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Cellulose mineralization in two-stage anaerobic digestion systems

by

Yuyun Shang

A dissertation submitted to the graduate faculty

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For the Graduate College

PERSISTENCE IS A KEY TO SUCCESS

This work is dedicated to my family, whose love and support has truly been a treasure to my whole life.

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NOMENCLATURE

Acid-methane	Acid-methane Anaerobic Digestion System
APHA	American Public Health Association
ASBR	Anaerobic Sequencing Batch Reactor
CFR	Code of Federal Regulation
COD	Chemical Oxygen Demand, mg/L or g/L
CSTR	Completely-Stirred Tank Reactor
EPA	Environmental Protection Agency
HRT	Hydraulic Retention Time, day
K_h	First-order Hydrolysis Rate, day^{-1}
MCC	Methane Converting Capacity
MPN	Most Probable Number
MSE	Mean Square of Error
MSR	Mean Square of Regression
MSW	Municipal Solid Waste
NFDM	Non-fat Dry Milk
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate, mg/L-reactor/day, or g/L/day
PCOD	Particulate Chemical Oxygen Demand, mg/L, or g/L
PSS	Primary Sewage Sludge
SCOD	Soluble Chemical Oxygen Demand, mg/L, or g/L
SRT	Solids/Sludge Retention Time, day
SS	Suspended Solids, mg/L, or g/L
SSE	Sum of Square Error
TCD	Thermal Conductivity Detector
Thermo-meso	Thermo-meso Anaerobic Digestion System
TPAD	Temperature-phased Anaerobic Digestion System

TVFA	Total Volatile Fatty Acids, mg/L as acetic acids
UASB	Upflow Anaerobic Sludge Blanket Reactor
VS	Volatile Solids, mg/L or g/L
VSS	Volatile Suspended Solids, mg/L or g/L
WAS	Waste Activated Sludge
WEF	Water Environment Federation
WPCF	Water Pollution Control Facility

ABSTRACT

The objective of this research was to evaluate the anaerobic degradation of a simulated high-strength solid waste/sludge, particulate cellulose, by two-stage anaerobic digestion systems. Cellulose hydrolysis and methanogesis were examined in both the thermo-meso and acid-methane two-stage systems under different hydraulic retention times (HRTs), feed concentrations and organic loading rates (OLRs). The emphasis of this reaerch was to characterize the similarities and differences between these two processes.

The acid-methane system consisted of two consecutive reactors, an acidogenic reactor and a methanogenic reactor with both operated at mesophilic temperatures (35 ^{0}C). The thermo-meso process consisted a thermophilic reactor (55 ^{0}C) followed by a mesophilic unit. All the reactors used were completely-stirred tank reactors.

This research demonstratesd that both thermo-meso process and acid-methane process had considerable potentials for the anaerobic degradation of cellulose with concentrations up to 60 g COD/L at system HRT of 13 to 30 days.

Methane production and methane yield of the thermo-meso system were in the range of 148 – 1,100 mL/L-reactor·d and 0.23 – 0.33 L/g COD added when the OLRs were 466 to 4,000 mg/L/d. Thermophilic reactor dominated the solids destruction and methane production in the thermo-meso system. An OLR of 18.3 gCOD/L-reactor·d in conjunction with 3-day HRT was a threshold loading limit for this reactor.

Under the similar OLRs, methane production rates of the acid-methane system were 71 – 776 mL/L·d and the methane yields were 0.16 – 0.27 L/g-COD. The first-stage acidogenic reactor demonstrated an average soluble organics yield of 0.352 g soluble COD/g cellulose COD added.

Results from the continues runs showed that the thermophilic reactor possessed 2-4 times higher methane production rate than the methanogenic reactor. The first-order hydrolysis rate of the thermophilic reactor was 0.79±0.22 day^{-1} which was higher than that of the acidogenic reactor (0.26±0.2day^{-1}).

Based on the system methane generation model obtained from operating the two-stage systems, thermo-meso system demonstrated higher methane productions if treating high solid wastes with a shorter hydraulic retention time needed when compared with the acid-methane two-stage system.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Background

Due to economic growth, waste production from municipal, agricultural and industrial activities has increased yearly for both the developed and developing countries (Eighmy and Kosson, 1996; Sparling *et al.*, 1997). It has been estimated that each year in the United States alone, 5.4 million tons of domestic wastewater sludge are generated. Management and disposal of these large amount of waste have become complex environmental issues and will continue to be a great challenge for environmental engineers.

In recent years, traditional waste disposal and stabilization methods, such as ocean dumping, the use of landfills and incineration have become less attractive due to increased costs and the associated environmental concerns. Instead, recovering resources from wastes, such as energy and nutrient, has brought tremendous benefits and has become more important as a long term waste management strategy.

For waste stabilization, biological anaerobic digestion is a common process employed in wastewater treatment plants and meanwhile produces energy in the form of methane. In addition, the digested sludge can be used as nutrients for plant growth. However, this stabilization process is typically archived by a conventional mesophilic single-stage anaerobic digester. Most studies performed at mesophilic conditions reported that a relative long hydraulic retention time (HRT) is needed to mineralize organic wastes by the single-stage digesters. Several advantages of anaerobic waste digestion at thermophilic temperatures, such as enhanced hydrolysis of particulate matters, increased reaction rate, and improved process efficiency have been reported (Hashimoto, 1982; Cecchi *et al.*, 1995 and Ahring, 1994). However, Buhr and Andrews (1977) stated that some disadvantages of the thermophilic digestion include poor process stability and effluent quality (high volatile fatty acids).

In order to improve the stability of anaerobic digestion and also to achieve a better process efficiency, several new approaches, including two-stage anaerobic digestion, where two consecutive

reactors were employed for stage separation, have been tried. In this research, two of the two-stage processes will be studied, one is an acid-methane system and the other is a thermo-meso process.

The acid-methane two-stage system, also referred to as the two-phase system, separates the acid and methane fermentation phases by employing two reactors, namely the acidogenic reactor and the methanogenic reactor. In this process, the first stage unit receives raw or preconditioned wastes and hydrolysis is encouraged here, while the second stage unit receives the effluent from the first stage unit and methanogenesis is encouraged. The purpose of this two-stage configuration is to provide optimal environmental conditions thus improving the activity of each group of microorganisms based on their different metabolic characteristics and growth rates, so the overall process efficiency is enhanced. Since its development, the acid-methane process has demonstrated great advantages over the single-stage system in stabilizing wastewater sludges (Pohland and Ghosh, 1971; Ghosh, 1987 and Ghosh, 1995).

