on anaerobic digestion of food waste

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INHIBITORY EFFECTS OF AMMONIUM, LIPIDS, AND SALT ON ANAEROBIC DIGESTION OF FOOD WASTE

ANG SUAY SIONG

**SCHOOL OF CIVIL & ENVIRONMENTAL ENGINEERING
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2005

Inhibitory Effects of Ammonium, Lipids, and Salt on Anaerobic Digestion of Food Waste

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A thesis submitted to Nanyang Technological University in
fulfillment of the requirement for the degree of
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SUMMARY

The main objective of this project was to investigate the stability of the hybrid anaerobic solid-liquid (HASL) system, which has been developed by the Nanyang Technological University, under the effect of different types of toxicity. The inhibition studied in this project included the effects that may be caused by high content of ammonium, lipids, and salt.

The first part of this project looked into the improvement of the HASL system treating high nitrogenous food waste. The HASL system enhanced with a submerged filter for ammonium removal was proposed. Application of the submerged filter in the HASL system increased concentrations of dissolved chemical oxygen demand (COD) and volatile fatty acids (VFA) in the acidogenic reactor (Ra). It has also improved the total COD removal and methane production in the methanogenic reactor (Rm). The nitrogen content in the biogas produced in the enhanced HASL system was higher than that in the conventional HASL system due to the denitrification process. However, methane production in the enhanced HASL system increased by 26% in comparison with the conventional HASL system.

The second part of the project examined the inhibitory effect of lipids, such as fat, oil, and grease (FOG) on the system. The four HASL systems treating food waste with different FOG concentrations, i.e., 0% (Control), 20%, 30%, and 40%, were used. Generally, hydrolysis and acidogenesis in Ra reactors were affected more by FOG addition than methanogenesis in the Rm reactors. The total solid (TS) and volatile solid (VS) removal efficiencies in the Ra reactors decreased as FOG concentration increased. The COD removal efficiencies in the four Rm reactors, except the 40% FOG reactor, were in the healthy range of 87.5% - 88.9%.

The last part of this project looked into the inhibitory effect of NaCl salt on the HASL system. The four HASL systems treating food waste with different NaCl concentrations, i.e., 0% (Control), 1%, 2%, and 3%, were investigated. It was shown that NaCl salt had affected the Ra reactor more than the Rm reactor. For the HASL system operated with 1% NaCl, there was little inhibition to the system. At 2% NaCl, inhibition was observed in the hydrolytic and acidogenic process. Lower COD and VFA production was observed in the Ra reactor. However, at 2% NaCl addition, little inhibition was observed in the Rm reactor as gas production decreased slightly and VFA removal efficiency was more than 99.5%. At 3% NaCl addition, inhibition in both the Ra and Rm was observed. COD and VFA production in Ra reactor had decreased by 70% and 75%, respectively.

In summary, a thorough understanding of these three types of inhibition, commonly found in the anaerobic digestion will enhance the stability of the HASL system. Preventive measures could be taken when critical level of toxicants were reached.

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CHAPTER 1

INTRODUCTION

1.1 Background

Anaerobic digestion of municipal solid waste is a relatively new concept that only in the past 15-20 years has found application in pilot and full scales. A number of different technologies have been demonstrated in full or pilot scale commercially in Europe, either in mesophilic or thermophilic temperature (Lusk *et al.* 1996). The technologies were operated as batch or continuous reactor, with some researchers (Wang *et al.* 2002; Xu *et al.*, 2002a) separated the process into acidogenic and methanogenic phases.

The Nanyang Technological University has been developing the two-phased hybrid anaerobic solid-liquid (HASL) digestion system for the past three years. The HASL system has proved to be effective and efficient for conversion of biodegradable food waste to biogas. The treatment efficiencies of 77-79% TOC removal, 57-60% volatile solids (VS) removal and 79-80% total COD reduction were achieved (Wang *et al.* 2002; Xu *et al.*, 2002a). Dilution of VFA prior to their entering into the methanogenic reactor is a feature of this hybrid two-phase digestion process, which made pH controlling unnecessary.

Another feature of the HASL system was the recirculation of the effluent from the methanogenic reactor to the acidogenic reactor. This was the advantage of the HASL system over other designs of anaerobic digestion system. Recirculation of the effluent did not require the addition of excess water to mix the solid, and hence did not need to treat wastewater produced during the digestion process. Besides, the higher alkalinity of the effluent helped increase the pH of the acidogenic reactor to a more neutral level

(Wang *et al.* 2002). This helps to accelerate the process of hydrolysis and acidification. The HASL system appeared to be technically feasible at its current state of development. However certain toxicity issues may arise as toxicants was accumulated in the HASL system due to recycling of the effluent from the methanogenic reactor. This was because toxins to anaerobic system, such as ammonium, high sodium concentration due to high salt content in the Asian cuisine, may accumulate in the closed HASL system and affect its operation. High lipids material, such as fat, oil, and grease (FOG), accumulated in the HASL system may also affect its anaerobic process.

1.2 Objectives

The main objective of this project was to investigate the stability of the HASL system under the effect of different types of toxicity. The inhibition studied in this project included the effects that may be caused by high content of ammonium, lipids, and salt.

1.3 Scope

The project was a laboratory scale study on the effect of toxicity on the HASL system. There were many types of factors, which may affect the operation of the HASL system. However, in this study, focuses were on the effect of ammonium accumulation, FOG concentration, and NaCl salt concentration. In order to prevent upsetting of the HASL system due to accumulation of ammonium, the use of aerated submerged biofilter to remove ammonium was also examined in this project.

1.4 Overview

This report comprises six chapters. Chapter 1 describes the anaerobic digestion technology, particularly the HASL system that is being developed at the Nanyang Technology University. The HASL limitations are also discussed and the scope of the project is also delineated in this chapter. The second chapter is a literature review of the research that has been conducted in this area, particularly the toxicity of various elements on the anaerobic processes. Other researchers' works on ammonium removal, effect of FOG, and salt concentration on anaerobic system are also discussed in this chapter. Chapter 3 presents and discusses the ammonia removal in the HASL bioreactor using submerged filter. Chapter 4 and Chapter 5 are presented on study of the influence of FOG and salt concentration on the operation of the HASL system, respectively. Conclusions and recommendation are presented in Chapter 6.

CHAPTER 2

LITERATURE REVIEW

2.1 The Hybrid (HASL) system

The hybrid anaerobic solid-liquid (HASL) system was proven to be suitable for treating waste with high concentration of organic matter (Wang *et al.* 2002; Xu *et al.* 2002a). Organic matter can be degraded into VFA in the acidogenic phase by hydrolytic and acidogenic bacteria, and then converted into biogas in the methanogenic phase.

The system consists of one acidogenic reactor (Ra) and one methanogenic reactor (Rm). One Upflow Anaerobic Sludge Blanket (UASB) reactor seeded with granules adapted to high strength wastewater was operated as the methanogenic phase of this enhanced anaerobic two-phase batch system. During the HASL operation, the leachate from the acidogenic phase was diluted with the effluent from the methanogenic phase. This mixture, as the new influent, was fed into the methanogenic reactor. The methanogenic reactor effluent was divided into two streams, S1 and S2. Typically, the flow rate ratio of S1:S2 was 1:4. S1 flew to the acidogenic reactor, while S2 was used to dilute the leachate of the acidogenic reactor and pump back into the methanogenic reactor. The system setup is illustrated in Figure 2.1.

Figure 2.1 Schematic diagrams of the HASL bioreactor

2.2 Toxicity

Anaerobic fermentation has a reputation of being sensitive to toxicants, and methanogenesis is reported to be the most sensitive step. A common misconception about anaerobic digestion is that the process cannot tolerate toxic substances and that the biomass will die when exposed to toxicants. However, studies on toxicity recovery of methanogenic strains indicated that some toxicants, found in agricultural and industrial wastes, exhibited a bacteriostatic or reversible effect on the methanogens, at the low concentrations normally encountered. Methanogenic bacteria were able to

acclimatize to levels of many times those causing inhibition to unacclimatized methanogens (Parkin and Speece, 1983).

Environmental conditions, such as pH, hydraulic retention time (HRT), total solids (TS) and organic loading rates (OLR), influence the sensitivity of bacteria, the response to toxicity and acclimatization characteristics (Hashimoto *et al.*, 1980). For example, long HRT increases the potential for acclimatization and, in general, minimizes the severity of the response to toxicity. Another important environmental factor involves toxicity of excessive quantities of many common, relatively non-toxic, organic or inorganic substances, which become inhibitory at high OLR values. The threshold toxic levels of inorganic substances vary, depending on whether these substances act singly or in combination. Certain combinations have a synergistic effect, whereas others display an antagonistic effect (Kugelman and McCarty, 1965a; Kugelman and Chin, 1971).

Inorganic cations, such as Ca^{++} , Mg^{++} , Na^{+} , K^{+} , Fe^{++} or NH_4^{+} , which have a stimulatory effect at low or normal concentration, exhibit inhibitory effect at higher concentrations. Inorganic ions, such as $\text{SO}_4^{=}$, and NO_3^{-} are potential inhibitors of methanogenesis in their ability to be alternative electron acceptors (Winfrey and Zeikus, 1977). Sulfide (S^{-}), which is essential for most methanogens, was toxic above 200 mg/L, and was insoluble when heavy metals were present (Zeikus, 1977; Stafford *et al.*, 1981).

Toxic compounds affect digestion by slowing down the rate of metabolism at low concentrations, or by poisoning or killing the organisms at high concentrations. The methanogenic bacteria are generally more sensitive, although all groups of microorganisms involved in digestion can be affected. Due to their slow growth, inhibition of the methanogens can lead to process failure in completely mixed systems, due to "washout" of the bacterial mass (i.e., the draining of bacteria through the outlet at a faster rate than their generation in the digester). In order to control and adjust the

operation, to minimize toxic effect, it is important to identify inhibition factors in its early stages. The two main indicators of inhibition are:

- Reduction in methane yield, indicated by two or more consecutive decreases of more than 10% in daily yield at a constant loading rate
- Increase in volatile acids concentration of the effluent, generally occurring when the total volatile acid (expressed as acetic acid) exceed the normal range of about 250 ppm to 500 ppm

The major toxicants usually encountered with natural feedstocks are ammonium, volatile acids, and heavy metals. High salinity, typically found in seafood wastewater and Asian food might be a cause of toxicity to the anaerobic digestion system. In addition, wastewater discharged from kitchens, restaurants and the food industry often includes lipid materials, such as Fat, Oil and Grease (FOG), which might also affect its operation. In this master thesis, the effect of ammonium toxicity, salinity toxicity, and FOG toxicity would be examined.

2.2.1 Ammonium toxicity

Ammonium toxicity is often a problem in feedstocks with high protein content. Ammonium is rapidly formed in a digester, by breaking down of protein constituents. Free ammonia has been found to be much more toxic than ammonium ion. It is found that the ammonium toxicity thresholds are very sensitive to pH below 7.0.

In general, free ammonia levels should be kept below 80 ppm, to prevent inhibition (Anderson *et al.*, 1982). A much higher concentration, about 1,500-3,000 ppm ammonium ion can be tolerated (Schmid and Lipper, 1969). Concentrations of free ammonia and ammonium ion are related by equilibrium reactions and pH. Table 2.1 shows the antagonistic and synergistic effect of ammonium on anaerobic digestion of solid waste at different concentration.

Table 2.1 Effect of ammonium nitrogen on anaerobic digestion (WPCF, 1987)

Ammonium concentration, NH_4^+ -N, mg/L	Effect
50-200	beneficial
200-1000	no adverse effect
1500-3000	inhibitory at pH over 7.4 -7.6
Above 3000	toxic

2.2.1.1 Nitritification

Biological nitrogen removal

The biological process of nitrogen removal is an important element of wastewater treatment (Figure 2.2). It involves two steps, nitrification and denitrification. Ammonium is first oxidized to nitrite by ammonium-oxidizers and then to nitrate by nitrite-oxidizers in the nitrification process. The overall stoichiometric reactions can be written as follows (Environmental Protection Agency, 1975).

Equations 2.1 and 2.2 are energy yielding reactions. Oxygen serves as the electron acceptor, while NH_4^+ -N and NO_2^- -N serve as the electron donors. Nitrate, which is the product of nitrification, is subsequently reduced to gaseous nitrogen by denitrifiers. The reaction can be generally express as Equation 2.3.

Figure 2.2 Biological nitrogen transformations
(Environmental Protection Agency, 1975)

NO_3^- -N serves as the terminal electron acceptor and some forms of organic matter serves as the election donor. Organic carbon sources are required to accomplish denitrification. When biodegradable organic carbon is not present in sufficient amounts, carbon from an external source must be added.

Factors

influencing

The processes of nitrification and denitrification are affected by several parameters including Dissolved Oxygen (DO) concentration, temperature, pH value, organic carbon loading, carbon/nitrogen ratio, and others. Successful operation of a wastewater treatment system depends upon knowledge of these parameters. Reviews on the

influence of these parameters on nitrifying and denitrifying bacteria or their activity have been widely reported (Painter, 1970; Sharma and Ahlert, 1977).

Dissolved oxygen

One of the most important factors affecting nitrification and denitrification is the level of dissolved oxygen in the reactor. Oxygen is utilized in the oxidation reactions carried out by nitrifying bacteria. The growth rate of nitrifiers is affected at low DO concentrations. Results from a number of studies on this effect are summarized in Table 2.2 (Sharma and Ahlert, 1977). Most denitrifiers are facultative. It has been well established that most denitrifying enzyme systems have to be induced first by growth of the organism in the presence of nitrate and absence of oxygen (Delwiche, 1956; Wainwright and Nevill, 1956).

Strictly anoxic process was initially considered to be most favorable for biological denitrification. This idea was supported by many researchers who have found that very low DO concentrations can cause almost immediate and complete cessation of denitrifying activity in cells (Krul, 1976; Nelson and Knowles, 1978). Kornaros and Lyberatos (1998) studied the changes of transient growth characteristics of *Pseudomonas dinitrificans* from anoxic to aerobic conditions. Each step of the denitrification pathway was affected differently by DO concentrations.

