TWO-PHASE ANAEROBIC DIGESTION OF SEMI-SOLID ORGANIC WASTES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
THE MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

FEBRUARY 2008

Approval of the thesis:

TWO-PHASE ANAEROBIC DIGESTION OF SEMI-SOLID ORGANIC WASTES

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ABSTRACT

TWO-PHASE ANAEROBIC DIGESTION OF SEMI-SOLID ORGANIC WASTES

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February 2008, 102 pages

The objective of this study is to illustrate that phase separation improves the efficiency of an anaerobic system which digests semi-solid organic wastes. Organic fraction of municipal solid waste (OFMSW) was the semi-solid organic waste investigated. In the first part of the study, the optimum operational conditions for acidified reactor were determined by considering the volatile solid (VS) reductions and average acidification percentages at the end of two experimental sets conducted. Organic loading rate (OLR) of 15 g VS/L.day, pH value of 5.5 and hydraulic retention time (HRT) of 2 days were determined to be the optimum operational conditions for the acidification step. Maximum total volatile fatty acid and average acidification percentage were determined as 12405 mg as HAc/L and 28%, respectively in the reactor operated at optimum conditions. In the second part, an acidification reactor was operated at the optimum conditions determined in the first part. The effluents taken from this reactor as well as the waste stock used to feed this reactor were used as substrate in the biochemical methane potential (BMP) test. The results of BMP test revealed that the reactors fed by acidified samples indicated higher total chemical oxygen demand (tCOD) removals (39%), VS reductions (67%) and cumulative gas productions (265 mL).

The result of this study indicated that the separation of the reactors could lead efficiency enhancement in the systems providing that effective control was achieved on acidified reactors.

Keywords: Acidogenesis, anaerobic, organic loading rate (OLR), phase separation, municipal solid waste (MSW), volatile fatty acid (VFA)

ÖZ

KATI İÇERİĞİ YÜKSEK ORGANİK ATIKLARIN İKİ FAZLI ANAEROBİK ORTAMDA BOZUNDURULMASI

Doğan, Eylem

Yüksek Lisans, Çevre Mühendisligi Bölümü
Tez Yöneticisi : Prof. Dr. Göksel N. Demirer

Şubat 2008, 102 sayfa

Bu çalışmanın amacı faz ayırımının, katı içeriği yüksek organik atıkları bozundurmada kullanılan anaerobik sistemin verimini artırdığını göstermektir. Evsel katı atığın organik kısmı, katı içeriği yüksek organik atık olarak incelenmiştir. Çalışmanın ilk kısmında, uçucu katı (UK) azalmaları ve ortalama asidifikasyon yüzdeleri göz önünde bulundurularak asidifiye edilmiş reaktör için optimum koşullar yapılan iki set deneyin sonunda belirlenmiştir. 15 gr UK/L.gün organik yükleme hızı (OYH), 5.5 olan pH değeri ve 2 gün hidrolik bekletme süresi (HBS) asidifikasyon aşaması için optimum işletim koşulları olarak saptanmıştır. Optimum koşullarda işletilen reaktörde maksimum toplam UYA ve ortalama asidifikasyon değerleri sırasıyla 12405 mg Hac/L ve 28% olarak bulunmuştur. İkinci kısımda, optimum koşulları ilk kısımda belirlenmiş olan asidifikasyon reaktörü işletilmiş ve bu reaktörden alınan çıkışlar ile reaktörün beslenmesi için kullanılan atık, biyokimyasal metan potansiyeli (BMP) testinde besin olarak kullanılmıştır. BMP testinin sonuçları asidifiye edilmiş numunelerle beslenen reaktörlerin daha yüksek toplam kimyasal oksijen ihtiyacı (KOİ) giderimi (39%), UK azalması (67%) ve toplam gaz üretimi (265 mL) gösterdiğini açığa çıkarmıştır.

Bu çalışmanın sonuçları, asidifiye edilmiş reaktörlerde etkili bir kontrol sağlandığında reaktörleri ayırmanın sistemde verim artışına neden olduğu göstermiştir.

Anahtar Kelimeler: Asidojenesis, anaerobik, organic yükleme hızı (OYH), faz ayırımı, evsel katı atık (EKA), uçucu yağ asitleri (UYA)

To My Family…

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my supervisor Prof. Dr. Göksel N. Demirer for his guidance, advice, trust and encouragement throughout this research.

I would like to thank the Supervising Committee Members, Prof. Dr. Celal F. Gökçay, Prof. Dr. Filiz B. Dilek, Assoc. Prof. Dr. Selim L. Sanin, Assoc. Prof. Dr. Ayşegül Aksoy for their constructive suggestions and helpful discussions.

I would like to thank Kemal Demirtaş and Gizem Uğurlu for their help in the laboratory.

My special thanks go to my office mates, Niğmet Uzal and Erkan Şahinkaya for their help, support, words of encouragement and sharing their experiences with me throughout my research. I would also thank Tuba Hande Ergüder, Evrim Doğan, Emrah Alkaya, Vedat Yılmaz, Recep Tuğrul Özdemir, Seçil Türkmenoğlu, and Fadime Kara for their helps.

Finally, my deepest thanks are dedicated to my family, especially to my mother, for all the patience, encouragement and love throughout my research.

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ABBREVIATIONS

AD : Anaerobic Digestion
BM : Basal Medium
BMP : Biochemical Methane Potential
BOD : Biochemical Oxygen Demand
CSTR : Completely Stirred Tank Reactor
GC : Gas Chromatograph
COD : Chemical Oxygen Demand
HAc : Acetic Acid
HPr : Propionic Acid
HRT : Hydraulic Retention Time
MLVSS : Mixed Liquor Volatile Suspended Solids
MSW : Municipal Solid Waste
OFMSW : Organic Fraction of Municipal Solid Waste
OLR : Organic Loading Rate
sCOD : Soluble Chemical Oxygen Demand
SRT : Solid Retention Time
VFA : Volatile Fatty Acids
tCOD : Total Chemical Oxygen Demand
tVFA : Total Volatile Fatty Acid
TS : Total Solids

CHAPTER 1

INTRODUCTION

1.1. General

The demand for energy and industrial materials is on a significant rise parallel to the rapid industrialization and population growth in many developing countries. The use of nonrenewable fossil-based resources for energy and industrial raw material generation results in several problems such as high cost, environmental pollution, etc. Converting organic wastes (organic fraction of municipal solid wastes (OFMSW), animal and agro-industrial wastes) into value-added products (biogas, organic acids, and fertilizer) may be a solution to all these problems.

Semi-solid (agricultural, food processing and the organic fraction of municipal solid waste) wastes have been shown to cause significant environmental and public health problems. These problems include, among others, nutrient enrichment of surface and ground waters, contamination of drinking water supplies, global warming enhancement, and odors (Ostrem, 2004).

Historically, safe and effective management of municipal solid waste (MSW) generated in heavily populated urban centers has challenged mankind to develop a number of strategies. These strategies included burial, burning, and ocean dumping, practices which lead to cross-media pollution between land, air, and water (Earle et al., 1990). In addition, municipal solid waste management is a complex task which depends on organization and cooperation between households, communities, private enterprises and municipal authorities. Moreover, it is an essential task which has important consequences for public health and well-being, the quality and sustainability of the urban economy (Schübeler, 1996).

Management and disposal of municipal solid waste is also a significant problem in Turkey. The production is 25 million tones / year in 2004 while the amount was 3-4 million tones in 1960 (TURKSTAT, 2007). In addition, according to the information obtained from TURKSTAT, per capita waste production was stated as 1.31 kg/day in year 2004. The statistics depicts that 7 and 0.4 million tones of the waste are treated in the controlled landfills and composting plants, respectively; whereas, 15.6 million tones / year are dumped to the dumping sites of the municipalities. Besides, totally 0.6 million tones / year of waste are burned in open areas, disposed to river or lakes. It is clear from the numbers that the majority of the solid waste is disposed somehow without any management and causing serious environmental problems in Turkey. Therefore, the MSW is an important concern which requires a management system consisting of collection, reuse and disposal rather than disposing at remote locations.

Landfilling may be the oldest technique that has been used for solid waste treatment and its main advantage is the low cost relative to other engineered techniques used for solid waste management. However, the European commission regards landfilling as the least favourable option for waste handling, since the waste is not used as a resource and it has negative impacts on environment. The produced landfill gas (mainly methane) can be used as a fuel; yet the conversion of waste into energy is only about one third from landfill compared to incineration.

Another technique used for solid waste management is incineration. In this technique, waste material is oxidized and becomes mainly heat, water vapor, nitrogen, carbon dioxide and oxygen, but also many other compounds (often toxic) depending on the composition of the incoming waste. Incineration demands high capital investment, and is therefore a very expensive waste management method.

Since the incinerator is designed for a certain composition of the waste, it is sensitive to changes.

However, anaerobic digestion (AD) has the opportunity to be an integral part of the solution to two of the most pressing environmental concerns of urban centers: waste management and renewable energy. Through AD, organics are decomposed by specialized bacteria in an oxygen-depleted environment to produce biogas and a stable solid. Each of these products can be used for beneficial purposes to close the loop in organic waste management. The biogas, which consists of up to 65% methane, can be combusted in a cogeneration unit and produce green energy. The solid digestate can be used as an organic soil amendment.

Because of the proven feasibility of the process and the multitude of environmental benefits, the use of AD technology has been firmly established in Europe, where over 160 facilities with an annual installed capacity more than 5 million tons generate electricity in excess of 600 MW. By this application, these countries reduce the volume of waste being sent to landfill, and therefore decrease methane emissions produced from its decay. The biogas generated at these sites is used to produce electricity and heat that is then sold to utilities, making the facilities profitable. Additional environmental gains include improvements in water and air quality over current practices of waste management (Ostrem, 2004).

Uncontrolled disposal of organic pollutants such as agro-industrial wastes and organic fraction of municipal solid waste which are produced in high amounts in Turkey result in significant environmental and public health problems. In addition, they should also be regarded as an economical loss. The conversion of the organic wastes produced in our country into renewable energy and industrial chemicals will reduce our foreign dependency, lead to important economical and ecological gains, refresh rural economies through new perspectives and investments create new employment opportunities, and make Turkey comply with international environmental agreements easier.

1.2 Objective of the Study

The main objective of the study is to demonstrate that anaerobic treatment of OFMSW might be enhanced with an innovative two-phase configuration system operated with relatively short retention times and high organic loading rates (OLR).

1.3 Scope of the Study

The application of AD processes for the treatment of MSW with one-phase systems is more common than two-phase systems. However, there are numbers of studies on digestion of MSW with two-phase systems in literature. Yet, in the majority of these studies, applied organic loading rates were relatively low since they were lab-scale applications (Table 2.1). In addition, the majority of the studies did not focus on the optimization for anaerobic acidification of OFMSW. Therefore, this study differs from literature in terms of high OLR application and focus on acidification phase optimization.

This study consisted of three main experimental parts. In the first part, objective was to determine the optimum OLR for maximum acidification condition in the treatment of OFMSW. For this purpose, necessary literature reviews were performed and optimum pH value was determined as 5.5 for the reactors. OLRs of 10, 15 and 20 g VS/L.day were chosen since there were not many studies conducted with high loads in lab-scale reactors and it was also intended to observe the effect of these loads on acidification performance. HRT of all acidified reactors were chosen as 2 days since short retention times enhance the acidification process according to the literature survey. The optimum reactor performance was chosen considering the VFA production, VS reduction, percent solubilization and acidification degrees.

In the second part of the study, the effect of different pH values on anaerobic acidification was investigated by applying optimum OLR and HRT values. One of the two reactors operated in this part was kept at pH of 6.5. The pH value of the other reactor was not controlled since it was intended to observe natural pH variation in the reactor and its effect on acidification performance. The performances were again compared by using the parameters stated above.

In the last part of the study, biochemical methane production (BMP) experiments were performed in order to figure out whether phase separation enhanced the efficiency of methanogenic activity or not. For this purpose, one more acidifications reactor was operated under optimum conditions. The effluents of this reactor and stock waste solution used to feed the reactor were taken and used as the substrate for BMP batch reactors. The batch reactors were operated as the methane producing reactor of a two-phase system. The performances were compared in terms of cumulative biogas productions, volatile solid (VS) and chemical oxygen demand (COD) reductions, and volatile fatty (VFA) concentrations.

CHAPTER 2

LITERATURE REVIEW

2.1. Anaerobic Digestion Process

In the past two decades, anaerobic treatment has become an effective biological process for treating many industrial organic wastewaters and food processing wastes. It is also one of the most promising wastewater treatment technologies for meeting sustainable development objectives outlined by the U.S. National Research Council (Wust, 2003).

Anaerobic digestion is a naturally occurring biological process that is carried out by a large number of bacteria working together in the absence of molecular oxygen. These bacteria (anaerobes) grow by converting the organic matter into methane and carbon dioxide (a mixture commonly called *biogas*) by going through several steps of biological treatment. Anaerobes can operate over a wide range of temperatures from $10^{o}C$ to over $50^{o}C$ and at atmospheric or varying pressures. The by-product or the digested solid is an environmentally friendly product that contains some protein and available form of nutrients, such as ammonia-nitrogen and other soil conditioners (Gas Technology, 2006).

The microbial conversion processes in anaerobic treatment are typically described in three main stages: hydrolysis, acidogenesis and methanogenesis.

The first phase of anaerobic digestion involves the decomposition or hydrolysis of insoluble organic polymers to simple soluble compounds such as triglycerides, fatty acids, amino acids, and sugars (Speece, 1996). Hydrolysis is catalyzed by enzymes excreted from the bacteria, such as cellulase, protease, and lipase. If the feedstock is complex, the hydrolytic phase is relatively slow. This is especially true for raw cellulolytic waste, which contains lignin (Ostrem, 2004).

Solid hydrolysis is the rate-limiting step in anaerobic digestion of cellulose. Therefore, to enhance the anaerobic degradation the conditions for hydrolysis have to be improved. The growth of hydrolytic microorganisms and the activity of their hydrolytic enzymes depend on pH. The concentration of solid substrate has a significant influence on activity and concentration of hydrolytic enzymes in the media. Therefore, a proper control of substrate concentration during hydrolysis will accelerate anaerobic degradation of solids (Kübler et al., 1994).

These simple soluble compounds are further degraded by the acidogenic bacteria in the acidogenesis or acid fermentation stage, where the simple sugars are converted into VFAs, mainly acetic, propionic, butyric, valeric and lactic acid. In the acid fermentation stage, there is minimal reduction of chemical oxygen demand (COD) or biological oxygen demand (BOD) since the complex compounds produced, such as short-chain fatty acids, alcohols and new bacteria cells exert an oxygen demand.

In the final stage, methanogenesis, methane-producing bacteria convert the acetate and hydrogen into methane and carbon dioxide, completing the decomposition process. There are two main genera of methane-producing microorganisms in engineered systems: Methanothrix and Methanosarcina. Usually one genus of methanogenic organisms will dominate depending on the conditions in the reactor (Speece, 1996).

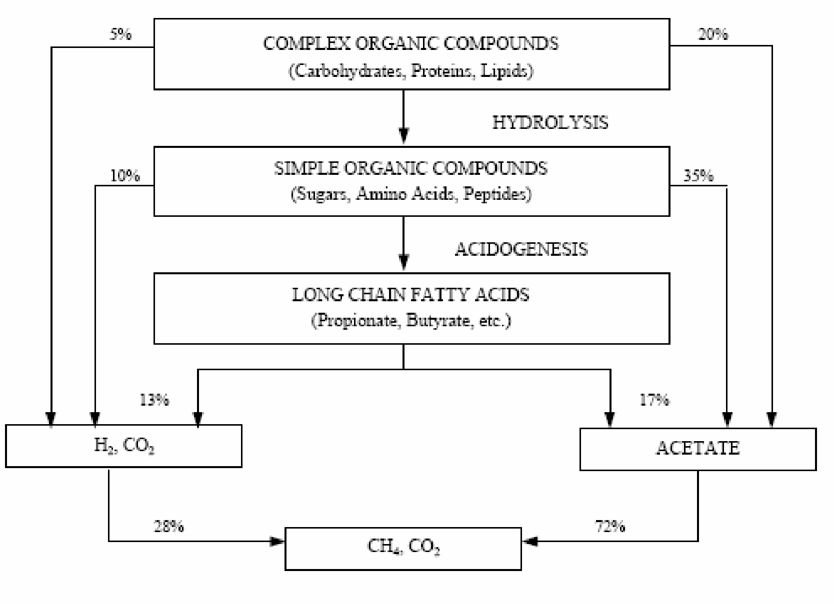

Figure 2.1: Series metabolism resulting in methanogenesis (Speece, 1996).

An approximate chemical formula for the mixture of organic waste is $C_6H_{10}O_4$ (Themelis and Verma, 2004). A hydrolysis reaction where organic waste is broken down into a simple sugar, in this case glucose can be represented following;

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2 \text{……………………Eqn-2.1}$$

Typical reactions in the acid-forming stages are shown in the following equations. In Equation 2.2, glucose is converted to ethanol and Equation 2.3 shows glucose is transformed to propionate.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \text{……………………..Eqn-2.2}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \text{…………..Eqn-2.3}$$

The next stage of acetogenesis is often considered with acidogenesis to be part of a single acid forming stage. BOD and COD are reduced through these pathways (Bouallagui et al., 2005)

In addition, the main products for carbohydrate fermentation have been found to be acetic, propionic, and butyric acids and ethanol. All the reactions occur naturally. However, due to differences of the acidogenic species as well as operating environment, end-products can be different during the fermentation of organic wastewater. In general, the two most common types of acidogenic fermentation for organic wastewater are butyric- and propionic-type fermentation. However, a third type, namely the ethanol-type fermentation, was discovered in a continuous flow reactor operated at a low pH of around 4.5. The fermentation products were ethanol, acetic acid, hydrogen, and carbon dioxide (Ren et al., 1997)

2.2. Anaerobic Digestion of Organic Fraction of Municipal Solid Waste (OFMSW)

Anaerobic digestion has become an established and proven technology for the treatment of organic fraction solid waste (De Baere, 2000). It reduces the volume of solid waste, generates saleable biogas (mainly methane) for energy recovery, and produces organic residue that can be used as soil conditioner/fertilizer (Wang et al., 2003).

Wastes from agriculture and food production are usually recycled through animal feeding, energy production or agricultural fertilizer. Nevertheless, large amounts originating from urban markets or generated by the variations in the production are carried to landfill sites or disposed of into the waste-pits with or without burying, generating smell and water pollution (Raynal J. et al., 1998).

Moving towards a sustainable waste management regime, the hierarchy of waste treatment methods has switched to minimization, recovery, incineration and landfill. Biological treatment of organic fraction of municipal solid waste (OFMSW) offers a waste management strategy that combines stabilization of the waste, i.e. the reduction of organic matter (volatile solids (VS)) with recovery of nutrients, if the end product is used as fertilizer (Hartmann, 2006b).

Recycling municipal solid waste (MSW) becomes more significant every day and, accordingly, many countries are starting new selective collection or source reduction programs to make this possible. Public awareness of the environmental hazards of incineration and landfilling are increasing the complexity of these operations and, consequently, their costs. The trend is then to minimize the amount of waste to be treated with these procedures (Vitura et al., 1995).

It was pointed out that when dealing with solid waste the recycling of reusable material and organics would always be the main advantage compared to incineration. Therefore the quality of the effluent should be one of the highest goals. And since the quality of the influent has a main impact on the effluent quality this should be always regarded as the key factor for a successful anaerobic digestion (AD) of solid waste (Hartmann et al., 2006a).

Compared to incineration, recovery of reusable material and organics makes AD highly superior in the context of a sustainable waste treatment concept. Moreover, AD has the potential to treat the wet fraction of MSW that is less amenable to incineration. Last, but not the least, the bottom ash of incineration treatment has to be deposited as hazardous waste. The content of chlorinated compounds in OFMSW is disadvantageous for incineration since it contributes to the formation of hydrogen chloride (HCl) and products of incomplete combustion (PICs) such as polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDFs) are formed (Hartmann et al., 2006b).

The main advantage of AD compared to composting is its positive energy balance. While composting represents an energy consuming process (around 30-35kWh is consumed per ton of waste input), anaerobic digestion is a net energy producing process (100-150 kWh per ton of input waste). Furthermore, area requirements are lower and odor problems are minimized since the whole treatment is performed in reactors (Hartmann et al., 2006b).

Baere et al. (1999) presented an overview of anaerobic digestion use for solid waste treatment. This study was limited to the plants in operation or under construction that were treating at least 10% organic solid waste coming from market waste and municipal solid waste. A total treatment capacity for solid waste organics, excluding the tonnage used for sewage sludge and manures, evolved from 122,000 ton per year in 1990 to 1,037,000 ton available or under construction by the year 2000 in 53 plants across Europe, showing an increase by 750%. Both mesophilic and thermophilic technologies have been proven, with about 62% of capacity being operated at mesophilic temperatures. Wet and dry digestions are almost evenly split, while a clear choice was made for one-phase systems instead of two-phase systems, which represent of 10.6% of the total capacity.

2.3. Two-phase versus One- phase Anaerobic Digestion

Anaerobic digestion can be operated in single or two phase configurations. Single phase incorporates both acid formation and methane production in the same reactor, while two phase operation attempts to separate acid formation from methane production, usually by providing two reactors (Speece, 1996).

Two-phase systems are especially suitable for wastewaters with high concentration of organic suspended solids, since they could be degraded to volatile fatty acids (VFA) in the first reactor by hydrolytic and acidogenic bacteria and finally converted into methane in the second reactor (Guerrero et al., 1999).

The main potential advantages expected of a two-step anaerobic treatment are better control of both acidogenic and methanogenic steps, smaller size of reactors, higher suspended solids removal efficiency, enhancement of acidogenic microorganisms' growth without disturbing methanogens, and a higher methanogenic specific activity in the second reactor. Moreover, potentially toxic compounds for methanogens that can be generated in the first steps of anaerobic digestion such as ammonia, long chain fatty acids or sulphide can be removed in an intermediate stage between both reactors. Some disadvantages have also be considered, as the higher investment costs or the poor granule formation in methanogenic sludge bed reactors treating acidified wastewater (Guerrero et. al., 1999).

It can be concluded that two-stage process can be beneficial for enhancing the overall degradation efficiency, but the process should be kept simple in order to avoid operational problems. A separate hydrolysis reactor can be advantageous for treatment of waste containing larger fractions of recalcitrant organic matter, while the separation into two stages can lead to overloading problems when using substrates with a high content of easily degradable organic matter. Generally, the separation into two reactors is most optimal if the two processes, hydrolysis and methanization, can be successfully separated. This means that a HRT in the hydrolysis reactor of 5 days or shorter should be applied in order to gain the highest overall VS reduction (Hartmann, 2006b).

Other advantages of the two-phase configuration are that it significantly increases the specific activity of methanogens and enables the disposal of the faster growing acid formers without loss of the former (the yield of acid formers is 8.15% while that of methanogens is only 1.2%). The acidification reactor can serve as a buffer system when the composition of the wastewater is variable, and can help in the removal of compounds that are toxic to methane bacteria (e.g., sulphide) (Dinopoulou et al., 1988).

On the other hand, Vitura et al. (1995) concluded that two-phase system does not seem appropriate to treat vegetables and fruit wastes unless it is equipped with some type of control of the hydrolytic step. At high organic loads it does not seem to be very effective. OLR affects the hydrolytic step. Furthermore, at higher loads, more methanization takes place in the hydrolyser than in methanizer, because VFA are in a lower concentration and, thus, phase separation is not so effective. At low organic loads, VFA concentrations are poor, because they are immediately metabolized and the yields per kg VS are high.

In the practice, however, the greater reliability of two-stage system has indeed at times been observed, at least in discontinuously-fed laboratory set-ups. For example, Pavan et al. (2000) compared the performances of the one and two-stage systems, using pilot-scale completely mixed reactors fed with very rapidly hydrolysable biowastes from fruit and vegetable markets. While the one-stage system failed at 3.3 kg VS/m^3.day, the performance of the two-stage plant remained stable at an overall system OLR of 7 kg VS/m^3.day.

2.4. Process Parameters Affecting Anaerobic Process

The primary factor in treating food waste is the physicochemical characteristics of substrate, including particle size and composition. Degradation of each component of food waste is affected by environmental conditions. Carbohydrate, cellulose, and protein have their optimum pH values and retention times for degradation. This means that the degradation of food waste could be enhanced by adjusting the environmental conditions depending on the state of degradation (Han and Shin, 2004).

The operational parameters, such as hydraulic retention time, organic loading rate, temperature, and pH also affect the content and composition of the acidification reactor effluent. It is important to know the effect of these parameters; because it can help engineering of the first phase towards more desirable products, and it can show to which parameters the system is most sensitive (Dinopoulou et al., 1988).

2.4.1. Hydraulic Retention Time (HRT)

HRT is a key parameter for the performance of a hydrolysis- acidification reactor, since it determines the solid solubilisation efficiency and the degree of acidification of the influent. The maximum process efficiency is usually obtained by operating the acidogenic step at short HRT, thus preventing methanogens development (Guerrero et al., 1999).

Penaud et al. (1997) and Cha and Noike (1997) confirmed the effects of HRT and temperature variations on changes in bacterial population level in the acid reactor. Penaud et al. (1997) observed that the bacterial forms exhibited variations with respect to the changes in HRT and temperature during acidogenesis of a pharmaceutical waste. Cha and Noike (1997) observed long and slender–shaped bacteria at an HRT of 48 h while short and thick stick-shaped bacteria were observed at short HRT and low temperature (Demirel and Yenigun., 2002). Demirer and Chen (2004) stated the pH drop was inversely proportional with the increase in HRT of the reactor and volatile solid removal during acidification showed an increase with increase in HRT at constant organic loading rate.

In addition, the concentration of total organic acids, hydrogen content and hydrogen production rate increased with an increase in HRT in the study conducted by Shin et al. (2005) to investigate the conversion of food waste into hydrogen by thermophilic acidogenesis.