The newly evolved thermo-meso process, also called temperature-phased anaerobic digestion system (TPAD), separates the stages into thermophilic and mesophilic ones through temperature controls. The combination of the thermophilic with mesophilic digesters allows this system to take full advantage of thermophilic digestion but eliminate its disadvantages. Results from bench-scale and full-scale operation of the thermo-meso process showed that a higher volatile solids destruction was achieved at a shorter or same HRT compared with the single-stage digester (Han and Dague, 1997; Schmit, 1998). Moreover, due to the thermophilic temperature employed in the first-stage digester, pathogen destruction was nearly complete in the thermo-meso process. Consequently, the digested sludge can meet the EPA 40 CFR Part 503 Class A biosolids requirements (Han and Dague, 1997; Han, 1997; Vandenburgh, 1998 and Chao, 1999). With more stringent environmental regulations being issued, the thermo-meso process, therefore, offers a great potential in defining the future of high-solid wastes decomposition.

Despite the successful implementations of acid-methane and thermo-meso systems in wastes stabilization, a lack of information on the mechanisms for organics degradation as well as important design and operational parameters provides an opportunity for further research in these

two-stage systems. Therefore, the emphasis of this research was to investigate cellulose degradation in the acid-methane and thermo-meso systems under different operating conditions. Furthermore, the similarities and differences between these two systems were characterized. Cellulose was selected as the sole substrate for this research because it constituts much of the organic content in various wastes and the degradation of cellulose is usually reported as a rate limiting step for anaerobic sludge digestion (Eastman and Ferguson, 1981; Pavlostathis *et al.*, 1991and Leschine, 1995).

For a two-stage process degrading organic wastes, HRTs, influent substrate concentrations and organic loading rates have been considered to be the important operational factors affect the digester efficiency and methane production (Dinopulou *et al.*, 1988; Elefsiniotis, 1994 and Grady, 1999). For this purpose, a set of different HRTs and cellulose concentrations were employed in planning the experiments for examining their effects on (1) the hydrolysis of cellulose, acidogenic and methanogenic bacterial activities in the acid-methane process; (2) methane generation from the thermophilic and mesophilic reactors in the thermo-meso system; and (3) comparative performances of the acid-methane system and the thermo-meso system for cellulose hydrolysis and methane generation.

Predictive polynomial quadratic equation and response surface methodology were adopted to provide a systematical and straightforward experimental data analysis. Methane converting capacity vial tests using each of acetate, propionate, iso-butyrate, and cellulose as the individual substrate were also conducted to evaluate the characteristics of the microorganism developed in each reactor of the two-stage systems and their maximum methane production rates were calculated.

1.2. Dissertation Organization

The purpose of the first introduction chapter is to describe the acid-methane and thermo-meso systems for anaerobic waste stabilization. More importantly, it introduces the three goals of this research. Chapter 2 is a literature review. It provides the background information and a general literature review for each system. The substrate characteristics *i.e.*, particulate cellulose along with its anaerobic degradation was also discussed.

Chapter 3 is a paper titled "Cellulose Mineralization in a Mesophilic Acid-methane Two-stage Anaerobic Digestion System". The main focus of this paper was to present the acid-methane system performance in cellulose hydrolysis and methane production under different operating conditions, more specifically under different hydraulic retention times (HRTs) and influent cellulose concentrations. Chapter 4 is a paper titled "Cellulose Mineralization in Thermo-meso Anaerobic Digestion System". This paper reported on studies of the cellulose degradation and methane generation in the thermo-meso system. Again, the HRT and feed cellulose concentration varied in each experimental run. The purpose of the research presented in Chapter 3 and 4 was to fully investigate the methane generation performances and cellulose degradation mechanism of the acid-methane and the thermo-meso system. The results from these sets of experiments were used as the basis for the process comparison as presented in Chapter 5. This chapter is a paper titled "Comparative Performances of Two-stage Anaerobic Digestion Systems for Cellulose Mineralization". The paper characterizes the similarities and differences between the acid-methane and thermo-meso systems.

Chapter 6 is a paper that summarizes the results obtained from laboratory and full-scale operation of the thermo-meso system for treating primary, secondary and municipal solid wastes. The collected data of volatile solids destruction and methane production under different operational conditions were analyzed to provide important guidelines regarding thermo-meso operating parameters such as feed strength, hydraulic retention time and organic loading rates. These parameters can be used for digester upgrading or new process designing.

Both papers in Chapter 4 and 5 were published in the Water Environment Federation Annual Conference Proceedings (WEFTEC 98 and 99). Four papers, presented in Chapter 3, 4, 5 and 6 respectively, will be further revised and submitted for journal publication.

The final chapter is a summary of this research and general conclusions drawn from the entire investigation. The references for each section are listed at the end of the corresponding chapter. At the end of this dissertation, raw experimental data are tabulated.

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CHAPTER 2. LITERATURE REVIEW

2.1. Anaerobic Digestion

Anaerobic processes have been used in wastewater treatment systems for more than a century, and were initially developed to stabilize the solids produced. The discovery of accelerating the digestion process by providing an uniform reaction environment and maintaining a constant temperature above 35 ^{0}C through mixing and heating has led to the development of the current high rate anaerobic digestion process. Now, anaerobic digestion remains as an extremely popular and widely used solids stabilization process (Grady *et al.*, 1999).

2.1.1. Fundamentals of Anaerobic Digestion

During anaerobic digestion, organic pollutants are converted to methane and carbon dioxide by a series of interrelated microbial metabolisms including hydrolysis/fermentation, acetogenesis, and methanogenesis as illustrated in Figure 1.