CHAPTER 2 LITERATURE REVIEW

Table 2.2 Effect of dissolved oxygen on nitrification (Sharma and Ahlert, 1977)

DO Concentration (mg/L)	Observation	Circumstances/ Method of Observation
Below 2	Limiting for Nitrosomonas growth	10-1 batches; water from Thames Estuary, England;
Below 4	Limiting for Nitrobacter growth	Determination made from a model
2, 4, 8	Degree of nitrification same: rate about 10% lower at 2mg/L	Small-scale plant
Below 3	Limiting for nitrification	Activated sludge
0.08	Critical	Pure culture of nitrosocystis oceanus (marine species)
>7.5	Inhibiting	
Below 1-1.5	Limiting for growth	Activated sludge
<0.1	Nitrification occurs	Pure culture of Nitrosocystis oceanus
0.5-0.7	Critical for nitrification	Activated sludge
<20% saturation	Limiting for nitrification	Batch test with activated sludge
1	Limiting for nitrification	Pilot plant; activated sludge
0.6-0.7	Limiting for nitrification	Percolating filter; receiving seawater; marine nitrifiers

pH value

pH another important factor that affects the nitrification and denitrification processes. There are three different effect that determine the optimal pH ranges for nitrification. They are:

- Activation-deactivation of nitrifying bacteria
- Nutritional effect associated with alkalinity
- Inhibition through free ammonia and free nitrous acids, and through heavy metals

Different researchers have reported that the optimum pH for nitrification ranged between 7.0 and 8.0 (Jones and Paskins, 1982; Painter and Loveless, 1983; Antoniou *et al.*, 1990). For *Nitrosomonas*, the optimum pH varied from 7.9 to 8.2 whereas it ranged from 7.2 to 7.6 for *Nitrobacter* (Alleman, 1984). Inhibition may occur out of these ranges. Free ammonia and free nitric acids were considered the most important among the three effects (Knowalski and Lewandoski, 1983; Suthersan and Ganczarczyk, 1986). Anthonisen *et al.* (1976) reported the threshold concentration of these matters over which ammonium and nitrite oxidizers were partially or totally inhibited. *Nitrobacter* was heavily inhibited by free ammonia concentration of 0.1-1.0 mg/L. Villaverde *et al.* (1997) stated that pH increase of one unit produced a 13% increase on the nitrification efficiency within a pH range of 5.0-8.5. Ammonium and nitrite oxidizing activity depended heavily on specific free ammonia concentration. Their activities increased with decreasing free ammonia concentration. On the other hand, high pH may cause nitrite buildup in nitrifying systems. Hao *et al.* (1994) reported that pH significantly affected effluent nitrite levels and maximum nitrite concentration occurred at around pH 8.7-8.8 in a submerged filter.

Organic carbon loading and chemical oxygen demand (COD)/N ratio

Organic carbon loading has a significant influence on nitrification and denitrification. Nitrification occurs under high dissolved oxygen and low organic matter concentrations. Autotrophic nitrifying bacteria compete for oxygen with carbon removing aerobic heterotrophic bacteria. Because the yield of new cell material is much lower for the autotrophic nitrifying bacteria than that for the later, heterotrophs grow accumulate much faster than autotrophic nitrifiers when conditions are conducive to both types of microorganisms. High chemical oxygen demand (COD) loading undoubtedly affect nitrification in this way.

Denitrification dominates under high organic matter and low oxygen levels. Carbon/Nitrogen ratio is a critical parameter that determines the rate of denitrification.

Low ratios of COD to nitrogen can lead to poor denitrification (Narkis *et al.*, 1979). Strand (1986) demonstrated that the rate of oxidized nitrogen removal in microbial films exposed to aerobic media could be as high as those observed in anoxic culture provided that the organic matter and microbial biomass are abundant. Wantanabe *et al.* (1995) indicated that an increase in the COD/N ratio increase the efficiency of denitrification under the same oxygen partial pressure in the air phase.

The minimum COD/N ratios required for denitrification are quite different in different systems. Wantanabe *et al.* (1995) noted that the maximum denitrification efficiency of 90% was obtained at COD/N ratio of 6 in a micro-aerobic biofilm system. According to Chui (1998), the minimum COD/N ratio of 5 was required to maintain nitrogen removal efficiency in submerged filters. Benthum (1998) reported a low average COD/N ratio of 2.6kg COD/kgN for denitrification when using acetate as the electron donor in an intermittently aerated airlift reactor. However, a much higher value of 13 to 15 was required for suspended growth systems with predenitrification (WPCF, 1987).

Temperature

Like other biochemical reactions, nitrification and denitrification rates are influenced by temperature. It was reported that nitrification can occur in the range of 4-50°C (Prakasam and Loehr, 1972), and denitrification can occur in the range of 5-60°C (Loehr, 1984). The rates are different at different temperatures even though they can occur over the wide range. Dawson and Murphy (1972) demonstrated that nitrate removal rate was very slow at 5-10°C. Yang *et al.* (1997) stated that the effect of low-temperature (10°C) on the efficiency of nitrogen removal was larger than that of carbon removal when using an entrapped-mixed-microbial-cell process. The optimal temperature for nitrification in pure cultures was reported to be in the range of 30-36°C (Alexander, 1965). An optimal value of 35°C for denitrification was reported in the immobilized sludge process (Chen and Lin, 1993).

2.2.1.2 ~~Refilm~~ ~~iglt~~

Aerated submerged filter, with aerobic and anoxic condition incorporated in the reactor by partial aeration, has demonstrated to be successful in removing ammonium from wastewater (Chui *et al.*, 2001). Their ability to sustain shock loadings because of high bacterial concentrations also makes them ideal for pretreatment of industrial wastewaters. Pilot-scale studies (Paffoni *et al.*, 1990; Dillion and Thomas, 1990; Vedry *et al.*, 1994; Tschui *et al.*, 1994) have confirmed the suitability of using aerated submerged filters for carbonaceous oxidation of settled sewage and tertiary nitrification of secondary effluent. Full-scale applications of biological aerated filters in conventional wastewater treatment facilities have also been reported (Rogalla and Sibony, 1992; Pujol *et al.*, 1994).

Biofilm

A biofilm is mainly composed of microorganisms and extra cellular polymers, which hold the cells together and to the surface of the media (Eighmy *et al.*, 1983). Biofilm composition, physical characteristics, and activity are important parameters for the successful operation and control of fixed film process in water and wastewater treatment. The spatial distribution of different microbial communities within the biofilm matrix and physical properties, such as biofilm thickness, density, and surface morphology, may influence biofilm function. The rates and extent of transport of electron acceptors and donors, and the metabolic products within or through the biofilm were largely dependent on the properties of the internal structure of the film (Christensen *et al.*, 1989).

Bibliography

Biofilm activity is not proportional to the quantity of fixed biomass, but increases with the thickness of biofilm up to a determined level termed the “active thickness”. Above this level, the diffusion of nutrient becomes a limiting factor. When characterizing biofilms and their performance, the distribution of active biomass is very important. This is because of the rates of biochemical transformation are dependent on microbial metabolic activity rather than on cell concentration. On the other hand, the ratio of active biomass to total biofilm amount may influence reactor clogging.

Masuda *et al.* (1991) discovered that the biofilm density and the number of heterotrophs, nitrifiers and denitrifiers per unit biofilm volume increased with the biofilm depth, but intrinsic reactions rates per unit biomass did not significantly change with the depth. Lazarova *et al.* (1994) summarized the specific activity of fixed biomass in airlift reactor, fixed bed, fluidized bed and turbulent bed. The specific activity of biomass fixed in mobile beds increased with biofilm growth. The values observed were up to three times higher than the activity of the enriched culture used for inoculation (4.5 kg $\text{NH}_4^+\text{-N/kg protein/day}$). This could be explained by better mass transfer induced by turbulence and the great mass contact surface areas in mobile particles. Maintaining a thin biofilm reduces diffusion limitation in the fixed biomass.

Okabe *et al.* (1996a) noted that more active cells were present in the surface than in the deeper biofilm. About 15% of total cells were still active in the deeper biofilm, indicating that whatever substrates (i.e., organic carbon and $\text{NH}_4^+\text{-N}$) and oxygen were transported to the deeper biofilm; they can be metabolized. It was also demonstrated that most of the morphologically distinct bacterial cells in the biofilms were physiologically active during the initial biofilm accumulation phase, and that inactive cells gradually accumulated up to 20-30% of total cell population in the biofilm with time (Okabe *et al.*, 1996b).

Miba 1 biofilm

Aerobic heterotrophs, autotrophic nitrifiers, and facultative denitrifiers may coexist in biofilms and accomplish the simultaneous transformation of organic carbon and nitrogen. Autotrophic nitrifying bacteria have less affinity for oxygen and lower maximum specific growth rate than heterotrophic bacteria. The heterotrophs will usually dominate the autotrophic nitrifiers under conditions that are conducive to both types of microorganisms. As a result, nitrifications in attached-growth systems occurs after the organic carbon concentration falls low enough to limit the growth of heterotrophs in the oxygenated zones of the biofilm. This was supported by observations that carbon oxidation occurred at the influent end of a tricking filter and rotating biological contractor systems, while nitrification took place near the effluent end after the Biochemical Oxygen Demand (BOD) levels fell less than about 25mg/L (Torpey *et al.*, 1972).

The favorable operating conditions required to achieve carbonaceous BOD removal and nitrification in a single-stage tricking filter are recommended to be low organic loadings, long residence times, and sufficient oxygen availability. Besides, consistency in hydraulic, organic and ammonia-nitrogen loading are favorable to the removal efficiencies. Surampalli *et al.* (1995) evaluated the nitrification process in the single-stage tricking filters of twenty-seven plants, and concluded that operating at loading levels less than 0.16 kgBOD/day/m³ assured an environment in which the autotrophic nitrifying bacteria could compete with the faster growing heterotrophic bacteria.

Recent investigation have attempted to examine how organic matter influence nitrification. Among the noted effect were a decrease in nitrification rate, nitrite build-up, and competition for oxygen. Rittmann and Manem (1992) reported that, with sufficient oxygen, an increase in acetate concentration would cause ammonium nitrogen fluxes to decrease because nitrifiers were forced deeper into the biofilm. High organic loading rate led to decrease in efficiency. Incomplete nitrification may occur

because of high growth rate of the heterotrophic bacteria, which outgrew the nitrifiers and swept them out of the biofilm (Manem and Rittmann, 1992; Okabe *et al.*, 1995). Leu *et al.* (1998) indicated that increasing organic matter concentration decreased the concentration of nitrite and total nitrogen, but an enormous build-up of nitrite occurred in the water column.

Beg *et al.* (1997a, b, c) contributed three studies on the effect of carbon feed on nitrification in an up-flow packed-bed biofilm reactor. The results showed that organic substrate was consumed rapid near the inlet of the reactor while most of the $\text{NH}_4^+\text{-N}$ was converted in the central region. The reactor effluent showed essentially complete nitrification during methanol fasting conditions. The concentration profiles of oxygen within the biofilm revealed significant variation during methanol fasting and nonfasting conditions, while keeping essentially unaffected during and after $\text{NH}_4^+\text{-N}$ fasting conditions. Moreover, when the inlet concentration of methanol was subjected to sinusoidal variation, the exit concentration of $\text{NH}_4^+\text{-N}$ also followed a sinusoidal pattern although its inlet concentration was constant. These observations indicated that the presence of organics impeded the nitrification process.

2.2.2 Effect of FOG on

Most studies on the effect of FOG on anaerobic digestion system focus on the anaerobic digestion of wastewater. There were limited studies on the effect of FOG on the digestion of solid waste. In this section, past studies on the effect of FOG on anaerobic digestion systems, mostly on anaerobic digestion of wastewater were reviewed.

The major toxicants usually encountered with natural feedstocks are ammonium, volatile acids, and heavy metals. In addition, wastewater discharged from kitchens, restaurants, and the food industry often includes lipid materials such as fat, oil, and

grease. Lipids are one of the major organic matters in wastewater (Yang and Anderson, 1993; Raunkjaer *et al.*, 1994). Most of the time, these types of wastewaters have received certain forms of preliminary physical-chemical treatment, e.g., trapping, intercepting, and flotation separation, before proceeding to the biological stages. Although these processes may attain effective removal efficiency up to 90% of the BOD as lipids, some practical impediments still remain, due to the small amount of partially unremoved emulsified and/or colloidal lipids (Forster, 1992). Economic recovery of lipids is often impracticable because of their association with tissue, soil or fecal matter. The treatment of the wastes with high lipid content is therefore a big technical problem. Large quantities of these highly concentrated lipid wastes (HCLW) are currently treated by combustion or landfill dumping. In recent years, there are concerns that combustion of wastes may lead to secondary air pollution, for example, dioxins (Chang *et al.*, 2000). Furthermore, shortage of land space complicates the landfilling of waste.

Biological wastewater treatment, which is conducted under ambient conditions, is feasible as it does not generate harmful substances, such as dioxins. Conventional activated sludge process, however, cannot treat high BOD-load wastewater, including lipid materials (Hsu *et al.*, 1983). Experience from sewage sludge digestion has proven that anaerobic systems have the ability to degrade fatty matter (Saw *et al.*, 1986). Biogas can be produced effectively by complete anaerobic degradation of fatty acids (Ozturk *et al.*, 1999). Although an advanced anaerobic system, such as the UASB, is known as an effective process for high BOD load wastewater, serious inhibition by long-chain fatty acids (LCFA) at millimolar concentrations also makes its application on HCLW difficult (Hwu *et al.*, 1996). Since the assimilation of oil by microorganisms was dependent on the solubility of oil in the medium (Loehr *et al.*, 1998), the physical properties of HCLW, such as low solubility in water, existence in the solid phase at ambient temperature and high fat concentration, were disadvantageous for biological treatment in a liquid system (Lefebvre *et al.*, 1998).