2.4.2. Organic Loading Rate

Organic loading rate (OLR) is a measure of the biological conversion capacity of the AD system. Feeding the system above sustainable OLR results in low biogas yield due to accumulation of inhibiting substances such as fatty acids in the digester slurry. In such a case, the feeding rate to the system must be reduced. OLR is a particularly important control parameter in continuous systems. Many plants have reported system failures due to overloading (Verma, 2002).

The volume loading rate must be a part of every process design. For instance, reactors fed with VFA substrates can be loaded 4-5 times higher than reactors fed with more complex substrates such as starches and proteins. This increased loading capability of VFA is caused primarily by the small yield coefficient for VFA compared to carbohydrate substrate, and secondarily by an increased fraction of methane bacteria in the biomass.

The loading rate (kg COD/ $m^3$of reactor –d) is often an economic issue. To achieve high loading rates, dilute wastewaters must be passed through the system in relatively short HRT's (Speece, 1996).

2.4.3. pH

Since pH affects growth rate, pH changes may cause drastic shifts in the relative numbers of different species in a heterogeneous population present in the acidogenic reactor. Many aspects of microbial metabolism are greatly influenced by pH variations over the range within which the microorganisms can grow. These aspects include utilization of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and various types of storage material, and release of metabolic products from cells. Moreover, pH variation can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena. A substantial number of studies have been carried out on the effect of pH on acidogenesis of carbohydrate rich wastes, but little attention has been paid to the influence of pH on acidogenesis of protein-laden wastes. However, many industrial and agricultural wastewaters also contain appreciable quantities of protein. Treating protein-rich wastewater often results in formation of scum accumulated inside the reactor, and causes sludge washout. In addition, proteins are degraded slower than carbohydrates under acidogenic conditions (Yu et al., 2003).

The influence of pH on the acidogenesis of a simple, soluble substrate, glucose, over the range from 4.0 to 7.9, and recommended the pH range of 5.7- 6.0 for the acid reactor to provide a stable and most favorable substrate for the methane reactor, while Elefsiniotis and Oldham (1994) reported that the variation in pH between 4.3 and 5.2 did not affect VFA production and COD solubilization, but higher pH levels (5.9-6.2) affected both parameters in acidogenesis of a complex substrate-primary sludge. Variations in higher pH levels from 6.0-8.0 were reported to be affecting the dominant microbial populations in the acid reactor (Demirel and Yenigun, 2002).

The distribution of effluent products was also substantially influenced by pH, and the relative amount of the four main VFA was strongly dependent on pH. Acetate, butyrate, and *i*-butyrate predominated above pH 6.0, whereas propionate predominated below pH 5.0, the region between pH 5.0 and 6.0 was the transition zone. Significant change in product distribution was also found for glucose acidogenesis. The change in dominant products might be due to either in the metabolism of the same population or a change in the population itself or a combination of these both changes. Furthermore, since the significant changes in product distribution occurred between pH 4.0–7.0, pH control should be important for the production of a stable effluent composition from an acidogenic reactor (Yu et al. 2003). Ren et al. (1997) stated the operating pH, in particular, plays a major role on the effluent composition of the acidogenic reactor. The optimum pH for acidogenesis was found between 6.0 and 6.5 to avoid an excessive yield of propionic acid; however, when the pH was below 4.5, CO_2 and H_2 were produced along with butyric and acetic acids plus ethanol.

Yu et al. (2002) states that as pH increased, partial pressure of hydrogen decreased accompanied by the increase of methane production. At pH 6.5, the biogas contained 31% methane and became free of hydrogen. This indicates that most of the hydrogen produced was consumed by the hydrogenotrophic methanogens at this pH. In order to effectively separate the acidogenic phase from the methanogenic phase, it is important to keep the pH low, 5.5 or less.

Puterbaugh et al. (2002) performed experiments with pre-sorted food processing waste using a computer controlled pH stat system. The system is comprised of sixteen 1- liter batch-fed mixed reactors. Hydrolysis rates were determined as a function of pH and organic loading rate (OLR). The pH values of 6.5, 5.5, 4.5, and uncontrolled (the pH was not manipulated in the uncontrolled reactors) were evaluated at OLRs of 5, 10, 20, and 30 g COD/L. All reactors were maintained at 35ºC. The hydrolysis constants show the best configuration for hydrolysis to be a pH of 6.5 and an organic loading rate of 5 g COD/L.

Yu et al. (2002) states the degradation of the three main constituents in dairy wastewater, i.e. carbohydrate, protein and lipid increased with pH. At pH 4, 69%, 34% and 16% of carbohydrate, protein and lipid were degraded. Degradation of carbohydrate increased to 95% at pH 5.5, reaching 98% at pH 6.5. Degradation efficiency of carbohydrate was pH sensitive at pH less than 5.5. Degradation of protein reached a maximum at pH 6.5. Compared with carbohydrate and protein, lipid had much lower degrees of degradation to any given pH, but the variation with pH had a trend similar to those of carbohydrate.

Horiuchi et al. (2002) conducted experiments with a chemostat culture in order to investigate the effect of pH on the production of VFAs at acidogenic phase. The study illustrated that under the conditions of pH from 5.0 to 7.0, the main soluble products were butyric and acetic acids, while the propionic acid concentration was rather low. The main products were acetic and propionic acids at pH 8, whereas the butyric acid concentration decreased at that pH value. The ethanol concentration was relatively low for all cases.

It is concluded that the selective production of organic acids in anaerobic acidogenesis was possible by pH control. The strong pH dependency of organic acid production in the acid reactor leads to the possibility of selective acid production in industries from the various organic wastes by pH control in the acid reactor. This will contribute to the composition improvement of organic acids which are used as a source for the production of biodegradable plastics (Horiuchi et al., 2002).

2.4.4. Temperature

Due to the strong dependence of digestion rate on temperature, temperature is perhaps the most critical parameter to maintain in a desired range. Anaerobic bacteria can survive in a wide range of temperatures, from freezing to 70°C, but thrive within two ranges: from 25°C (77°F) -40°C(104°F), the mesophilic range, and from 50°C (122°F) to 65°C (149°F), the thermophilic. The optimum temperature for mesophilic digestion is 35°C (95°F) and a digester must be maintained between 30°C and 35°C for most favorable functioning (Ostrem, 2004).

Anaerobic digestion will take place at usable rates across a broad temperture range of 15-65 ^{0}C. The mesophilic range, from about 25-40 ^{0}C is generally considered as optimum for heated digesters. It is the rate rather than the extent of the reactions that is affected by temperature, and a lower digester temperature can be compensated for a longer time (Wheatley, 1991). On the other hand, an abrupt drop in temperature results in a corresponding abrupt increase in VFA and COD in the effluent.

In most cases, methanogenesis is the rate-limiting step for the overall degradation process in one-phase systems; anaerobic reactor should be operated around 37 ^{0}C or 55 ^{0}C to ensure methanogens to grow at their optimum temperatures. However, acidogens are not sensitive to temperature changes as methanogens. Operation at 45

^{0}C or 50 ^{0}C does not result in a lower degree of acidification or VFA/alcohol formation rate compared with at 37 ^{0}C.

Temperature effect on the maximum substrate utilization rates of methanogens has been observed. Lowering operational temperature generally leads to a decrease in the maximum specific growth and substrate utilization rate. In addition, methanogenic sludge yield has been shown to decrease with decreasing temperature. Temperature also affects the maintenance requirements of methanogens. Specific maintenance rate has been shown to give linear Arrhenius plots over a limited temperature range. However, the temperature effect studies have been focused on overall anaerobic degradation process or methanogenesis, rather than acidogenesis (Yu et al., 2003).

Good methanization yields can be obtained in a two-phase system with a mesophilic temperature of the hydrolytic reactor and a thermophilic temperature in the methanogenic reactor and with an overall hydraulic retention time of around 12 days, treating the source selected OFMSW. Safe ranges of operating conditions in which the yields are similar are the following: HRT in the hydrolytic reactor 2-3 days (mesophilic temperature); HRT in the methanogenic reactor 8-9 days (thermophilic temperature). The increase of the temperature in the hydrolytic reactor up to the thermophilic levels apparently does not improve either the yields or the kinetics (Pavan et al., 2000). It was also reported that more hydrogen could be produced at thermophilic conditions than mesophilic conditions (Shin et al., 2005).

Veeken and Hammelers (1994) determined the rates of hydrolysis for six components of biowaste (whole wheat bread, leaves, bark, straw, orange peelings and grass). The first-order hydrolysis kinetic constants ranged from 0.003-0.15 d^{-1} at 20°C to 0.24-0.47 d^{-1} at 40°C, values which are consistent with those reported for carbohydrates and food waste mixtures. However, biodegradability of biowaste components ranged from 5% to 90% without dependence on temperature.

Maharaj et al. (2001) investigated the effects of low temperature on acidogenesis. The study indicated that net VFA concentration was highest at 25°C (at 30 h) and decreased at 16°C and 8°C in reactors. However, despite the drop in VFA production at lower temperatures, the stability of operation and the concentration levels produced indicate both the feasibility as well as the potential of the process at low temperatures.

Table 2.1 summarizes the anaerobic digestion studies carried out with organic fraction of municipal solid waste (OFMSW) in terms of varying temperature, hydraulic retention time (HRT), organic loading rate (OLR) and corresponding volatile solid (VS) reductions were depicted.

Table 2.1 indicates that each study has its unique operation procedure, reactor configuration, HRT, OLR and temperature value and these parameters affect the system outputs; thus, the VS reduction levels are different. However, the VS reduction range varies 26-91%. In addition, it can be concluded that the VS reduction degrees increase as the OLRs decrease with the same HRT values (Diaz et al., 1981; Cecchi et al., 1992).

Table 2.1: Process parameters and biogas yields in previous studies of AD of OFMSW

Waste type	**T (^{0}C)**	**HRT (d)**	**OLR (kg VS/m^3.day)**	**VS reduction (%)**	**Reference**
Processed MSW	35	15	6.4	46	Diaz et al.(1981)
		15	4.8	78	
		15	3.2	75	
		15	1.6	53	
		15	1.6	61	
		30	1.1	77	
		30	1.1	61	
Organic Household	55	15-21	10-16.5	55	Baeten and Verstraete(1988)
Processed MSW	37	14	3.4	70	Rivard et al. (1990)
		20	2.4	75	
		30	1.6	81	
Source Sorted(SS) or Mechanically Sorted (MS) OFMSW	35	14	4.2	67	Malta-Alvarez et al.(1990)
		18	3.2	68	
		25	2.1	69	
Food Market Waste	35	12	2.8	90-91	Malta-Alvarez et al.(1992)
		14	2.4		
		16	2.1		
		20	1.7		

Table 2.1: Process parameters and biogas yields in previous studies of AD of OFMSW (Cont'nd)

Waste type	T (^{0}C)	HRT (d)	OLR (kg VS/m^3.day)	VS reduction (%)	Reference
Mechanically Sorted (MS) OFMSW	48-55	6	19.9	26	Cecchi et al.(1992)
		6	18.5	27	
		8	13.5	37	
		9	14.4	32	
		12	6.9	43	
Simulated OFMSW	55	15	17.9	64	Kayhanian and Tchobanoglous (1993)
		20	13.2	65	
		30	8.9	67	
Food market waste	35	5	12.6	27	Mtz-Viturtia et al.(1995)
		6	9.4	38	
		9	6.3	53	
		18	3.1	72	
Source-sorted OFMSW	50-56	6	12.8	38	Kübler (1994)
		9	8.6	52	
		12	6.4	45	
		21	3.7	55	
Fresh OFMSW	36	12	5	50	Krzystek et al.(2001)
Sourcesorted OFMSW, food and vegetables	55	22	2.3	78	Hartmann and Ahring (2005)
	55 + 68	18	3	80	
		18	3.4	82	

2.5. Anaerobic Digestion Enhancement

There is a link between successful pre-treatment and digester yields, that is, pretreatments, such as biological, mechanical or pysico-chemical, improve the performance of digesters. The economic aspects of digestion enhancement are very important to industry, a point not usually covered in the studies (Mata-Alvarez et al., 2000).

2.5.1. Mechanical pre-treatment

Size reduction of particles and the resulting increase in the specific surface available to the medium improves the biological process. Two effects have been reported: first, if the substrate has high fiber content and low degradability, their combination leads to improved gas production; and second, size reduction can lead to more rapid digestion (Mata-Alvarez et al., 2000).

2.5.2. Co-digestion

An interesting option for improving yields of anaerobic digestion of solid wastes is co-digestion. That is, the use of a co-substrate, that in most cases improves the biogas yields due to positive synergisms established in the digestion medium and the supply of missing nutrients by the co-substrates. In addition, economic advantages derived from the fact of sharing equipment are quite significant. Sometimes the use of a co-substrate can also help to establish the required moisture contents of the digester feed. Other advantages are the easier handling of mixed wastes, the use of common access facilities and the known effect of economy of scale. However, some drawbacks also exist, mainly due to slurry transport costs and the problems arising from the harmonization of different policies of the waste generators digestion (Mata-Alvarez et al., 2000).

2.5.3. Pre-composting

Among biological methods of improvement the influence of a pre-composting treatment on the start-up and performance of dry anaerobic digestion of pulp mill sludge was reported. The effect was clearly visible through methane yields and consequently solids reduction which were greater than in the digestion of untreated sludge (Mata-Alvarez et al., 2000).

2.5.4. Solubilization by Other Means

Anaerobic digestion of solid wastes is rate limited by the hydrolysis step, and so physico-chemical treatments are often used to promote solubilisation of organic matter. However, the substrate solubilisation step limited the anaerobic digestion of an industrial microbial biomass. A thermochemical pre-treatment based on sodium hydroxide addition can be used to enhance COD solubilization. It is stated that, with increasing pressure and temperature, the organic part of the waste is split up into short-chain fragments that are biologically well-suited to micro-organisms (Mata-Alvarez et al., 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1. Chemicals and Laboratory Devices

Chemicals: NH_4Cl, $MgSO_4.7H_2O$, KCl, $Na_2S.9H_2O$, $CaCl_2.2H_2O$, $(NH_4)_2HPO_4$, $FeCl_2.4H_2O$, $CoCl_2.6H_2O$, KI, $MnCl_2.4H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $AlCl_3.6H_2O$, $NaMoO_4.2H_2O$, H_3BO_3, $NiCl_2.6H_2O$, $NaWO_4.2H_2O$, Na_2SeO, cysteine were used for the preparation of the basal medium. Calibration of pH-meter and pH-controllers were done by EUTECH Instrument pH buffer solutions. Formic acid was used to decrease the pH values of the samples taken from the reactors for the Gas Chromatograph (GC) analysis. Acetic acid, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-caproic, caproic and heptanoic acids were used for the calibration of GC.

Laboratory Equipments: The laboratory devices used in the experiments were as follows; Trace GC Ultra (Thermo Co.) equipped with a flame ionization detector (FID) and with a length of 30 m Zebron column (0.25 mm I.D., 0.25 μm film thickness) and thermal conductivity detector (TCD) and 15 m Porapak Q, 5 mm I.D column, pump (Cole-Parmer C/L Variable-Speed Dual- Channel Compact Pump), pH meter (Hanna Instruments HI 8314 Membrane), pH controller (Alpha pH200 1/8 DIN pH/ORP Controller), pH probe (HI 1230), Photometer (Aqualytic PC Multidirect), Cole-Parmer 1200 Spectrophotometer.

3.2. Basal Medium (BM)

Basal Medium was added to each reactor every day in order to supply nutrients for the microorganisms. The composition of basal medium used in experiments was as follows (mg/L): NH_4Cl (1200), $MgSO_4.7H_2O$ (400), KCl (400), $Na_2S.9H_2O$ (300), $CaCl_2.2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2.4H_2O$ (40), $CoCl_2.6H_2O$ (10), KI (10), $MnCl_2.4H_2O$ (0.5), $CuCl_2.2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_2O$ (0.5), $NaMoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_2O$ (0.5), $NaWO_4.2H_2O$ (0.5), Na_2SeO (0.5), cysteine (10) (Uzal et al., 2003).

3.3. Experimental Set-up

There were three sets of experiments used in this study. In the first set (Set-1), the effect of OLR on anaerobic acidification was studied by running three acidifying anaerobic reactors at different OLRs (10, 15, 20 g VS/L.day). The second set (Set-2) was conducted in order to find out whether a control mechanism on pH value was necessary or not for highest acidification. For this purpose, two acidifying anaerobic reactors were operated. A constant pH value of 6.5 ± 0.1 was used in the first reactor while no pH control was applied in the second one. In the last set (Set-3), one more CSTR reactor was operated at optimum acidification conditions (optimum pH and OLR value). After this reactor reached its steady state, effluents of it were given to the batch reactors in biochemical methane potential (BMP) experiment. Meanwhile, raw solid waste used to feed this acidifying reactor was also given to different batch reactors in BMP test. Batch reactors fed by acidified samples were operated as the second reactor of a two-phase system, whereas the batches fed by raw solid waste were used as one- phase reactors (Figure 3.1). At the end, the performances of these batches were compared.

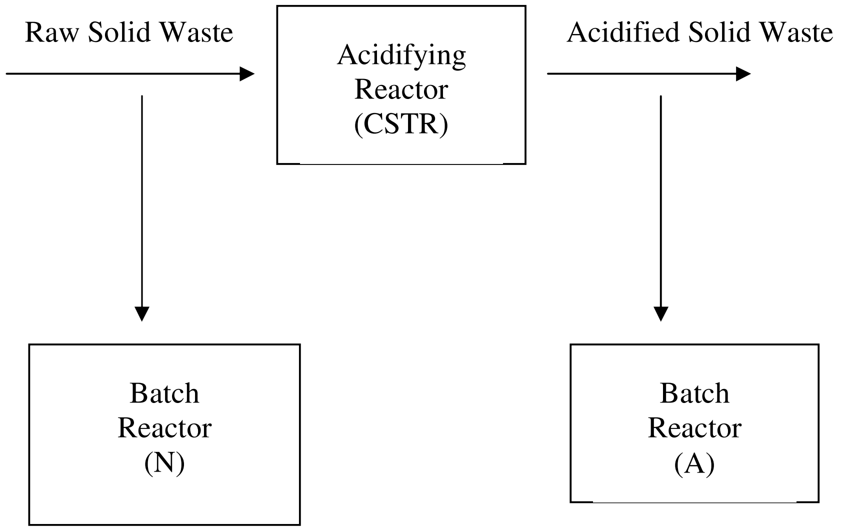

Figure 3.1: Graphical presentation of processes applied in Set-3

3.3.1. The Operational Conditions of the Reactors

For the Set-1 of this study, three 1-L acidogenic fed-batch continuously stirred reactors were operated with HRT of 2 days at pH of 5.50 ± 0.1 and temperature of 35 ±1 ^{0}C. Organic loading rates were 10, 15, and 20 g VS /L.day for Reactor-1, Reactor-2 and Reactor-3, respectively. Basal Medium (BM) containing no alkalinity was added to each reactor every day for the nutrient demand of the microorganisms. 3 N, 4 N and 5 N NaOH solutions were used for the pH adjustment. The daily feeding volume was 500 mL in total, containing 100 mL BM and 400 mL stock waste solution. For the Set-2 experiments, the same reactor configurations were used (Reactor-4 and Reactor-5) as in Set-1. The OLR of 15 g VS/L.day, HRT of 2 days were applied to both reactors where the pH value was 6.5 for Reactor-4. pH control for Reactor-5 was not practiced.

At the start-up (Set-1 and Set-2), 800 mL of sludge and 200 mL five times concentrated BM were added into a 1-L flask on first day and they were flushed with N_2 gas to supply anaerobic conditions. The feeding procedure was applied on second day of the experiment and 25, 37.5, and 50 g VS/L stock waste solutions were prepared and used in order to obtain OLR of 10, 15, 20 g VS/L. day in each reactor.

BMP experiments were performed for Set-3 and two different COD concentrations (4000 mg/L and 5000 mg/L) were applied. The control reactors (with no substrate) and blank reactors (with no seed) were also set up in order to eliminate the background gas production that can be observed in the reactors. The pre-acidified samples taken from the effluents of acidifying reactor were used as substrate for reactors A1 and A2, whereas the raw waste used as feed for the acidifying reactor was the substrate for reactors N1 and N2. All reactors were operated in duplicates for 40 days. Daily gas productions were measured throughout the experiment. Effluents of BMP reactors were taken at the end of operation period and tCOD, VS, and VFA analysis were conducted in duplicates.

3.3.2. Configuration of the System

The reactor configuration for Set-1 and Set-2 experiments was based on pH-stat continuous stirred tank reactor (CSTR) (Demirer and Speece, 1999). The pH-stat CSTR system consisted of a magnetically stirred glass erlenmeyer of 1 L effective volume with a headspace of 500 mL (Figure 3.2). The erlenmeyer was sealed with rubber stopper containing ports for pH-probe, NaOH solution addition, feeding, and gas venting. Sludge recycle was not applied in the system. The pH controller had connection with the pH probe and peristaltic pump. The pH value decreased as microorganisms utilized the substrate. When the pH of the culture remained under the pH set point value of 5.5 and 6.5, the controller operated the pump and NaOH solution was delivered to the reactor until the pH value increased again up to 5.5 and 6.5. The pH value of the reactor was kept at 5.5 ± 0.1 and 6.5 ± 0.1 in this way.

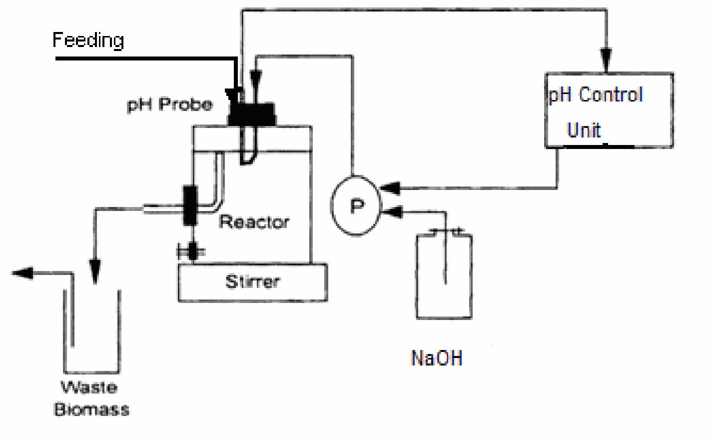

Figure 3.2: Illustration of the experimental set-ups (Set-1 and Set-2)

In Set-3, 250 ml serum bottles with effective volume of 150 mL were used as batch reactors. The reactors which contained BM were flushed with N_2 gas for 5 min to maintain anaerobic conditions after seeding. All the reactors were sealed with rubber stoppers and plastic screw-caps and mixing were applied at 200 rpm. The gas production was measured with water replacement device. The device consisted of U-tube displacement made from an inverted burette. The U-tube consisted of a 50 ml burette on the one side, a plastic tube having the same internal diameter of burette on the other side. The tip of the burette was connected small tubing attached on a syringe needle (Yılmaz, 2007).

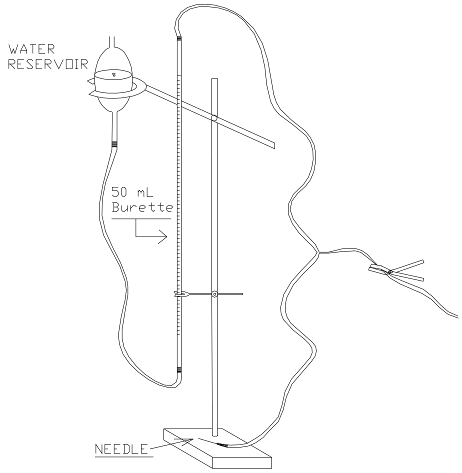

Figure 3.3: Illustration of water replacement device (Set-3)

The details of the experimental set-up of the Set-3 were tabulated in Table 3.1. Two COD concentrations (5000 mg/L and 4000 mg/L) were used in different reactors as shown in Table 3.1. High COD concentrations were not applied in the reactors since the high values may cause inhibition in the reactors due to overloading. The concentrations were chosen to obtain a food to microorganism (F/M) ratio between the ranges 0.2-1.35 which are suitable for BMP tests as stated in literature (Prashanth et al., 2006).

Table 3.1: Experimental set-up information of the BMP reactors

Reactor	SEED mL	Substrate(mL)	COD (mg/L)
A1	15	16	5000
A2	15	13	4000
N1	15	15	5000
N2	15	12	4000
BA1	-	16	5000
BA2	-	13	4000
BN1	-	15	5000
BN2	-	12	4000
Control	15	-	-

A: Reactors with substrate from effluent acidified reactor, **N:** Reactors with substrate of crude OFMSW, **BA:** Blank for A reactors, **BN:** Blank for N reactors, **C:** Control reactors

3.4. Inocula

The mixed anaerobic sludge culture from the anaerobic digestors of the Ankara Central Wastewater Treatment Plant was used as inoculum. The volatile suspended solid (VSS) concentration of the sludge was 8017 ± 1438 mg VSS/L. The seed sludge was screened through a 1 mm size sieve before used in order to remove debris, fibers etc.

3.5. Characterization of OFMSW

The waste used for the experiments were food and kitchen waste collected from houses, vegetables and fruits wastes collected from markets, and all these wastes were separated from glasses, plastic materials. In Set-1 and Set-3; 5 kg of vegetable, fruit and kitchen food waste was mixed and 1 kg of paper was added in order to simulate the municipal solid waste composition in Turkey. In Set-2 the waste was mainly composed of 6 kg of fruit, vegetable, and kitchen food waste. In addition, 1.2 kg of paper was added to this mixture to achieve the paper content observed in the typical waste composition.

The collected solid waste was shredded by meat grinder to achieve a particle size of about 4 mm and was mixed manually for homogenization. The waste mixture was kept at deep-freeze to prevent natural decay. Table 3.2 summarizes the characteristics of the OFMSW used for Set-1, Set-2 and Set-3 in the experiments.