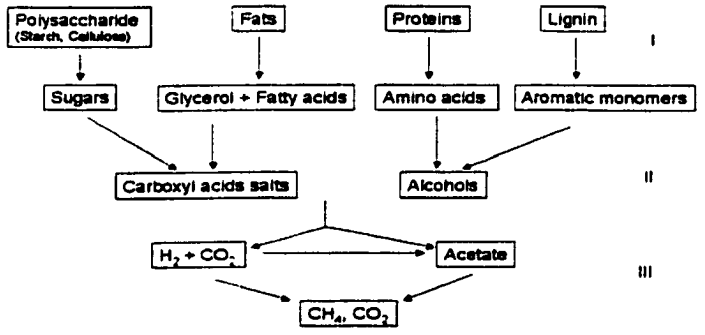

Figure 1. Anaerobic degradation of organic pollutants in wastes (source from Masuda *et al.*)

Three major groups of bacteria are involved in methane production from sludge, (1) the hydrolytic and fermentative bacteria; (2) the acetogenic bacteria, and (3) the methanogenic bacteria. The hydrolytic and fermentative bacteria convert organic polymers such as proteins, lipids and carbohydrates to amino acids, long-chain fatty acids and sugars, respectively, which are then

fermented to carboxylic acids, alcohols, carbon dioxide and hydrogen. The acetogenic bacteria further ferment these intermediates to acetate. The methanogenic bacteria use the acetate, formate, H_2, and CO_2 produced to form methane. The production of methane and carbon dioxide from complex organic wastes represents complete stabilization.

2.1.2. Factors Affecting Digester Performance

Several environmental and operational parameters play important roles in anaerobic digestion. pH and temperature are the two main environmental factors, solids retention time, hydraulic retention time, feed strength and volumetric organic loading rate are the common operational factors.

2.1.2.1. pH

pH has significant impact on the performance of anaerobic processes. Bacterial activity generally decreases as the pH deviates from an optimal value. A pH range of 6.8 to 7.4 provides optimal conditions for the methanogens. The effect of pH on the acidogenic bacteria is less significant and its primary influence is on the nature of fermentation products.

2.1.2.2. Temperature

Temperature greatly affects almost all the biological processes. Optimal performance is typically obtained by operating the digester in the two higher temperature ranges, *i.e.*, 30 - 40 ^{0}C for mesophilic and 50 - 60 ^{0}C for thermophilic. These two regions generally represent the optimal growth for methanogens. Therefore, most of the anaerobic digestion processes are designed operating in either the mesophilic or the thermophilic temperatures. For substrates that consist of large amounts of complex organic compounds or particulate materials, the effect of temperature on hydrolysis and acidogenesis are the primary concern. The purposeful use of high temperature for destroying pathogens in municipal wastewater solids is a new and evolving application.

2.1.2.3. Solids Retention Time (SRT) and Hydraulic Retention Time (HRT)

Solids retention time (SRT) is the average time that the solids reside in the reaction vessel. It controls the types of microorganisms that can grow in the process and the extent to which various reactions will occur (Grady *et al.*, 1999). Hydraulic retention time (HRT) is the average time that a

water molecule stays in the reactor. For a flow-through system as studied in this research, SRT is equal to HRT, and the determination of SRT/HRT is straightforward, which is equal to the active volume of the bioreactor divided by the flow rate.

2.1.2.4. Feed Concentration and Volumetric Organic Loading Rate (OLR)

Even though the OLR and feed concentration are not fundamental parameters that determine the performance of anaerobic treatment systems, they are related to SRT through the active biomass concentration in the bioreactor. Knowledge of the OLR that a particular process typically can achieve helps quantifying how effectively the bioreactor's volume is being utilized (Grady *et al.*, 1999). More concentrated feed and high organic loading rate reduce the reactor volume and also the heating requirement.

2.2. Anaerobic Digestion System

For sludge stabilization, conventional single-stage digesters are commonly employed in the municipal wastewater treatment plants. However, in recent years, two-stage systems, especially the thermo-meso processes have demonstrated great advantages over the single-stage processes. Due to their ability of meeting the more stringent environmental regulations, two-stage systems hold great potential in defining the future of anaerobic sludge digestion. In this thesis, two-stage process is defined as a system in which anaerobic digestion occurs in two separate consecutive reactors. It was proposed and introduced with the aim of improving digester stability and efficiency. In this research, two two-stage processes were examined, one is an acid-methane process, and the other is a thermo-meso system.

2.2.1. Acid-methane Two-stage System

Acid-methane two-stage system, also referred to as a two-phase system, separates the acid and methane fermentation phases by employing two reactors, namely the acidogenic reactor and the methanogenic reactor. In this process, the first stage unit receives raw or preconditioned wastes where hydrolysis is encouraged, while the second stage unit receives the effluent from the first stage unit and methanogenesis is encouraged here. The purpose of this two-stage configuration is to

provide optimal environmental conditions thus improving the activity of each group of microorganisms based on their different metabolic characteristics and growth rates, so the overall process efficiency is enhanced. This kind of arrangement is different with the conventional single-stage process where hydrolysis/fermentation, acetogenesis, and methanogenesis all co-exist in a microbial consortium.

The advantages and disadvantages of the acid-methane two-stage process were summarized by Fox and Pohland (1994). The main cited advantages are: isolating and optimizing the potential rate-limiting steps, improving reaction kinetics and process stability to shock loads, and selecting for fast-growing microbes. The disadvantages are: disruption of syntrophic relationships; more difficult to implement, engineer and operate; the lack of process experience and applicability to a variety of wastes; and the uncertainty of linkage between substrate type and reactor configuration (Fox and Pohland, 1994).

According to Fox and Pohland (1994), phase separation enhances the treatment of carbohydrate wastes. The following two sections will review the studies performed on the first stage acidogenic as well as the two-stage process as a whole for carbohydrates wastes degradation, mainly cellulose, sewage sludge and municipal solid wastes.

2.2.1.1. First stage of the acid-methane anaerobic digestion process

For an acid-methane system, the performance of the first stage acidogenic reactor is important because it provides intermediates which can be easily converted to methane in the following methanogenic reactor. Therefore, it is common to engineer the operation of the first stage reactor towards maximizing its efficiency and rate of formation of the desired product, *i.e.*, soluble organics, including volatile fatty acids.