FOG has a high COD despite its small volume (Quemeneur *et al.*, 1994). A wastewater treatment plant treating high FOG content wastewater are responsible for clogging of wastewater pipes and have resulted in many problems in the wastewater treatment process. A FOG overload will leads to a decrease in treatment efficiency. Usually, the overloading occurred through the proliferation of the filamentous microorganisms and the settling problem in the clarifier (Lefebvre *et al.*, 1998).

2.2.2.1 ~~Dig~~FOG

It has been established since 1950s that the complete degradation of lipids to CH₄ and CO₂ can be achieved in a well mixed anaerobic digester (Heukelekian and Mueller, 1958). Modern concepts of anaerobic microbiology indicate that the methane fermentation of FOG is achieved by the action of three groups of bacteria, namely the hydrolytic fermentative, syntrophic acetogenic, and the methanogenic bacteria.

During the hydrolysis process extracellular enzymes split the carbohydrates, proteins, and lipids of the influent into glycerol and LCFA. This is necessary to enable further breakdown by the bacteria. The speed of the hydrolysis depends on several factors, such as the size and the surface-volume ratio of the particles to split. By taking the sum of all these different processes, a first-order equation may be suitable to describe the total process of hydrolysis (Gujer and Zehnder, 1983).

The most important mechanism of the acetogenesis process is the β -oxidation as demonstrated below (Novak and Carlson, 1970). Due to this mechanism, long chain fatty acids are degraded to shorter fatty acids, mainly acetic acid and H₂.

During the degradation, two carbon acetate groups are removed in each step. Equation 2.4 shows the activation with co-enzyme A. Depending on the chain length (14-20 C, 4-13 C, 2 and 3C) different enzymes are responsible for this activation.

Dehydrogenation follows, i.e. the removal of two hydrogen atoms and the formation of a double bond between the second and the third C atom as illustrated in Equation 2.5.

This double bond is broken as demonstrated by Equation 2.6 and a hydroxyl group is fixed at the third C atom.

In a second hydrogenation as shown in Equation 2.7, two hydrogen atoms are removed from the third C atom and a ketone group is formed.

Subsequently, the coenzyme splits the chain now into another LCFA, but two chain links shorter, and acetic acid as shown in Equation 2.8. These steps are repeated until the LCFA is fully degraded. (Novak & Carlson, 1970)

Unfortunately, full degradation by β -oxidation cannot be achieved in some LCFA, which have an odd number of C atom. In the last step, propionic acid (3 C) will remain

(Novak & Carlson, 1970). The degradation pathway of propionate is still not fully understood. A syntrophic oxidation to H_2 , formate, acetate and, reductive carboxylation to butyrate were just two probable mechanisms, but the detailed biochemical rate was still questionable (Lens *et al.*, 1996). On the other hand 90% of the LCFA in wastewater are myristic acid, palmitic acid, stearic acid, oleic acid, and linolenic acid. These acids have a chain length of 18, 16, and 14 C atoms, i.e., a full degradation should be possible (Novak and Carlson, 1970). β -oxidation is considered to be a limiting step in the anaerobic degradation of complex organic substrates because LCFA oxidizing bacteria were slow growers (growth rate was 0.1-0.3 d⁻¹). Furthermore, as syntrophic substrates, like VFA, their anaerobic microbial degradation was limited by high hydrogen (H_2) partial pressure.

Acetate and hydrogen produced by hydrolytic fermentative bacteria and syntrophic acetogenic bacteria were finally converted into biogas by methanogenic bacteria. In these complex biological reactions, the first step proceeded easily and fast and was not usually the rate-limiting step in the anaerobic digestion of lipids (Heukelekian and Mueller, 1958; Novak and Carlson, 1970). The overall conversion rate is considered to be limited either by the physical process of dissolution and mass transfer of insoluble lipids or by the biological conversion of the LCFA (Novak and Carlson, 1970; Hanaki *et al.*, 1981).

2.2.2.2

Fatty

OG

Lipids in wastewaters are normally recalcitrant to biological treatment because of the tendency of lipids to form floating aggregates by the formation of a light lipid layer around biomass particles. The low surface area to volume ratios of this aggregate slows down their degradation by microorganism (Gujer and Zehnder, 1983). For an effective degradation of lipids, it is very important to disperse the lipids in the substrate for making an uniformity feed. In addition to the above factors, a sufficient mixing for maintaining a good contact between the bacteria and influent lipids in the digester is also necessary (Heukelekian and Mueller, 1958; Renzema *et al.*, 1993). These two points are the most important physical factors affecting FOG degradation.

pH and H₂ partial pressure

In general, a pH of 6-8 should be achieved for anaerobic digestion. However, the pH range for methanogens is somewhat smaller. Optimum condition for methanogens occurs at a pH range of 6.8 to 7.4 (Yang *et al.*, 1990). The H₂ partial pressure has a great influence on the methanogenesis as well as on the acetogenesis processes. As shown in Section 2.2.2.1, during β -oxidation, the LCFA are dehydrogenated two times. Hence, a high H₂ partial pressure inhibits the β -oxidation, and as a result LCFA accumulate while the production of acetic acid decreases. At the same time, methane production decreases since the methanogenesis depends on the degradation of acetate. Subsequently, the lower pH caused by the accumulation of LCFA and VFA lowers the methane production of H₂-oxidizing methanogens. This can trigger a chain reaction, because a lower activity of H₂-oxidizing methanogens means a further increase of H₂ concentration. (Grady *et al.*, 1999)

A H₂ partial pressure of 10⁻⁴ atmosphere is referred to as a common maximum value (Yang *et al.*, 1990; Grady *et al.*, 1999). However, Lee and Zinder (1988) reported much higher H₂ partial pressure in thermophilic (T = 60°C) acetate-oxidizing methanogenic coculture. During the acetate oxidation H₂ partial pressure of 0.02-0.04 atmosphere occurred. Their explanation was due to the higher temperature. While the acetate

oxidation required 1046 kJ at 25°C, it requires only 88 kJ at 60°C. It seemed that thermophilic conditions during methanogenesis allowed a higher hydrogen partial pressure than during mesophilic conditions.

Temperature

The choice and control of temperature is of major importance during the process of lipid degradation. Anaerobic digestion systems can be operated in three temperature ranges, namely the psychrophilic, mesophilic, and thermophilic regions. The mesophilic range (20°C to 40°C) is commonly adopted in full scale applications.

It appears that thermophilic anaerobic condition (45°C to 75°C) improves the degradation process. The higher temperature allows higher loading rates, and shorter solid retention time (SRT) as microbes grow at a rapid rate. This will also results in higher methane production. Further advantage lies in the pathogen inactivation at higher temperatures due to the shorter HRT. However, a decrease in process stability is often noted during thermophilic treatment. Due to the higher growth rate and shorter SRT, the maintenance of the biomass system proved to be more difficult as the microorganisms either accumulate too fast or are washed out of the reactor. Although the higher organic loading rate is said to be one of the advantages of thermophilic treatment, problems caused by incomplete degradation may occur. Yu and Fang (2000) observed an incomplete acidification of lipids in an acidogenic reactor of a two-phase system.

Toxicity

LCFA are well known inhibitors of various microorganisms at low concentration, and consequently, cause some serious problems in anaerobic digestion. LCFA affects either the acidogenesis or the methanogenesis or even both processes of anaerobic digestion at one time. It has been established that a concentration of 1 g/L LCFA in anaerobic

digester sludge is sufficient to cause severe inhibition of the digestion process (Hanaki *et al.*, 1981; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994; Chu *et al.*, 1997). It is reasonable to expect that both methanogenic bacteria and acidogenic bacteria, the two consortia related to LCFA degradation, suffer from LCFA inhibition by protecting the accumulation of LCFA. For this purpose, both increasing the total biomass concentration and promoting methanogenic rate in the methanogenic reactor are effective measures because the former way is expected to increase the LCFA degrading bacteria and the latter may contribute to improve beta-oxidation reactions, and aids in the removal of hydrogen and acetate as shown in the Equation 2.9:

Oleic acid is a major derivative of lipid hydrolysis and one of the most common LCFA. It causes serious problems in biologically anaerobic systems treating FOG wastewaters. Oleate inhibited flocculent sludge more than granular sludge. The toxicity is irrelevant to the specific acetate-utilizing methanogenic activity but significantly dependent on temperature. Acetate-utilizing methanogenesis under thermophilic (55°C) conditions are found to be more susceptible to oleate toxicity than under mesophilic conditions (30°C to 40°C). Fifty percent inhibition concentrations of oleate ranged between 0.35-0.79 mM at 55°C, 0.53-2.27 mM at 40°C, and 2.35-4.30 mM at 30°C (Hwu *et al.*, 1997). Oleate was over 12-fold more toxic to thermophilic flocculent sludge than to mesophilic granular sludge. LCFA toxicity varied with the type of anaerobic sludge and was more correlated to their physical characteristics, specific surface area, and size distribution, than to their biological ones.

Selection of inoculums for full scale reactor treating LCFA or lipids containing wastewater should be based on sludge size rather than on wastewater type. Granular sludge from full scale treatment plant was suggested as appropriate inoculum.

Based on the above fundamental consideration, the HASL system was employed to investigate the effect of FOG on the hydrolysis, acidogenesis, acetogenesis, and methanogenesis on the HASL food waste anaerobic digestion system.

2.2.3 ~~Sludge~~ ~~high~~

For the past decade, anaerobic treatment on wastewater has been a preferred method among many other available technologies, mainly due to its capability in tolerating higher concentrations of inhibitory compounds such as sodium salt. However, the presence of sodium salt has nevertheless hindered this anaerobic technique on a wide range of wastewaters (e.g., wastewaters from chemical production, vegetable canning, edible oil refining, dairy industry, seafood processing, etc.). As the current trend in examining the effect of salinity on anaerobic digestion systems are mainly on the anaerobic treatment of wastewater, it is hoped that similar comparison may be applied to the anaerobic digestion of solid waste. In the following sections, the effect of salinity affecting anaerobic digestion performance on the treatment of wastewater is reviewed.

2.2.3.1 ~~High~~ ~~Salinity~~

Application of anaerobic digestion on wastewaters with high concentration of salt is affected by several parameters. These include the inhibitory and toxicity effect of cations, temperature, pH value, effect of metals, microbial activity, and others. Sufficient knowledge of these parameters is essential as they may be extended to very important conditions for anaerobic solid waste digestion.

Inhibitory and toxicity effect of cations

Research on anaerobic digestion of wastes with high sodium content had been conducted since the late 1960s and results in the establishment of many studies on the effect of sodium salt on methanogenesis. From these studies a general consensus concerning the relative importance of cations and anions (e.g., Na^+ , Cl^- , and SO_4^{2-}) in salt toxicity had been concluded. It was observed that the inhibitory effect of salts on microorganisms was mainly related to the cations (McCarty and McKinney, 1961a). However, many conflicting results were also published depicting the maximum allowable sodium concentration in anaerobic digestors as shown in Table 2.3.

The presence of other cations may cause antagonism or synergism, resulting in differences in the tolerable sodium concentration of at least one order of magnitude. The different species of methanogenic bacteria probably have a different sensitivity for sodium (Liu *et al.*, 1985). Furthermore, it appeared that the sensitivity of bacteria could change as a result of adaptation (Kugelman and McCarty, 1965b). The differences in the test method and the definition of the reference point may inevitably affect the interpretation of the results obtained.

Further studies have been conducted on the response of anaerobic biological treatment systems to wastewater containing high concentrations of sodium cations (Na^+), since sodium salts are commonly adopted in industrial processes. McCarty and McKinney (1961b), and Kugelman and McCarty (1965b) conducted benchmark studies on cation toxicity and the antagonistic or synergistic role of the major cations such as sodium, potassium, calcium, and magnesium. They established that nominally 0.2 M concentrations of these cations caused significant inhibition of methanogens. In their preliminary studies, Li and Speece (1994) found that (1) with proper acclimation, methanogens can function at sodium concentrations of 0.8 M, and (2) careful supplementation of 1 mg/L concentrations of iron, cobalt, and nickel may antagonize the inhibitory effect of sodium at concentration of 10,000 to 20,000 mg/L.

McCarty and McKinney (1961b) showed that cation toxicity may be the cause of anaerobic digester failure, and the addition of only 3,930 mg/L (67.2 mM) of NaCl caused a significant decrease in anaerobic reactor activity. Kugelman and McCarty (1965b) showed that sodium at concentrations near 230 mg/L (10mM) was strongly inhibitory. High sodium concentrations may cause a delay in methane formation in batch reactors and increase the time necessary to achieve the theoretical stoichiometric methane evolution volume from a known mass of substrate. Kugelman and McCarty (1965b), and Kugelman and Chin (1971) showed that the toxic effect of sodium can be antagonized somewhat by potassium and synergised (magnified) by high concentrations (10 mM to 50 mM) of magnesium, calcium, and ammonium.

Where halophilic methanogens were added in the anaerobic digestion of high saline wastewater, the methanogens had the ability to function in a broad range of sodium concentrations at 35°C. The highest initial and maximum specific methanogenic activity (SMA) was found in salt concentrations of 30-35 and 20g/L, respectively. Lower or higher salt concentrations than these resulted in reduced SMA. Increasing salt concentrations also caused the extension of the acclimation time.