Table 3.2: Characterization of OFMSW for Set-1, 2 and 3

PARAMETER	**VALUE (Set-1and Set-3)**	**VALUE (Set-2)**
Compact Density (kg/m^3)	1131.0±6.5	1022.0±8.5
Bulk Density (kg/m^3)	969.0±7.2	963.0±9.2
Porosity (%)	25.0±1.0	25.0±1.0
Total Solids (g/kg)	173.0±1.2	299.0±6.4
Volatile Solids (g/kg)	152.0 ±1.9	262.0±3.7
Total COD (g/kg)	220.0±3.8	241.0±2.5
Total N (g/kg)	4.40±0.50	4.00±0.50
Total P (g/kg)	3.56±0.10	2.00±0.10
pH	4.59±0.20	5.18±0.20

3.6. Analytical Methods

pH and Temperature: The pH values were measured by pH-meter and pH probe and the temperature of the constant temperature room was checked with a thermometer.

Total Solids (TS) and Total Volatile Solids (TVS): The suspended solid and volatile suspended solid of the sludge samples were performed according to the Standard Methods-2540B and 2540E (APHA, 2005)

Total Phosphorous (TP) and Total Kjeldahl Nitrogen (TKN): The TP and TKN analysis of the waste were done according to the Standard Methods-4500 N_{org} (APHA, 2005).

Nitrate (NO_3^-): Nitrate concentrations were measured according to the Standard Methods-4500 NO_3 (APHA, 2005).

The Total Chemical Oxygen Demand (tCOD) and Soluble Oxygen Demand (sCOD): The tCOD and sCOD analysis were carried out using HACH COD (0-1500) vials at 150 ^{0}C for 2 hours and the COD values were measured by HACH photometer. The sCOD of the samples were filtered from 0.45 Millipore filter papers before the analysis.

Volatile Fatty Acids (VFA): VFA measurements were conducted by using a Trace Gas Chromatograph (GC) Ultra (Thermo Co.) with a Zebron ZB-FFAP column, with a length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 μm, injector temperature of 250 ^{0}C. Helium was the carrier gas in the system. The effluent samples were filtered from 0.45 μm filter (Milipore) first, and then filtrates were passed from 0.22 μm filter paper in order to prevent column clogging. Formic acid (%98) was added to the filtered samples in order to decrease the pH below 3 to convert the volatile fatty acids to their undissociated form.

Gas Analysis: The gas composition was measured by a (GC) unit (Thermo Electron Co.) equipped with thermal conductivity detector (TCD). Methane, nitrogen and carbon dioxide were separated through a 15 m Porapak Q, 5 mm I.D column and it was operated with helium as the carrier gas at a constant pressure of 20 kPa at 40 ^{0}C. The injector was maintained at 100 ^{0}C, and the detector temperature was set to 100 ^{0}C. An individual standard for each gas was used for the calibration procedure.

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the experimental results obtained are presented. The effects of OLR on the production of VFA and on acidification of each reactor are given. The effect of OLR and pH on performance of the reactors are discussed. After acidification processes, biochemical methane potential (BMP) test was conducted with the effluent of acidified reactor that was chosen as optimum and waste stock in order to make a comparison between one-phase and two-phase systems.

4.1. Results of the Set-1 Experiments

In this set, effect of OLR on anaerobic acidification was investigated in three reactors with different OLRs of 10, 15, 20 g VS/L.day. The reactors were operated with an HRT (or SRT) of 2 days and monitored for influent and effluent tCOD, sCOD, TS, VS, VFA concentrations and corresponding acidification degrees were estimated. The experimental data obtained from the reactors with selected OLR operated at pH of 5.5 and at a temperature of 35 ± 1 ^{0}C are presented Figure 4.1. Figure 4.1 depicts the pH variation in the feed-stock solution throughout the experiment. The pH values varied between 4.30 and 6.25 for Reactor-1; whereas, the value changed between 4.30 and 5.84; 4.37 and 5.83 for Reactor-2 and Reactor-3, respectively.

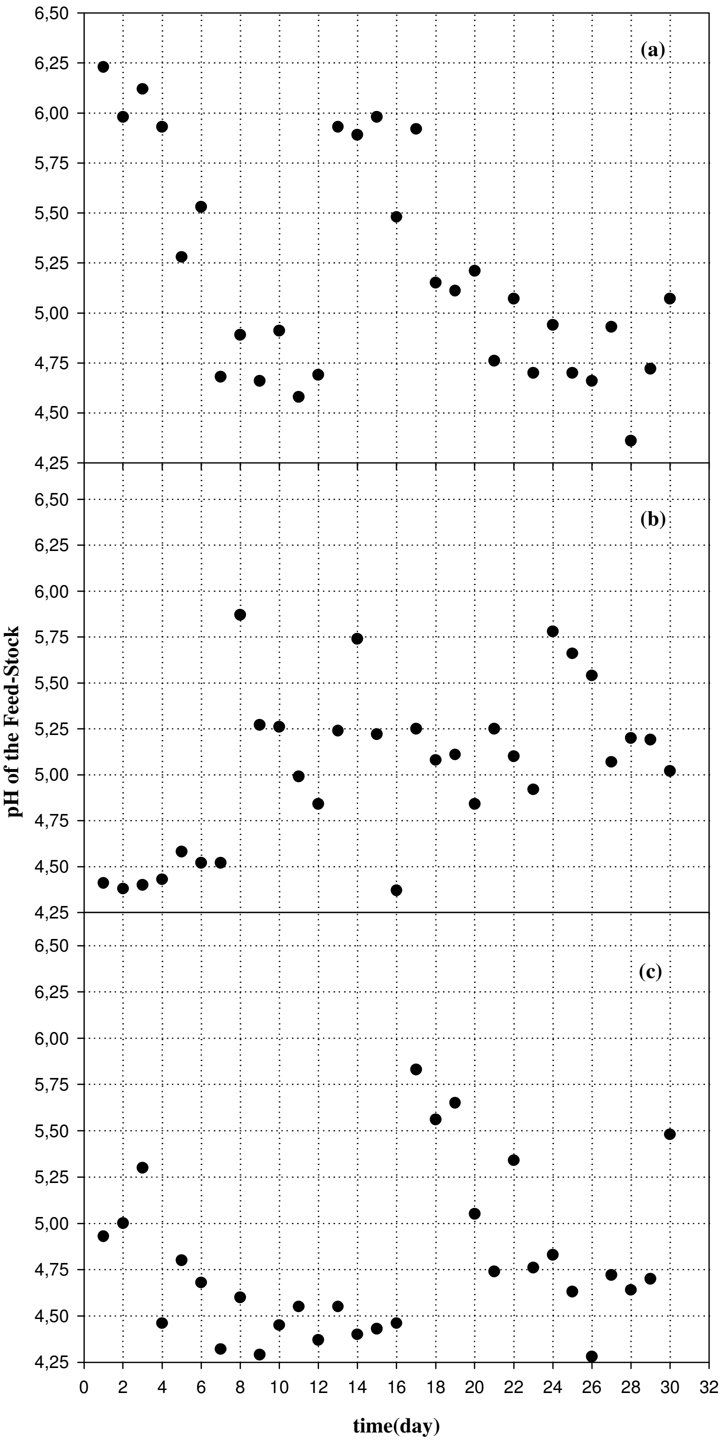

Figure 4.1: pH variation of feed-stock of OFMSW (a) Reactor-1, (b) Reactor-2 and (c) Reactor-3

The feed-stock was kept at deep-freeze (-20 ^{0}C) before feeding; however frozen waste could not be used directly for the feeding procedure without thawing; thus, thawing process and keeping thawed waste in the fridge for a week for the feeding procedure resulted in decrease in pH value due to natural pre-acidification. Although the feed-stock used for all reactors was the same, keeping the diluted stock in the fridge for one week resulted in the pH change.

4.1.1. Total COD (tCOD) and Soluble COD (sCOD) Concentration Variations

The influent and effluent tCOD and sCOD concentration variations were depicted in Figure 4.2a-c. Maximum tCOD concentration observed in Reactor-1 was about 40000 mg/L; whereas the minimum was around 25000 mg/L as seen from Figure 4.2a. The effluent sCOD concentrations were higher than influent sCOD concentrations which was the indication of hydrolysis of the particulate organics in the reactor. The maximum tCOD concentration reached to 75000 mg/L in Reactor-2; yet, the values fluctuated between 40000 and 60000 mg/L during the course of the reactor operation (Figure 4.2b). The concentration increased to 30000 mg/L on Day-4; then decreased to 20000 mg/L and then remained constant at this value with small fluctuations until the end of the experiment in Reactor-2 (Figure 4.2b). In Reactor-3, the maximum tCOD concentration was measured as around 72000 mg/L and the minimum appeared as 21900 mg/L; on the other hand, the maximum and minimum sCOD concentrations determined in the reactor were 20250 and 32500 mg/L (Figure-4.2c). The value that sCOD concentration reached in Reactor-3 was higher than those observed in Reactor-1 and Reactor-2. The average percent increases in sCOD concentration estimated as 30%, 34% and 40% for Reactor-1, 2 and 3, respectively. Hence, it can be concluded that as the OLR increased, the concentration of soluble COD increased. The sCOD rise in effluents for all reactors was due to the hydrolysis of organic matter as stated in the literature (Argelier et al. 1998; Guerrero et al., 1999; Wang et al., 2002). In other words, conversion of organics into VFAs appeared as increase in sCOD concentrations.

Although some differences existed between the influent and effluent tCOD concentrations of reactors (Figure 4.2.a-c), the results were practically very close to each other when the standard deviations of the COD analysis are considered; which indicates tCOD removals were not achieved in the reactors. The differences were probably due to the heterogeneous composition of the waste. High particulate composition of the influent and effluent samples led to deviations in the measurements of tCOD concentrations; thus, high standard deviations were observed. Therefore, the conversion of particulate organics into soluble COD occurred in the acidifying reactors but the total COD was not reduced. It is already stated in literature that there is minimal reduction of COD in the acid fermentation stage since the complex compounds such as short-chain fatty acids, alcohols produced, and new bacteria cells exert an oxygen demand (Speece, 1996). In addition, Guerrero et al. (1999) observed no significant COD removal in the acidified reactors operated at HRT of 24 hr with organic solids and protein indicating that the end products remained as solubilised compounds in the effluent. Therefore, the results of this study were compatible with the literature work (Speece, 1996; Guerrero et al., 1999) since no reduction in tCOD was observed in the acidifying reactors.

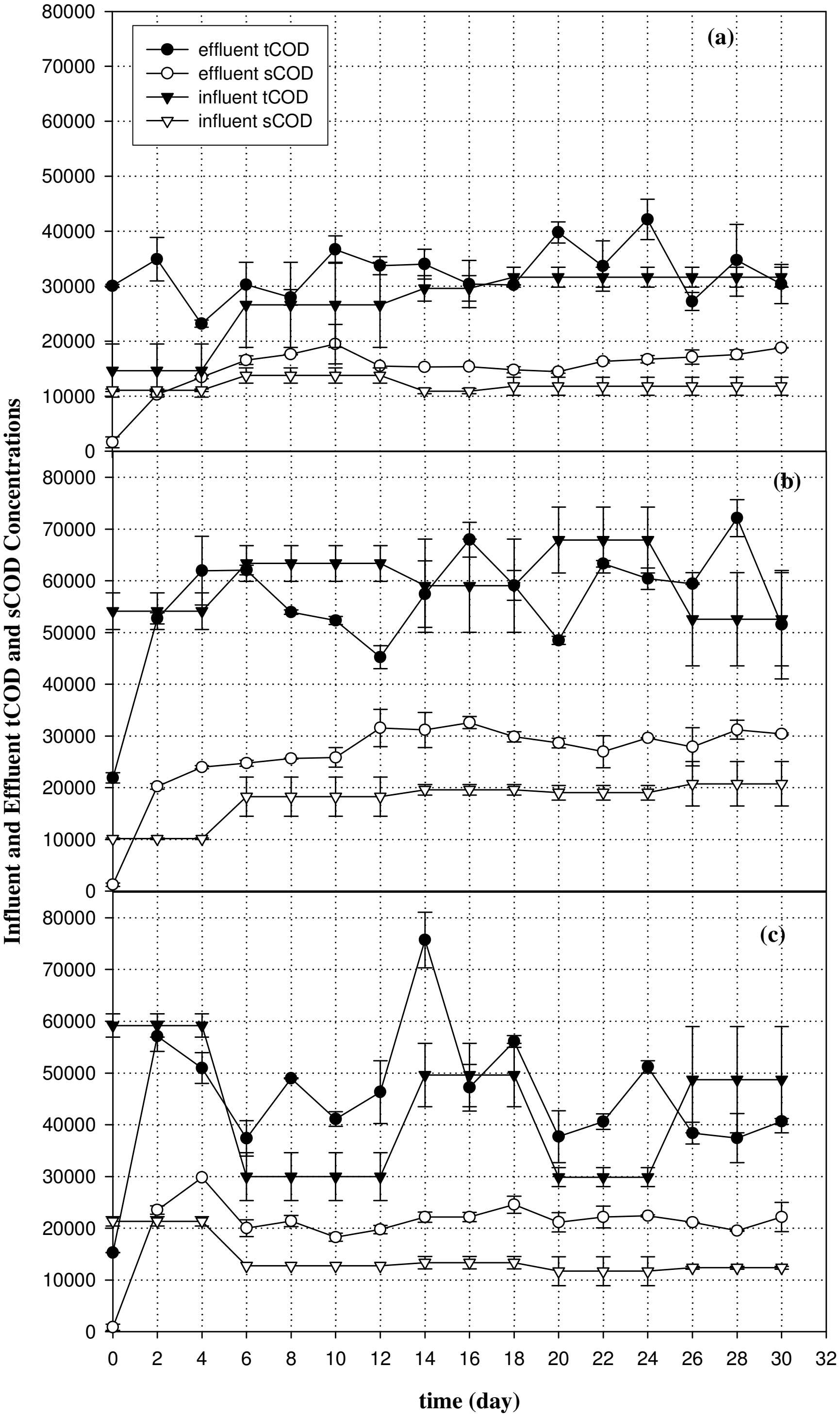

Figure 4.2: Influent and effluent tCOD and sCOD (a) Reactor-1 (b) Reactor-2 and (c) Reactor-3

In addition, the ratio between sCOD and tCOD is also an indicator for the evaluation of the reactor performances. The average influent sCOD/tCOD ratios were 43%, 33% and 30%; whereas, the average ratio percentages of effluents were calculated as 48%, 48% and 46% for Reactor-1, 2 and 3, respectively (Figure 4.3). Consequently, increases in the ratio were 5%, 15% and 16% for the Reactors 1, 2 and 3, respectively. The results revealed that percent solubilization performances increased as the organic load increased throughout the experiment since increase in load might enhance the hydrolysis process (Raynal et al.,1998). The results were also compatible with average VS reductions; that is, increase in VS reduction resulted in rise in the soluble COD concentration which led to increase in sCOD /tCOD ratio. Traverso et al. (2000) conducted experiments with a pilot scale mesophilic anaerobic completely stirred fermenter with mixture of vegetable and fruits, and the percent of sCOD/tCOD ratio in reactor was estimated as %43.47 which is a value close to the ones estimated in this study.

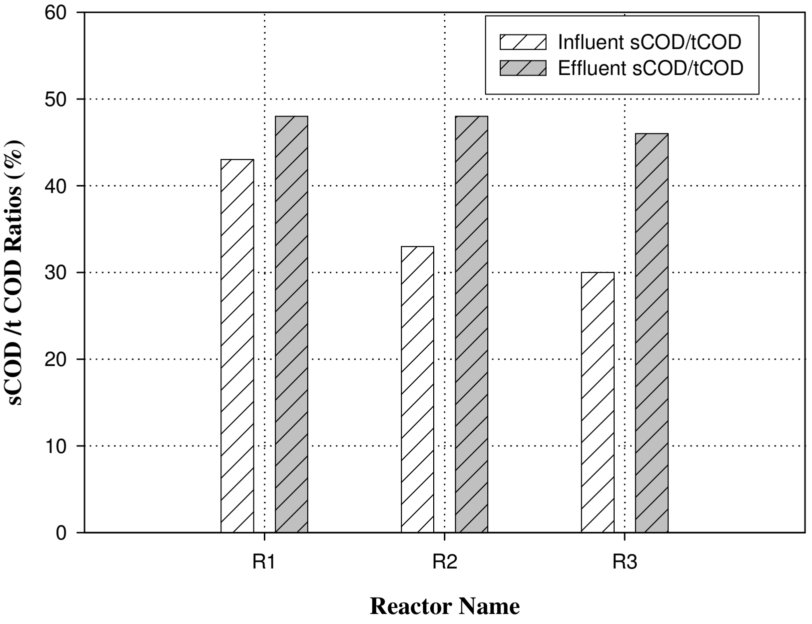

Figure 4.3: Influent and effluent sCOD/tCOD ratios of reactors

No reduction in tCOD along with increase in sCOD concentrations were observed in this study. In the majority of the studies (Speece, 1996; Argelier et al., 1998; Guerrero et al., 1999; Wang et al., 2002), tCOD reduction was not observed in the acidified reactors; yet, the conversion of organic matter into solubilised form, that is, increase in sCOD, occurred. Therefore, results obtained in this study were consistent with the literature work.

A CSTR model investigation on tCOD in the acidified reactors was applied in order to understand the behavior of the tCOD variation (Figure 4.4). The effluent tCOD concentrations of the reactors were calculated by model in case there was no removal in the reactors. The results depicted that the theoretical effluent tCOD concentrations reached 36914, 55412, 73882 mg /L for Reactor-1, 2 and 3, respectively when there was no microbial utilization of the substrate. Even though the experimental measurements for effluent tCOD concentrations were lower than the modeled values on some days (Figure 4.4 a-c) which may indicate the removal of tCOD in the reactors, their values were nearly the same when the standard deviations were taken into considerations. Therefore, it was also proved by model that there occurred no removal in tCOD concentrations in each reactor.

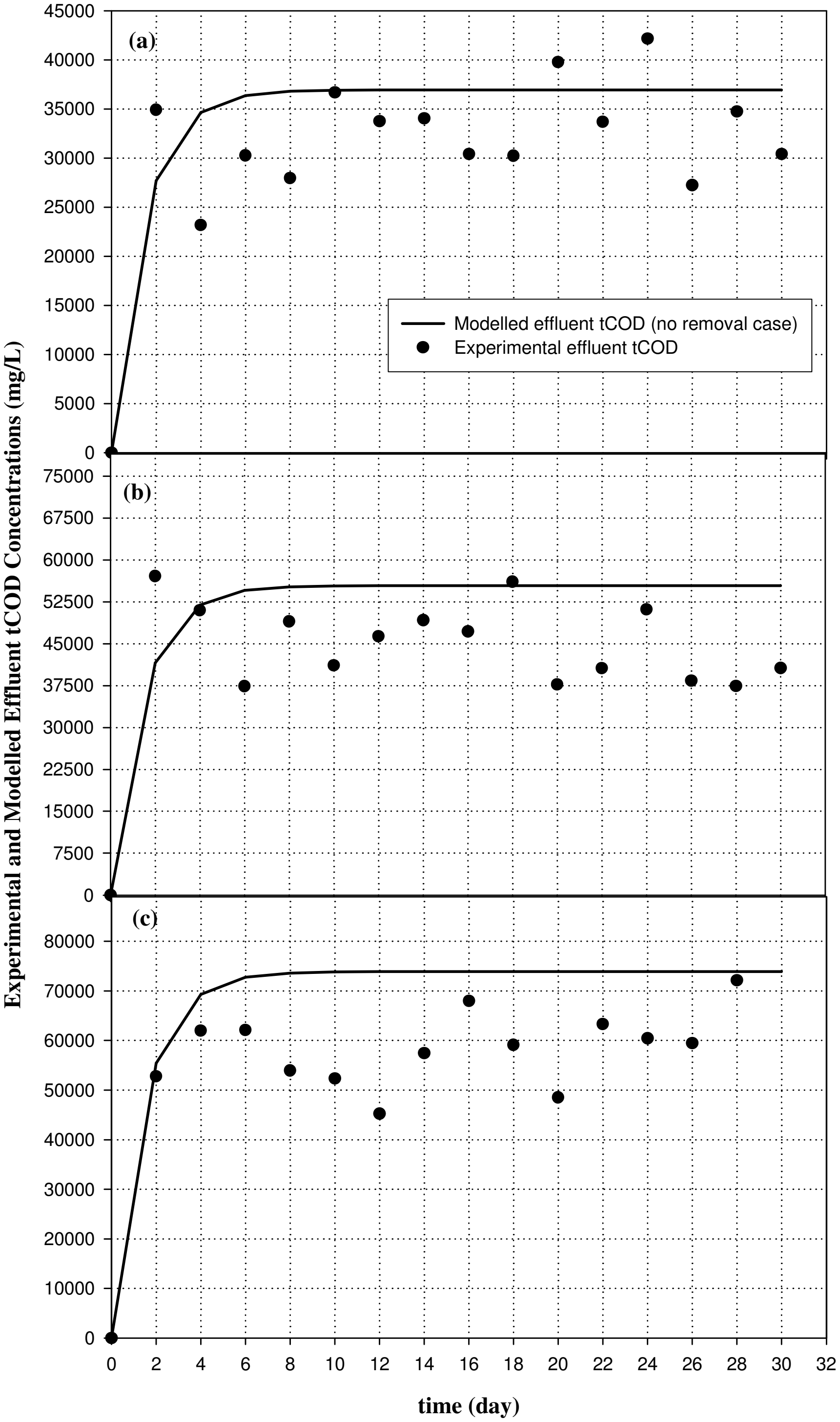

Figure 4.4: Theoretical tCOD concentrations for (a) Reactor-1, (b) Reactor-2 and (c) Reactor-3

Hydrolysis rate constants of the reactors are other indicators of hydrolysis performance. Linke (2006) studied the kinetic of anaerobic digestion of solid wastes from potato processing with fed-batch CSTR. The equation was based on a mass balance in a CSTR and first order kinetic was used for the calculation of the hydrolysis rate constants of the reactors (See Appendix).

The hydrolysis rate constants were estimated as 0.0105 day^{-1}, 0.05200 day^{-1} and 0.0721 day^{-1} for Reactor-1, Reactor-2, and Reactor-3 respectively. The results indicated that as the load increased, the value of hydrolysis rate constant increased similar to the studies reported in the literature (Raynal et al.,1998). The results were also consistent with the VS reduction trends of this study; that is, the VS reduction in Reactor-3 was higher than that observed in Reactor-2; similarly the value in Reactor-2 was higher than in Reactor-1. Vieitez et al. (2000) studied biphasic fermentation to a simulated laboratory-scale landfill with OFMSW and the first order hydrolysis rate constant was evaluated as 0.017 day^{-1}. Veeken et al. (1999) stated the hydrolysis of all biowaste components could be described by first-order kinetics and the study indicated that the composition of the waste strongly affected the rate. The hydrolysis rates were estimated as 0.195, 0.215, 0.076, 0.087, 0.264, 0.090 day^{-1} for wastes of bread, leaves, bark, straw, orange peelings and grass, respectively. An exact literature value for rate constant could not be stated as an example for the comparison since the content of the waste stock was complex in this study; that is it consisted of different types of waste at a unique combination. However, the rate constants depict different values for different type of wastes in the literature and the rate values estimated in this study are acceptable since they are in the range stated in the literature studies (Veeken et al., 1999; Vieitez et al., 2000).

4.1.2. Total Solid (TS) and Volatile Solid (VS) Concentration Variations

Effluent and influent TS and VS concentration of the reactors were illustrated in Figure 4.5. The effluent TS concentrations were lower than influent TS values except the days between 10 and 20 in Reactor-1. Influent TS concentrations of the feedstock varied between 25000 and 30000 mg/L; whereas, the effluent TS concentration varied between 25000 and 32000 mg/L (Figure 4.5a). A similar situation was observed in Reactor-2; that is, effluent TS and VS concentrations were lower than influent values. The maximum TS concentration observed in the influent was around 49000 mg/L; whereas, the concentration was measured as 45000 mg/L for influent VS (Figure 4.5b). The effluent TS and VS concentrations were around 45000 and 25000 mg/L throughout the experiment. On the other hand, the influent TS and VS concentrations measured around 55000 mg/L and 50000 mg/L for Reactor-3 where effluent values were lower than those measured in influent as depicted in Figure-4.4c. The concentrations were varied between 30000 to 50000 mg/L and 20000 to 35000 mg/L for TS and VS concentrations, respectively. The numbers revealed that the effluent VS concentrations were lower than influent concentrations for all reactors which depict the reductions in VS concentration in all reactors. The average VS reductions were estimated as 28%, 34% and 40% in Reactor-1, Reactor-2 and Reactor-3, respectively.

When the literature studies tabulated in Table 2.1 were investigated, the numbers for VS reduction varied between 26% and 91% and clearly the VS reductions affected by HRT, OLR and temperature values applied to the reactors. Low OLRs, high HRT and temperature values generally result in high reduction percentages, although some exceptions were observed. Liu et al. (2006) observed 18.7 % VS reduction in the first reactor (continuously stirred fed-batch) of a two-phase system utilizing household solid waste with OLR, HRT and pH value of 37.5 g VS/L.day, 2 days and 5.5, respectively. The VS reduction (18.7%) obtained in the study of Liu et al. (2006) was lower since the OLR (37.5 g VS/L.day) was quite higher than those applied in this study. It is obvious high loads in OLR may decrease the VS removal efficiency, since extra load increases the stress on microorganisms (Oktem et al., 2006). Therefore, the VS reduction values in this study make sense since OLRs (10, 15 and 20 g VS/L. day) were lower than that of the study of Liu et al. (2006).