Studies conducted on acid-phase digestion of primary sewage sludge with a detention time of 9 to 72 hours showed that hydrolysis of the solid sludge particles was the rate-limiting step of the overall acidogenic phase (Eastman and Ferguson, 1981). Since then, many studies have been aimed at investigating the effects of pH, HRT, substrate concentration and temperature on the hydrolysis of particulate substrate and volatile fatty acids production by this acidogenic reactor. Perot studied the effects of pH, temperature and agitation speed on anaerobic sludge hydrolysis-

acidification. In this study, a mixture of primary and waste activated sludge with suspended solid concentration of 29 g/L was used as the feed. The author concluded that the best running conditions for hydrolysis were at pH = 6.8, temperature = 50 ^{0}C, and agitation speed of 545 rpm (Perot *et al.*, 1988).

Study of acidogenesis using beef extract as the substrate showed that the degree of acidification increased with HRT and decreased with the influent substrate concentration and organic loading rate, while the opposite held true for the product formation rate. Furthermore, it was demonstrated that acidification was primarily dependent on HRT and the rate of product formation was controlled by the influent substrate concentration. The optimum pH and temperature were 7 and 40 ^{0}C respectively (Dinopoulou *et al.*, 1988).

The effect of particle size, pH and HRT on the anaerobic acidogenesis of cellulose was investigated by Chyi (Chyi and Dague, 1994; Chyi and Levine, 1992). Chyi used the mesophilic complete mixed reactors (CSTRs) with pH controls. An optimum pH of 5.6 was found for the acidification of cellulose and the solubilization of cellulose was greatest with an HRT of 72 hours. Based on the results obtained from this study, the authors suggested that the hydrolysis of cellulose was the primary rate limiting step for cellulose degradation. This is in agreement with earlier research by Eastman and Ferguson (1981).

Elefsiniotis and Oldham (1994) investigated the effect of pH on the acidogenesis of primary wastewater solids using two bench scale continuous-flow reactors. Two different configurations were used: 1) a completely mixed reactor with solids recycling from a clarifier; and 2) a high-rate upflow anaerobic sludge blanket (UASB) reactor. Results indicated that, in either system, the specific rates of VFA production and COD solubilization were not affected by variations in pH between 4.3 and 5.2. However, at higher pH values (5.9 – 6.2), a 25 – 30% decrease in the rates was observed. These authors also investigated the effect of hydraulic retention time (HRT) on the acidogenic anaerobic digestion of primary sludge in another study (Elefsiniotis and Oldham, 1994). Results showed that both VFA production and COD solubilization increased significantly with the increasing of HRT up to 12 hours, but these values dropped moderately at a longer HRT. At HRT of 15 hours, onset

methanogenesis was observed and turned out to be the main reason accounting for reactor performance drop. This study also found that carbohydrates were solubilized to the greatest extent, followed by lipids and then proteins (Elefsiniotis and Oldham, 1994).

2.2.1.2. Acid-methane two-stage anaerobic digestion process

The acid-methane two-stage process was developed by Pohland and Ghosh (1971). The early research performed was using this process to treat a variety of waste streams including wastewater sludge. A study by Ghosh used two CSTRs operating in series at 35^{0}C to treat wastewater sludge. In this study, the first stage acidogenic reactor was operated at a pH of 5.7 with HRTs from 10 to 24 hours and the second stage methanogenic reactor was operated at 6.5-day HRT. Volatile solids (VS) destruction of 40% was obtained and the methane yield was up to 0.97 L/g VS reduced (Ghosh *et al.*, 1975).

Another study on acid-methane system for cellulose refuse fermentation obtained a total volatile fatty acids (VFA) yield up to 0.2 g/g VS and methane yield of 0.31 m^3/Kg VS added (Ghosh, 1984).

A later research by Ghosh focused on the temperature effect on the single-stage and acid-methane two-stage system treating mixed primary and activated sludges with the volatile solids content varied between 67 and 77 wt% of total solids. Bench-scale study showed that mesophilic acid-methane digestion afforded higher gasification kinetics and efficiency than the mesophilic or thermophilic single-stage digestion (Ghosh, 1986). In another research for treating mixed primary and activated sludge, Gosh reported that the mesophilic acid-methane system exhibited about the same methane yield and solids reduction at a 3-day HRT as those of the single-stage high-rate digestion at 15 and 17-day HRTs. The enhanced stability of this two-stage digestion relative to the single-stage digestion increased as the system loading and HRT increased. Optimal hydrolysis and acidification occurred at pH equal to 6 (Ghosh, 1987).

Another study by Ghosh was conducted in order to alleviate sever foaming problem and overloading conditions in the anaerobic digesters of DuPage County, IL wastewater treatment plant. Moreover, the research was performed at high-loading rates and low HRTs. In this study, acid-

methane systems were used to stabilize the waste activated sludge (WAS) generated from this wastewater treatment plant. Ghosh summarized the results and experiences from operating the pilot- and full-scale acid-methane systems. In the pilot scale studies, the acidogenic digester was operated at both mesophilic (36.8 ^{0}C) and thermophilic (49.8 ^{0}C) temperatures. Although the thermophilic acidogenic digester exhibited higher methane yields and VS destruction than the mesophilic digester, the creation of strong odors from the increased production of butyric, iso-butyric, and iso-valeric acids made thermophilic operation for the first-stage undesirable. The optimal results for mesophilic acid-methane system were obtained when the two-stage process operated at a 12-day total system HRT, with the first stage of 3 days and the second stage of 9 days. For a feed solids concentration of 7.5% TS and an OLR of 4.7 g VS/L/d, the methane yield was 0.29 L/g VS added with a VS reduction more than 70% without an evidence of digester foaming. Full scale data showed that the performance of the mesophilic full-scale two-stage system was as good or better than that of the pilot process.

However, Bhattacharya compared the volatile solids reduction of acid-methane system with conventional single-stage digester at mesophilic temperatures (35^{0}C). CSTRs reactors were used for the treatment of a 1:1 mixture of PS:WAS and WAS only (Bhattacharya *et al.*, 1996). Five studies were performed with feed solid concentrations ranging from 2.6 to 4.1% total solids and system HRTs ranging from 12 to 12.7 days. The methanogenic reactor was operated at an HRT of 10 days. The results showed that when treating WAS only, the VS destruction efficiency was up to 8.7 percent higher than conventional digestion. VS destructions were 1.9 to 6.0% higher for acid-methane processes as compared to the single-stage processes when treating a 1:1 ratio of PS:WAS mixture. The authors stated that the small increase in efficiency may not worth the extra cost of operating two-stage systems.