CHAPTER 2 LITERATURE REVIEW

Table 2.3 Previous studies on sodium inhibition in anaerobic digestion
(Rinzema *et al.*, 1987)

Reference	Test System	Reference concentration (g Na ⁺ l ⁻¹)	Sodium concentration (g Na ⁺ l ⁻¹) leading to % inhibition		
			10	50	100
Kugelman and McCarty (1965a)	CSTR (15 d), C ₂ , shock exposure enrichment culture, presumably <i>M. sarcina</i> (volumetric CH ₄ production rate)	1.15	4.8	7.2	~10
Kugelman and Chin (1971)	CSTR (15d), C ₂ , contin. exposure	0.46	6.1	7.4	~8
	CSTR (70d), C ₂ , contin. exposure enrichment culture, presumably <i>M. sarcina</i> (volumetric CH ₄ production rate)	0.46	7.0	>8	N.A
Van den Berg <i>et al.</i> (1976)	Batch assay, C ₂ , shock exposure enrichment culture, presumably <i>M. thrix</i> (CH ₄ production rate, 1 day)	0.54	3.6	6.6	N.A
Yang <i>et al.</i> (1979)	Batch assay, C ₂ , shock exposure enrichment culture, presumably <i>M. thrix</i> (CH ₄ production rate, 1 day)	2.20	~6.7	8.7	N.A
Georgacakis and Sievers (1979)	Batch assay, dairy waste, shock exposure lagoon sludge (gas production rate, 1 day)	0.13	~3	6	10-11
Lettinga and Vinken (1981)	UASB reactor, sauerkraut waste, continuous exposure	10	13-16	21-24	N.A
Toldra <i>et al.</i> (1984)	CSTR (42 d), tomato waste	0.02	4	7	~10
	CSTR (70 d), tomato waste continuous exposure (volumetric CH ₄ production rate)	0.02	6.5	9.2	N.A
De Baere <i>et al.</i> (1984)	Anaerobic filter, C ₂ + EtOH	1.7	12.5	15	N.A
	shock exposure continuous exposure enrichment culture, <i>M. sarcina</i> / <i>M. thrix</i> (TOC reduction, CH ₄ production)	1.7	260	39	N.A
Dolfing and Bloemen (1985)	Batch assay, C ₂ , shock exposure granular sludge, <i>M. thrix</i> (specific activity test < 1 day)	2.74	4.5	6.3	~9

N.A: Not Applicable

Metals

Methanogenic bacteria require substantial metal ions for growth including sodium, potassium, magnesium, nickel, cobalt, molybdenum, selenium, and tungsten. Trace metals (particularly iron, cobalt, and nickel) were able to stimulate the activity of specific bacteria at specific salt concentration. Iron slightly stimulated halophilic methanogens and the mixed culture at all salt concentrations. Cobalt was able to stimulate maximum SMA of halophilic methanogens and mixed culture at salt concentrations of 0 and 30-50 g/L, respectively. Nickel had the ability to stimulate the mixed culture with sodium content of 30-50 g/L. Minor benefits were found when nickel was added to halophilic methanogens and digester sludge.

Microbial activity

Previous research has discovered that a rapid increase in salt concentration may lead to a significant inhibition of microbial activity in anaerobic treatment systems. The degree of inhibition can be lowered by slowly acclimating microbial cultures to higher salt levels (Burnett, 1974). High salt levels will cause bacterial cells to dehydrate because of osmotic pressure. Some microorganisms are more susceptible to osmotic pressure than others. Methanogenesis in marine or salt marsh sediments, which contain approximately 3.5% NaCl, has been fairly well documented, mostly in relation to sulfate reduction (Mountfort *et al.*, 1980). The inhibitory effect of sodium has been investigated by Kugelman and McCarty (1965b).

As compared to other metal cations, sodium proved to be the strongest inhibitor on a molar basis. Sodium showed moderate inhibition at 3.5% and strong inhibition at 8% (Kugelman and McCarty, 1965b). Numerous researchers (Yancey *et al.*, 1982; Yerkes *et al.*, 1997) have found that the presence of compatible solutes, or solutes which accumulate to high intracellular concentrations but do not interfere with normal cell

metabolism within bacterial cells, can help to mitigate the osmotic stresses of high sodium concentrations in the external cell environment.

When a microbe is in a non-saline environment, available water can pass through the membrane of the microbe easily. In the non-saline environment, water inside and outside of the cell comes into equilibrium because of diffusion. This implies that the amount of water moving out of the cell is the same as water moving into the cell. This must happen for the organism to survive. However, when salt was added to the water to produce a saline environment, it will create an isotonic condition for the cell. It means that there is more water moving out of the cell than moving into the cell. This results in slower growth for the microbe or even death. As a result of the drying effect of salt, addition of salt to food has been adopted for thousands of years as a preservation method. It usually takes about 20% NaCl concentration to inhibit microbes. White (1982) and Vairavamurthy *et al.* (1985) have also found that several sulfur compounds manufactured by bacteria in response to osmotic stress caused by high exogenous sodium concentrations. These compounds are continuously reduced to dimethylsulfide, a compound, which may serve as an indicator of sodium stress or other forms of toxicity and yield valuable insight into anaerobic reactor performance.

Temperature

There is an increasing interest of introducing a closed treatment cycle in wastewater industries. Therefore it is of utmost importance to evaluate the possibility of anaerobic digestion at elevated temperature under high salinity condition. Extensive studies on the effect of sodium on anaerobic digestion for the treatment of seafood process wastewater (Guerrero *et al.*, 1997) were studied. The activities of methanogenic bacteria were reported to cease at sodium concentration of 1.0% to 1.6% NaCl. However, other studies shows that mesophilic and thermophilic methanization of these wastewaters are not hindered at NaCl concentrations ranging from 1.5% to 2.5%.

pH

The specific pH range affecting inhibitory effect of sodium salts has been studied extensively by many researchers (Rinzema *et al.*, 1988). They discovered that at neutral pH, sodium concentrations of 1.2%, 2.4%, and 3.4% NaCl cause 10%, 50%, and 100% inhibition, respectively, relative to the maximum specific acetoclastic methanogenic activity of the granular sludge used (Rinzema *et al.*, 1988). These values reflected the sensitivity of methanogen towards sodium (Wantanabe *et al.*, 1995). The pH did not significantly affect the inhibitory action of sodium in the range of 6.5-7.2, but at pH level near 8.0 the inhibitory effect was more pronounced. At equal sodium concentrations, sodium chloride has a somewhat stronger effect on the specific activity than sodium sulfate, but the difference between these two sodium salts was of minor importance for design purposes. The results obtained with short-term activity measurements can be used reliably for predicting the effect of sodium salts on continuously fed UASB reactors.

CHAPTER 3

AMMONIUM REMOVAL IN THE HASL SYSTEM USING SUBMERGED BIOFILTER

3.1 Introduction

The operation of the hybrid anaerobic solid-liquid (HASL) system included an acidogenic reactor to treat the solid food waste and an Upflow Anaerobic Sludge Blanket (UASB) methanogenic reactor to treat liquid leachate from the acidogenic reactor. Part of the effluent from the methanogenic reactor was used to dilute the effluent from the acidogenic reactor to obtain optimal pH for methanogenesis. The rest of the effluent from the methanogenic reactor was recycled into the acidogenic reactor to minimize fresh water consumption, and hence prevent wastewater production (Wang *et al.*, 2002).

However, the effluent from the methanogenic reactor contains ammonium and the residual organic carbon. Ammonium was accumulated in the HASL system due to recycling of the effluent from the methanogenic reactor. Accumulation of the ammonium may have negative impact on the anaerobic digestion of food waste. It is known that ammonium is an inhibitor of methanogenesis (Bitton, 1994). It was reported that methanogenic activity dropped to 10% at the concentration of 1670-3720 mg NH_4^+ -N/L, to 50% at 4090-5550 NH_4^+ -N/L, and to zero at 5880-6600 mg/ NH_4^+ -N/L (Lay *et al.*, 1997).

Therefore, the objective of this study was to investigate the performance of the HASL system, enhanced by the addition of the aerated submerged biofilter for ammonium removal.

3.2 Materials and methods

3.2.1 Reactor setup

The enhanced system consisted of an acidogenic reactor (Ra), a methanogenic reactor (Rm), and a submerged biofilter. The reactors were operated in a temperature-controlled room at $35 \pm 1^\circ\text{C}$. Ra reactor was a transparent perspex cylinder with an inner diameter (ID) of 140 mm and a height of 500 mm. The total effective volume was 5.4 L. An UASB reactor, the Rm, was seeded with granules adapted to high strength wastewater. It was operated as the methanogenic phase of the enhanced HASL system. The working volume of Rm reactor was 3.0 L with ID of 90 mm, and a height of 600 mm. A laboratory scale upflow submerged biofilter was cultivated in this study. The submerged biofilter was a reactor 90 cm high with an ID of 75 mm. Siporax glass rings with a diameter of 25 mm were used as carriers. It was piled up in the column to a depth of 70 cm. The biofilter was aerated by compressed air pump through an air stone located at the bottom of the reactor. The biofilter was aerated by compressed air pump through an air stone located at the bottom of the reactor. The system setup is illustrated in Figure 3.1.

Figure 3.1 Schematic diagrams of the enhanced HASL bioreactor for ammonium removal using submerged biofilter

3.2.2 Experimental procedures

The submerged biofilter reactor for simultaneous chemical oxygen demand (COD) and ammonium removal was started up by seeding with 1.5 L of activated sludge collected from Ulu Pandan Water Reclamation plant and 0.2 L of enhanced aerobic nitrifying granules (Tay *et al.*, 2002). It was continuously fed with synthetic wastewater after start up. The chemical composition of the synthetic wastewater is listed in Table 3.1. Ethanol and peptone were used as carbon sources. Ammonium chloride was used as nitrogen source. Some essential nutrients and micro-elements were also provided. Synthetic wastewater was prepared daily, stored in a chiller and fed through the base of the reactor by means of a peristaltic pump. The reactor was operated at a hydraulic retention time of 20 hours. Dissolved oxygen concentration was kept in the range of 3-6 mg/L. The pH value was maintained in the range of 7-8 by adding sodium bicarbonate. Total nitrogen loading was kept in the range of 0.4-0.5 kg-N/m³/d with an influent concentration of 400 mg-N/L. The submerged biofilter reactor was initially operated at organic loading of 1.2 kg COD/m³/d and gradually increased to 3 kg COD/m³/d. The reactor was operated in a constant room temperature at 35°C.

Table 3.1 Chemical composition of synthetic wastewater for submerged biofilter

Constituent	Concentration (mg/L)	Micro-elements	Concentration (mg/L)
Ethanol + Peptone	Adjustable	ZnCl ₂	1
Ammonium chloride	1530	CuCl ₂	0.6
Sodium bicarbonate	Adjustable	MnSO ₄ ·H ₂ O	1
K ₂ HPO ₄	70	H ₃ BO ₃	1
CaCl ₂ ·6H ₂ O	60	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	1
FeCl ₃ ·6H ₂ O	32	AlCl ₃	1
MgSO ₄ ·7H ₂ O	80	CoCl ₂ ·6H ₂ O	1
Na ₂ HPO ₄	150	NiCl ₂	1

After a short period of two months, with the addition of nitrifying granules, strong biofilm capable of simultaneous carbon removal and nitrification was cultivated on the carriers. The submerged biofilter was then connected to the HASL system.

Table 3.2 shows the characteristics of the food waste used in this experiment. In this study, 800 g of food waste were used. The food waste was realistically representative of the Singapore food waste. The food waste was shredded into particles with an average size of 6.0 mm using Robot-Coupe Shredder (CL50 Ultra, France).

The shredded food waste was placed in the reactor. One litre of digested sludge, used as an inoculum for the acidogenic reactor, was collected from an anaerobic digester at the Ulu Pandan Water Reclamation Plant, Singapore. The concentrations of suspended solid (SS) and volatile suspended solid (VSS) in the digested sludge were 4.6 g/L and 3.1 g/L, respectively. Furthermore, 250 ml of distilled water with 2g CaCO_3 was added as pH buffer.

Daily sample was taken from Rm effluent for analysis. Gas production was recorded daily. Gas composition was analysed in terms of nitrogen (N_2), carbon dioxide (CO_2), and methane (CH_4). pH value, concentrations of COD, Volatile Fatty Acids (VFA), NH_4^+ , NO_2^- , and NO_3^- were also studied. Besides, the following parameters were monitored during the experiment period:

- Total solids (TS) and volatile solids (VS) of the food waste
- Recirculation rate in the recycle reactor

In this study, VFA concentration and pH were chosen as indicators of the microbial status in the HASL bioreactors, whereas concentrations of NH_4^+ , NO_2^- , and NO_3^- were measured for the submerged biofilter. COD was selected as an indicator to represent the strength of the leachate. The concentration of nitrogenous compound was used to indicate the performance of the submerged biofilter.

Table 3.2 Characteristics of food waste used in experiments

Food waste	Weight (%)	TS (%)	VS (%TS)	MC (%)
Vegetable roots	50	6.65	71	93.35
Orange peels	20	23.93	97	76.07
Rice	15	9.51	56	90.49
Noodles	15	45.3	96	54.7

3.2.3 Chemical analysis

The concentration of COD and nitrogenous compound were monitored during the course of the experiment. Influent and effluent liquid samples were collected from the reactors. All samples were filtered by 0.45 μm membrane filter paper. The concentrations of COD and ammonium were determined in accordance to the standard method (APHA, 1998). Nitrite and nitrate were analyzed by Flow Injection Analysis (FIA). All data were measured in triplicates.

pH

The pH value was measured using a pH meter (BECKMAN Φ 71) equipped with a glass electrode. Normal range of accuracy was 0.01 pH unit. Sample pH was measured immediately to minimize the pH increase by escaping of dissolved carbon dioxide.

COD

The method used to test the COD value in this experiment was a modified procedure of Standard Method (APHA, 1998) Section 5220D. The samples were diluted as required by the test. 2 ml of each diluted sample was withdrawn and placed in a test tube. 2 ml of digestion solution and 3 ml of sulfuric acid reagent solution were then dispensed into the test tubes. Blanks and standards were tested in duplicate at a minimum. After

heating at 150°C for 2 hours, the COD samples were removed and allowed to cool to room temperature. Upon cooling, the COD values were measured at a wavelength of 600 nm using a spectrophotometer (UV-1201V Shimadzu).

VFA

VFA are perhaps the most common inhibitors of anaerobic system since they are generated as intermediates during the decomposition of organics. It has been identified that the extent of VFA inhibition is influenced by the system pH (Veeken *et al.*, 2000). Samples were filtrated from Whatman 0.2µm cellulose nitrate membrane filters and were then measured by HPLC (Perkin Elmer, Series 200).