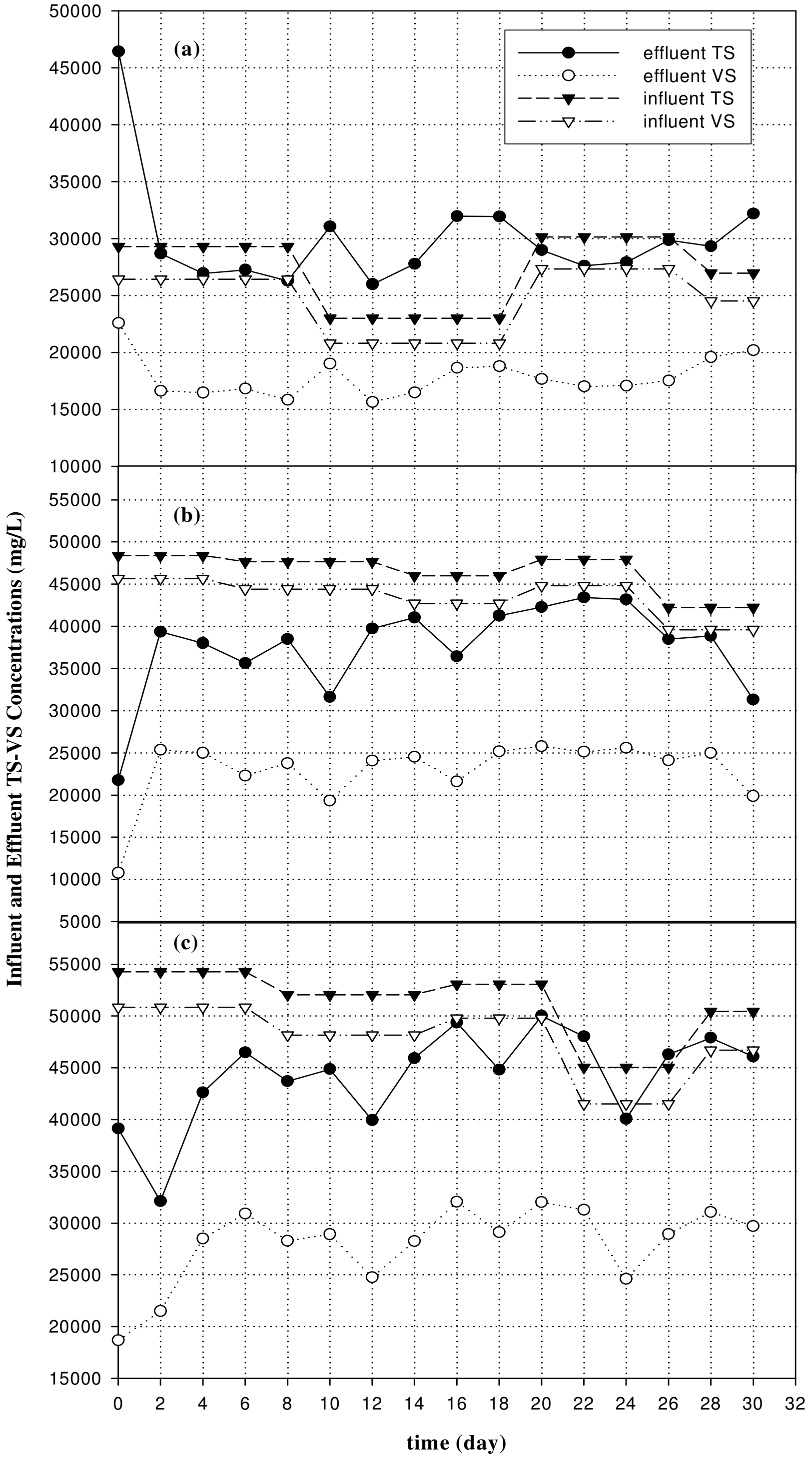

Figure 4.5: Influent and effluent TS and VS (a) Reactor-1 (b) Reactor-2 and (c) Reactor-3

In addition, Scherer et al. (2002) calculated the VS reduction as 46% in the acidified reactor with a HRT and OLR of 4 days and 10.6 g VS/L. day treating organic fraction of municipal solid waste. Hence, although some of the operational conditions were different, the VS reductions observed in this study were in the range stated in the literature (Liu et al., 2006; Scherer et al., 2002). However, it should be noted that every study had its unique system configuration and parameters, and therefore, different VS reduction percentages were unavoidable. Hence, one to one comparison between the values could not be made.

In addition to that, the VS reduction values for the reactors are compatible with the increases in solubilization percentages (Figure 4.3); that is, as the VS reductions increased in the reactors, the solubilization percentages increased simultaneously due to the conversion of solids into the solubilized form.

In order to understand the VS reduction behaviors of reactors and to make a comparison between experimental and theoretical data, a CSTR model was applied (Figure 4.6 a-c). It was assumed in the model that no destruction / degradation of the substrate occurred in the reactor which was under feeding and wasting process. As can be seen in Figure 4.6(a-c), removals in VS concentrations were achieved in all reactors since experimental effluent VS concentrations were lower than those estimated by model. Therefore, it was clear removals occurred in the reactors due to the hydrolsis.

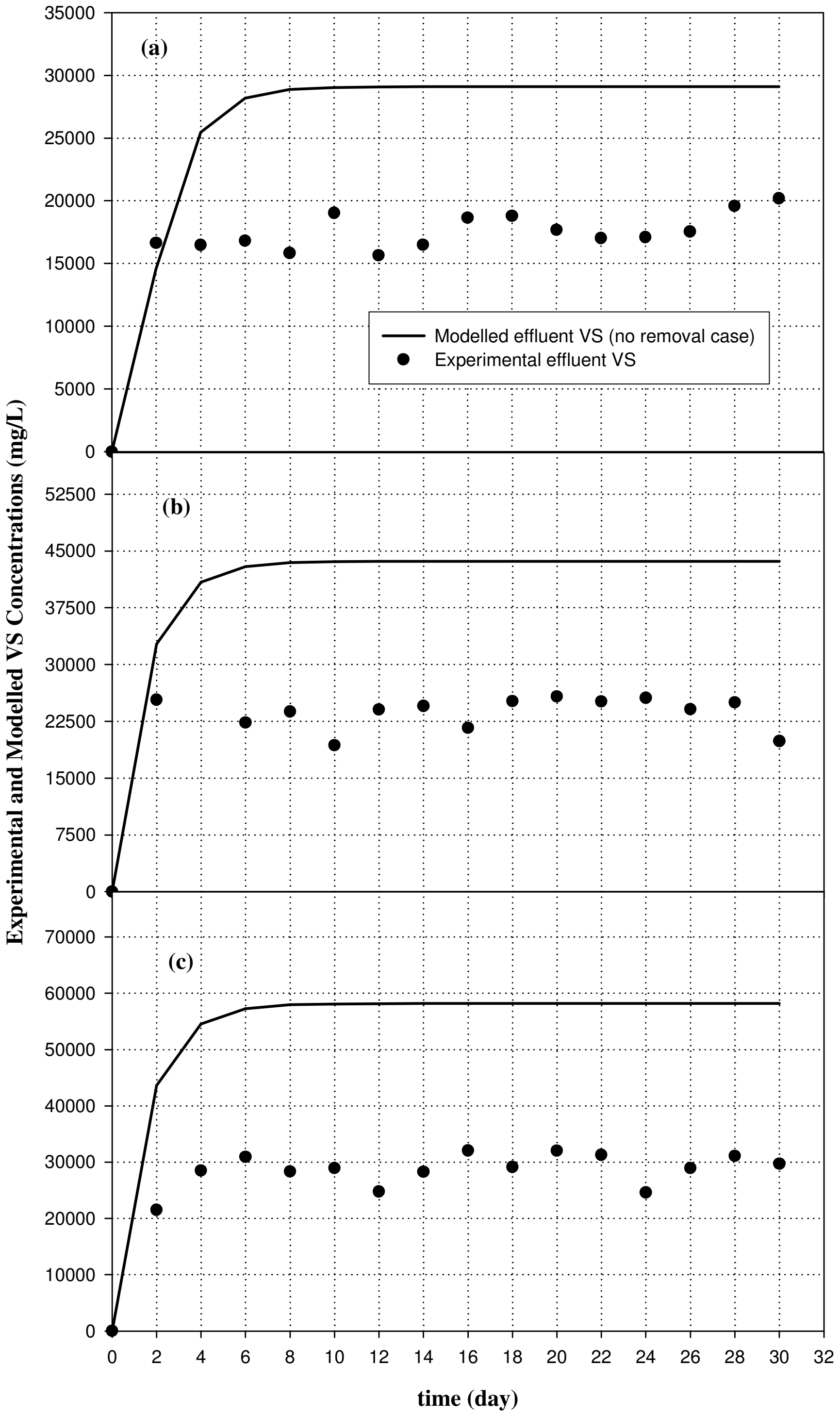

Figure 4.6: Theoretical VS concentrations for (a) Reactor-1, (b) Reactor-2 and (c) Reactor-3

The maximum effluent VS concentrations estimated by model for Reactor-1, Reactor-2 and Reactor-3 were 30000 mg/L, 44000 mg/L and 58000 mg/L, respectively. On the other hand, the average effluent VS concentrations measured around 17867 mg/L, 22825 mg/L and 28032 mg/L in the experimental studies. It is clear from Figure 4.6 that the experimentally measured effluent VS concentrations were lower than those estimated by model. This result proved that removals in the reactors occurred throughout the operation period.

4.1.3. Volatile Fatty Acid (VFA) Concentration Variations

The VFA variations were estimated during the experiments and the results were illustrated in Figure 4.7-4.9. The total VFA production in the reactors increased as the organic load increased throughout the experiment (Figure 4.7). The average values calculated as 6390, 9590, 12287 mg as HAc/L for the Reactor-1, Reactor-2 and Reactor-3, respectively. The concentrations increased up to a certain point in the first eight days of the experiment, and then stayed constant with little fluctuations for all reactors. In addition, the maximum tVFA concentrations were 8419, 12405 and 15244 mg as HAc/L in Reactor-1, Reactor-2 and Reactor-3, respectively. It is stated that total VFA production increases as the OLR increases in an acidified reactor (Dinopoulou et al*.,* 1988 and Demirel and Yenigün*.,* 2004). Hence, the production of VFAs increased as the OLR increased in the reactors for this study as expected.

The rise in VFA concentrations was compatible with the increase in sCOD concentrations and in VS reductions of the reactors. In other words, tVFA concentrations in the reactors rose as the load increased and this situation resulted in increases in sCOD concentrations for all reactors. The average percent increase in sCOD between Reactor-1 and Reactor-2 was calculated as 32%, whereas the value was estimated as 28% between the Reactors-2 and 3. On the other hand, average COD equivalence of tVFAs was determined and then, percent increases for tVFA productions between reactors were estimated. The values were 33% and 28%

between the Reactors-1 and 2, and Reactors-2 and 3, respectively. As a result, the percent sCOD rises were consistent with those calculated for VFAs. The 33% rise in tVFA concentration, for instance, resulted in 32% increase in sCOD concentration since tVFA in an acidifying reactor means sCOD. The trend of tVFA concentrations was also reasonable when considering the VS reductions; that is, the VS removals appeared as rises in the tVFA concentration when the organic matter was solubilized in the reactors.

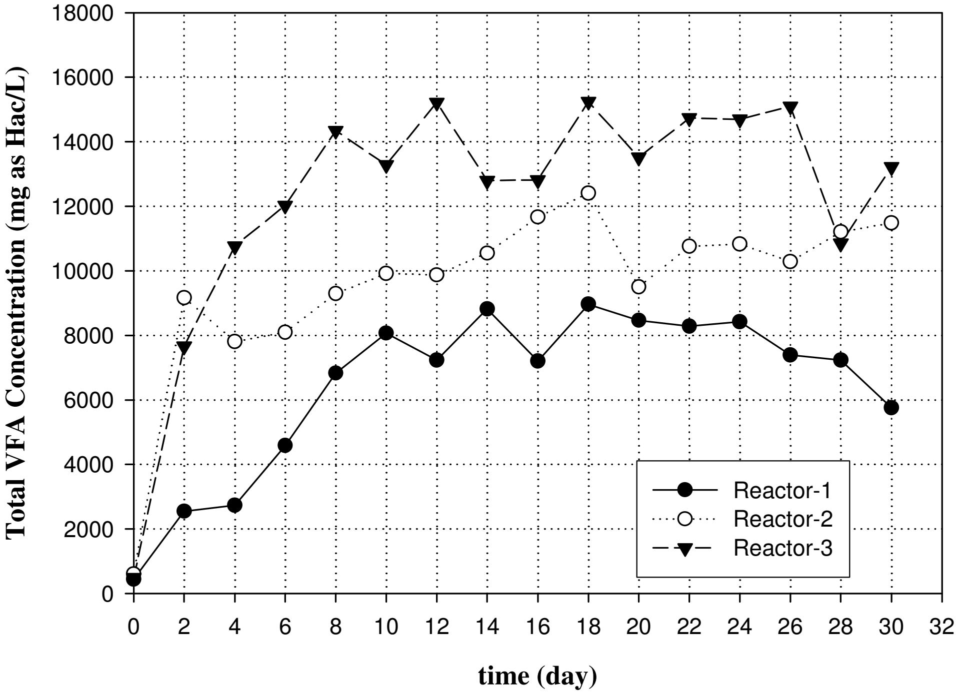

Figure 4.7: Total volatile fatty acid concentrations observed in reactors

The VFA profiles in the reactors mainly consisted of acetic acid as depicted in Figure 4.8. The VFA concentrations in Reactor-1 increased until Day-14, and then stayed constant with little fluctuations in concentrations (Figure 4.8a). The acetic acid concentration reached to 5500 mg/L, whereas the maximum butyric and propionic acid concentrations were 3000 and 2500 mg/L as illustrated in Figure

4.8a. On the other hand, the acetic acid concentration in Reactor-2 reached to 9000 mg/L on Day-18 in a continuous increasing trend, and then decreased to the concentration of 8000 mg/L and little fluctuations were observed in the concentration till the end of the experiment. The concentrations of remaining VFAs were increased on Day-2, and then stayed constant throughout the experiment (Figure 4.8b). The VFA profile in Reactor-3 was similar to those observed in the other reactors. Main organic acid measured was acetic acid and the concentration reached approximately to 12000 mg/L on Day-12 then remained at this level with little fluctuations. The remaining VFAs also reached to their maximum value on Day-12, then, remained constant until the end of the operation (Figure 4.8c). Wang et al. (2002) conducted studies with a two-phase system consisting of a solid waste (food waste) acidified reactor and the predominant VFAs were 4400 mg/L acetic acid, 4300 mg/L butyric acid and 2800 mg/L propionic acid. Therefore, the existence of acetic, propionic and buytric acids was an expected result in the process of solid waste acidification which was applied in this study.

Ethanol is classified as one of end-products of anaerobic acidification process of solid waste. The existence of glucose fermentation may result in a number of possible fermentation products apart from organic acids in anaerobic digesters, mainly lactate and ethanol. Yet, since the lactate is subsequently degraded very quickly, primer one stays as ethanol in the reactors (Batstone et al., 2002). The existence of glucose in the solid waste used in this study was most probably the reason for the ethanol production. The maximum ethanol concentrations detected in Reactor-1 was 766 mg/L; whereas, 1120 mg/L was measured in Reactor-2. The production made its peak in Reactor-3 with a concentration of 1686 mg/L. However, the concentrations fluctuated throughout the experiments (Figure 4.8a-c) and average ethanol concentration calculated as 247, 432 and 971 mg/L.

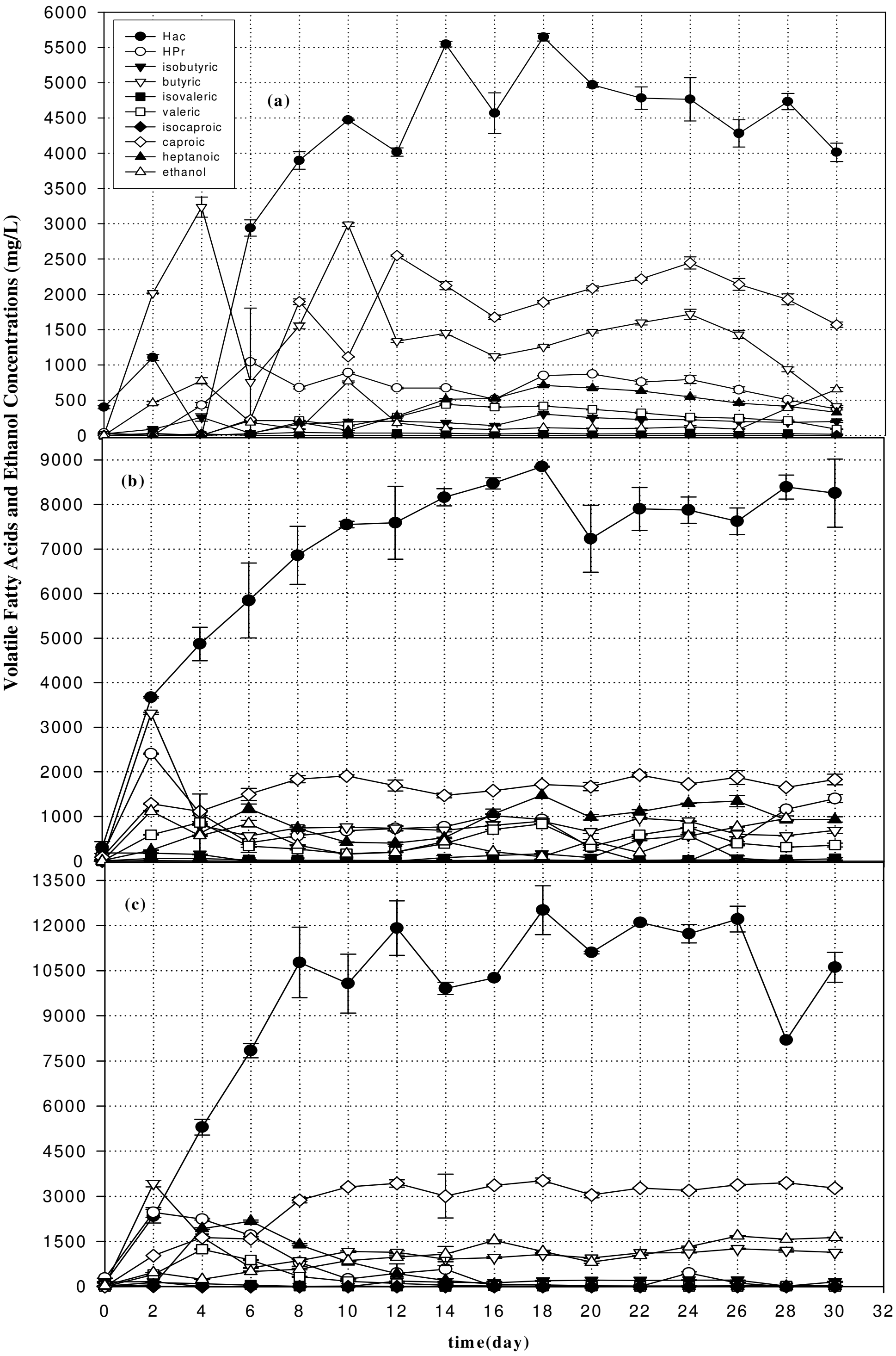

Figure 4.8: Volatile Fatty Acid (VFA) concentration in (a) Reactor-1, (b) Reactor-2 and Reactor-3

It is obvious that the concentration of ethanol rose as the load increased since glucose content increased. Zhang et al. (2007) determined the maximum ethanol concentration as 254 mg/L in the 25 mL batch reactors treating cornstalk wastes. The values obtained in this study were higher than the values stated in literature and the reason could be the difference in the experimental conditions such as substrate type, reactor volume, SRT and especially OLR.

In literature, the butyric-type fermentation is characterized by the production of butyrate and acetic acids plus carbon dioxide and hydrogen gases, whereas the propionic type fermentation produces mainly propionate, acetate, and some valerate, with no significant gas production (Ren et al*.,* 1997). Therefore, both butyric-type and propionic type of fermentation (Ren et al*.,* 1997) were achieved in this study since the production of butyric and acetic acid with carbon dioxide and hydrogen gas production; and, propionic, valeric acid formation was detected in the reactors throughout the experiments. In addition, the ethanol-type fermentation was observed in the reactors due to the presence of ethanol in the reactors.

The compositions of VFAs were also estimated and the results were summarized in Figure 4.9. The main VFAs observed in Reactor-1 were acetic, propionic, butyric, and caproic acids with the percentages of 59, 7, 15 and 12%, respectively. Around 2% of isobutyric, valeric, and heptanoic acids were determined in Reactor-1 as depicted in Figure-4.9. On the other hand, 71% of acetic acid, 8% of caproic acid, 6% of butyric and propionic acid and 4% of heptanoic acid were determined in Reactor-2 throughout the experiment. The percentages of VFA measured in Reactor-3 were 75, 12, 6, 4, and 1% for acetic, caproic, butyric, propionic and valeric acids, respectively.

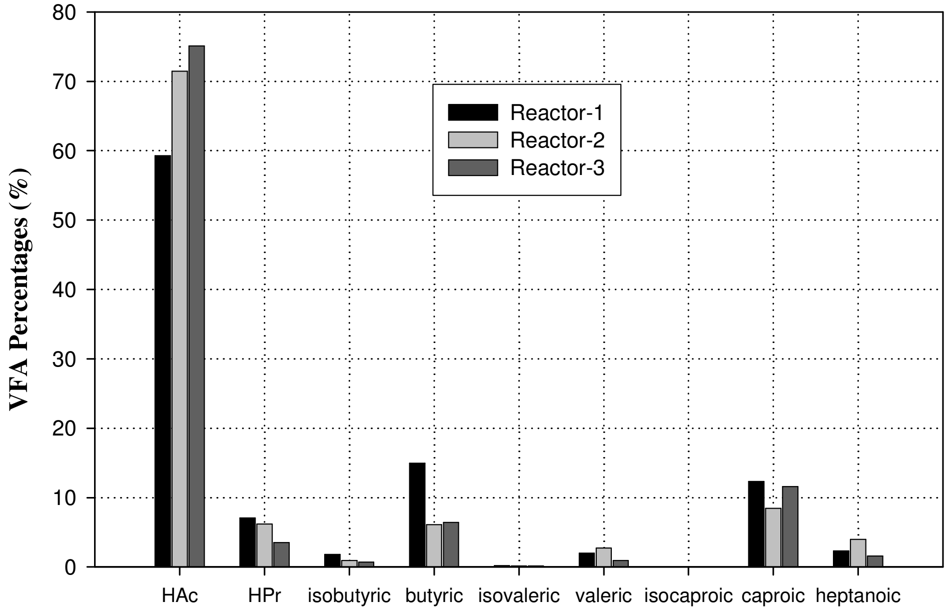

Figure 4.9: Percentages of volatile fatty acids observed in reactors

Traverso et al. (2000) performed experiments with a pilot scale mesophilic acidogenic fermenter and used source separated mixture of vegetable and fruits as substrate. The main VFAs measured in the reactor were acetic, propionic, isobutyric, butyric, isopentanoic, pentanoic, exanoic and heptanoic acids. Liu et al. (2006) observed that acetate and butyrate were the main VFAs species in this experiment which was conducted by household solid waste and it was stated 90% of the VFA was acetate at pH value of 5.2. Other volatile fatty acids measured in the study of Liu et al. (2006) were propionic, n- and iso-butyric, valeric, and iso-valeric acids. According to the literature findings as stated above, types of VFA produced as result of solid waste acidification vary due to the complex nature of the waste; however the main products, the ones with high concentrations relative to other types, are acetic, butyric and propionic acids. The production of acetic, butyric and caproic acids was higher than the other products in the reactors as depicted in Figure 4.9. The major organic acid determined in the process of anaerobic acidification of solid waste is acetic acid as stated and the main acid observed in this study was also acetic acid (Figure 4.9) which supports the fact that short-chain fatty acids are observed in higher concentrations in the anaerobic reactors fed by complex substrate like solid waste (Guerrero et al., 1999).

The percent of acetic, heptanoic and valeric acids increased from 59% to 71%, 2% to 4% and 2% to 3% as the OLR increased from 10 to 15 g VS /L.day. Further increase in OLR to 20 g VS /L.day resulted in a rise in acetic acid percentage from 71% to 75% and decrease in heptanoic and valeric acid percentages from 4% to 2% and 3% to 1%, respectively (Figure 4.9). On the other hand, the percent of butyric, caproic and propionic acids decreased from 15% to 6%, 12% to 8% and 7% to 6% by increase in OLR from 10 to 15 g VS/L.day in the study. However, the percentages remained constant for butyric acid, and decreased to 4% for propionic acid; finally increased to 12% for butyric acid with the increase in OLR from 15 to 20 g VS/L.day in Reactor-3. Viturtia et al. (1995) studied two-phase anaerobic digestion of fruit and vegetable wastes and the main VFAs observed in the acidified reactor were acetic, propionic, butyric and valeric acids with the percentages of 27, 25, 23 and 25%, respectively. The enhancement of acidification step may be achieved by increasing organic load and application of high loads probably results in the production of higher acetic acid since its formation is more feasible due to its short chain structure. Higher loads resulted in rise in the acetic acid percentages in this study, whereas the percent productions for butyric and propionic acids decreased meanwhile.

The VFA concentration and VFA composition data may be useful in deciding which pH and organic load is optimum for the methane producing reactor. Since complex organic acids are not easily utilized by microorganisms, the simple ones, such as acetic acid, are more suitable for utilization and giving effluents of an acidified reactor containing high acetic acid most probably increase the efficiency in the methane reactor as stated in literature (Wang et al., 2006).

The ratio of VFA produced per influent tCOD gives information about the production of VFAs due to anaerobic acidification of substrate (Figure 4.10). The amount of VFAs increased on Day-2 and then a sharp decline observed on Day-6. After Day-6 an increase occurred again in the reactor except Days 14 and 22 and then the ratio remained constant for Reactor-1 (Figure 4.10). A similar trend was achieved for Reactor-2; that is, the ratio was increased until Day-12; afterwards, a decrease on Day-14 was observed followed by increase in the ratio again. The production in Reactor-3 increased till Day-12, and then depicted fluctuations throughout the experiment.