A lab-scale, acid-methane UASB-UASB (upflow sludge blanket reactor) was used to digest synthetic primary and secondary sludge at 35 ^{0}C with the specific pH controls of 5 for the acidogenic reactor and 7 for the methanogenic reactor. A process failure was reported due to the combination of hydraulic and organic overloading of the methanogenic reactor (Fongastitkul *et al.*, 1994).

It is worth to mention that, for acid-methane system, several kinetical models were developed to simplify the complicate anaerobic digestion process by considering only two key steps, hydrolysis/acidification and methanization. Viturtia adopted first order model for the hydrolytic step and Monod one for the methanization step. A good correlation between the model and the experimental results was obtained for treating shredded mixture of fruit and vegetable wastes at mesophilic temperatures (Vituria *et al.*, 1995). A dynamic simulation model was also developed for an acid-methane system treating solid wastes (Mata-Alvarez, 1987).

2.2.2. Thermo-meso Two-stage System

Thermo-meso system, also called temperature-phased anaerobic digestion system (TPAD), is a two-stage process with the first stage unit operated at the thermophilic temperatures and the second stage maintained at mesophilic temperatures. The combination of the thermophilic unit with a mesophilic digester allows this system to achieve high organic loads but with short hydraulic retention times (HRTs) needed. In thermo-meso system, both the thermophilic first stage and mesophilic second stage reactors are operated as methanogenic units and thus the syntrophilic relationships between different bacterial groups are maintained in both units.

Thermo-meso two-stage system was developed at Iowa State University by Dague and coworkers (Han and Dague, 1997; Schmit and Dague, 1997; Welper and Dague, 1996; Kaiser *et al.*, 1995; Steinbach, 1994) at the middle of 1990s. The evolution of the thermo-meso process was trying to obtain a better effluent quality. Early studies on the thermo-meso system was to characterize the process advantages by treating synthetic nonfat dry milk wastewater. High-rate bioreactors including biofilter and anaerobic sequencing batch reactor (ASBR) were selected either as the first stage or second stage unit for the thermo-meso system (thermo biofilter/meso biofilter; thermo ASBR/meso ASBR; and thermo biofilter/meso ASBR). The results from these studies demonstrated the great advantages of this system over the high-rate single-stage mesophilic process in terms of much higher COD removal at equivalent system HRTs (Kaiser *et al.*, 1995; Steinbach 1994; Welper and Dague, 1996; Schmit and Dague, 1996). The details and results of these initial investigations on thermo-meso process have been described and summarized by Schmit (Schmit, 1998).

Using thermo-meso process for stabilizing wastewater sludges came after the work of Han and Dague (1997). The thermo-meso system they used consisted two completely stirred tank reactors (CSTRs) operating in series to treat primary wastewater solids (PS). A higher VS reduction was obtained and less than 1,000 MPN/g TS of fecal coliforms were present in the effluent of thermo-meso system. With the start of new federal regulations (40 CFR, Part 503), thermo-meso process became an attractive waste management method to meet the pathogen destruction requirement of Class A biosolids due to the thermophilic temperatures employed. Han and Dague (1997) later used the same system to treat the mixture of PS and waste activated sludge (WAS).

Thorberg extended the work of Han and Dague (1997) and investigated at longer hydraulic retention time of bench scale thermo-meso stabilizing the mixture of PS and WAS (Thorberg, 1998). Later research by Vandenburgh studied the effect of increasing feed volatile solids concentration on the thermo-meso process. The tested feed VS concentrations were from 33.4 to 57.8 g VS/L and the system HRT was 20 days, 7.4-day for the first stage and 12.6-day for the second stage. Optimal feed concentration of 37.8 g VS/L was reported (Vandenburgh, 1998). Schmit (1998) first compared the thermo-meso process with acid-methane system and single-stage digester for co-digestion of synthetic municipal solid waste with primary sewage sludge. Using thermo-meso process to stabilize the waste activated sludges generated from industrial wastewater treatment plant has been investigated by Chao (1999). The bench-scale study showed that more than 40% volatile solids reduction was obtained at a feed VS concentration of 4.5% and HRT of 3 days for the thermophilic unit, 9 days for the mesophilic reactor. This promising result was to be used for the design of a full-scale thermo-meso system at Western Lake Superior Sanitary District (WLSDD) of Duluth, MN.

Conversion of the conventional single-stage anaerobic digesters of Newton Water Pollution Control Facility (WPCF) to the thermo-meso two-stage system represented the first full-scale thermo-meso operation in the United States (Streeter, 1996). Newton WPCF later received the Environmental Protection Agency's (EPA) 1st place award in 1996 for outstanding beneficial use of biosolids. Interested in generating Class A biosolids as Newton WPCF, several other wastewater treatment plants have converted their existing single-stage systems to thermo-meso processes. The

operation of these full scale thermo-meso systems demonstrated increased volatile solids reduction and methane production. Moreover, stable process performance and Class A pathogen reduction were also reported. Vik (1997) and Streeter (1997) described the full-scale operations of thermo-meso system in the United States. A list of the utilities that are either currently using, designing, or contemplating use of the this process can be found elsewhere (Schmit, 1998). Loess (1997) summarized the development of two-stage thermophilic/mesophilic sludge digestion in Germany. Design value of 2-3 days retention time in the thermophilic stage followed by 12-15 days retention in the mesophilic stage was suggested. Important recommendations on digester temperature and solid concentration regarding full-scale thermo-meso system application and operation were addressed. A similar hydraulic retention time was confirmed by Dichtl with the consideration of disinfection aspect in two-stage digestion (Dichtl, 1997).

A summary of all the results from previous bench-scale studies of thermo-meso process treating municipal wastewater solids or organic fraction of municipal solid wastes is shown in Table 1 of Chapter 6. This table also includes the available results from the operation of full-scale thermo-meso processes.

One of the known drawbacks of the thermo-meso system is the extra energy input for operating the first stage reactor. However, if increased methane production due to the alleviated temperature can compensate this energy requirement, then the two-stage system will bring an extra benefit of greater pathogen destruction. Pathogen destruction is very critical for those existing facilities treating wastewater solids in an effort to comply with stricter environmental regulations.