Ammonium, nitrite, and nitrate nitrogen

Concentration of nitrogenous compounds such as NH_4^+ , NO_2^- , and NO_3^- of the effluent from the UASB reactor and submerged biofilter were determined as described in Standard Methods (APHA, 1998).

Gas production and composition

The principal gases produced during anaerobic digestion are CO_2 and CH_4 . Lower methane production rates, compared to the influent mass application rate of organic matter, give a warning of the soluble acid products accumulation. Monitoring the gas composition offers insight of the variations in the relative proportions of both gases. The imbalance in the process should be avoided. The gas production of the reactors were recorded daily from a wet gas meter (Ritter TG 05) and the gas composition was analysed by a Hewlett Packard HP5890A (HACH Company, USA) for methane, carbon dioxide, and nitrogen.

Other parameters

TS and VS of the food waste and digested residue were determined on well-mixed samples in triplicates, as described in Standard Methods (APHA, 1998).

3.3 RESULTS AND DISCUSSIONS

This section demonstrates the differences in the performance of the two HASL systems. The first was the control system with only Ra and Rm. For the enhanced HASL system, there was an additional submerged biofilter to Ra and Rm for ammonium removal.

3.3.1 pH

Bacteria inside the anaerobic reactors are usually sensitive to pH. This is particularly true for the methanogens in the Rm reactors. Generally, variations of pH in the Ra reactors reflect the different phase of the acidification process whereas the pH in the Rm is a rough indication to the performance of the Rm reactor. Normally, for a batch HASL system Ra reactor, there will be a drop in pH initially followed by a return to the neutral pH (Xu *et al.*, 2002a). The drop in the pH was due to the rapid accumulation of VFA during the initial stage. As the VFA in the acidogenic reactor was metabolised in the methanogenic reactor, the pH in the acidogenic reactor will return to the neutral pH.

The pH of the leachate from acidogenic reactor in control system was 8.2 at the beginning of the experiment, and it dropped rapidly to 4.8 at the end of day 2 (Figure 3.2). After that, the pH increased steadily to about 7 through the whole operation. The pH change in the enhanced HASL system showed the same trend as the control did, but it was lower than that of the control system. This indicated that hydrolysis and acidogenesis process in the enhanced HASL system were more active than the control system. The pH of the effluents from methanogenic reactors were 7.5 ± 0.5 and 7.8 ± 0.2

for control and the enhanced HASL systems, respectively. The neutral pH of the methanogenic reactors indicated that they were in stable and healthy condition.

Figure 3.2 pH and TVFA changes in leachate from acidogenic reactors of the control and the enhanced HASL systems. (■), pH in leachate of control HASL; (▲), pH in leachate of enhanced HASL; (●), TVFA in leachate of control HASL; (○), TVFA in leachate of enhanced HASL.

3.3.2 Volatile fatty acids (VFA) and chemical oxygen demand (COD)

The highest concentrations of total VFA and COD in the leachate from acidogenic reactor were 12,800 mg/L and 36,080 mg/L in control system, respectively, while in the enhanced HASL system they were 24,090 mg/L and 40,795 mg/L, respectively (Figure 3.2 and Figure 3.3). The higher COD and VFA generation in the acidogenic reactor indicated that with the addition of submerged biofilter, the HASL system had improved on the hydrolysis and acidification. The average concentration of COD in the

effluents from methanogenic reactors of control and enhanced systems were 741 mg/L and 554 mg/L, respectively (Figure 3.4). The COD concentration in the effluent from submerged biofilter was 75 mg/L (Figure 3.4), which was below the allowable limits for trade effluent discharge into a public watercourse (100 mg COD/L) according to the Ministry of the Environment, Singapore (<http://www.nea.gov.sg>). Therefore, organic carbon removal was effective in the submerged biofilter. Overall COD removal efficiency of 99% was achieved in the enhanced HASL system in comparison with 84% in the control HASL system. The TVFA concentrations in the effluent from methanogenic reactors were 52 mg/L and 11 mg/L for control and the enhanced HASL system, respectively.

Figure 3.3 The COD changes in leachate from acidogenic reactors of the control and the enhanced HASL systems. (▲), COD in leachate of control HASL; (◆), COD in leachate of enhanced HASL

Figure 3.4 The COD changes in effluent (□), COD in effluent of control HASL; (○), COD in effluent of enhanced HASL; (■), COD in effluent from submerged biofilter.

3.3.3 Ammonium concentration

Ammonium concentrations in the effluents from the methanogenic reactors in control and the enhanced HASL systems are presented in Figure 3.5. In the methanogenic reactor of the control HASL system, ammonium concentration increased to 1,016 mg $\text{NH}_4^+\text{-N/L}$ on Day 7. The accumulation of ammonium in the control system was expected. This was because in a strictly anaerobic condition, biological method of nitrogen removal using nitrification and denitrification did not take place.

However, stable ammonium concentration, around 40 mg $\text{NH}_4^+\text{-N/L}$, was detected in the effluent from the methanogenic reactor in the enhanced HASL system. Concentration of ammonium was approximately 20 mg $\text{NH}_4^+\text{-N/L}$ in the effluent from submerged biofilter. Ammonium was maintained at a low concentration because

nitrification, which converted ammonium to nitrite and nitrate, was active in the submerged biofilter. As a result, there was no accumulation of ammonium in the system. No nitrate was detected in the effluent from methanogenic reactor (i.e., influent of submerged biofilter), meanwhile concentration of nitrate changed during the experiment from 68 mg NO_3^- -N/L (on Day 2) to 12 mg NO_3^- -N/L (at the end of the experiment) in the effluent from the submerged biofilter (Figure 3.6). Therefore, denitrification was active in the enhanced HASL system.

Figure 3.5 Concentrations of ammonium, (NH_4^+ -N mg/L), in effluent from the methanogenic reactors of the control (◆) and the enhanced (■) HASL systems, and in effluent from the submerged biofilter (▲).

Figure 3.6 Concentrations of nitrate, (NO_3^- -N mg/L), in influent (◆) and effluent (▲) of the submerged biofilter.

3.3.4 Gas production and gas composition

Gas production in the enhanced HASL system was 86 L on Day 14, while it was 54 L in control system (Figure 3.7). This was probably due to the denitrification process within the methanogenic reactor. Nitrate, produced by nitrification in the submerged biofilter, was recirculated and reduced to nitrogen gas in the methanogenic reactor, as shown by increased nitrogen gas content in the biogas. The volume ratio of CH_4 : CO_2 : N_2 was 71: 17: 11 for control, and 56: 13: 31 for the enhanced HASL system. However, the total volume of produced methane (48 L) was higher in the enhanced HASL system than in the control HASL system (38 L). Overall, a methane yield of 0.33 L/g TS added was observed in the control, whereas a methane yield of 0.42 L/g TS added was added in the enhanced system.

Figure 3.7 Biogas and methane production in control and enhanced HASL systems. (◆), biogas production in control HASL; (▲), methane production in control HASL; (□), biogas production in enhanced HASL; (○), methane production in enhanced HASL.

3.4 CONCLUSIONS

The submerged filter was able to maintain low ammonium concentration of less than 150 mg/L in the system. The low ammonium concentration had improved on the performance of the HASL system. The application of submerged biofilter in the HASL system increased COD and VFA generation in the acidogenic reactor, total COD removal, total biogas, and methane production in methanogenic reactor. COD removal efficiency was 99% in the enhanced HASL system, which demonstrated a significant improvement in comparison with 84% in the control HASL system. Methane production in the enhanced HASL system increased by 26% compared with the conventional HASL system. However, methane content in the biogas of enhanced HASL system was lower and nitrogen content was higher than that in a conventional HASL system due to the denitrification process.

CHAPTER 4

INFLUENCE OF FAT, OIL, AND GREASE (FOG) ON THE OPERATION OF THE HASL SYSTEM

4.1 INTRODUCTION

In many Asian countries, the food waste generated is usually accompanied by a high concentration of lipids, such as fat, oil, and grease (FOG). This high FOG trend in Asian food waste is more significant as compared to the western counterparts. In this study, the hybrid anaerobic solid-liquid (HASL) system has been extended to observe the effects of FOG on the hydrolysis, acidogenesis, acetogenesis, and methanogenesis of food waste digestion. Problems affecting anaerobic digestion of food waste with the presence of FOG were highlighted with a laboratory-scale HASL system operation. Understanding the degradation mechanism will enable a better provision in enhancing the treatment of FOG-rich food waste using the HASL system.

4.2 MATERIALS AND METHODS

4.2.1 Reactor setup

The experiment was carried out simultaneously in four identical HASL systems (referred to as Control, 20% FOG, 30% FOG, and 40% FOG in this report; the percentage was in term of dry weight). Each of the HASL systems consisted of one acidogenic reactor (Ra) and one methanogenic reactor (Rm). The HASL system reactors were operated in a temperature-controlled room at $35\pm 1^{\circ}\text{C}$. Each of the Ra reactors was a transparent perspex cylinder with an Inner Diameter (ID) of 140 mm and a height of 500 mm. Four UASB reactors seeded with granules adapted to high strength

wastewater were operated as the methanogenic phase of the HASL systems. The working volumes of each Rm (UASB) reactors were 4.4 L each with the ID of 90 mm and height of 600 mm. The system setup of the acidogenic reactors is illustrated in Figure 4.1. Figure 4.2 shows the actual experimental setup of the HASL batch systems.

Figure 4.1 Schematic diagrams of the acidogenic reactors

Figure 4.2 The HASL batch systems in the laboratory (FOG)

4.2.2 Experimental procedures

Start-up of the HASL system

Before the actual HASL operation, the four Rm reactors with mature sludge granular beds were operated for one week at an organic loading rate of 10,000 mg/L/day. Throughout the one-week operation, the Rm reactors were fed with synthetic wastewater with a concentration of 5,000 mg COD/L. Table 4.1 shows the chemical composition of the synthetic wastewater. Synthetic wastewater was prepared daily, stored in a chiller and fed through the base of the reactor by means of a peristaltic pump.

The reactor was operated at a hydraulic retention time of 12 hours. The pH value was maintained in the range of 7-8 by adding sodium bicarbonate. The reactor was operated in a constant room temperature at 35°C. Subsequently, an effluent concentration of less than 500 mg COD/L (COD removal > 90%) and average methane content of biogas of 75% were observed in all four reactors. This implied that the Rm reactors were active in methanogenesis.

Table 4.1 Chemical composition of synthetic wastewater for the HASL reactors

Constituent	Concentration (mg/L)	Micro-elements	Concentration (mg/L)
Peptone	1000	ZnCl ₂	1
Glucose	3400	CuCl ₂	0.6
Meat extract	700	MnSO ₄ ·H ₂ O	1
Ammonium chloride	800	H ₃ BO ₃	1
Sodium bicarbonate	2000	(NH ₄)	1
K ₂ HPO ₄	200	AlCl ₃	1
CaCl ₂ ·6H ₂ O	48	CoCl ₂ ·6H ₂ O	1
FeSO ₄ ·7H ₂ O	40	NiCl ₂	1
MgSO ₄ ·7H ₂ O	54		

Next, 800 g of synthetic food waste was prepared for each acidogenic reactor. The mixed food waste comprises vegetable roots, orange peels, rice, and noodles. The food waste was shredded by a meal scraps processing machine. Table 4.2 shows the characteristics of the food waste. The portions of FOG added to the reactors were in the dosage of: Control - 0 g, 20% - 26.08 g, 30% - 39.12 g, and 40% - 52.16 g. (weight of FOG/weight of TS of food waste).

On the actual day of the experiment, 1 L of 3% NaHCO₃ was mixed with the food waste as buffer to control pH. The total alkalinity in each reactor was 25 g CaCO₃/kg food wastes. Finally, 1 L of anaerobic sludge was added as inoculum for each reactor. The digested sludge was taken from an anaerobic digester of the Ulu Pandan Water Reclamation Plant, Singapore.

Operation of the HASL Bioreactor

The leachate from the acidogenic reactors were pumped into the methanogenic reactors at a long hydraulic retention time (HRT) of 72 hours due to the high COD concentration of the leachate. The methanogenic reactors were operated at a recirculation rate five times the inlet influent rate.

Samples from Ra reactors leachate and Rm reactors effluent were extracted daily for analysis of pH value, concentrations of COD, and VFA. Gas production for each of the HASL system was recorded daily and the gas sample was obtained to determine its gas composition in terms of carbon dioxide (CO₂), methane (CH₄), and nitrogen (N₂). Experiments on the content of lipids in the initial and final products were carried out to study the efficiency of FOG degradation. Biological samples on the Rm reactors were collected every three days and analyzed to study the methanogenic activity in the reactors. The total solids (TS)/ volatile solids (VS), initial/final weight of the food waste and residue and their respective moisture content were also monitored.

Table 4.2 Characteristics of food waste used in experiment

Food waste	Weight percentage (%)	TS (%)	VS (%TS)
Vegetable roots	50	5.47	60.22
Orange peels	20	43.68	89.60
Rice	15	26.70	94.76
Noodles	15	42.48	89.64

4.2.3 Chemical analysis

pH, COD, VFA and gas production and composition were carried out during the course of the FOG experiment. The procedures for the experiment stated above were described in Section 3.2.3. Lipid extraction and chemical content were also carried out in the experiment and were described in the following sections.

Lipid extraction

The following procedures were used to measure the concentration of lipid in the food waste and residue before and after the experiment. Approximately 10 g of dry samples were required for the extraction. Samples were transferred into tared thimbles. Next, the thimbles were placed into soxhlet apparatus using 200 ml of diethyl ether as solvent. The round bottom flask of the apparatus were then placed into the water bath set at 38°C for 24 hours to extract the lipid content from the samples into the flasks. Subsequently, the water bath was set to 80°C for 12 hours duration to evaporate off the solvent before obtaining the lipid content.