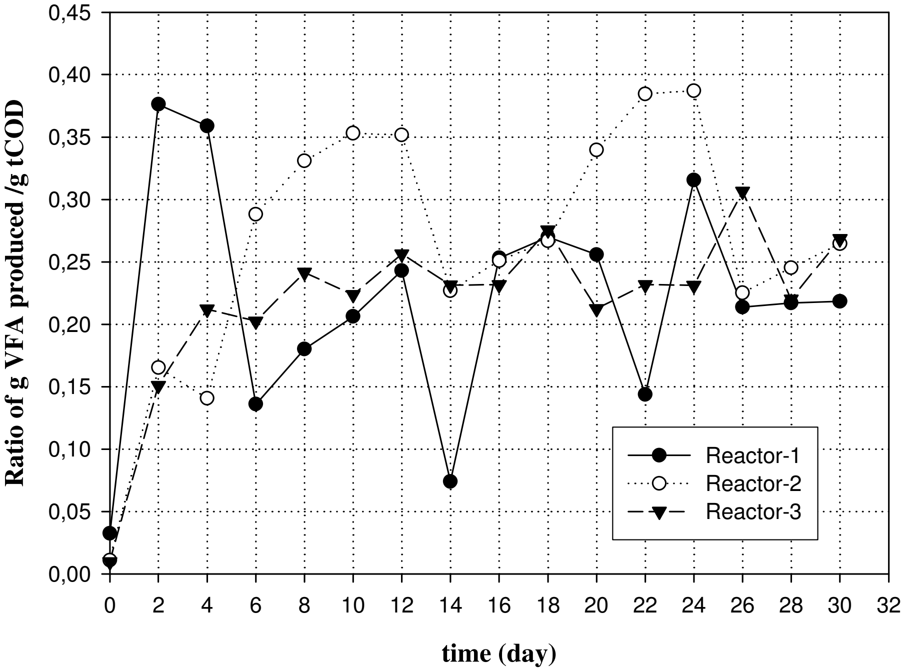

Figure 4.10: Ratio of g VFA produced/g influent tCOD for reactors

The fluctuations in the ratio are mainly due to the fluctuations occurred in the influent tCOD concentrations for all reactors. It is clear from Figure 4.10 that the production g VFA per g of influent tCOD in Reactor-2 was higher than that produced in Reactor-1. Although total VFA production was the highest in Reactor-3 due to the highest load, the production per tCOD was not the highest in this reactor. As a result, achieving maximum tVFA amounts does not mean having maximum production efficiency in a reactor. Load increases may lead rises in acid productions yet this does not mean that increases in acid production per gram of substrate will also be achieved. Therefore, it can be concluded that the g VFA production per g of substrate increased as the load increased up to a certain value; and then the production decreased with further increase in load in this study. It may be explained by the inefficiency of microorganisms to convert the substrate into organic acids due to overloading. Parawira et al. (2004) stated that the ratio may vary from study to study and the ratio may change between values from 0.4 to 0.9 g/g. The values varied from 0.1 to 0.4 g/g in this set which were lower than stated. However, OLRs, therefore influent tCOD, concentrations were higher in this study and this may resulted in lower ratios. In addition, since the ratio mostly depends on VFA production and the production of VFA also depends on OLR, HRT, and pH values, the differences between studies are inevitable.

4.1.4. Acidification Degree of the Reactors

The degree of acidification is calculated by taking the ratio of COD-equivalent of acidogenic products and the soluble COD of the waste (Ince, 1998; Parawira et al., 2004; Oktem et al., 2006 and Wang et al., 2006) (Method a). On the other hand, Dinopoulou et al. (1988), Fang et al. (2001) and Demirel and Yenigun (2004) calculated acidification degree as ratio of COD-equivalent of acidogenic products and the total COD (Method b). In this study, both calculation methods were applied and results were compared.

The daily acidification degrees were depicted in Figure 4.11 for three reactors. The trend of the acidification curves were similar for all reactors with two methods; that is, the degrees increased up to a certain point, and then, stayed constant with small fluctuations throughout the experiment. The degree values were higher in Reactor-2 which illustrated the rise in acidification as organic load increased from 10 g VS/ L.day to 15 g VS/ L.day; however, further rise in the load resulted in decrease in Reactor-3 for both methods. The degree reached to 76%, 90% and 83% for Reactor-1, Reactor-2 and Reactor-3 respectively (Figure 4.11, Method a). However, the values appeared as 29%, 38% and 26% for Method b. Since the purpose is to achieve maximum acidification degree in the acidified reactors, best time period must be chosen for maximum efficiency. As seen in Figure 4.11, all reactors reached their maximum acidification peaks in short time periods since the HRT was short (2 days). Until Day-24, the percentages remained as a plateau at maximum level with small fluctuations; however, after this day decreases were observed for all reactors with both methods. Therefore, the effluents of the acidified reactors should be given into the second reactor (in which methanogenesis occurs) within first 24 days of operation period for the maximum conversion of VFAs to methane.

The average acidification percentages of the reactors calculated by Method a and b were illustrated in Figure 4.12. The percentages were estimated as 60 ± 21%, 81 ± 18% and 76 ± 8 % for Reactor-1, Reactor-2 and Reactor-3, respectively (Figure 4.12, Method a). On the other hand, average acidification degrees estimated by Method b were 26 ± 5%, 28 ± 8% and 23 ± 4% for reactors (Figure 4.12, Method b). The results revealed that the degree increased with an increase in organic load; however, further increase resulted in a decrease in the efficiency for both methods. The analysis of variance was applied for all acidification values to check the reliability of data (Appendix, A.2); that is, to check increases or decreases in the degree were really significant. Demirel and Yenigun (2004) conducted experiments with dairy wastewater to study the effect of OLR and HRT on the VFA production of acidogenesis. The study indicated that net VFA production, consequently both degree of acidification and rate of product formation increased with increasing OLR and acidification degree increased from 20% to 60% with increase in OLR from 6 kg COD/ m^3.day to 10 kg COD/m^3.day, respectively. It is also realized from the study of Raynal et al. (1998) that as the OLR increased the acidification yield increased in all reactors having constant HRT. Therefore, increase in acidification observed with rise in OLR from 10 to 15 g VS/L. day was consistent with the literature results.

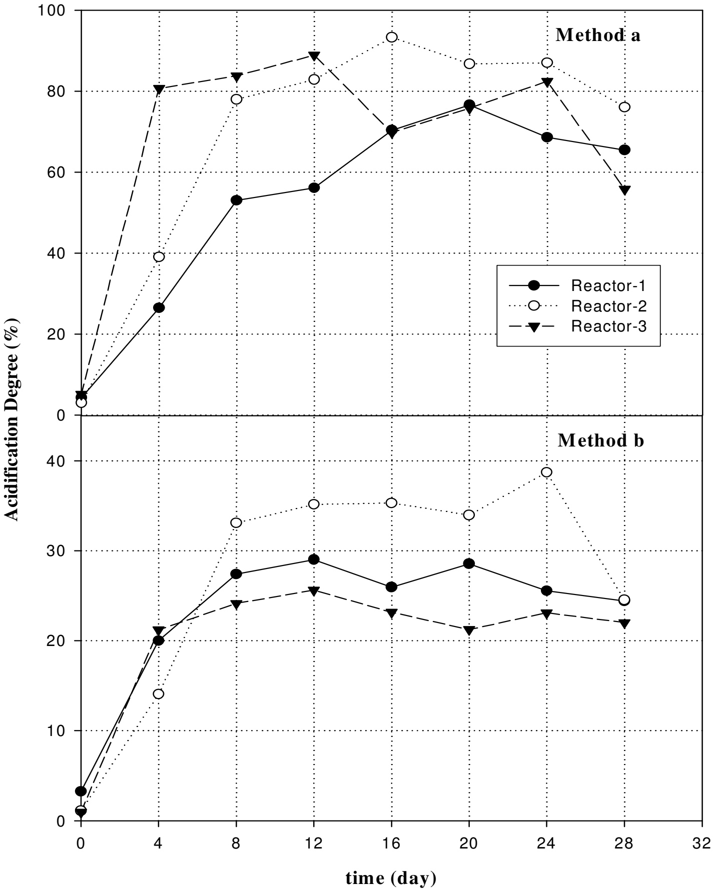

Figure 4.11: Acidification percentages obtained in the reactors

On the other hand, Dinopoulou et al. (1988) stated the degree of acidification was found to increase with the hydraulic retention time and decrease with the increase in the influent substrate concentration and organic loading rate. As a result, it can be concluded that the degree of acidification increased as the load increased up to a certain value; and then a decrease occurred in the reactors with further increase in the load in this study which can be explained by stress on acidogenic bacteria with extra organic load (Oktem et al., 2006).Moreover, it was stated that acidification of solid waste was inhibited at high total VFA concentrations (Vieitez and Ghosh, 1999); therefore, the high tVFA concentration in Reactor-3 may also cause the acidification degree decline due to partial inhibition.

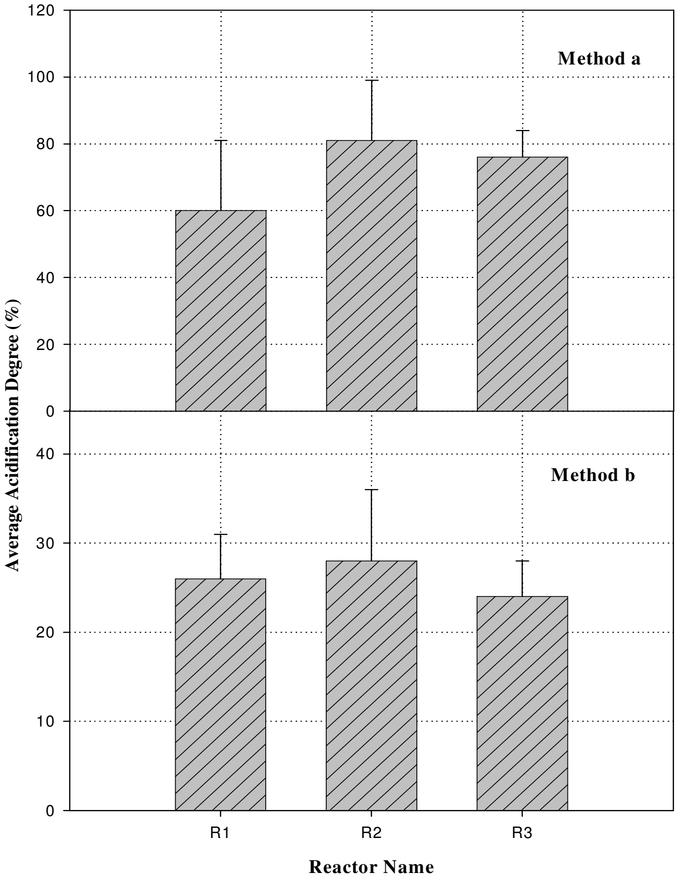

Figure 4.12: Average acidification percentages of the reactors

Table 4.1 summarizes the acidification degrees observed in different studies, of which substrate compositions were close to the one used in this study. Guerrero et al. (1999) estimated maximum acidification efficiency value as 32.5% for the hydrolysis of solids and proteins by using Method b with HRT and OLR of 2 days and 106.6 kg COD/m^3.day, respectively. Raynal et al. (1998) conducted experiments with different types of solid wastes namely potato peelings, salad leaves, green peas-carrots and apple pomace and the acidification degrees for these wastes were calculated with Method b and found as 40, 36, 44.5, 29.7% for OLR of 4 g tCOD / L.day.

Table-4.1: Acidification Degrees (%) of Different Studies Estimated by Method a and b

Waste Type (a)	**Waste Type (b)**	**Degree(%) Method a**	**Degree(%) Method b**	**Reference (a)**	**Reference (b)**
dairy wastewater	complex wastewater	60	60	Ince et al. (1998)	Dinopoulou et al.(1988)
Solid potato waste	solid waste	54-67	29.7-44.5	Parawira et al.(2004)	Raynal et al. (1998)
pharmaceutical wastewater	food industry wastewater	39-43	32.5	Oktem et al.(2006)	Guerrero et al.(1999)
Food waste	wastewater	10-20	61	Wang et al. (2006)	Fang et al. (2001)
-	Fruit and vegetable wastes	-	40.3	-	Bouallagui et al.(2004)
OFMSW	OFMSW			This Study	This Study
OFMSW	OFMSW	60	26	Reactor-1	Reactor-1
OFMSW	OFMSW	81	28	Reactor-2	Reactor-2
OFMSW	OFMSW	76	23	Reactor-3	Reactor-3

The average degrees estimated in this study by Method b were lower than the values stated in the literature (Table 4.1). The reason may be the higher organic load application. It is apparent that, at most of the studies conducted in lab scale, the low loads were chosen and these low loads were easily degraded by microorganisms and high acidification percentages were clearly achieved. On the other hand, the percentages estimated by Method a were clearly much higher than literature values. In this study, the sCOD concentrations of the waste were relatively lower than tCOD concentrations and this led to obtain higher degrees since the denominator values in the equation low during calculations. However, literature values were not above 60% which may be due to the high sCOD content of the waste used. Yet, it should also be noted that since every study has its unique system configuration and operational parameters, a complete and suggestive comparison can not be made during the course of data interpretation.

The difference between the results of Method a and Method b can be explained by high particulate organic content of the feedstock. The influent tCOD concentrations were always much higher than sCOD values in the waste stock which resulted in significant differences between the acidification degree calculations conducted by Method a & b. However, using influent tCOD values; that is, using Method b, is more accurate since VFA productions in the reactors are mainly due to the conversion of tCOD into sCOD.

4.1.5. Gas Productions and Compositions

The daily gas production variations depicted in Figure 4.13. As can be seen from Figure 4.13(a) daily gas production in Reactor-1 varied throughout the experiments and maximum production was 1100 mL, whereas the minimum measured around 400 mL. The variations in the production remained until Day-24 and after this day the gas production was constant about 900 mL/day. The cumulative gas production reached to 30000 mL for Reactor-1. The daily variation in gas production for Reactor-2 was depicted in Figure 4.13(b) and the production reached steady state on Day-18 with a value of 1390 mL. The maximum production was achieved as 1590 mL, yet the minimum observed as 390 mL. In addition, the maximum and cumulative gas productions in Reactor-2 were higher than that observed in Reactor-1. The value reached nearly to 40000 mL in Reactor-2; whereas, 30000 mL cumulative gas production was achieved in Reactor-1. The gas production trend in Reactor-3 was similar to those observed in Reactor-1 and Reactor-2. The maximum gas production was measured nearly as 2000 mL; whereas the minimum appeared as 1235 mL. However, the gas production stayed constant at the value of 1640 mL (Figure 4.13c). In addition, the highest cumulative gas production was observed in Reactor-3 with a value of 49000 mL. As a result, it can be concluded that increase in organic load resulted in a rise in gas production.

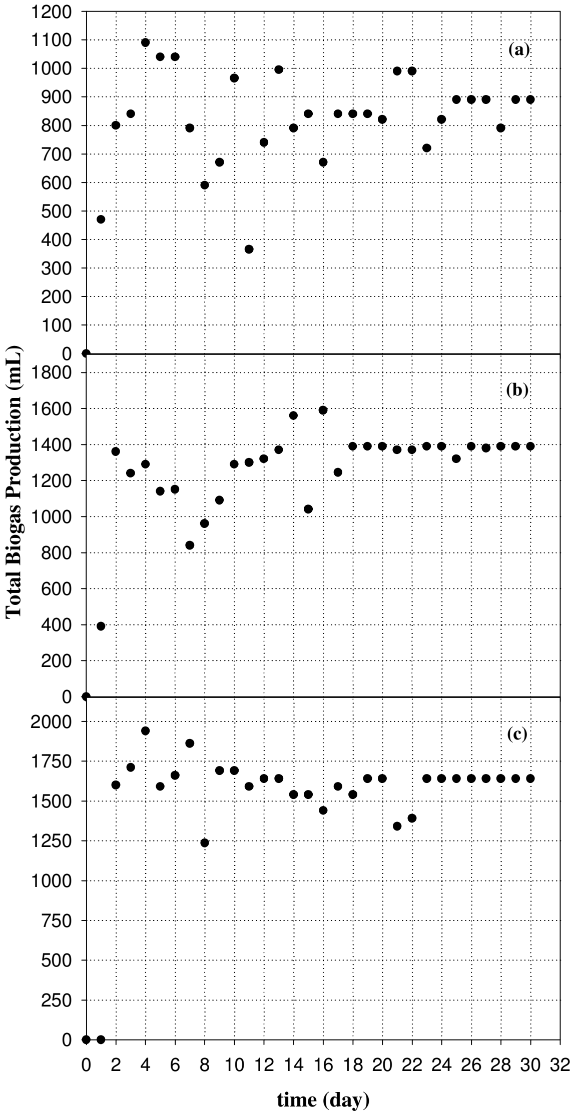

Figure 4.13: Daily Gas Production of the Reactors (a) Reactor-1, (b) Reactor-2 and (c) Reactor-3

There is also a relation between gas production amounts and VS reduction percentages of the reactors. The VS reductions increased by the increase in organic load as stated in Section 4.1.2. These reductions may be related to the increase in sCOD and tVFA concentrations and also increase in gas productions. The amount of gas productions rose with the rise in the VS reduction percentages in the reactors.

The gases observed in the reactors were nitrogen, carbon dioxide and hydrogen. As can be seen from Figure 4.14(a), the majority of the gas production in Reactor-1 was N_2 gas. However, the amount of N_2 decreased from 952 mL to 92 mL, while the CO_2 and H_2 production increased throughout the experiment for Reactor-1. The maximum amount of nitrogen was 952 mL; yet 614 mL and 38 mL were measured for CO_2 and H_2 gas productions, respectively. On the other hand, the gas productions in Reactor-2 differed from Reactor-1 in terms of CO_2 production. The CO_2 production in Reactor-2 was higher than that observed in Reactor-1 except the last week of the operation. Nitrogen production was again high in quantity and the amounts showed fluctuations throughout the experiment. Maximum nitrogen production was 1341 mL in the reactor where 49 mL CO_2 and no H_2 were determined (Figure 4.14b). The amount of gas production measured in Reactor-3 showed variations throughout the experiments. The maximum H_2 production was 25 mL; yet the minimum was calculated as 4 mL. The productions of N_2 and CO_2 gases were again higher than the production of H_2 in Reactor-3 as observed in Reactor-1 and Reactor-2. 1154 mL nitrogen gas production was measured as maximum value; whereas, the minimum value was 639 mL in Reactor-3 (Figure-4.14c). Carbon dioxide amounts (in mL) were lower compared to production of nitrogen gas. The produced amount varied between 88-693 mL as depicted in Figure 4.14(c).

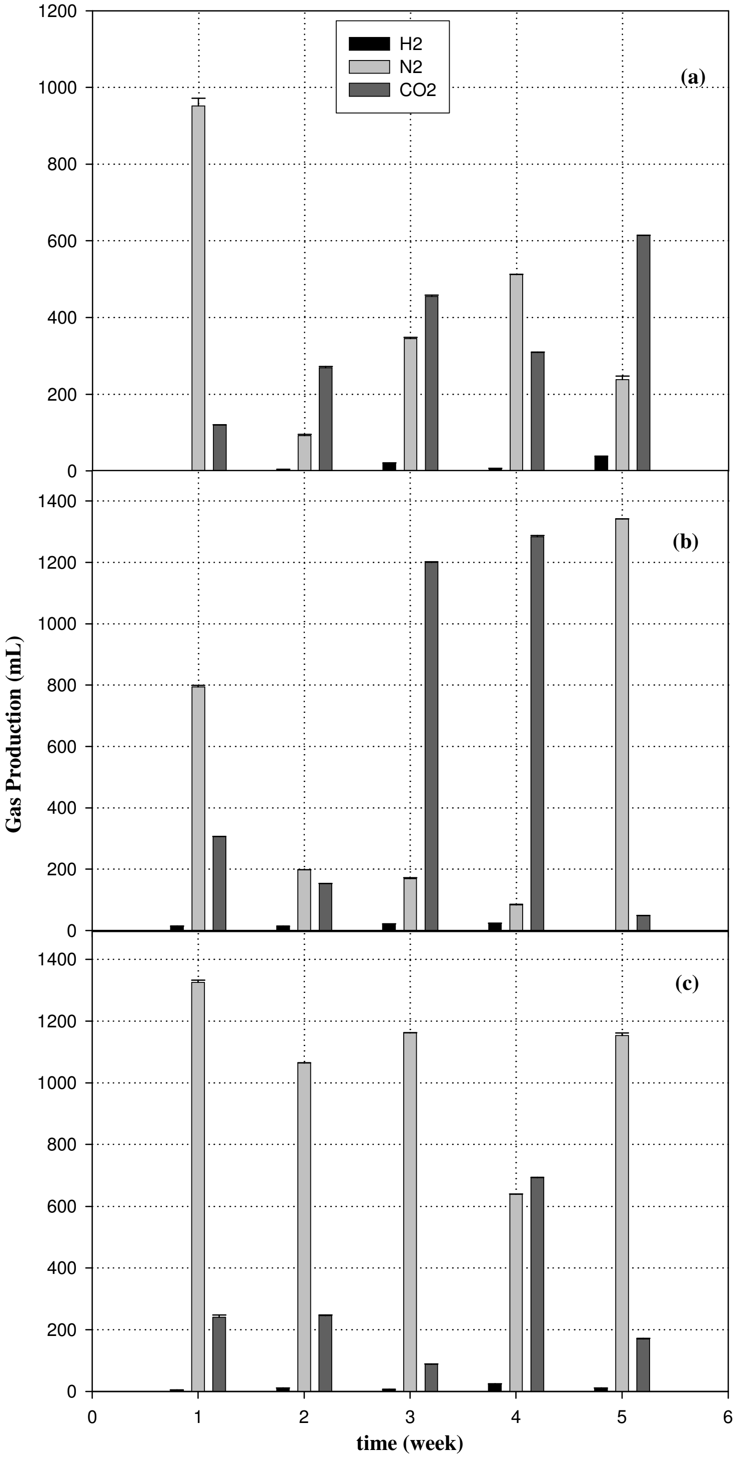

Figure 4.14: Amount of Weekly Gas Compositions of the Reactors (a) Reactor-1, (b) Reactor-2 and (c) Reactor-3

Vieitez et al. (1999) studied biogasification of solid wastes by two-phase system and solid-bed head gas consisted of 75% CO_2, 20% N_2, 2% H_2 and 3% CH_4. Wu et al. (2004) stated that hydrogen gas production was highly pH dependent. The hydrogen gas contents ranged from 58 to 60% when pH in the range 6-8; whereas, no hydrogen was detected at pH 4-5. Fermentation product data show that at pH 4-5, carbon was removed and converted into VFA and alcohol, but not H_2 gas. Zhang et al. (2007) studied enhanced biohydrogen production from cornstock wastes and found that when the initial pH value of the reactor was below 4.0 the production of hydrogen gas ceased and the production increased as the load increased in the reactor with pH values higher than 4.0. Hence, small rise in hydrogen gas production in Reactor-2 was due to the increase in organic load and the reason of low hydrogen production in reactors was the low pH value.

The results revealed that increase in organic load resulted in rise in the production of the N_2, CO_2 and H_2 gases. The production of CO_2 and H_2 and absence of methane proved that the fermentation was achieved successfully in the reactors. In addition, the production of N_2 was an indication of denitrification in the acidification phase. Vigneron et al. (2007) and Rustrian et al. (1998) stated denitrification could be achieved in the acidogenic reactor and nitrogen gas was the end product. Therefore, it can be concluded denitrification took place in the reactors since the nitrogen gas production was observed. The studies related with anaerobic acidification of OFMSW do not especially focus on the denitrification and nitrogen gas production; therefore a complete analysis could not be conducted on limited results stated in the literature.

Although there were not many studies in the literature about the gas production and types, the gas percentages obtained in this study may be accepted as consistent with the values stated in the limited studies of the literature (Vieitez et al., 1999) for Reactor-1 and Reactor-2 except some values. However, the percentages observed in Reactor-3 were not close to the values stated in the literature. The reason may be the different types of reactors, operational conditions etc. Yet, as stated earlier, the number of studies was few; therefore, a more detailed comparison cannot be done.

As a conclusion, although the VS reduction was the highest in Reactor-3 with an OLR of 20 g VS/L.day, the optimum acidified reactor was chosen as Reactor-2 with an OLR of 15 g VS/L.day since the maximum acidification percentages were achieved in this reactor at the end of the analysis conducted in Set-1. In addition, the gram tVFA produced per gram waste was greater than other reactors and increase in solubilization percent (%15) was relatively close to those observed in Reactor-3 (%16). Moreover, the amount of NaOH used to keep the pH at desired values (5.5 and 6.5) was the highest in Reactor-3 and it would not be economical if the system was applied in large scales. Hence, it was feasible to choose Reactor-2 as optimum one due to the reasons stated above. Therefore, optimum pH determination experiments were conducted at OLR of 15 g VS/L.day.

4.2. Results of the Set-2 Experiments

In this part of the study, the effect of pH control on anaerobic acidification of OFMSW was investigated. In Reactor-4, pH was kept constant at 6.5; whereas, in Reactor-5, no pH control was practiced. The reactors were compared in terms of effluent sCOD, tCOD, TS, VS, VFA productions, acidification degrees and VS removals.