2.3. Substrate Characteristics

The substrate used for this research was particulate cellulose with the particle size of 20 μm. The following section describes the cellulose containing wastes, the characteristics of cellulose and its anaerobic degradation along with the different groups of microorganisms involved.

2.3.1. Cellulose Containing Wastes

Cellulose, mainly synthesized by plants, is the most abundant organic polymer on earth. Each year photosynthetic fixation of CO_2 yields more than 10^{11} tons of dry plant material worldwide, and almost half of this material consists of cellulose (Bailey and Ollis, 1986). Due to its abundance in the natural environment, cellulose is a big component of biomass and counts as a major source for various wastes. Table 1 and 2 show cellulose distribution in biomass and its composition in various wastes.

2.3.2. Anaerobic Mineralization of Cellulose

Each year, cellulosic wastes are produced in increasing amount either as municipal solid waste or as agricultural and industry wastes. Lacking of landfill area and concerning of global warming caused by a rapid increase of greenhouse gases in the atmosphere has brought an added incentive for the development of energy derived from wastes. As an alternative, anaerobic conversion of these wastes into methane and carbon dioxide by bacterial consortia is gaining increasing acceptance as the solution and has attracted the continuing interest of environmental engineers.

Table 1. Distribution of cellulose, hemicellulose, and lignin in biomass and waste resources (%)

Material	Cellulose	Hemicellulose	Lignin
Hardwoods stems	40~55	24~40	18~25
Softwoods stems	45~50	25~35	25~35
Grasses	25~40	25~50	10~30
Leaves	15~20	80~85	~0
Cotton seed hairs	80~95	5~20	~0
Newspaper	40~55	25~40	18~30
Waste papers from chemical pulps	60~70	10~20	5~10

Table 2. Cellulose composition in various wastes (% of dry matter)

MSW	OFMSW	PSS	WAS	Cattle manure	Chicken manure	Paper & pulp WAS
35 ~ 37	32.9	32.2	9.7	17	28.3	12

MSW: municipal solid waste OFMSW: organic fraction of municipal solid waste
PSS: primary sewage sludge WAS: waste activated sludge
Sources were from Baily, Pavlostathis, Schmit and Chao

2.3.2. Anaerobic Mineralization of Cellulose

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2.3.2.1. Characteristics of Cellulose

Cellulose is a complex carbohydrate. Each cellulose molecule is a long, unbranched chain of D-glucose subunits with an unit molecular weight ranging from 50,000 to over one million. The glucose is connected by the β-1, 4 glycosidic linkage bonds as shown in Figure 2. Few living creatures can hydrolyze the β-1, 4 bonds of cellulose.

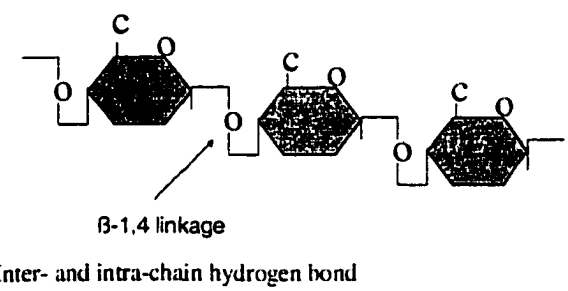

Figure 2. The glucose chain of cellulose

The resistance of cellulose to natural and process degradation derives more from the crystalline structure of cellulose and upon its biological "packaging" than its use of β-1, 4 glycosidic bonds. Cellulose molecules are strongly associated through inter- and intra-molecular hydrogen bonding and van der Walls forces. This hydrogen bonding makes cellulose chains combine to give crystallites, larger structures, which are visible in the electron microscope. Intrachain hydrogen bonding occurs between the C-3 hydroxyl and oxygen in the pyranose ring.

The crystalline structure of cellulose can be divided into two regions, crystalline and amorphous regions. Most of the cellulose is organized into highly ordered crystalline regions, in

which cellulose chains or fibrils are so tightly packed that even water molecules scarcely penetrate. Cellulose is, accordingly, water insoluble. Less ordered portions of the assembly, called amorphous regions, comprise typically about 15% of the cellulose microstructure. The amorphous regions are easily hydrolyzed by, for example, acids; the crystalline regions on the other hand are much more difficult to decompose. Cellulose almost never occurs alone in nature. The association with hemicellulose and lignin makes its natural degradation even more difficult.

2.3.2.2. Cellulose Anaerobic Decomposition

In the natural environment with the absence of oxygen and other exogenous inorganic electron acceptors, cellulose is decomposed by the anaerobic community into CH_4, CO_2 and H_2O through a complex microbial food chain. Figure 3 illustrates the cellulose degradation by microbial communities in soils and freshwater sediments. It is similar in most anaerobic soils and sediments and in anaerobic digesters (Leschine, 1995).

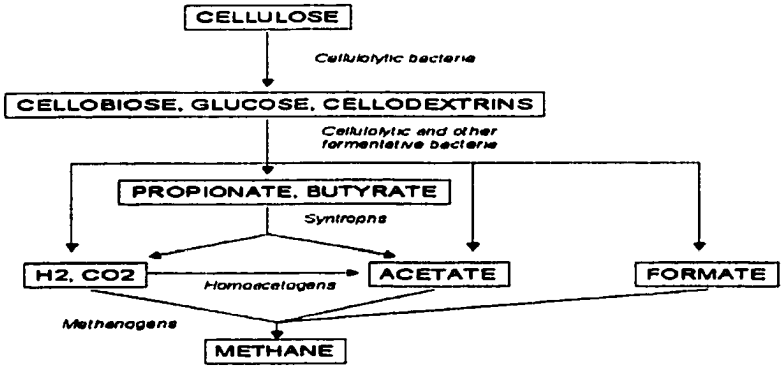

Figure 3. Cellulose anaerobic degradation by microbial communities in soils and freshwater sediments (Leschine, 1995)

Cellulolytic microbes produce enzymes that depolymerize cellulose, thereby formed the hydrolysis products of cellobiose, cellodextrins, and some glucose. These sugars are then fermented by cellulolytic and other saccharolytic microorganisms. By keeping cellobiose concentrations low, and thus preventing the inhibition of the cellulase system by this product of cellulose hydrolysis,

noncellulolytic cellobiose-fermenters play a very important role in this step. Fermentation of the produced sugars yields CO_2, H_2, organic acids (*e.g.* acetate, propionate, butyrate) and alcohols. It should be pointed that the fermentation products vary with the types of bacteria as well as with the cultural conditions such as temperature, pH and redox potential etc.