Figure 4.3 Image of the apparatus for lipid extraction

Chemical content

The chemical content of the food waste samples were determined by Elemental Analyzer CHNS/O 2400 (Perkin Elmer, USA). Samples were first oven-dried at 105°C for 24 hours, and then ground to particle size of less than 2 mm. 1.0 mg to 1.5 mg of the ground samples were then put into the sample collector of the elemental analyzer.

Other parameters

TS and VS of the food waste and digested residue were determined on well-mixed samples in triplicates, as described in Standard Methods (APHA, 1998).

4.2.4 Microbiological analysis

The microbial population of methanogens can be enumerated using autofluorescence of co-enzyme F₄₂₀ (Solera *et al.*, 2001) and the measurement of this autofluorescence was used as a relative parameter of methanogenic biomass (Ashby *et al.*, 2001). The measurement of co-enzyme F₄₂₀, which is an essential component of methanogenic biomass, was made using a Luminescence Spectrometer LS-50B (Perkin Elmer, UK) in 3 ml quartz cuvette. The F₄₂₀ was extracted from 0.1 g of the sample into 10 ml of distilled water by boiling for 10 min. An extract was centrifuged and analysed by synchronous scan regime fluorescence spectrometry. Constant difference between excitation and emission wavelengths was 20 nm. The excitation wavelength was changed from 360 to 480 nm and the emission wavelength was synchronously changed from 380 to 500 nm.

4.3 RESULTS AND DISCUSSIONS

Table 4.3 summarises the performance of the four HASL systems with respect to the various chemical parameters used in determining their biodegradation efficiency. It can be seen that the treatment efficiency of 30% FOG is slightly better as compared to its counterparts (i.e., Control, 20% FOG, 40% FOG) in terms of gas production, VS removed and COD / VFA production levels. Further interpretation of the results is demonstrated in the sections that follow.

Table 4.3 Characteristic of the HASL system under different FOG concentration

	Total COD consumed (g)	Total VFA produced (g)	Total gas produced (L)	VS removed/g	gas (L)/g VS removed
Control	51.9±2.1	7.13	33.7	98.3±1.2	0.34
20% FOG	55.8±1.2	7.27	36.0	87.4±1.1	0.41
30% FOG	58.3±3.2	7.42	38.3	66.1±0.7	0.58
40% FOG	51.2±1.3	6.43	33.7	53.7±0.3	0.63

4.3.1 TS, VS and lipid/COD removal rate

Table 4.4 illustrates the contents of TS, VS, and lipid of each acidogenic reactor at the initial and final stages, while Table 4.5 highlights their removal rates. From the tables shown below, the Control obtained the highest TS and VS removal efficiency while 30% FOG attained the largest lipid removal efficiency. TS and VS removal rates in Control, 20% FOG, 30% FOG, and 40% FOG were in the ranges of 43.5%-76% and 41.7%-88.3%, respectively.

Generally, the results presented imply that FOG was less biodegradable compared to the other types of VS. This was shown by an increase in the proportion of lipid in the final residue after 13 days of anaerobic digestion. High content of FOG in the final residue, which tended to degrade when applied to soil, was not ideal for plant growth. This meant that by using the HASL treatment, high FOG food waste (FOG>40%) may not be a suitable source of solid waste for the production of high quality organic fertilizer. This was further elaborated in section 4.3.6.

The lipid removal rate for the Control reactor of 1.78% was the lowest compared to the other three reactors (i.e., 20% FOG, 30% FOG, and 40% FOG). The low lipid removal rate for the Control was due to its low initial lipid content. Besides, the type of lipid found inherent to the food waste in the Control reactor was less degradable. This type of lipid was less exposed to the leachate compared to the FOG that was dosed into the food waste of the other three reactors. This led to a difficulty in hydrolysing the lipid in the Control reactor during the experiment.

The COD removal rate decreased as the FOG concentration in the food waste increased. The decrease was due to two reasons. First, the presence of FOG resulted in a higher initial COD concentration. The high COD influent led to a lower performance of the methanogenic reactors. The second reason was due to the fact that generally FOG consists of more complex molecular structure and less biodegradable. Therefore, under the same HRT, the COD removal rate for the Rm with higher FOG influent was lowered.

Figure 4.4 shows pictures of total solids remaining in the acidogenic reactors on Day 13 of the experiment. By comparison, degradation of food waste for the 20%, 30%, and 40% FOG reactors were less efficient as compared to Control reactor as remnants of food waste were still visible in the TS of the FOG reactors. This phenomenon is also strongly supported by the TS removal rate (Table 4.5). The presence of FOG could have caused inhibition of the digestion process as lipids have the tendency to form

floating aggregates by the formation of a light lipid layer around biomass particles. The low surface area to volume ratios of the aggregate may eventually slow down the degradation process by microorganism (Gujer and Zehnder, 1983). This condition may be further aggravated by an increase in FOG concentration as a decreasing TS removal efficiency trend was observed for the four Ra reactors.

Figure 4.4 Influence of FOG concentration on the food waste end products

CHAPTER 4 INFLUENCE OF FAT, OIL, AND GREASE (FOG) ON THE OPERATION OF HASL SYSTEM

Table 4.4 Initial and final TS/VS/lipid

Food waste	Control		20% FOG		30% FOG		40% FOG	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TS,%	17.0±0.3	8.8±0.1	18.5±0.2	13.1±0.1	18.9±0.4	13.9±0.3	19.4±0.4	12.0±1.4
VS,% of TS	90.9±0.6	60.2±0.8	91.2±0.7	74.9±0.1	92.1±0.7	89.6±0.8	94.3±0.2	89.6±1.4
Lipid, % of TS	2.5±0.6	5.5±0.8	12.1±0.2	24.9±0.5	18.2±0.1	33.1±0.1	22.1±0.8	37.1±0.5

Table 4.5 TS/VS/lipid/COD removal rate

Food waste	Control (%)	20% FOG	30% FOG	40% FOG
TS removal (%)	76.0	66.9	57.9	43.5
VS removal (%)	88.3	71.5	56.6	41.7
Lipid removal (%)	1.78	9.68	23.4	5.00
COD removal (%)	88.9	88.1	87.5	78.2

4.3.2 pH

pH stability played a fundamental role in the HASL system as any variation of pH will affected the performance of the anaerobic bacteria during both acidogenic and methanogenic processes. Generally, variation of pH in the Ra reactors reflects the different phase of the acidification process, while the pH in the Rm reactor indicated the stability of the anaerobic system during methanogenesis. Normally, for a batch HASL system, leachate in the Ra reactor experienced an initial drop in pH followed by a return to the neutral pH. In the case of a healthy Rm reactor, the optimum condition for methanogens falls in a pH range of 7.5 to 8 (Yang *et al.*, 1990). Figure 4.5 and 4.6 highlight the pH variation in both Ra and Rm reactors with different FOG concentrations.

From Figure 4.5, shows that during the start-up period, rapid acidogenesis for all four Ra reactors occurred as the general trend showed a drop in leachate pH value. This finding was consistent with the actual HASL system operation (Xu *et al.*, 2002a). According to Tchobanoglous *et al.* (1993), the drop in pH was due to the breakdown of the food waste to the volatile organic acids (VFA), especially to the smaller chain acetic acid.

pH of the leachate for the Control Ra reactor decreased significantly from 7.87 to 5.68 within the first three days before rising steadily to 8.20 on Day 13. The leachate in the 20% FOG and 30% FOG Ra reactors yielded a similar trend. There was a rapid drop in pH till Day 3 before increasing gradually to about 8.7 on Day 13.

The decrease in leachate pH value during the start of the experiment was caused by the hydrolysis and acidification of the food waste, which led to the accumulation of VFA. Subsequently, the Ra reactors experienced a decrease in VFA concentration as the methanogens in Rm reactors converted VFA into biogas. This led to a general increase in pH readings from Day 4 to Day 13.

The faster dip in pH values for the leachate in the Control Ra reactor as compared to the other three Ra reactors (20% FOG, 30% FOG, and 40% FOG) suggested that the presence of FOG inhibited the hydrolysis and acidification of food waste.

Figure 4.6 shows that the pH values of the effects of the Control, 20% FOG, 30% FOG and 40% FOG Rm reactors were relatively constant in the range of 7.78-8.71. This implied that the four UASB reactors were healthy during methanogenesis. Furthermore, the hydraulic retention time of 72 hours was satisfactory as the methanogens were allowed to utilize the accumulated VFA at their pace. Thus, neutral pH readings for the four Rm reactors indicated operational stability during the whole HASL process.

Figure 4.5 Influence of FOG concentration on pH of Ra reactors

Figure 4.6 Influence of FOG concentration on pH of Rm reactors

4.3.3 Chemical oxygen demand (COD)

COD is a very important parameter for biological degradation process. The COD trends for the acidogenic and methanogenic reactors indicated the effectiveness of organic breakdown during anaerobic digestion. Variations of COD in the Ra and Rm reactors are illustrated in Figures 4.7 and 4.8 respectively.

From Figure 4.7, similar trends of COD are observed for the leachates in Control, 20% FOG, 30% FOG, and 40% FOG Ra reactors. Peak COD concentrations for the four Ra reactors were clearly visible on Day 2 followed by a decline from Day 3 to Day 13. The COD concentration of the leachate in the Control Ra reactor was 7,770 mg/L on Day 0. It increased rapidly to a peak concentration of 36,290 mg/L on Day 2. Following that, COD concentration dropped consistently and stabilized at 5,300 mg/L towards the end of operation.

The 20% FOG, 30% FOG, and 40% FOG Ra reactors were observed to have displayed a similar trend, having reached an average peak COD concentration on Day 2 before experiencing a gradual drop till Day 13.

Further interpretation of the data suggested that with an increased in FOG content, a substantially high COD concentration was obtained in the leachates of the Ra reactors. This was consistent with the findings of Quemeneur *et al.* (1994). They observed that lipids contained a high COD despite its small volume. By displaying such a trend, it implied that with the presence of FOG in food waste, the COD concentration in the leachate of the Ra reactors would increase significantly.

Figure 4.8 shows the influence of FOG on the COD concentration of Rm reactors. The COD removal rate in the four Rm reactors, except 40% FOG, remained efficient. They were in the range of 87.5%-88.9%. However, the COD removal rate for 40% FOG Rm reactor was less efficient at 78.2%. The COD concentrations of the effluent for the Control, 20%, and 30% FOG Rm reactors peaked on Day 2 before decreasing to approximately 800 mg COD/L on Day 13. On the other hand, the COD concentration of the effluent peaked at a high of 5,000 mg COD/L on Day 4, before decreasing to a healthier level. This suggested that the microorganism in the 40% FOG Rm reactor required a longer acclimatisation period as compared to the other three Rm reactors.

The COD removal efficiencies was calculated by the (total amount of COD going into the methanogenic reactor - total amount of COD going out of the methanogenic reactor) and divided by (total amount of COD going into the methanogenic reactor).

Figure 4.7 Influence of FOG on the COD concentration of Ra reactors

Figure 4.8 Influence of FOG on the COD concentration of Rm reactors

4.3.4 Volatile fatty acids (VFA)

Volatile fatty acids (VFA) are important intermediates obtained from the breakdown of organic compounds to acetate and finally to methane. The accumulation of VFA may inhibit the production of methane during the anaerobic digestion processes.

Figure 4.9 illustrates the influence of different FOG concentrations on the Ra reactors VFA production. The four Ra reactors accumulated high levels of VFA particularly during the first three days of operation. This effect was due to the constant acid production during hydrolysis and acidogenesis. Subsequently, no further increases in VFA concentration were observed throughout the rest of the experiment. This may have resulted from the conversion of certain portion of VFA by the acidogenic and methanogenic microorganisms in the Rm reactors.

The acidogenic reactors with 0% FOG addition, 20% FOG addition, 30% FOG addition, and 40% FOG addition released a total VFA of 7.13 g, 7.28 g, 7.42 g, and 6.42 g respectively during the 13 days operation. It was noted that the rate of VFA production decreased with an increased in FOG concentration in the food waste. During the first two days of operation, for the Control Ra reactor, the VFA concentration increased rapidly to 1,724 mg/L. In the case of the Ra reactors with 20% FOG, 30% FOG, and 40% FOG, their VFA concentrations on the second day were 1,584 mg/L, 1445 mg/L and 709 mg/L, respectively. This could be attributed to the inactivation of acidogenic bacteria when FOG was present in higher concentration.

Figure 4.9 Influence of FOG concentration on Ra VFA production

Table 4.6 Total VFA-Ra production

Food Waste	Total VFA produced (g)
Control	7.13
20%	7.28
30%	7.42
40%	6.42

4.3.5 Gas production and composition

Methane is one of the major biogas generated from anaerobic digestion process. The amount of biogas production and its composition are two important parameters in determining the effectiveness of an anaerobic treatment system. Past research conducted on the HASL system had shown that methane generated in Rm reactor had constituted up to 99% of total methane yield of the HASL bioreactor (Xu *et al.*, 2002). This was probably because Rm reactor effluent could not bring enough methanogenic bacteria to Ra reactors. By using an UASB reactor, Rm reactor retained its biomass in the form of sludge granules, and biomass wash out was scanty (Young and Dahab, 1983; Thaveesri *et al.*, 1994).

Figure 4.10 shows the variations in the biogas production when different amount of FOG were added to the food waste. Within 24 hours of the commencement of the experiment, methane production rate and accumulative methane yield in the four Rm reactors increased rapidly at an increasing rate before declining on Day 4. It was observed that for the Control Rm reactor, gas was produced readily when the leachate from the Control Ra reactor was fed to the Rm reactor. However, for the Rm reactors containing FOG concentration, there was an acclimatisation period as their initial gas production rate was slow. This phenomenon occurred probably due to the tendency of lipids to form a light lipid layer around biomass particles which hindered the methanogenesis process. The gas production gradually increased at an increasing rate as the methanogen became acclimatised to the food waste leachate on Day 4.