4.2.1. pH Variation in Reactor-5

No pH control was applied to the Reactor-5. The pH value in Reactor-5 decreased sharply from 7.57 to 4.05 in four days (Day 0-4) and then further decreased to 3.51 in 21 days (Day 5-25) where it remained nearly constant until the end of the operation period (Day-37) (Figure 4.15). Bouallagui et al. (2004) stated the pH value of an anaerobic sequencing batch reactor (ASBR) system in acidification stage decreased to 4.2 when pH was not controlled during the treatment operation of fruit and vegetable wastes and an inhibition of hydrolysis was observed in very low pH values. However, Parawira et al. (2004) observed a sharp decrease in pH values from 7.0 to 4.0 and even lower values were observed at higher organic loads but an inhibition due to low pH values was not stated in the study in which acidification of solid potato waste was conducted. As a result, it can be figured out that the low pH values may decrease the performance efficiency but pH below the value of 3.0 may lead inhibitions in the systems.

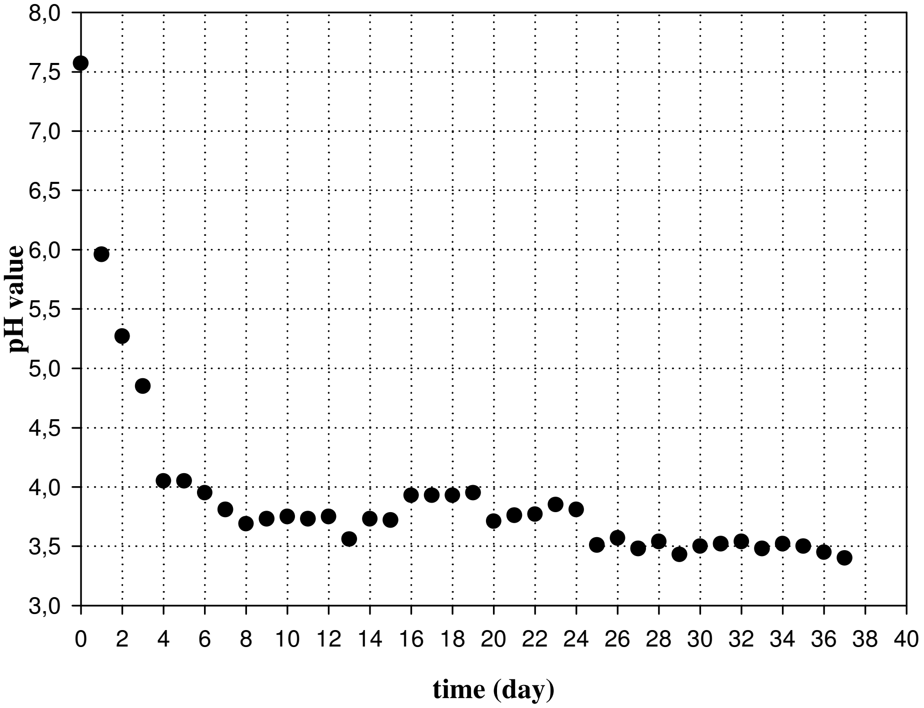

Figure 4.15: pH variation in Reactor-5

4.2.2. Total COD (tCOD) and Soluble COD (sCOD) Concentration Variations

Influent and effluent total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) concentrations of the Reactor-4 and 5 are presented in Figure 4.16.

Figure 4.16 (a) depicts the tCOD and sCOD variations of Reactor-4. The concentrations varied during operation period and the fluctuations in concentration were observed for influent tCOD values due to the heterogeneous structure of the waste. These fluctuations naturally led to the variations in the effluent tCOD concentrations. The maximum effluent tCOD concentration was around 50000 mg/L yet the minimum appeared as 40000 mg/L. As can be seen from Figure 4.16 (a), the effluent tCOD concentrations were nearly the same and the results depict that removal of tCOD was not held in the reactor. On the other hand, the effluent sCOD concentrations were higher than influent values which proves that solubilization of organics was achieved in Reactor-4.

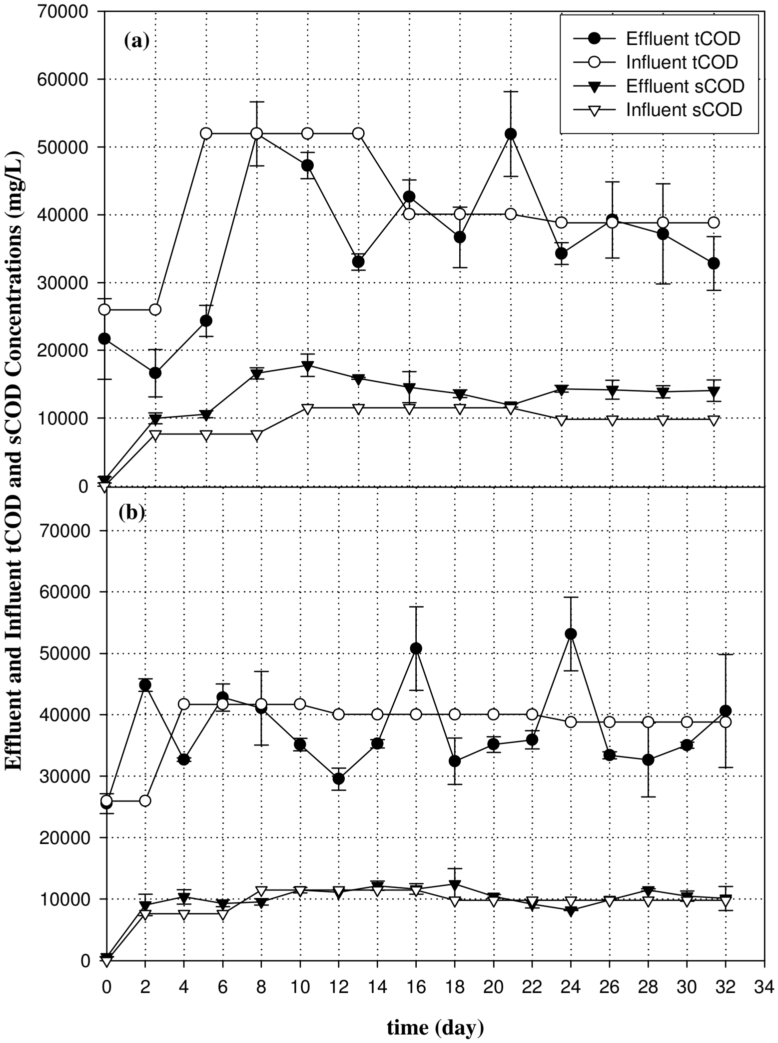

Figure 4.16: Influent and effluent tCOD & sCOD of (a) Reactor-4, (b) Reactor-5

Figure 4.16 (b) illustrates the tCOD and sCOD variations in Reactor-5. Both tCOD and sCOD concentrations increased after first day since the feeding operation started after that time. Effluent and influent tCOD concentrations were again very close to each other showing that removal in tCOD was not achieved in Reactor-5. The effluent sCOD concentrations were little higher than influent sCOD concentrations on some days; however, the concentrations were close to the influent sCOD values in general which depicts insufficient solubilization in the reactor due to effect of low pH on hydrolysis as Bouallagui et al. (2004) explained in their study.

According to the results, the effluent sCOD concentrations in Reactor-4 were higher than that observed in Reactor-5 which indicates the solubilization of organics was achieved more successfully in Reactor-4 than in Reactor-5. The VFA production concentrations support this result in such a way that the VFA production was higher in Reactor-4 than in Reactor-5 in terms of concentration; that is, low VFA concentrations were measured in Reactor-5. The VFA concentration increased as the soluble COD increased in Reactor-4.

In both reactors the trends for tCOD and sCOD concentrations were similar; that is, reduction was not observed in tCOD; however solubilization was achieved which resulted in increase in effluent sCOD concentrations. Wust et al. (2003) studied two-phase treatment of cheese-whey and little or no tCOD reduction was expected in the acidogenic reactor; whereas, an increase was achieved in the sCOD concentration as the VFA production increased. Ghosh et al. (1975) found that there was no reduction in total COD of the non-acidic soluble organics in an acid digester fed raw municipal activated sludge, but actually a 25% increase in sCOD during acidogenesis and finally, Bouallagui et al. (2004) stated no mechanism for tCOD removal in acidified reactor. It is obvious that tCOD removal mechanism is not likely observed in acidifying reactors as stated in literature and the results achieved in this study are consistent with the literature information.

The influent and effluent sCOD/tCOD ratios are also an indication for solubulization performances of the reactors. The influent ratio were calculated as 24% for both reactors; whereas, the effluent values appeared as 36% and 27% for Reactor-4 and Reactor-5, respectively. As a result, increases in percent solubilization were 12% and 3% for the reactors and clearly, the solubilization efficiency was higher in Reactor-4 than in Reactor-5. The reason again was the low pH value in Reactor-5 relative to Reactor-4 (Figure 4.17).

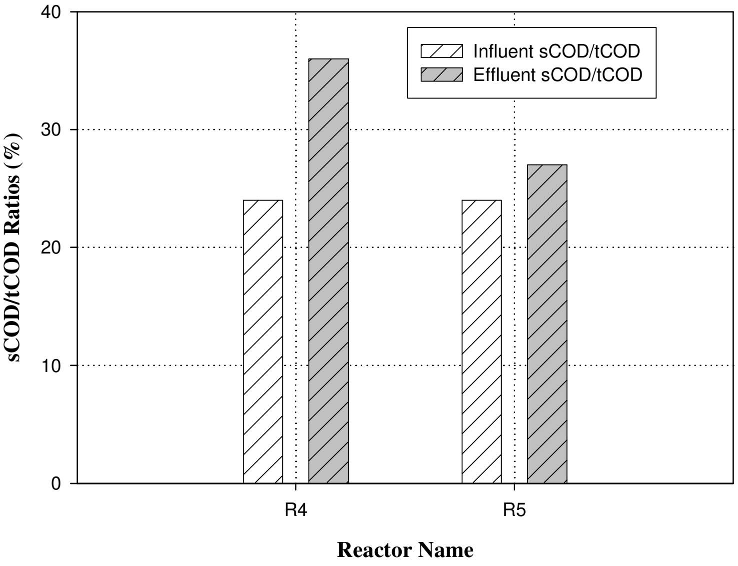

Figure 4.17: Influent and effluent sCOD/tCOD ratios for reactors

In this set, the average values for hydrolysis rate constants were estimated as 0.096 day^{-1} and 0.047 day^{-1} for Reactor-4 and Reactor-5, respectively by conducting mass balance for a fed-batch CSTR and calculating the rate with final achieved equation**.** The results depicted again that the pH drop affected the hydrolysis rate negatively. The hydrolysis rate constant for Reactor-5 declined nearly by half than Reactor-4. Linke (2006) estimated rate constant as 0.089 day^{-1} for anaerobic digestion of solid waste. Borja et al. (2005) calculated the kinetic hydrolysis constant as 0.054 day^{-1} in the experiments in which anaerobic digestion process of olive pomace was studied. As a result, the rate values obtained in this study seemed reasonable when compared with the literature value.

4.2.3. Total Solid (TS) and Volatile Solid (VS) Concentration Variations

The influent and effluent TS and VS concentrations were illustrated in Figure 4.18. Effluent values showed variations throughout the experiment with the variation in influent TS and VS concentrations of the feedstock. Although the same feed-stock was used for all feeding processes, the bulky and heterogeneous structure of the stock resulted in the variation in the solid concentrations. The average VS removal efficiencies were 24% and 10% for Reactor-4 and Reactor-5, respectively. It can be observed from Figure 4.18 (a) that the effluent TS and VS concentrations were lower than the influent TS and VS concentration until Day-6 for Reactor-4. On the following 10 days, the effluent concentration started to increase although the TS and VS content of the influent feedstock decreased which indicated that there was no TS and VS reductions in reactor in that time period. After that period, the effluent concentrations decreased to 18810 mg/L until Day-28, then increased to 32220 mg/L again.

Figure 4.18 (b) depicts the variations in effluent and influent TS and VS concentrations during experiments for Reactor-5. In the first 12 days, effluent TS and VS concentrations were lower than influent values indicating the removal in the reactor in this period. After Day-12, influent and effluent concentrations of the feedstock decreased; however, effluent concentrations appeared to be equal or higher than influent concentrations.

Chen et al. (2007) investigated the enhancement of hydrolysis and acidification of solid waste by a rotational drum system and VS reduction determined as 16.5% to 21.1%. Moreover, Viturtia et al. (1995) found VS removal as 27% for reactor with OLR and HRT of 12.6 kg VS /m^3.day and 5 days as tabulated in Table 2.1. As a result; the VS reduction values estimated in this study were in the range stated in the literature studies conducted with solid waste (see Table 2.1). Yet, it should again be stated that each study has its unique configuration and operational parameters, thus VS reductions may change due to these parameters.

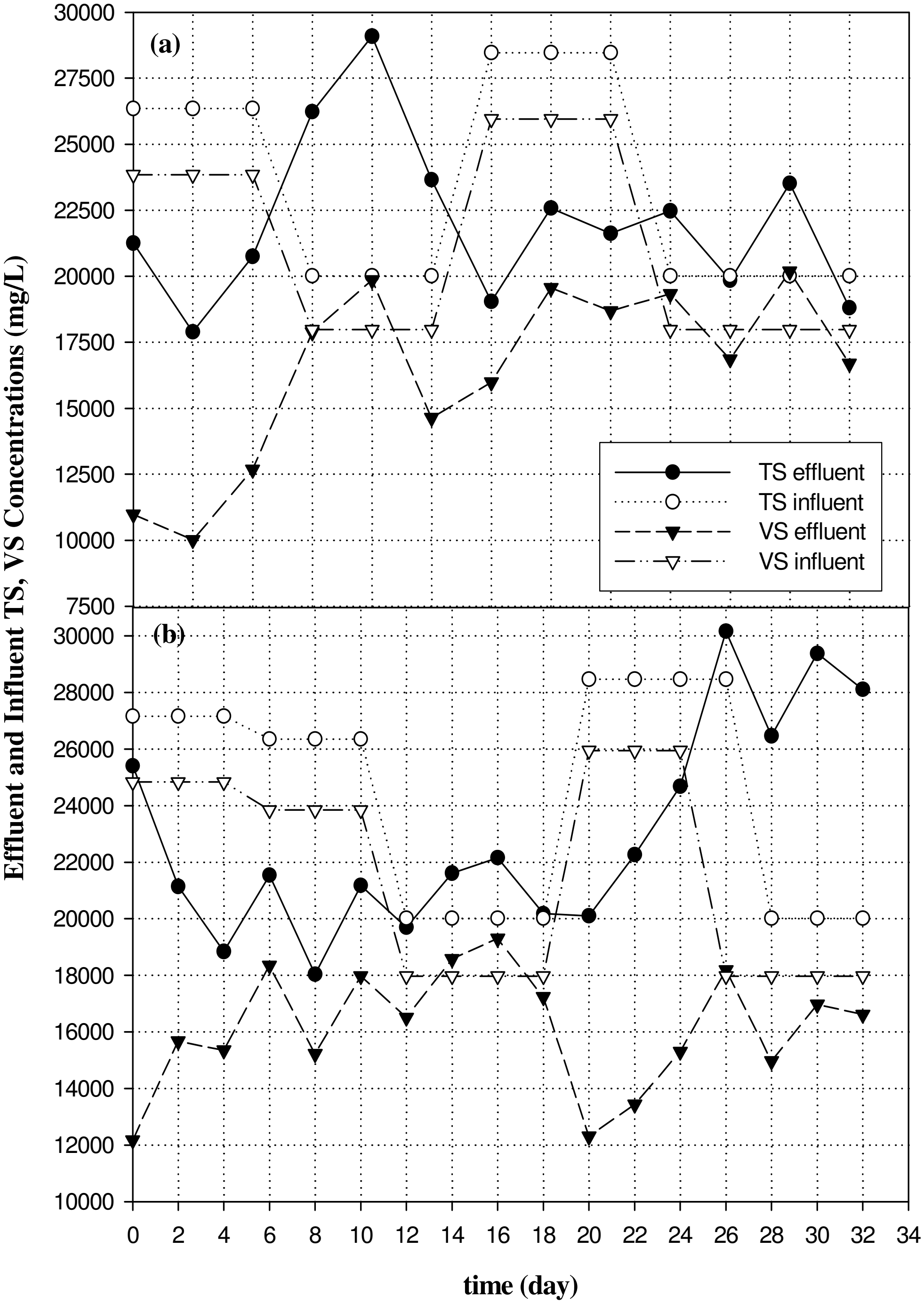

Figure 4.18: Influent and effluent TS & VS of (a) Reactor-4, (b) Reactor-5

4.2.4. Volatile Fatty Acid (VFA) Concentration Variations

The VFA concentrations were measured throughout the experiment and profiles are depicted in Figures 19-22. The total VFA production reached to 9000 mg as Hac/L for Reactor-4, yet the concentrations were under 3000 mg as Hac/L for Reactor-5. The reason of low tVFA production in Reactor-5 was probably low pH values observed in the reactor. The average tVFA concentration was calculated as 5168 mg (as Hac)/L for Reactor-4, and 1794 mg (as Hac)/L of average tVFA was determined in Reactor-5.

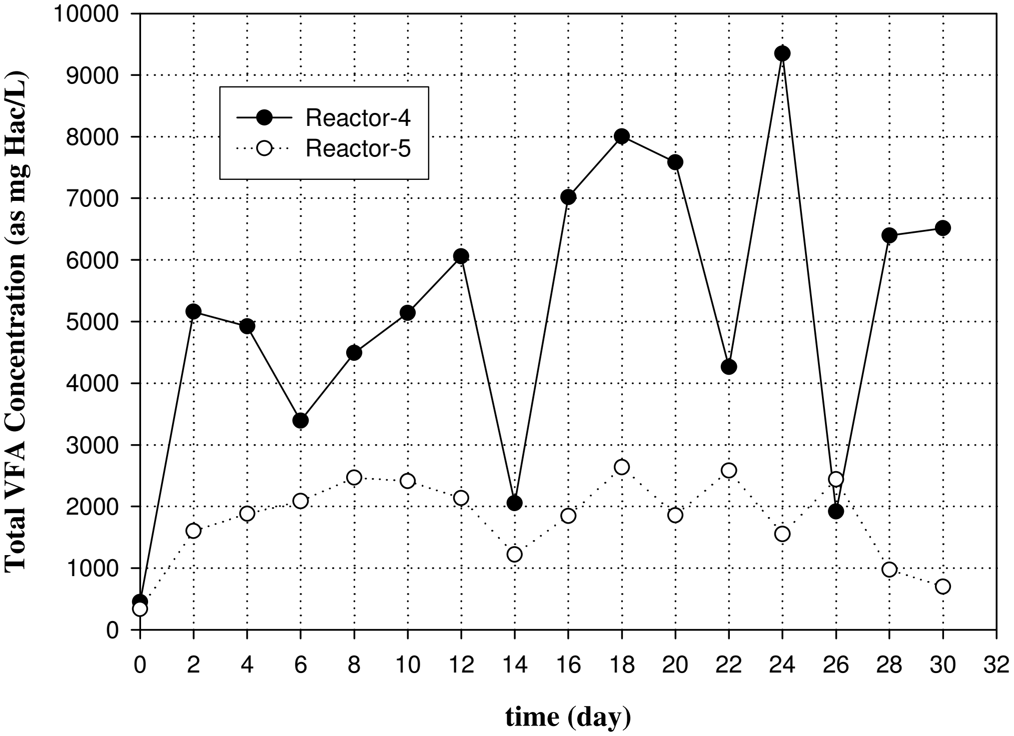

Figure 4.19: Total volatile fatty acid concentrations observed in reactors

Volatile fatty acid (VFA) production of each reactor was measured and the results also are depicted in Figure 4.20 and Figure 4.21. The main organic acid observed was acetic acid in both reactors. The acetic acid concentration was around 4500 mg/L and 2250 mg/L for Reactor-4 and Reactor-5, respectively. Bouallagui et al. (2004) conducted experiments with vegetable and fruit wastes with ASBR system with HRT of 3 days. In the reactor with OLR of 3.7 g COD/L. day and with no pH control; butyric and valeric acids were the major acids observed. In this study; acetic, propionic, and butyric acids were the ones measured in Reactor-5 with no pH control. In the studies of Wang et al. (2002) conducted with food waste, the majority of the VFAs consisted of acetic, propioic and butyric acids. Therefore, it can be concluded that food wastes which are mainly composed of carbohydrates result in the formation of acetic and propionic acids. In addition, the occurrence of the individual VFAs decreased as the chain length increased. The higher molecular weight VFAs, including valeric and isovaleric, were present at low concentrations in the acidification process of potato waste (Parawira et al., 2004). These VFAs are mainly associated with the fermentation of proteins and thus, low concentrations in valeric acid were expected due to the complex and low protein content of the solid waste in this study.

In addition, ethanol production was observed in both reactors throughout the experiment (Figure 4.20). The maximum ethanol concentration was 1179 mg/L in Reactor-4, whereas the value appeared as 1363 mg/L in Reactor-5. Thus, the production increased as the pH value dropped below 5.5. Rodriguez et al. (2006) stated at lower pH values (< 5.6) the butyrate decreased and ethanol became the dominant products. Moreover, it was determined that when the pH was below 4.5, CO_2 and H_2 were produced along with butyric and acetic acids plus ethanol (Ren et al., 1997). Therefore, increase in ethanol and acetic acid concentration and decrease in butyric acid concentration in Reactor-5 (with low pH) was probably due to the low pH value in the reactor.

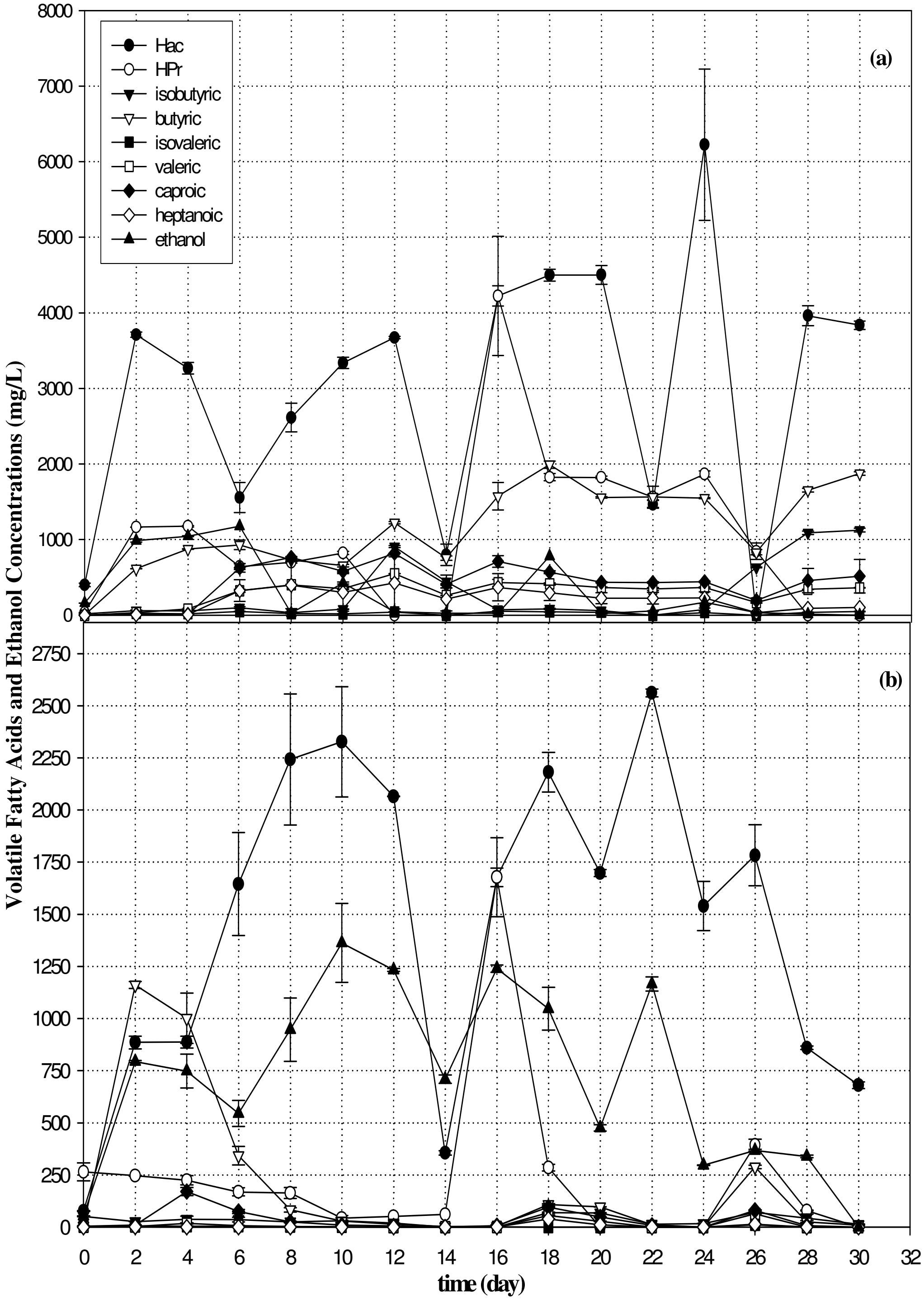

Figure 4.20: Volatile Fatty Acid (VFA) concentration in (a) Reactor-4, (b) Reactor-5

The composition percentages of VFAs were also determined and it is concluded from Figure 4.21 that the main VFAs measured were acetic acid, propionic acid, and butyric acid. The remaining VFA components observed in the reactors were iso-butyric, valeric and caproic acids. There were differences between the reactors in terms of VFA compositions. While the acetic acid percentage was 58% in Reactor-4 having the pH value of 6.5, the percentage increased to 81% in Reactor-5 with no pH control. On the other hand, propionic, butyric, valeric and caproic acid concentration percentages decreased while the pH decreased as depicted in Figure 4.21. Low pH value favored acetic acid production; whereas high pH favored the butyric acid production (Wu et al., 2004). Hence, increase in acetic acid percentage in Reactor-5 was mainly due to the pH drop in the reactor.