The produced fatty acids will be further fermented to acetate by the syntrophic bacteria, and then methanogens, specifically classified as hydrogenotrophic methanogens (*i.e.*, hydrogen-utilizing chemolithotrophs), will use H_2 produced during fermentation process as the electron donor to reduce CO_2 to CH_4. Hydrogen produced from the fermentation process is also immediately consumed by homoacetogens to produce acetate. Aceticlastic methanogens use acetate produced by fermentors or by homoacetogens through the acetoclasic cleavage to CH_4 and CO_2. In sewage sludge digesters, about 65 to 70% of the methane produced is via reduction of acetate to methane, and one third of the methane produced comes from the reduction of CO_2 by H_2. Through the combined activities of several major physiological groups of microbes, cellulose is completely dissimilated to CO_2 and CH_4.

Since syntrophic bacteria grow very slowly, and thus the fermentation of fatty acids could be one potential rate-limiting step in anaerobic decomposition of cellulose. Inerspecies H_2 transfer and utilization is very important in regulating the rate of H_2-producing reactions, under relatively high H_2 partial pressure, acetate formation is reduced and the substrate is converted to propionic acid, butyric acid and ethanol rather than methane. There is a symbiotic relationship between acetogenic bacteria and methanogens. Methanogens help achieve low hydrogen tension required by acetogenic bacteria.

As we have discussed previously, hydrolysis of cellulose is usually reported as the primary rate limiting step for cellulose dissimilation, the following section will look more closely of this step.

2.3.2.2.1. Cellulose Hydrolysis

Hydrolysis of cellulose is an enzyme-catalyzed reaction. Enzymatic hydrolysis is generally considered consist of three steps: the adsorption of cellulase enzymes onto the surface of the cellulose, the subsequent breakdown of cellulose to fermentable sugars through the synergistic action of the cellulase enzymes, and desorption of the cellulase enzymes from the lignocellulosic residue

into the supernatant. Like all the enzyme-catalyzed reaction, the kinetics of the enzymatic hydrolysis of cellulose depends upon three groups of factors: the nature of the enzyme that the system employed, the structure of cellulose itself, and the inhibitory effect of substrate, intermediate or final product. Operating conditions such as pH and temperature will affect the enzyme activity. The influence of the pH can be represented by using extended Michaelis-Menten equation. Arrhenius equation is often used to describe the temperature effect on the reaction rate.

Substantial research and development efforts worldwide have focused on enzymatic hydrolysis of cellulose. Most of the research were conducted with pure strain to evaluate the enzyme activity and to obtain kinetical parameters by fitting the experimental data into enzyme-catalyzed reaction equations. However, in anaerobic waste digestion, it is often hard to do the same things as allowed in the research with pure strain. Therefore, some models have been developed to simplify this complex reaction happened in the digester to evaluate the extent of hydrolysis of the particulate matter in the wastes. Among them, first-order rate with the particulate substrate is the most popular one. Table 3 summarizes the apparent first-order hydrolysis/fermentation rate constants for cellulose.

2.3.2.3. Microorganisms for Cellulose Decomposition

Various groups of bacteria are involved in cellulose dissimilation, Table 4 lists some species which are in favor of thermophilic temperatures.

2.4. Summary

This chapter gave an overview of the fundamentals for anaerobic digestion. Several environmental and operational factors which play important roles in digester performance were discussed. General descriptions along with their performances of two two-stage systems investigated in this research, *i.e.*, the mesophilic acid-methane and thermo-meso systems were provided. Previous studies indicated that both systems provided increased waste stabilization as compared to the single stage processes. The purpose of the research at hand was to further characterize these two-stage systems and a direct comparison of the thermo-meso two-stage process with the acid-methane two-stage system was quantified for cellulose degradation. Characteristics of the substrate utilized for this study, cellulose, were provided along with its anaerobic degradation process.

Table 3. Summary of the first-order hydrolysis rate constant from literatures

Temp. (^{0}C)	Process type	Culture	Rate (day^{-1})	Reference
30-35	semi-continuous	mixed	0.05	Singh, R. *et al.*
28	batch	mixed	0.17	Heukelekian, H.
35	semi-continuous	mixed	0.28-0.52	Speece, R. E. *et al.*
35	batch	mixed	0.12	Greco, R. L. *et al.*
37	batch	*Ruminococcus albus*	2.88	Stack, R. J. *et al.*
37	continuous	*R. albus*	1.18	Pavlostathis, S. G. *et al.*
60	batch	*Clostridium thermocellum*	0.42	Tailliez, P. *et al.*
60	continuous	*C. thermocellum*	0.15	Lynd, L. R. *et al.*

Table 4. Microorganisms important in anaerobic cellulose dissimilation (Kristjansson, 1991)

Organism Name	Temp. ^{0}C	Growth Substrates	Major Products
Clostridium thermocellum	40-68	Cellulose, hexoses	Acetate, ethanol, lactate, H_2, CO_2
Clostridium stercorarium	45-70	Cellulose, hexoses, pentoses	Acetate, ethanol, lactate, H_2, CO_2
Clostridium thermohydrosulfuricm	40-78	Sugars	Ethanol, lactate, acetate, H_2, CO_2
Thermoanaerobium brockii	40-80	Sugars	Ethanol, lactate, acetate, H_2, CO_2
Thermoanaerobacter ethanolicus	40-75	Sugars	Ethanol, acetate, H_2, CO_2
Clostridium thermoaceticum	45-65	Sugars, H_2-CO_2, CO_2	Acetate
Acetogenium kivui	50-70	Sugars, pyruvate, H_2-CO_2	Acetate
Methanobacterium thermoautotrophicum	45-75	H_2-CO_2	CH_4
Methanococcus thermolithotrophicus	30-70	H_2-CO_2	CH_4 (CO_2)
Methanococcus jannaschii	50-86	H_2-CO_2, formate	CH_4
Methanogenium thermophilicum	37-65	H_2-CO_2,	CH_4 (CO_2)
Methanogenium frittonii	26-62	H_2-CO_2, formate	CH_4 (CO_2)
Methanothermus fervidus	65-97	H_2-CO_2, formate	CH_4
Methanosarcina sp. Strain TM-1	<37-57	H_2-CO_2	CH_4, CO_2
Methanothrix spl strain CALS-1	40-65	Acetate, methanol, methylamines Acetate	CH_4, CO_2