Interestingly, the biogas production rate and methane percentage increased with the higher influent FOG concentration in the influent. The results showed that lipids had a higher gas production potential as compared to carbohydrates and proteins. The cumulative biogas production of Rm reactors for the Control and 20% FOG addition were 33.66 L and 35.96 L, respectively. 30% FOG addition produced the highest cumulative gas production of 38.27 L. Table 4.7 shows the respective average gas

composition generated by each Rm reactors. The methane content for biogas generated from the Control Rm reactor was 78%, while the 30% FOG and 40% FOG reactors contributed 83.5% and 69.5%, respectively. It was clear that effective treatment performance for FOG-rich food waste was obtained up to a content of 40%. This was reflected by the biogas production rate of 40% FOG, which obtained the lowest cumulative biogas generation of 33.73 L and an average methane gas content of 67.9% throughout the experiment.

Figure 4.10 Influence of FOG concentration on gas production

Table 4.7 Average gas composition generated by the methanogenic reactors Rm

Gas Composition (%)	CH ₄	CO ₂	N ₂	L-CH ₄ /g-COD
Control	78.0	13.3	8.7	0.35
20% FOG	69.5	21.2	9.3	0.33
30% FOG	83.5	7.2	9.3	0.33
40% FOG	67.9	25.9	6.2	0.32

4.3.6 Chemical composition in CHNS of the HASL end products

Table 4.8 shows that the HASL end products have a reasonably high nitrogen value between 2.4% and 3.7%. This would enable the digestion residue be used as a valuable organic fertilizer. Table 4.8 also presents the differences in the C/N ratio when different amount of FOG were added to the food waste. Under the controlled conditions, C/N ratio of the compost can be used as an indicator of stability. Generally, the initial C/N ratio must be at or near of 30, while the finished compost C/N ratio drops to below 21.

It was shown that as the amount of FOG added to the food waste increased, the C/N ratio of the digestion end products also increased. The Control digestion end product had a C/N ratio of 13.6, while the digestion residue with 40% FOG addition had a C/N ratio of 24.3. The increase in the C/N ratio was an indication of the instability in the end product when FOG was added. When FOG was added to the food waste, the digestion process was not as complete as compared to the case with no FOG added. Unstable end product was not desirable because under humid and warm condition, unstable end product would continue to undergo active microbial degradation. Generally, C/N ratio of compost was an indication of the compost stability for the production of organic fertilizer. However, further tests are needed for more complete examination of the compost.

Table 4.8 Chemical composition of the HASL end products

Food Waste	Composition				C/N ratio
	C	H	N	S	
Control	49.8±1.2	7.4±0.3	3.7±0.3	0.9±0.1	13.6
20% FOG	51.2±1.2	7.7±0.3	3.2±0.3	0.9±0.3	15.9
30% FOG	52.7±0.4	8.1±0.4	2.8±0.01	1.0±0.03	19.5
40% FOG	57.1±0.3	9.3±0.2	2.4±0.1	1.0±0.1	24.3

4.3.7 Methanogenic activity analysis

The F_{420} analysis was adopted for a rapid assessment of the potential methanogenic activity of anaerobic granule, indicating possible inhibition of the methanogenesis. Figure 4.11 illustrates the methanogen count for the biomass of the four Rm reactors using F_{420} autofluorescence spectrometry. The F_{420} content of the four UASB reactors were measured in order to determine their specific methanogenic activity during the experiment.

From Figure 4.11, a typical growth curve was not fully observed for the Rm reactors. A typical growth curve has several distinct phases: lag phase, log phase, stationary phase, and death phase. Lag phase is a time for the adaptation of the bacteria to new conditions. Log phase (or exponential phase) is a period of fast growth. Stationary phase is when the bacteria population is stagnant due to the equilibrium reached between growth rate and decay rate of bacteria. Death phase occurs when food was depleted and bacteria population decreased.

Lag phase was not observed on the Control and 20% FOG Rm reactor. This indicated that no acclimatization period was required. Higher percentage of FOG present in Ra reactor leachate may have induced the lag phase until the culture was adapted. This was observed in the 30% FOG Rm reactor. Similar observations were shown in the case of other inhibiting compounds such as high ammonia concentrations (Van Velson, 1979). However, an adverse effect was obtained on the methanogen count for the biomass in 40% FOG Rm reactor. A moderate drop in methanogen count was observed from Day 0 to Day 5. The reactor recovered to its original condition after Day 5, which as can be seen from the increase in methanogen count.

Further interpretation of the results highlighted the presence of FOG as a possible inhibitor during methanogenesis as the biomass in the Control Rm reactor obtained the highest relative methanogen count during 13 days of batch operation. Subsequently, a

progressively higher FOG content appeared to have resulted in lower methanogenic activity of the UASB reactors. The biomass in the Control Rm reactor obtained a peak relative methanogen count of 1.53 on Day 2, while the peak relative methanogen count for the remaining three reactors fell within the range of 1-1.38.

Figure 4.11 Influence of concentration of FOG on methanogens determined by autofluorescence F_{420}

4.4 CONCLUSIONS

This research work was aimed to understand the influence of different FOG concentrations in food waste on the performance of the HASL system. To achieve this goal, food waste FOG up to 40% was used as the feedstock for the HASL system. The following conclusions can be drawn from this study.

- Generally, the methanogenic reactor of the HASL system was not affected by FOG addition up to 40% FOG addition. COD removals in the four methanogenic reactors, except 40% FOG, remained efficient, which falls within the range of 87.5%-88.9%. The good performance of these methanogenic reactors were further reflected by the stable pH range of 7.78-8.71 throughout the operation. However, the higher effluent COD for the 40% FOG methanogenic reactor (Rm) suggested that a longer acclimatisation period was required as compared to the other three Rm reactors.
- Generally, the Rm reactors of the HASL system were not affected by FOG addition up to 40% FOG addition. The COD removals in the four Rm reactors, except 40% FOG, remained efficient, which falls within the range of 87.5%-88.9%. The good performance of these methanogenic reactors were further reflected by the stable pH range of 7.78-8.71 throughout the operation. However, the higher effluent COD concentration for the 40% FOG Rm reactor suggested that a longer acclimatisation period was required as compared to the other three Rm reactors.
- FOG concentration would affect the hydrolysis and acidification in Ra reactors more than methanogenesis in the Rm reactors. This was shown as TS and VS removal efficiencies decreased as the concentration of the FOG dosage increased. The VS removal efficiency decreased from 88.3% in the Control Ra reactor to 41.7% in the 40% FOG Ra reactor.

- Poor degradation of FOG in high FOG concentration food waste may result in unstable organic fertilizer. It was suggested that food waste with FOG>20% was not a suitable source of solid waste for the production of high quality organic fertilizer.
- Lipids had a higher gas production potential as compared to carbohydrates and proteins. The biogas production per gram of VS consumed increased from 0.34 L/gVS removed in the Control to 0.63 L/gVS removed in the 40% FOG.
- Presence of FOG was a possible inhibitor to methanogenesis. This was supported by the biological analysis for the four Rm reactors. The biological analysis showed a gradual decreased in methanogenic activity as influent FOG content increased.

CHAPTER 5

INFLUENCE OF HIGH SALT CONTENT ON THE OPERATION OF THE HASL SYSTEM

5.1 INTRODUCTION

The hybrid anaerobic solid-liquid (HASL) system was designed to digest a variety of food waste, including leftovers, which may contain high salt contents, fat, oil, and grease (FOG). This was especially prevalent in the Asian cuisines. To ensure a smooth operation of the HASL system, the issues of possibly high salt concentration in the food waste was looked into. The laboratory scale HASL systems were operated to test the influence of salt presence on the process of anaerobic digestion of food waste. A range of salt concentrations, i.e., 0% (Control), 1%, 2%, and 3% of NaCl salt were dosed to the food waste. The objective of this study was to monitor the stability of the system under unfavorable conditions.

5.2 MATERIALS AND METHODS

5.2.1 Reactor setup

The lab-scale HASL system consisted of one acidogenic reactor (Ra) and one methanogenic reactor (Rm). The reactors were operated in a temperature-controlled room at $35\pm 1^{\circ}\text{C}$. Ra reactor was a transparent perspex cylinder with an Inner Diameter (ID) of 140 mm and a height of 500 mm. The total effective volume was 5.4 litre. Upflow anaerobic Sludge Blanket (UASB) reactor that was seeded with anaerobic granules adapted to high strength wastewater was operated as the methanogenic phase

of this two-phase anaerobic system. The working volume of Rm reactor was 4.4 L with ID of 110 mm and a height of 600 mm. The system setup is illustrated in Figure 5.1.

Figure 5.1 Schematic diagram of the HASL system

5.2.2 Experimental procedures

Four different concentrations of NaCl salt were examined in this study to assess the influence of different NaCl concentrations on the HASL system. The HASL system comprised of a methanogenic reactor, Rm, which was operated for one week at an organic loading rate of 10,000 mg/L/day prior to the beginning of the experiment.

During the one week operation, the Rm reactors were fed with synthetic wastewater with a concentration of 5,000 mg COD/L. The chemical composition of the synthetic wastewaters listed in Table 4.1. Ethanol and peptone were used as carbon sources. Ammonium chloride was a source of nitrogen. Alkalinity was provided by sodium bicarbonate. Some essential nutrients and micro-elements were also provided. Synthetic wastewater was prepared daily, stored in a chiller and fed through the base of the reactor by mean of a peristaltic pump. At the end of the one week, an effluent concentration of less than 500 mg COD/L (COD removal>90%) and average methane content of biogas of 75% were observed. This indicated that Rm reactors were highly active in methanogenesis.

Next, 800 g of synthetic food waste was prepared for each setup. Characteristics of the food waste are shown in Table 4.2. 1 L of anaerobic sludge was added as inoculum. 1 liter of 3% NaHCO₃ was added as a buffer to control pH. The total alkalinity in the food waste was 25 g CaCO₃/kg food waste. NaCl salt was added to the food waste in the dosage of 0% (0 g), 1% (28 g), 2% (56 g), and 3% (84 g). The percentage referred to weight of salt/weight of substrate.

Leachates from the Ra were pumped to the Rm at a long hydraulic retention time (HRT) of 72 hours due to the high COD concentration of leachate. The Rm reactors were operated at a recirculation rate five times of the inlet effluent rate.

Samples from Ra reactors leachate and Rm reactors effluent were taken daily for analysis. Gas productions were recorded daily and gas compositions were analysed in terms of carbon dioxide (CO₂), methane (CH₄), and nitrogen (N₂). pH value, COD and VFA were also studied. The TS, VS of the food waste and recirculation rate in the recycle reactor were also monitored during the experiment period.

5.2.3 Chemical analysis

Concentration of NaCl, COD, VFA production, pH, gas production, and gas composition in the HASL system were monitored during the course of the experiment. Influent and effluent liquid samples were collected from the reactors. All samples were filtered by 0.45 μm membrane filter before testing. The pH, COD, VFA, gas production and composition tests were described in Section 3.2.3.

NaCl concentration

The NaCl concentration was measured using a conductivity meter (Horiba ES-14E) equipped with a glass electrode. Normal range of accuracy was 0.01% unit. Samples were measured immediately.

Other parameters

TS and VS of the food waste and digested residue were determined on well-mixed samples in triplicates as described in Standard Methods (APHA 1998).

5.3 RESULTS AND DISCUSSIONS

Table 5.1 summarises the performance of the four HASL systems with respect to the various chemical parameters that were used in determining their biodegradation efficiency. It can be shown from the table that additional of salt indeed had an adverse effect on the HASL system. The COD production, VFA production and gas production all decreased as the concentration of the salt increased. Further interpretations of the results are discussed in the following sections.

Table 5.1 Characteristic of the HASL system under different NaCl concentrations

	Total COD consumed (g)	Total VFA produced (g)	Total gas produced (L)	VS removed/g	gas (L) / g VS removed
Control	80.1±1.2	8.30	48.1	135.2±2.1	0.36
1% NaCl	79.1±0.9	8.09	47.2	118.7±3.2	0.40
2% NaCl	75.0±1.1	4.09	48.5	106.3±1.5	0.46
3% NaCl	45.6±2.1	2.05	30.8	80.4±3.1	0.38

5.3.1 pH

Methanogens are particularly sensitive to pH in the Rm reactors. Normally, for a batch HASL system Ra reactor, there will be a decrease in pH initially followed by an increased to the neutral pH, whereas for a healthy Rm reactor, the pH shall maintain fairly constant at neutral level (Xu *et al.*, 2002a). Figure 5.2 and Figure 5.3 shows the variations of pH in the Ra and Rm reactors, respectively when different concentrations of NaCl salt were dosed to the food waste.

In Figure 5.2, all four concentrations of NaCl show a decrease in the pH initially before they rose to the neutral level. This agreed with the past observation of the HASL system operation. The drop in the pH was due to the breakdown of food waste to VFA, especially to the smaller chain acetic acid (Tchobanoglous *et al.*, 1993).

However, among the four Ra reactors, leachate from the Control Ra reactor showed the greatest decrease in pH. The pH of the leachate in the Control Ra reactor decreased significantly from 8.10 to 4.78 within the first 2 days, after which the pH remained fairly constant at neutral level. For the Ra reactor with the addition of 1% NaCl salt to the food waste, the performance of the pH variation was quite similar to that of the

Control Ra reactor. The pH reading decreased from 8.45 on Day 0 to 4.9 on Day 2 and stabilized at about 7.5 thereafter. It implied that with the addition of 1% NaCl salt to the food waste, there was no significant influence on the acidification process. For the leachate in the 2% NaCl Ra reactor and 3% NaCl Ra reactor, the pH decrease in the first two days were less drastic than that of the leachate in the Control Ra reactor and 1% Ra reactor. The minimum pH for the leachates in the 2% NaCl Ra reactor, and 3% NaCl Ra reactor were 5.58 and 6.49, respectively. The decrease in the pH value was due to hydrolysis and acidification of the food waste which had caused VFA to accumulate. Therefore, the faster decrease of the pH values in the Control Ra reactor and 1% NaCl Ra reactor compared to the 2% NaCl Ra reactor and 3% NaCl Ra reactor suggested that food waste with the addition of more than 2% NaCl reactor inhibited hydrolysis and acidification.