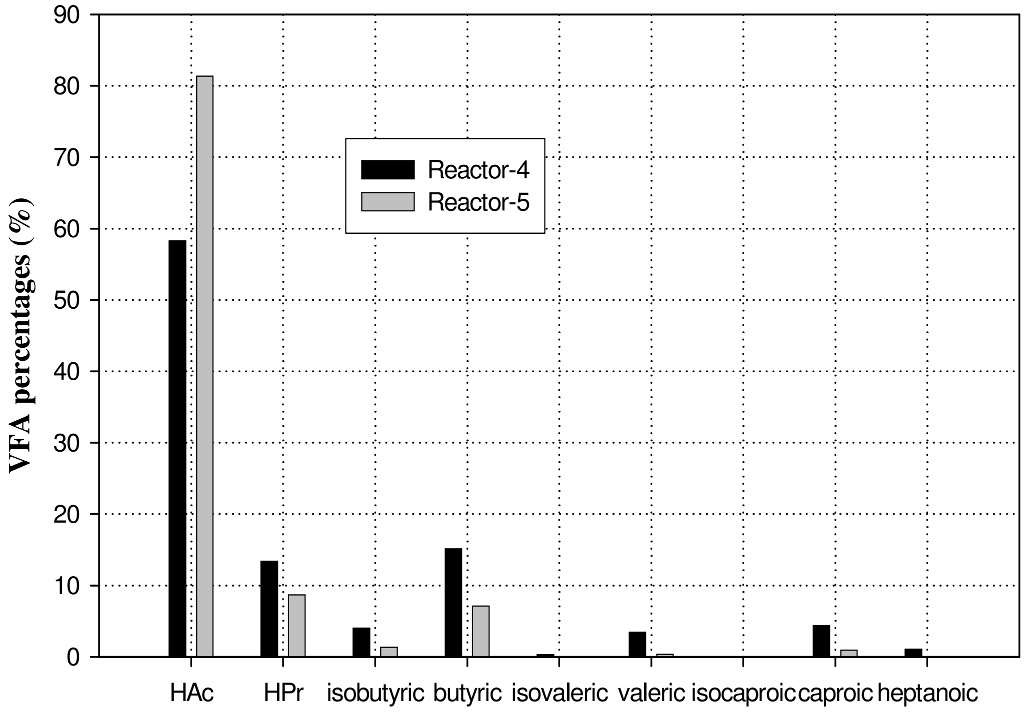

Figure 4.21: Percentages of volatile fatty acids observed in Reactor-4 and Reactor-5 throughout the experiments

The mass of volatile fatty acid produced in grams per gram of influent tCOD was also calculated. As seen from Figure 4.22 the production of VFA per gram of tCOD was higher in Reactor-4 than that observed in Reactor-5. It can be concluded from the results that as the pH of the reactor decreased the amount of VFA produced affected negatively; that is, the amount decreased. The reason might be the partial inhibition of low pH value on the microorganisms.

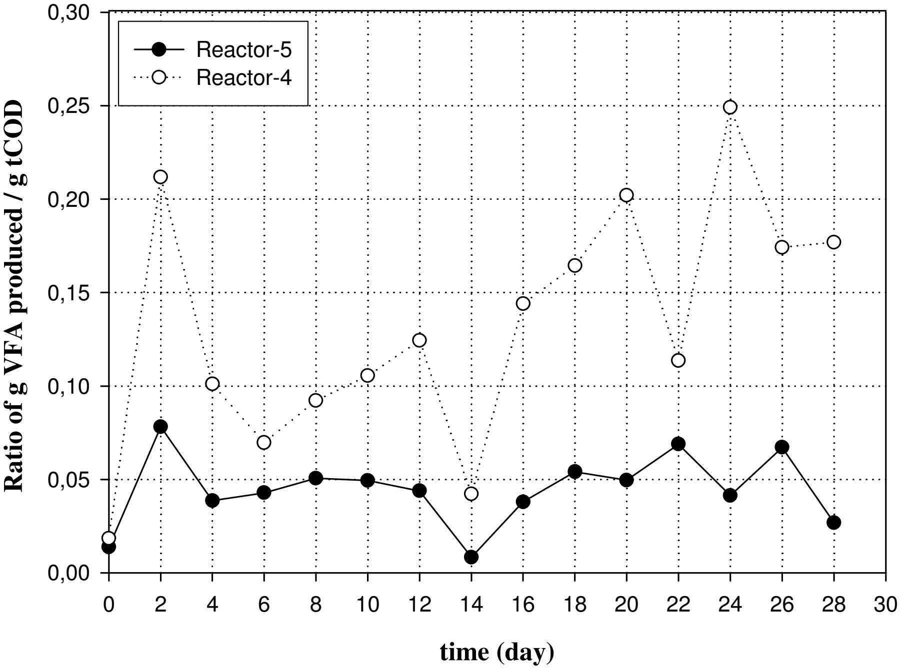

Figure 4.22: Ratio of g VFA produced/g influent tCOD (a) Reactor-4, (b) Reactor-5

As stated Section-4.1.3, the results from various acid-phase studies showed a great deal of variation in the proportion of COD in the form of VFAs with mean values ranging from 0.4 to 0.9 g/g. The values in this set ranged between 0.05 and 0.25 for reactors (Figure 4.22) and these low ratios were mainly due to pH effect on reactors. Low pH value in Reactor-5 brought about low VFA concentrations, hence low VFA/tCOD ratios was observed. It is also apparent that pH value of 6.5 did not enhance the acidification process in Reactor-4 which led to low VFA production as compared to production occurred in Reactor-2 (with pH value of 5.5) in Set-1. Therefore, low ratios for Reactor-4 were again due to low production of VFA.

4.2.5. Acidification Degree of the Reactors

As mentioned in Section 4.1.4, the method for the estimation of the acidification degree differs from study to study. Two methods named as Method a and Method b have been also used in this section to quantify level of acidification in the reactors. The acidification percentage of Reactor-4 decreased on Day 8, and then showed an increasing trend until Day-24 for both Method a and b (Figure 4.23). On the other hand, the percentage in Reactor-5 increased until Day-8, after that, it decreased and finally nearly stayed constant in the remaining part of the operation. The percentages reached to 87% and 20% (Figure 4.23, Method a); whereas, the values depicted as 25% and 5% (Figure 4.23, Method b) for Reactor-4 and 5, respectively.

It is obvious from Figure 4.23 that the acidification percentages were about to increase after Day-8 and decrease after Day-24 of the operation period. Therefore, in order to achieve maximum efficiency in the methane producing reactor, this time period should be preferred to give the effluents to the second reactor in a two-phase system.

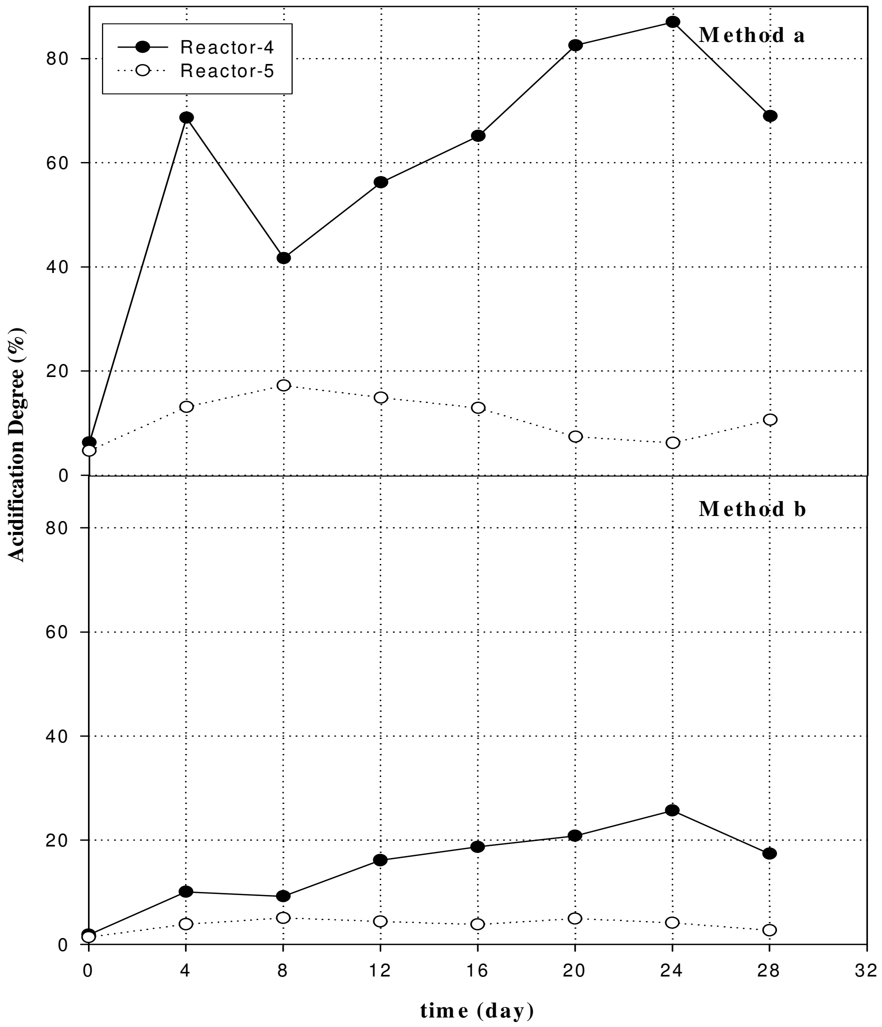

Figure 4.23: Acidification percentages of the Reactor-4 and 5

Figure 4.24 represents the average acidification degrees of the reactors. The average percentages of Reactor-4 and Reactor-5 calculated by were 55% ± 24 and 14% ± 7, respectively (Figure 4.24, Method a); however, the values were 14% ± 7 and 5% ± 2 for Method b (Figure 4.24, Method b). It is concluded that, the pH drop affected the process negatively; that is, the anaerobic acidification degree decreased as the pH value of the reactor decreased.

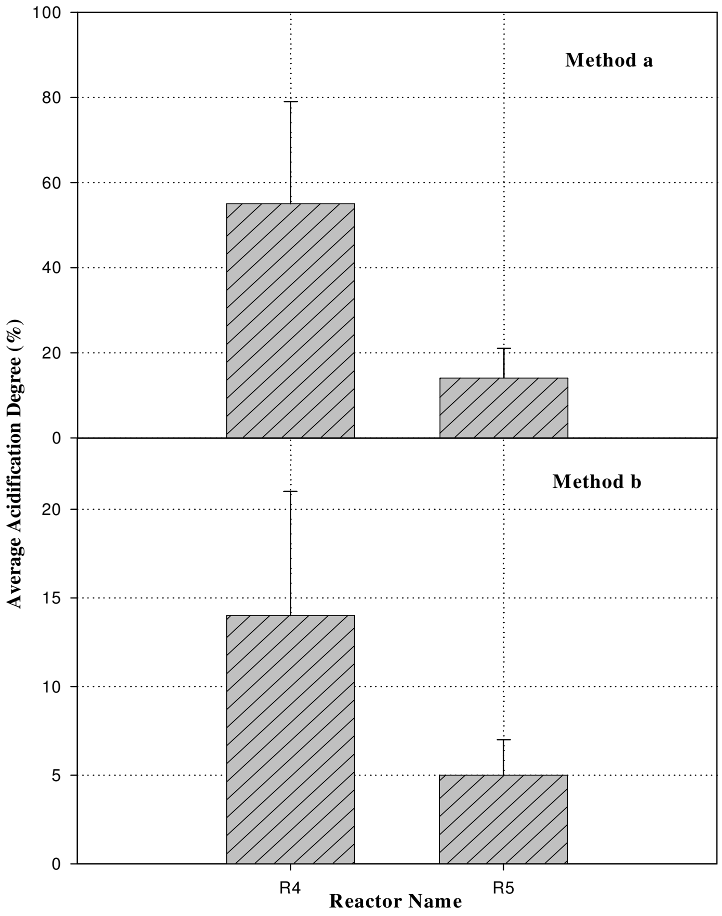

Figure 4.24: Average acidification percentages of the Reactor 4 and 5

The degrees obtained in this set were lower than the values stated in the literature as depicted in Table 4.1. The reason is probably pH values applied in this set. The literature studies mainly emphasize that proper pH range for optimum acidification process is around 5.0-5.5 and the values below or above this range result in decline in acidification performance.

Finally, it is obvious from Figure 4.23 and 4.24 that the acidification degrees calculated by Method b is different than that calculated by Method a. The results depicted that the degrees became lower when the estimations were conducted by

Method b. Yet, it should be noted as an argument that the acidification degree decreased as the pH of the reactor decreased during experiment, although the values were different between two methods.

Table 4.2 depicts the results achieved from Reactor-2, Reactor-4 and Reactor-5 to make a comparison between the performances of the reactors and to figure out pH effect on acidification phase.

Table 4.2: Experimental comparison results of Reactors-2, 4 and 5

Reactor	**pH value**	**Increase in solubilization (%)**	**VS reduction (%)**	**tVFA (mg/L)**	**Acidification Degree (%)**
Reactor-2	5.5	15	34	9590	28
Reactor-4	6.5	12	24	5168	14
Reactor-5	No control (< 4.0)	3	10	1794	5

It is clear from Table 4.2 the pH value of 5.5 was the best value for the maximum efficiency in terms of solubilization increase, VS reduction, tVFA production and finally degree of acidification. Values of all performance indicating parameters decreased as pH increased from 5.5 to 6.5. The same situation was also observed as the pH value was not controlled and let to decrease naturally. In the literature, different pH values were determined as optimum for the acidification stage of anaerobic treatment; however, the applicable range varied between 4.5 and 6.5. Yet, Yu et al. (2002) stated at pH value of 6.5 biogas contained methane and for effective separation of acidogenic phase from the methanogenic phase, pH must be kept at 5.5 or less. As a result, it can be concluded different pH values are applicable for acidification stage but the value of 5.5 was the optimum one as proved in this study.

4.3. Results of the Set-3 Experiments

In the experiments conducted in Set-1 and Set-2, the objective was to determine optimum conditions for maximum acidification process. In this part of the study, it was intended to figure out what would be the performance of batch reactors when they were fed with acidified and unacidified raw OFMSW samples.

4.3.1. Cumulative Gas Production Variations

Cumulative gas productions were indicators of the reactor performances. As depicted in Figure 4.25, the gas productions in reactors fed by acidified samples had higher values than the reactors fed by stock waste. The production reached to 265 mL and 160 mL in the reactors A1 and N1, whereas the values were 212 mL and 110 mL for reactors A2 and N2. The results also support the tCOD and VS reduction percentage profiles observed between the reactors; that is, rises in gas production were consistent with the tCOD reductions held in the reactors. The gas productions inclined apparently as tCOD and VS reduction increased.

The occurrence of higher gas production values in the reactors fed with acidified substrate was consistent with outcomes of literature studies (Demirer and Chen, 2004). Moreover, higher productions were observed in the reactors with the same substrate content but different tCOD concentration. The production was higher in reactor A1 than A2; similarly in reactor N1 than N2 (Figure 4.25). It was apparent that when the tCOD load increased, the gas production simultaneously increased as stated in the literature (Uzal et al., 2003; Demirer et al, 2000). As a result, it is clear that acidification stage improves both tCOD and VS reduction, and gas production.

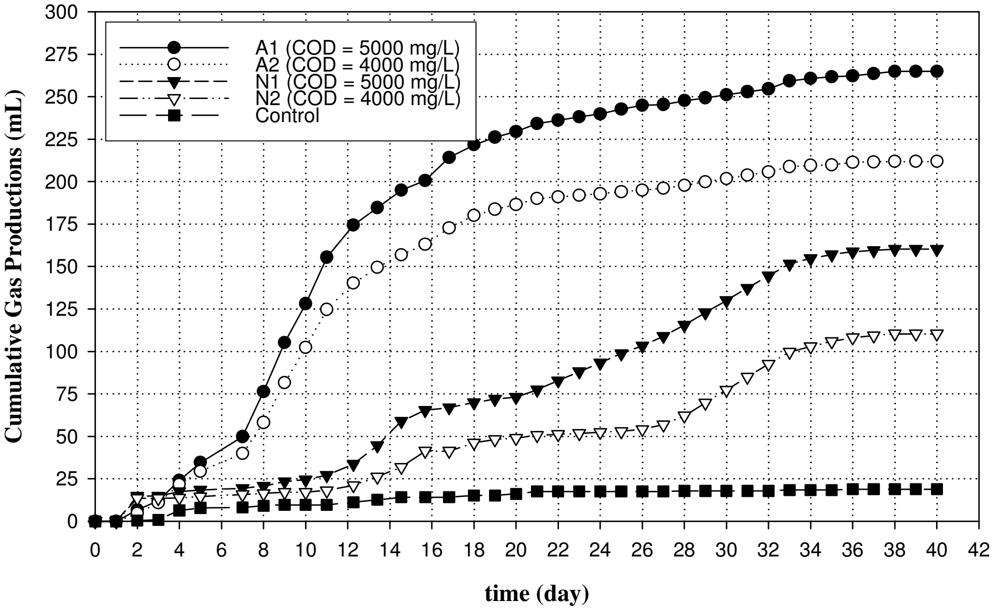

Figure 4.25: Cumulative gas productions measured throughout the experiments

In addition, an acclimation period was not observed for the microorganisms in reactors A1 and A2 and, therefore, direct conversion of substrate to biogas was achieved (Figure 4.25). On the other hand, a lag phase, in which microorganisms needed to acclimate to the waste, occurred in the reactors fed by unacidified samples (N1 and N2)

4.3.2. tCOD and VS Concentration Variations

The COD and VS reductions of the reactors were illustrated in Figure 4.26. The reductions of tCOD were 39% and 29% for the reactors A1 and N1 with initial tCOD value of 5000 mg/L. The numbers clearly depicted that the tCOD reduction percentage was higher in the reactor fed with acidified waste. The same situation was also observed between the reactors A2 and N2 which contained 4000 mg/L of tCOD. The reduction percentage was determined as 36% for A2 whereas the value appeared as 27% for N2. It can be concluded from the numbers that reactors fed with acidified samples showed higher tCOD removal efficiencies than other reactors since acidified samples contained readily biodegradable sCOD (VFAs) and the removal was more likely to be achieved in that reactors for the same time durations. In other words, the conversion of waste to VFAs occurred in N1 and N2 first, and then the utilization of those acids achieved; however, in reactors A1 and A2, VFAs utilization directly occurred which led to rise in process efficiency.

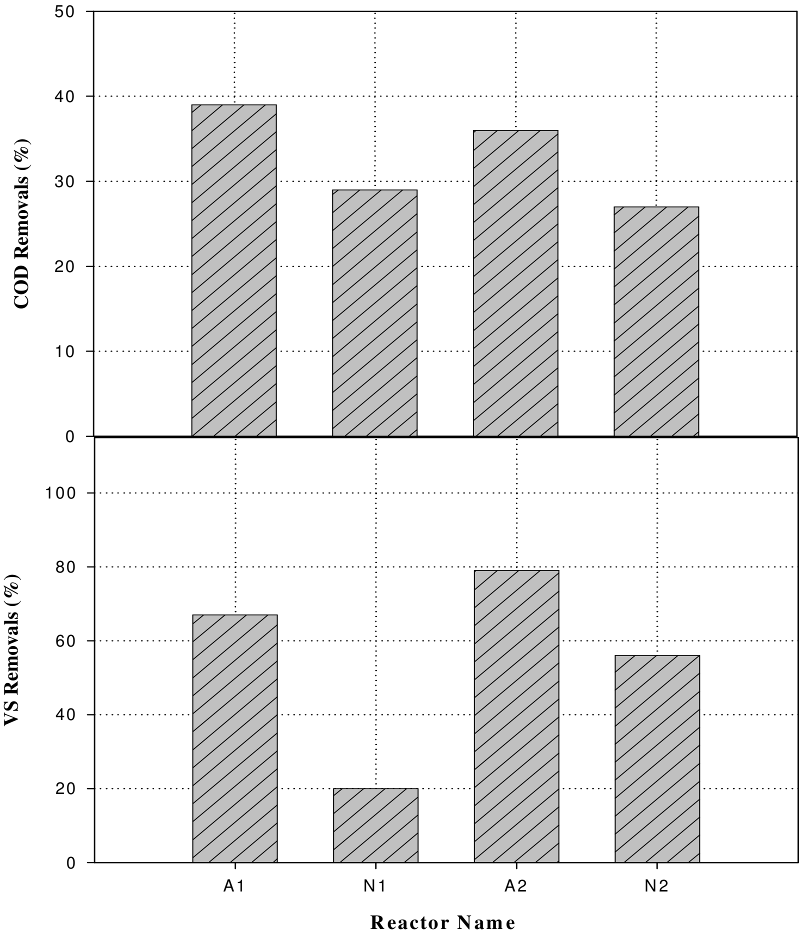

Figure 4.26: COD and VS reductions observed in the reactors

The VS removals were also determined and efficiencies were calculated as 67% and 20% for reactors A1 and N1, respectively. On the other hand, the values were

79% and 56% for reactors A2 and N2 with tCOD concentration of 4000 mg/L (Figure 4.26). The VS removal percentages were again higher in the reactors fed by pre-acidified waste and it improves that phase separation enhanced the performance of the methane reactor.

4.3.3. Volatile Fatty Acid (VFA) Concentration Variations

Total VFA concentrations were also measured at the end of the operation period and the values were found as 30, 146, 99, and 197 mg/L for reactors A1, N1, A2 and N2, respectively (Figure 4.27). The tVFA concentration was lower in the reactor fed by acidified sample (A1) than the one fed by stock waste (N1) due to rapid utilization of acids by microorganisms since the acidified sample contained readily available acids. The same outcome was also observed between the reactors A2 and N2; the concentration of total volatile fatty acid was lower in reactor A2 than that measured in reactor N2.

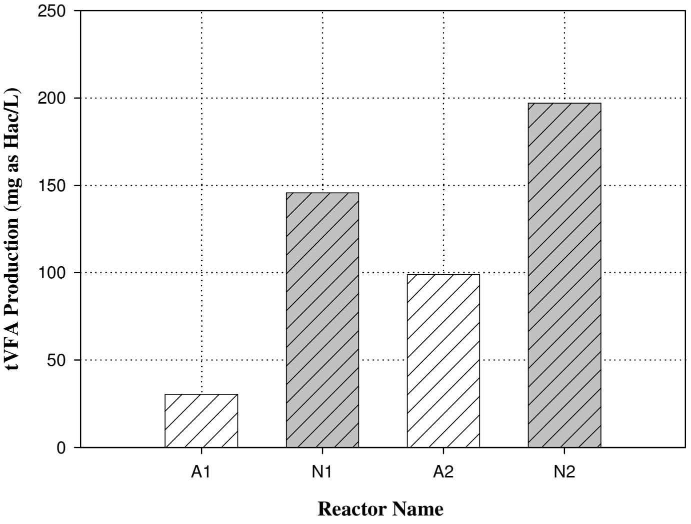

Figure 4.27: tVFA production of the reactors

The gas productions also support this result such that an increase in production started on Day-8 for the reactors A1 and A2; whereas, same situation was observed on Day-22 for N1 and N2 (Figure 4.25). The VFA production was probably occurred until Day-22 in reactors N1 and N2 first, and then produced VFA was utilized by microorganisms leading to rise in gas production. However, the utilization took place on Day-8 for reactors A1 and A2 because the feed had already contained VFAs due to pre- acidification. In this sense, the low VFA concentrations in reactors A1 and A2 was due to direct utilization of acids in the reactors.

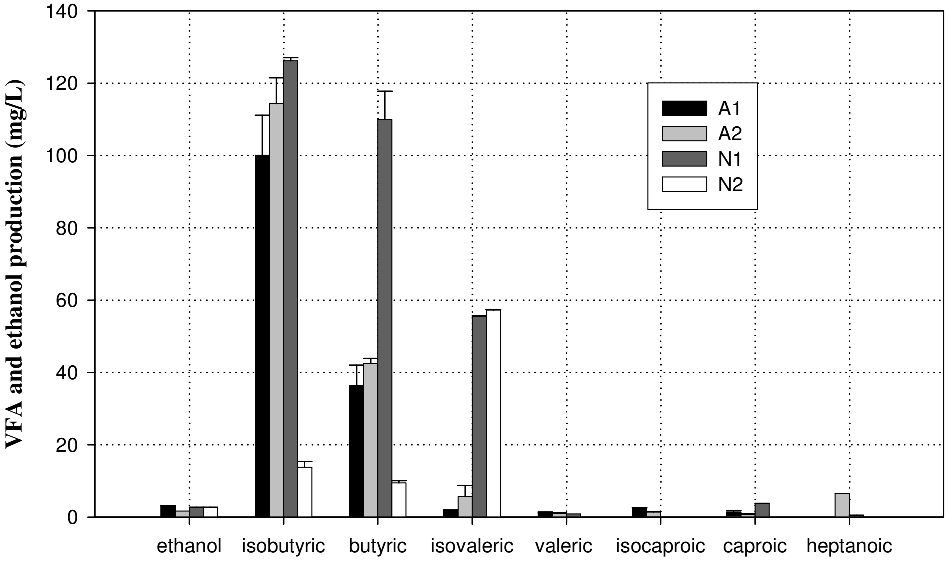

Figure 4.28: VFA compositions in the reactors

The VFA compositions observed in the reactors were also determined and depicted in Figure 4.29. The main organic acids measured were butyric and isobutyric for all reactors and isovaleric for reactors N1 and N2. The reactors contained no acetic acid which was mostly due to the utilization of this acid during acetogenesis step. Viturtia et al. (1995) stated that lower acetic acid concentrations, compared with other acids, were indication of methanogenic activity; hence, methanogenic activity, which resulted in gas production, took place in this study. Moreover, the propionic acid was not detected in the reactors since it was again immediately utilized by microorganisms due to its simple structure (Speece, 1996). Han et al. (2005) conducted experiments by using a UASB reactor to treat the fermented food wastewater containing VFA and alcohol and, they stated that even-numbered carbon fatty acids were degraded more easily than odd-numbered ones in the methane reactor. In this study, although two acids namely butyric ($C_4H_8O_2$) and iso-butyric ($C_4H_8O_2$) have even-numbered carbon, their concentrations were high in the reactors which means a complete degradation did not occur. The reason might be the conversion pathway of these acids as stated in literature (Han et al., 2005). In other words, the degradation of substrate to butyric and iso-butyric acids is achieved first in the reactors; and then a complete conversion of those acids to acetic acid is observed and finally acetogenesis step comes. However, most probably the conversion to acetic acid to methane did not take place in the reactors and this caused high concentrations. On the other side, since iso-valeric acid ($C_5H_{10}O_2$) has odd-numbered carbon, the conversion to acetic acid was not achieved easily in reactors N1 and N2 after the formation of it from waste. The reason of low concentration of iso-valeric in reactors A1 and A2 might be due to successful conversion of it to acetic acid, then utilization of produced acetic acid.