Figure 5.3 shows the stable pH level of the Rm reactor effluents, which ranged from 7 to 8. This was a rough indication that the performances of Rm reactors were stable. It also suggested that the Rm reactors were generally less sensitive when compared to the Ra reactors.

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Figure 5.2 Influence of NaCl concentration on the pH readings of Ra reactors

Figure 5.3 Influence of NaCl concentration on the pH readings of Rm reactors

5.3.2 Chemical oxygen demand (COD)

The influence of different NaCl concentrations in the food waste on the COD concentration of the Ra reactors leachates are illustrated in Figure 5.4, while the COD removal efficiencies in the Rm reactors are illustrated in Figure 5.5.

Figure 5.4 shows the COD concentration variations of the leachate from the Ra reactors for the 13 days batch operation. It was shown that leachate from the Control Ra reactor had the highest COD concentration at 36,080 mg/L on Day 2 of the operation. For the leachate from 1% NaCl Ra reactor, the peak concentration was 30,587 mg/L, which was slightly less than the Control Ra reactor. This indicated that food waste with an addition of 1% NaCl, the inhibition to hydrolysis was not significant. The result also showed that the inhibition to hydrolysis was more significant at 2% NaCl addition and 3% NaCl addition. At 2% NaCl addition, the leachate peak concentration was 20,720 mg/L, which was only 57% of the peak concentration of the Control leachate. For the food waste with 3% NaCl addition, the leachate peak concentration was 11,100 mg/L, which was only 30% compared to the leachate peak concentration of the Control Ra reactor. Thus it showed that when the concentration of NaCl increased, the COD concentration in the leachate decreased. It was more significant at concentration more than 2% NaCl. This could be attributed to the poor hydrolysis of the food waste when excess NaCl salt was presented. The total COD hydrolysed from 800g of food waste were 217 g, 183 g, 94 g, and 60 g for the Control, 1% NaCl, 2% NaCl, and 3% NaCl Ra reactors, respectively.

As for the Rm reactors, the average COD removal efficiency for the Control reactor was the highest of 93.8%, followed by the removal efficiency of the 1% NaCl Rm reactor 92.8%. At 2% NaCl addition, the removal efficiency was 88.9% and at 3% NaCl addition, the removal efficiency was 85.1%. These results were comparable to the literature, which stated 10% inhibition in COD removal at 3% NaCl under continuous exposure (Lettinga and VinKen, 1981).

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Table 5.2 Peak COD concentrations and percentage inhibition of NaCl on the HASL system

	Control	1% NaCl	2% NaCl	3% NaCl
Peak COD concentration (mg/L)	36,080	30,587	20,720	11,100
Percentage Inhibition (%)	0	15.2	42.6	69.2

Figure 5.4 Influence of NaCl concentration on the COD concentration of Ra reactors

Table 5.3 COD consumption and removal efficiency by the HASL reactors

	Control	1% NaCl	2% NaCl	3% NaCl
COD consumed (g)	80.1	79.1	75.0	45.6
COD removal efficiency (%)	93.8	92.8	88.9	85.1

Figure 5.5 Influence of NaCl concentration on the Rm COD removal efficiency

5.3.3 Volatile fatty acids (VFA)

Figure 5.6 shows the influence of different amount of NaCl addition to the Ra reactor VFA production. It clearly indicated that when the concentration of NaCl increased, VFA production in the Ra reactor was decreased. The Ra reactors with 0% NaCl addition, 1% NaCl addition, 2% NaCl addition, and 3% NaCl addition released a total VFA of 62.9g, 61.3g, 31.0g, and 15.5g, respectively, during the thirteen days of operation. From the results, it can be deduced that 1% NaCl addition to the food waste had marginal influence on the HASL system, whereas 2% NaCl addition would cause approximately 50% inhibition on VFA production and 3% NaCl would cause 75% inhibition. It was also noted that the rate of VFA production was also slower with an increase in NaCl concentration to the food waste. During the first two days of operation, the VFA concentration in the Ra reactor increased rapidly to 12,800 mg/L for the Control Ra reactor. But for the Ra reactor with 1% NaCl addition, 2% NaCl addition, and 3% NaCl addition, the VFA concentration on Day 2 was 8,200 mg/L, 5860 mg/L, and 3750 mg/L, respectively. This could be attributed to the inactivation of acidogenic bacteria when NaCl was present in larger amount.

The VFA concentrations in the effluents of the Rm reactors remained low except for the case of 3% NaCl. This was an indication that the methanogenic reactors were stable during the 13 days of the HASL operation when the concentrations of NaCl were low. However, when the NaCl concentrations in the food waste increased, the VFA of the effluent also increased. At 2% NaCl addition to the food waste, the peak VFA concentration detected in the effluent was 135 mg/L. This amount increased dramatically to 1,053 mg/L for the case of 3% NaCl. This indicated that the Rm reactor was sensitive when 3% NaCl was added to the food waste. Overall, the use of the UASB reactor was considered efficient in treating the influents with the removal efficiency more than 99%, with the exception of 3% NaCl. From 2% NaCl to 3% NaCl, the VFA removal efficiency decreased from 99.5% to 73.3%. This showed that inhibition occurred at 3% NaCl.

Table 5.4 VFA produced and percentage inhibition of Ra reactors

	Control	1% NaCl	2% NaCl	3% NaCl
VFA produced (g)	8.30	8.09	4.09	2.05
Percentage Inhibition (%)	0	2.54	50.7	75.4

Figure 5.6 Influence of NaCl concentration on the VFA Ra production

Figure 5.7 Influence of NaCl concentration on VFA in Rm effluent

5.3.4 Gas production and gas composition

The amount of biogas production and its composition is an important parameter to determine the effectiveness of an anaerobic digestion system. Past research on the HASL system had shown that methane generated in Rm reactors had constituted up to 99% of total methane yield of the HASL bioreactor (Xu *et al.*, 2002a). This was probably because Rm reactor effluent could not bring enough methanogenic bacteria to Ra reactor. As a UASB reactor, Rm reactor retained its biomass in the form of sludge granules, and biomass wash out was scanty (Young and Dahab, 1983; Thaveesri *et al.*, 1994). Consequently, in this study on the influence of NaCl concentration in the food waste to the HASL system, the focus was on the biogas production of the Rm reactors.

Figure 5.8 shows the changes in the biogas production when different amounts of NaCl were added to the food waste. It was noted that the total biogas production in the Rm

reactors with 0% NaCl, 1% NaCl, and 2% NaCl were comparable at about 47 L to 49 L. The performance of the Rm reactors for the Control and 1% NaCl addition were quite similar. It indicated that there was little inhibition of NaCl at 1%. At 2% NaCl, although the total biogas produced was only slightly less than that of the Control, the methane composition in the biogas was lower at $65\pm 6\%$ compared to the Control with methane composition at $72\pm 4\%$. This might be due to the inactivation of the more sensitive methanogen. The rate of biogas production in Control and 1% NaCl reactors were also much higher than that of 2%. As for the biogas production in the 3% NaCl, it was much lower at 30.77 L. The methane composition was similar to 2% NaCl at $63\pm 6\%$. Once again, it showed that inhibition occurred at 3% NaCl.

Table 5.5 Gas production and gas composition of Rm reactors

	Control	1% NaCl	2% NaCl	3% NaCl
Total Gas Production/L	48.1	47.2	48.5	30.8
CH ₄ (%)	72.0	71.0	65.0	63.0
CO ₂ (%)	6.5	6.8	8.3	7.0
N ₂ (%)	21.5	22.2	26.7	30.0

Figure 5.8 Influence of NaCl concentration on gas production

5.3.5 NaCl concentration

Reference concentration referred to the concentration of NaCl that was initially present in the food waste. As shown in Figure 5.9, the reference concentration of NaCl in the food waste which comprised of rice, noodle, orange peels and vegetable roots was approximately 0.57%. 1% NaCl was referred to the addition of 1% NaCl salt to the food waste on top of the reference concentration. i.e., 1.57% of NaCl was actually in the food waste. Similarly, 2% NaCl and 3% NaCl concentrated had 2.57% and 3.57% NaCl in the food waste initially.

It was noted that as the experiment proceeded, the NaCl concentration in the leachate of the Ra reactors decreased while that of the effluent of the Rm reactor would increase. This can be shown in Figure 5.9 and Figure 5.10. Ultimately the concentration of the NaCl in the Ra reactor leachate and Rm reactor effluent would reach equilibrium. This was expected in a closed loop HASL system. For the

equilibrium point in the Control reactor, it was 0.51%. For the case of 1%, 2%, and 3% bioreactors, the equilibrium points were 0.84%, 1.11%, and 1.49%, respectively.

Figure 5.9 NaCl concentrations in Ra vs. time

Figure 5.10 NaCl concentrations in Rm vs. time

5.4 CONCLUSIONS

This research work was to understand the influence of different NaCl concentrations in food waste on the performance of the HASL system. To achieve this goal, food waste with addition of NaCl up to 3% was used as the feedstock for the HASL system. The following conclusions can be drawn from this study.

- NaCl affected the acidogenic reactor (Ra) more than the methanogenic reactor (Rm). For the HASL system operated with 1% NaCl addition, there was little inhibition on the system as there was minimal changes to the pH, total COD production, total VFA production, gas production and gas composition.
- But as the NaCl concentration increased to 2%, inhibition was observed in the hydrolysis and acidogenesis. Lower COD and VFA production were observed in the Ra reactors.
- However, at 2% NaCl addition, Rm reactor was not really affected by the salt as gas production only decreased slightly and the pH had remained in the neutral range. Moreover, the VFA removal efficiency for the Rm reactor was more than 99.5%. This had shown there were little inhibition on the Rm reactor at 2% NaCl addition to the food waste.
- However, at 2% NaCl addition was the methane content in the biogas decreased from $72\pm4\%$ to $65\pm6\%$. This suggested that at 2% NaCl addition, NaCl began to have an effect on the more sensitive methanogen in the UASB reactor.
- At 3% NaCl addition, inhibition on both the Ra and Rm reactors was observed. Compared to the Control COD and VFA production in Ra reactor dropped by 70% and 75%, respectively. For the Rm reactor, although pH remained quite constant at neutral range, the quality and amount of biogas decreased

significantly. The amount of biogas produced dropped by 37% and the gas composition for methane decreased from $72\pm4\%$ to $63\pm6\%$.

CHAPTER 6

CONCLUSIONS

The objective of this research was to investigate the influence of high ammonium concentration, lipids such as fats, oil, and grease (FOG), and NaCl salt concentration on the hybrid anaerobic solid-liquid (HASL) system during the biodegradation of food waste. Based on the results from this study, several conclusions can be drawn:

Ammonium removal in the HASL system using submerged filter

- The submerged filter was able to maintain a low ammonium concentration of less than 150 mg/L in the system. The low ammonium concentration improved the operation of the HASL system.
- The application of submerged biofilter in the HASL system increased chemical oxygen demand (COD) and volatile fatty acid (VFA) generation in the acidogenic reactor (Ra), total COD removal, total biogas and methane production in the methanogenic reactor (Rm).
- COD removal efficiency was 99% in the enhanced HASL system, which demonstrated a significant improvement in comparison with 84% in the control HASL system.
- Methane production in the enhanced HASL system increased by 26% in comparison with the conventional HASL system. However, methane content in the biogas of the enhanced HASL system was lower and nitrogen content was higher than that in the control conventional HASL system due to the denitrification process.

Influence of FOG on the HASL

- Generally, the Rm reactors of the HASL system were not affected by FOG addition up to 40% FOG addition. The COD removals in the four Rm reactors, except 40% FOG, remained efficient, which fell within the range of 87.5%-88.9%. The good performance of these methanogenic reactors were further reflected by the stable pH range of 7.78-8.71 throughout the operation. However, the higher effluent COD concentration for the 40% FOG Rm reactor suggested that a longer acclimatisation period was required as compared to the other three Rm reactors.
- High FOG concentration may affect the hydrolysis and acidification in Ra reactors more than methanogenesis in the Rm reactors. This was shown as TS and VS removal efficiencies decreased as the concentration of the FOG was increased. The VS removal efficiency decreased from 88.3% in the Control Ra reactor to 41.7% in the 40% FOG Ra reactor.
- Lipids had a higher gas production potential as compared to carbohydrates and proteins. The biogas production per gram of VS consumed increased from 0.34 L/gVS removed in the Control to 0.63 L/gVS removed in the 40% FOG.
- Presence of FOG was a possible inhibitor to methanogenesis. This was supported by the biological analysis for the four Rm reactors. The biological analysis showed a gradual decreased in methanogenic activity as influent FOG content increased.

Influence of NaCl salt on the HASL

- NaCl affected the Ra reactors more than the Rm reactors. For the HASL system operated with 1% NaCl addition, there was little inhibition to the system as there was minimal changes to the pH, total COD production, total VFA production, gas production, and gas composition.
- Hydrolysis and acidogenesis of food waste were inhibited at 2% NaCl addition as lower COD and VFA production were observed in the 2% NaCl Ra reactor.
- However, at 2% NaCl addition, there was only marginal effect on the Rm reactor. The gas production decreased slightly and the pH remained in the neutral range. Moreover, the VFA removal efficiency for the Rm reactor was more than 99.5%. This shown little inhibition on the Rm reactor at 2% NaCl addition to the food waste.
- It was observed that at 2% NaCl addition, the methane content in the biogas decreased from $72\pm4\%$ to $65\pm6\%$. This suggested that at 2% NaCl addition, NaCl began to have an adverse effect on the more sensitive methanogen in the UASB.
- At 3% NaCl addition, inhibitions in both the Ra and Rm reactors were observed. COD and VFA production in Ra reactors decreased by 70% and 75%, respectively, compared to the Control reactor. For the Rm reactors, although pH remained quite constant at neutral range, the quality and amount of biogas decreased significantly. The amount of biogas produced decreased by 37% and the gas composition for methane decreased from $72\pm4\%$ to $63\pm6\%$.

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