4.3.4. Initial and Final pH Values

The initial and final pH values of the reactors were also measured. The initial pH values were 6.80, 6.90, 7.10 and 7.40 for reactors A1, A2, N1 and N2 which were close to each other. However, the values increased to 7.20 for reactors A1 and A2, whereas, the values of 6.00 and 6.10 were measured for reactors N1 and N2. The reason of decrease in reactors N1 and N2 was higher VFA content as stated in the literature (Carucci et al., 2005). However, if the digestion period was extended, the pH values would probably increase due to the utilization of produced acids in the reactors. Utilization of already existed VFA in reactors A1 and A2 resulted in increase in the pH value due to the natural alkalinity.

CHAPTER 5

CONCLUSIONS

In this study, organic fraction of municipal solid waste was first converted to volatile fatty acids (VFA) at optimum acidification conditions in pH controlled fed-batch completely stirred tank reactors and then the conversion of these acids to methane was achieved in batch reactors of biochemical methane potential (BMP) experiments.

Based on the results of this study, the following conclusions could be made;

- As the organic loading rate (OLR) increased from 10 g VS/L.day to 15 g VS/L.day, the average acidification percentage was increased from 26% to 28%. However, further increase in the load to 20 g VS/L.day resulted in a decline in the acidification percentage to 23%. Although the volatile solid (VS) reductions and increase in solubilization percentages were close to each other in reactors having OLRs of 15 and 20 g VS/L.day, respectively; the amount of NaOH added to keep the pH at desire value was highest in reactor with OLR of 20 g VS/L.day which made the condition unfeasible for large scale applications in terms of operational economy. Therefore, the optimum OLR for acidification load was chosen as 15 g VS./L.day in this study.

- The best pH value was found as 6.5 at the end of the experiments conducted to determine the best pH value since maximum VFA production and average acidification percentage were achieved in the reactor having pH value of 6.5 compared to the reactor to which pH control was not applied.

- According to the results obtained in the experiments conducted for the determination of optimum OLR and best pH value, pH value of 5.5 and OLR of 15 gVS/L.day were chosen as optimum considering solubilization, VS reductions, tVFA productions and acidification degrees.

- The optimum operational conditions for the maximum acidification efficiency for the waste composed of food, kitchen, vegetable, fruit and paper were OLR of 15 g VS/L.day, pH value of 5.5 and HRT/SRT of 2 days.

- N_2 gas productions may be the indication of denitrification process occurred in the acidification stage of an anaerobic system. This must be further investigated.

- The results of BMP experiments proved that separation of the anaerobic reactor into two, and application of optimum acidification conditions enhanced the performance in the methane producing reactor in terms of tCOD and VS reduction, and cumulative gas production. 10% and 23% increases in tCOD and VS removals were achieved, respectively, by phase separation. Therefore, phase separation was applicable to improve the performance of the anaerobic system for the treatment of OFMSW.

REFERENCES

American Public Health Association (APHA), 2005. *Standard Method for the Examination of Water and Wastewater,* 21st Edition., Washington D.C.

Argelier S., Delgenes J.-P., Moletta R.,1998. “Design of acidogenic reactors for the anaerobic treatment of the organic fraction of solid food waste”, *Bioprocess Engineering*, Vol. 18, pp. 309-315.

Batstone D. J., keller J., Angelidaki I., Kalyuzhnyi S. V., Pavlosttahis S. G., Rozzi A., Sanders W. T. M., Siegrist H., Vavilin V. A., 2002. “ Anaerobic digestion moden No.1 (ADM 1)”, *Water Scienece and Technology,* Vol. 45(1), pp. 65-73.

Borja R., Marti A., Sanchez E., Rincon B., Raposo F., 2005. “Kinetic modelling of the hydrolysis, acidogenic and methanogenic steps in the anaerobic digestion of two-phase olive pomace (TPOP)”, *Process Biochemistry*, Vol. 40, pp. 1841–1847.

Bouallagui H., Torrijos M, Godon J.J., Moletta R., Cheikh R. Ben, Touhami Y., Delgenes J.P., Hamdia M., 2004. “Two-phases anaerobic digestion of fruit and vegetable wastes: bioreactors performance”, *Biochemical Engineering Journal*, Vol. 21, pp.193–197.

Bouallagui H., Touhami Y., Cheikh R. B., Hamdia M., 2005. “Bioreactor performance in anaerobic digestion of fruit and vegetable wastes”*, Process Biochemistry*, Vol. 40, pp. 989-995.

Bowman A.W and Robinson D.R., *Introduction to Statistics,* Adam Hilger, Bristol, 1987.

Carucci G., Carrasco F., Trifoni K., Majone M., and. Beccari M., 2005. " Anaerobic Digestion of Food Industry Wastes: Effect of Codigestion on Methane Yield ", *Journal of Envıronmental Engıneerıng,* pp. 1037-1045.

Chen L., Jiang W. Z., Kitamura Y., Li B., 2007. "Enhancement of hydrolysis and acidification of solid organic waste by a rotational drum fermentation system with methanogenic leachate recirculation" *Bioresource Technology*, Vol.98, pp. 2194–2200.

De Baere L., 2000. "Anaerobic digestion of solid waste: state-of the art", *Water Science and Technology*, Vol. 41(3), pp. 283-290.

Demirel B. and Yenigun O., 2002. "Two-phase anaerobic digestion processes: a review", *Journal of Chemical Technology and Biotechnology*, Vol.77, pp. 743-755.

Demirel B. and Yenigun O., 2004. "Anaerobic acidogenesis of dairy wastewater: the effects of variations in hydraulic retention time with no pH control", *Journal of Chemical Technology and Biotechnology,* Vol. 79, pp. 755–760.

Demirer G.N. and Speece R, 1999. " Inhibitory effect and biotransfrmation of acrylic acid in computer-controlled pH-stat CSTRs", *Biotechnology and Bioengineering,* Vol. 62 (2), pp. 200-207.

Demirer G. N., Duran M., Ergüder T. H., Güven E., Ugurlu Ö. and Tezel U., 2000. "Anaerobic treatability and biogas production potential studies of different agro-industrial wastewaters in Turkey", *Biodegradation,* Vol.11, pp.401–405.

Demirer G. N. and Chen S, 2004. "Effect of retention time and organic loading rate on anaerobic acidification and biogasification of dairy manure" *Journal of Chemical Technology and Biotechnology*, Vol.79(12), pp. 1381–1387.

Demirer G. N. and Chen S., 2005. “Two-phase anaerobic digestion of unscreened dairy manure”, *Process Biochemistry,* Vol.40, pp.3542-549.

Dinopoulou G., Rudd T. and Leste J. N., 1988. ”Anaerobic Acidogenesis of a Complex Wastewater: 1. The Influence of Operational Parameters on Reactor Performance”, *Biotechnology and Bioengineering*, Vol.31, pp. 958-968.

Earle J.F.K., Chynoweth D.P., Nordstedt R.A., 1990. “Anaerobic Bioconversion: Biogasification of Municipal Solid Waste”, *University of Floria*, Institue of Food and Agricultural Sciences.

Fang H. H. P., and Yu H. Q., 2001. “Acidification of lactose in wastewater”, *Journal of Environmental Engineering*, Vol.127 (9), pp. 825-831.

Gas Technology, 2006.
Avaliable online:
http://www.gastechnology.org/webroot/app/xn/xd.aspx?it=enweb&xd=4reportspubs% C4_8focus%5Chimetfocus.xml. Last accessed: August 2006

Guerrero L., Omil F., Mendez R., Lema J. M., 1999. “Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein”*, Water Research*, Vol.33(15), pp. 3281-3290.

Ghosh S. and Klass D. N., 1977. “ Two phase anaerobic digestion”, *United State Patent,* 4022665.

Han S. and Shin H., 2004. “Performance of an innovative two-stage process converting food waste to hydrogen and methane”*, Air and Waste Management Association*, Vol.54, pp. 242-249.

Han S., Kim S., Shin H., 2005. "UASB treatment of wastewater with VFA and alcohol generated during hydrogen fermentation of food waste", *Process Biochemistry*, Vol.40, pp. 2897–2905.

Hartman H. and Ahring B.K., 2006a. "Status of ADSW 2005"*, Water Science and Technology*, Vol. 53(8), pp. 1-5.

Hartman H. and Ahring B.K., 2006b. "Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview"*, Water Science and Technology*, Vol. 53(8), pp. 7-22.

Horiuchi J.-I., Shimizu T., Tada K, Kanno. T.,. Kobayashi M., 2002. "Selective production of organic acids in anaerobic acid reactor by pH control"*, Bioresource Technology,* Vol. 82, pp. 209–213.

Ince O., 1998. "Performance of two-phase anaerobic digestion system when treating dairy wastewater", *Water Research,* Vol.32(9), pp. 2707-2713.

Jiang W.Z., Kitamura Y., Li B. (2005). "Improving acidogenic performance in anaerobic degradation of solid organic waste using a rotational drum fermentation system", *Bioresource Technology*, Vol. 96, pp. 1537–1543.

Kübler H., Schertler C., 1994. "Three-phase anaerobic digestion of organic wastes", *Water Science and Technology*, Vol.30(12), pp. 367-374.

Linke B., 2006. "Kinetic study of thermophilic anaerobic digestion of solid wastes from potato processing", *Biomass and Bioenergy,* Vol.30, pp. 892–896.

Liu D., Liu D., Zeng R. J., Irini A., 2006. "Hydrogen and methane production from household solid waste in the two-stage fermentation process"*, Water Research*, Vol. 40(11), pp. 2230-2236.

Maharaj I., Elefsiniotis P., 2001. "The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters*", Bioresource Technology,* Vol.76, pp. 191-197.

Mata-Alvarez J., Macé S., Llabrés P., 2000. "Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives", *Bioresource Technology*, Vol.74, pp. 3-16.

Oktem Y. A., Ince O., Donnelly T., Sallis P., Ince- Kasapgil B., 2006. "Determination of optimum operating conditions of an acidification reactor treating a chemical synthesis-based pharmaceutical wastewater", *Process Biochemistry,* Vol.41, pp. 2258–2263.

Ostrem Karena , 2004. *Greening Waste: Anaerobic digestion for treating the organic fraction of municipal solid wastes,* Department of Earth and Environmental Engineering Foundation of School of Engineering and Applied Science, Columbia University.

Parawira W., Murto M., Read J. S. and Mattiasson B., 2004. "Volatile fatty acid production during anaerobic mesophilic digestion of solid potato waste", *Journal of Chemical Technology and Biotechnology*, Vol.79, pp. 673–677.

Pavan P., Battistoni P., Cecchi F., Mata-Alvarez J., 2000. "Two-phase anaerobic digestion of source sorted OFMSW (Organic Fraction of Municipal Solid Waste): performance and kinetic study"*, Water Science and Technology*, Vol. 41(3), pp. 111-118.

Prashanth S., Kumar P., Mehrotra I. 2006. "Anaerobic Degradability: Effect of Particulate COD", *Journal of Environmental Engineering*, Vol.132 (4), pp. 488-496.

Puterbaugh S. and DiStefano T.D., 2002. *Preliminary Analysis of Hydrolysis and Acidification of Food Processing Waste,* Civil and Environmental Engineering Department, Bucknell University.

Raynal J., Delgenes J.P., Moletta R., 1998. “Two-phase anaerobic digestion of solid waste by multiple liquefaction reactors process”, *Bioresource Technology*, Vol.65, pp. 97-103.

Ren N., Wang B. H., Ju-Chang, 1997. “Ethanol-Type Fermentation from Carbohydrate in High Rate Acidogenic Reactor” *Biotechnology and Bioengineering,* Vol. 54 (5), pp. 428-433.

Rustrian E., Delgenes J. P., Bernet N. and Moletta R., 1998. “Simultaneous removal of carbon, nitrogen and phosphorus from wastewater by coupling two-step anaerobic digestion with a sequencing batch reactor”, *Journal Chemical Technology and Biotechnolog.*, Vol.73, pp. 421-431.

Rodriguez J., Kleerebezem R., Lema J. M., van Loosdrecht M.C.M.,2006. “Modeling Product Formation in Anaerobic Mixed Culture Fermentations”, *Biotechnology and Bioengineering*, Vol. 93 (3), pp. 593-606.

Scherer, P.A., Vollmer, G. R., Fakhouri, T. and Martensen, S., 2000. “Development of methnogenic process degrade exhaustively the organic fraction of municipal “grey waste” under thermophilic and hyperthermophilic condition”, *Water Science and Technology,* Vol.41 (3), pp. 83-91.

Schübeler P., August 1996. “Conceptual Framework for Municipal Solid Waste Management in Low-Income Countries”, *UNDP/UNCHS (Habitat)/World Bank/SDC Collaborative Programme on Municipal Solid Waste management in Low-Income Countries*, Working Paper, No.9.

Shin H. and Youn J., 2005. “Conversion of food waste into hydrogen by thermophilic acidogenesis”, *Biodegradation*, Vol.16, pp. 33–44.

Speece. R E., Anaerobic *Biotechnology for Industrial Wastewaters,* Archae Press, Nashville, TN, 1996.

Themelis, N.J., Verma, S., 2004. “The better option: anaerobic digestion of organic waste in MSW”*, Waste Management World.*

Traverso P., Pavan P., Bolzonella D., Innocenti L., Cecchi F. and Mata-Alvarez J.,2000. “Acidogenic fermentation of source separated mixtures of vegetables and fruits wasted from supermarkets”, *Biodegradation*, Vol.11, pp. 407–414.

TURKSTAT, *Turkish Statistical Institue,*
Avaliable online: http://tuik.gov.tr/VeriBilgi.do, Last accessed: October 2007.

Uzal N., Gökçay C. F., Demirer G.N., 2003. “Sequential (anaerobic/aerobic) biological treatment of malt whisky wastewater”, *Process Biochemistry*, Vol.39, pp.279-286

Wang J.Y., Xu H.L., Tay J.H., 2002. “ A hybrid two-phase system for anaerobic digestion of food waste”, *Water Science and Technology,* Vol.45(12), pp.159-165.

Wang J.Y., Zhang H. Tay J-H., 2003. “Semi-continuous anaerobic digestion of food waste using a hybrid anaerobic solid-liquid bioreactor”*, Water Science and Technology*, Vol. 48(4), pp. 169-174.

Wang Junqin, Shen Dongsheng, Xu Yanhua, 2006. “Effect of acidification percentage and volatile organic acids on the anaerobic biological process in simulated landfill bioreactors”, *Process Biochemistry,* Vol. 41, pp. 1677–1681.

Wheatley Andrew, *Anaerobic Digestion: a Waste Treatment Technology,* Elsevier Science Publishers Ltd., England, 1991, pp.120-121.

Wu J. H., Lin C.Y., 2004. "Biohydrogen poduction by mesophilic fermentation of food wastewater", *Water Science and Technology,*Vol.49 (5-6), pp.223-228.

Wust Elizabeth L., S*ingle-phase and Two-phase Cheese Wastewater Treatment by Anaerobic SBRs,* Wisconsin, May, 2003.

Verma Shefali, *Anaerobic Digestion Of Biodegradable Organics in Municipal Solid Wastes*, Ms. Thesis, Department of Earth and Environmental Engineering, Columbia University, May 2002.

Veeken A., Hamelers B. (1999). "Effect of temperature on hydrolysis rates of selected biowaste components", *Bioresource Technology*, Vol. 69(3), pp. 249-254.

Vieitez E.R., Ghosh S., 1999. "Biogasification of solid wastes by two-phase anaerobic fermentation", *Biomass and Bioenergy,* Vol. 16, pp. 299-309.

Vieitez E.R, Mosquera J, Ghosh S., 2000. "Kinetics of accelerated solid-state fermentation of organic-rich municipal solid waste", *Water Science and Technology,* Vol. 41 (3), pp. 231-238

Vigneron V, Ponthieu M, Barina G, Audic J.M, Duquennoi C., Mazeas L., Bernet N., and Bouchez T., 2007. "Nitrate and nitrite injection during municipal solid waste anaerobic biodegradation"*, Waste Management*, Vol.27 (6), pp. 778-791.

Viturtia A. Mtz., Mata-Alvarez J., Cecchi F., 1995. "Two-phase anaerobic digestion of fruit and vegetable wastes*", Recourses, Conversion and Recycling*, Vol.13, pp. 257-267.

Yılmaz V., 2007. *Enhancing the Performance of Anaerobic Digestion of Dairy Manure through Phase-Separation,* PhD Thesis, Department of Environmental Engineering, Middle East Technical University.

Yu H.Q. and Fang H.H.P., 2002. “Acidogenesis of dairy wastewater at various pH levels*”,Water Science and Technology*, Vol.45 (10), pp. 201-206.

Yu H.Q. and Fang H.H.P., 2003. ” Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature”, *Water Research*, Vol.37, pp. 55–66.

Zhang M., Fan Y., Xing Y., Pan C., Zhang G., Lay J., 2007.”Enhanced biohydrogen production from cornstalkwastes with acidification pretreatment by mixed anaerobic cultures”, *Biomass and Bioenergy*, Vol.31, pp.250–254.

APPENDIX

A.1. Calculation of Hydrolysis Rate Constant

The simple model was named as semi-continuous reactor model. The mass balance equation with equal mass flow of input and output (mass of biogas is neglected) can be written as;

$$V_R\,(dC/dt) = M_o * C_o - M_o * C + V_R * r(C) \text{..........Eqn.A-1}$$

Where M_0 = flow in and out of reactor (L/ day)

C_0 = influent concentration (mg/L)

C = effluent concentration (mg/L)

V_R = volume of reactor (L)

The substrate removal rate r(C) as a function of C is express as first order kinetic with;

$$-dC/dt = r(C) = -kC \text{.........Eqn.A-2}$$

Combining Eqn.A-1 and Eqn-A-2 with $V_R = M_o * HRT$, at steady state for V_k $(dC/dt) = 0$, it is obtained;

$$V_R\,(dC/dt) = M_o * C_o - M_o * C + V_R\,(-kC) \text{......Eqn.A-3}$$

$$dC/dt = [M_o\,(C_o - C)/V_R] - kC \text{.............Eqn.A-4}$$

at steady state the term dC/dt is equal to zero, then;

$$0 = [1/HRT\ (C_o\text{-}C)]\text{- } kC \ \rightarrow \mathbf{k = [1/HRT\ (\ C_0/C\ \text{-}1)]}$$ **…Eqn.A-5**

(Linke, 2006)

All the hydrolysis rate constants were estimated according to the Eqn.A-5 in this study and one of the calculations was illustrated in Table A-1.

Table A.1: An example of calculation for hydrolysis rate constant

C_0/C	(C_0/C)-1	1/HRT	Rate (day^{-1})
2.472603	1.4726027	0.5	0.7363014
1.027027	0.027027	0.5	0.0135135
1.020942	0.0209424	0.5	0.0104712
1.175243	0.1752434	0.5	0.0876217
1.21147	0.2114695	0.5	0.1057348
1.401327	0.4013267	0.5	0.2006633
1.028086	0.0280862	0.5	0.0140431
1.399845	0.3998453	0.5	0.1999227
1.072275	0.0722749	0.5	0.0361374
1.123526	0.1235258	0.5	0.0617629
1.020087	0.0200873	0.5	0.0100437
		Average	**0.0721**

A.2. Statistical Application for Analysis of Variance (Student t Test)

Independent Two-Sample t-test, Equal sample size

This equation is only used when the two sample sizes (that is, the n number of participants of each group) are equal.

$$t = (X_1 - X_2)/S_d \quad \text{where } S_d^2 = S_1^2/n_1 + S_2^2/n_2 \text{..........Eqn.A-6}$$

where X_1 = average of sample 1
X_2 = average of sample 2
S_1 = standard deviation of sample 1
S_2 = standard deviation of sample 2
n = sample size
degrees of freedom = 2n-2

If the calculated t values exceed the tabulated value (Table A.2), it is said that the means are significantly different at that level of probability (Bowman and Robinson, 1987).

Example:

The average acidification degrees were calculated as 26 ± 5% and 28 ± 8% in Set-1. By using the Eqn.A-6;

$t = (28-26)/ \text{sqrt} [(5^2 + 8^2)/30] = 1.1611$

The degree of freedom, 2*30-2 = 59, when the numbers in Table A.2 were considered in row 50, the calculated t value (1.1611) was higher than the value 1.047; which means the averages were 70% (1-0.3 = 0.7) different than each other.

Table A.2: t table for Student t test

	0.5	**0.4**	**0.3**	**0.2**	**0.1**	**0.05**	**0.04**	**0.02**	**0.01**	**0.005**	**0.002**	**0.001**
1	1.00			3.08	6.31	12.71		31.82	63.66			636.62
2	0.82			1.89	2.92	4.30		6.96	9.92			31.60
3	0.765	0.978	1.250	1.638	2.353	3.182	3.482	4.541	5.841	7.453	10.210	12.920
4	0.741	0.941	1.190	1.533	2.132	2.776	2.999	3.747	4.604	5.598	7.173	8.610
5	0.727	0.920	1.156	1.476	2.015	2.571	2.757	3.365	4.032	4.773	5.893	6.869
6	0.718	0.906	1.134	1.440	1.943	2.447	2.612	3.143	3.707	4.317	5.208	5.959
7	0.711	0.896	1.119	1.415	1.895	2.365	2.517	2.998	3.499	4.029	4.785	5.408
8	0.706	0.889	1.108	1.397	1.860	2.306	2.449	2.896	3.355	3.833	4.501	5.041
9	0.703	0.883	1.100	1.383	1.833	2.262	2.398	2.821	3.250	3.690	4.297	4.781
10	0.700	0.879	1.093	1.372	1.812	2.228	2.359	2.764	3.169	3.581	4.144	4.587
11	0.697	0.876	1.088	1.363	1.796	2.201	2.328	2.718	3.106	3.497	4.025	4.437
12	0.695	0.873	1.083	1.356	1.782	2.179	2.303	2.681	3.055	3.428	3.930	4.318
13	0.694	0.870	1.079	1.350	1.771	2.160	2.282	2.650	3.012	3.372	3.852	4.221
14	0.692	0.868	1.076	1.345	1.761	2.145	2.264	2.624	2.977	3.326	3.787	4.140
15	0.691	0.866	1.074	1.341	1.753	2.131	2.249	2.602	2.947	3.286	3.733	4.073
16	0.690	0.865	1.071	1.337	1.746	2.120	2.235	2.583	2.921	3.252	3.686	4.015
17	0.689	0.863	1.069	1.333	1.740	2.110	2.224	2.567	2.898	3.222	3.646	3.965
18	0.688	0.862	1.067	1.330	1.734	2.101	2.214	2.552	2.878	3.197	3.611	3.922
19	0.688	0.861	1.066	1.328	1.729	2.093	2.205	2.539	2.861	3.174	3.579	3.883
20	0.687	0.860	1.064	1.325	1.725	2.086	2.197	2.528	2.845	3.153	3.552	3.850
21	0.686	0.859	1.063	1.323	1.721	2.080	2.189	2.518	2.831	3.135	3.527	3.819
22	0.686	0.858	1.061	1.321	1.717	2.074	2.183	2.508	2.819	3.119	3.505	3.792
23	0.685	0.858	1.060	1.319	1.714	2.069	2.177	2.500	2.807	3.104	3.485	3.768
24	0.685	0.857	1.059	1.318	1.711	2.064	2.172	2.492	2.797	3.091	3.467	3.745
25	0.684	0.856	1.058	1.316	1.708	2.060	2.167	2.485	2.787	3.078	3.450	3.725
26	0.684	0.856	1.058	1.315	1.706	2.056	2.162	2.479	2.779	3.067	3.435	3.707
27	0.684	0.855	1.057	1.314	1.703	2.052	2.150	2.473	2.771	3.057	3.421	3.690
28	0.683	0.855	1.056	1.313	1.701	2.048	2.154	2.467	2.763	3.047	3.408	3.674
29	0.683	0.854	1.055	1.311	1.699	2.045	2.150	2.462	2.756	3.038	3.396	3.659
30	0.683	0.854	1.055	1.310	1.697	2.042	2.147	2.457	2.750	3.030	3.385	3.646
40	0.681	0.851	1.050	1.303	1.684	2.021	2.123	2.423	2.704	2.971	3.307	3.551
50	0.679	0.849	1.047	1.295	1.676	2.009	2.109	2.403	2.678	2.937	3.261	3.496
60	0.679	0.848	1.045	1.296	1.671	2.000	2.099	2.390	2.660	2.915	3.232	3.460
80	0.678	0.846	1.043	1.292	1.664	1.990	2.088	2.374	2.639	2.887	3.195	3.416
100	0.677	0.845	1.042	1.290	1.660	1.984	2.081	2.364	2.626	2.871	3.174	3.390
1000	0.675	0.842	1.037	1.282	1.646	1.962	2.056	2.330	2.581	2.813	3.098	3.300
Inf.	0.674	0.841	1.036	1.282	1.640	1.960	2.054	2.326	2.576	2.807	3.091	3.291

↓ degrees of freedom → alpha level