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CHAPTER 3. CELLULOSE MINERALIZATION IN A MESOPHILIC ACID-METHANE TWO-STAGE ANAEROBIC DIGESTION SYSTEM

A paper to be submitted to Waste Management Research

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ABSTRACT

A mesophilic two-stage acid-methane anaerobic digestion system was operated over a range of feed cellulose concentrations (7.5 to 60 g chemical oxygen demand (COD)/L) and hydraulic retention times (HRTs, 15 to 30 days) to evaluate process performance. The two-stage acid-methane system consisted of two completely stirred tank reactors connected in series, and the pH of the first-stage reactor was controlled at about 5.6. The experimental results demonstrate that methane production of the first-stage was from non-detectable to 56 mL/L_reactor·d and from 84 to 970 mL/L reactor per day for the second-stage reactor. This suggests that the second-stage reactor governed system methane formation and COD destruction, while the first-stage reactor played the major role in hydrolyzing/fermenting cellulose to volatile fatty acids. The results obtained with the first-stage reactor indicated that when cellulose loading rate was increased from 1,367 to 13,025 mgCOD/L-reactor/day, soluble organics production rate ranged from 722 to 4,452 mgCOD/L-reactor/day, and a yield of 0.352 g soluble COD per gram cellulose COD added was obtained. Experimental results imply that as the system loading rates increased, the second-stage reactor not only shared in the hydrolysis of cellulose, but also played an important role in polishing the organics to methane.

Several vial tests were carried out on the mixed liquors, withdrawn from the two-stage digester at quasi-steady state, to measure the methane converting capacity (MCC) of specific substrates, including acetate, propionate, iso-butyrate, and cellulose. MCC data proved that methanogens existed in the first-stage reactor, whereas less methane formation resulting was from the operational suppressions of the methanogenic activities. According to MCC values of the second-

stage reactor, fermentation/conversion of propionic and butyric acids to methane was not limiting for cellulose mineralization. The rate controlling step for the microorganisms converting cellulose to methane in the second-stage reactor could be the hydrolysis/fermentation of cellulose.

INTRODUCTION

Interest in utilizing anaerobic bacteria for methane generation from various wastes increased in the early 1970s because of the oil shortage. Now, due to increased waste disposal costs and growing environmental concerns related to incineration or land disposal of wastewater sludges, this method may be an excellent way to deal with the world's increasing production of sewage sludge and municipal solid waste (MSW). For waste stabilization, cellulose is a major component in the organic fraction of MSW and sewage sludges. The conversion of cellulose to methane by microbial fermentation, therefore represents a partial solution to organic waste accumulation and depletion of hydrocarbon fuel reserves (Zeikus, 1980).

In anaerobic waste stabilization, a common process employed at municipal wastewater treatment plant is conventional single-stage digestion. Due to the hard-degradable nature of sludges, most studies conducted with single-stage digester was operated with relative long sludge/hydraulic retention time (SRT/HRT); as a result, the digester were under low organic loading rate. Sometimes, poor digester stability due to the imbalance between the acid and methane formations has also been reported (Ghosh, 1998). In order to improve the efficiency and stability of anaerobic digestion, acid-methane two-stage system which separates the acid and methane forming stages by employing two reactors connected in series, was introduced by Pohland and Ghosh (1971). Through this separation, the acivities of each group of the microorganisms involved in anaerobic digestion, namely acidogens, acetogens and methanogens, will be optimized based on their specific metabolic characteristics and growth rates. Therefore, the acid-methane two-stage system is expected to overcome the disadvantages of the conventional single-stage mixed-phase digestion.

Although considerable work including both fundamental and full-scale demonstration plants for sludge stabilization has been done since its development, a lack of information as well as a

systematical analysis of the acid-methane system degrading particulate cellulose provides an opportunity for further research. For a two-stage process degrading high-solids, HRT and influent substrate concentration have been considered to be important operational factors affecting the efficiency of the process. In this study, a set of HRTs and cellulose concentrations were employed in planning the experiments for learning their effects on the hydrolysis/fermentation of cellulose in the first stage reactor using predictive polynomial quadratic equation with response surface methodology (Box *et al.*, 1978). Methane generation from the second stage reactor by treating the effluent of the first stage one was also investigated. Methane converting capacity (MCC) batch tests using acetate, propionate, iso-butyrate, and cellulose were conducted to evaluate the maximum methane producing characteristics of the microorganisms developed in the first- and the second-stage reactors, respectively.

METHODS AND MATERIALS

Experimental Setup and Procedure

Acid-methane Two-stage Process

The experimental setup of the two-stage process is shown in Fig.1. This process was composed of two completely stirred tank reactors. The first-stage reactor was a 4.7-liter plexiglass rectangular tank with 6 inch long (L), 6 inch wide (W) and 8 inch high (H). The second-stage reactor compartment was an 8"L×8"W×10"H rectangular tank with a total volume of 10.5 liters. Heat-water jackets were employed to maintain both reactors at a mesophilic condition of 35±0.5 °C. An automatic pH controller controls the pH of the first-stage reactor at 5.6±0.2, a reported optimal value for cellulose fermentation (Chyi and Dague 1994). The amount of biogas produced from the first- and second-stage reactors were recorded daily with two wet gas meters (Precision Scientific). For each reactor, a biogas sampling port was installed between the meter and the reactor to allow direct biogas sampling with a syringe. The headspace pressure of the reactor was equalized using an inflatable biogas collection ball while decanting. Masterflex positive displacement pumps controlled by timers

(Chrontrd) provided semi-continuous (10 times a day) influent and effluent flows for the first- and the second-stage reactors in order to adjust to the appropriate HRTs as listed in Table 1. The system was fed with a nutrient mineral medium containing particulate cellulose (Sigma, 20μm, S3504) as the sole carbon and energy source with concentrations varied from 7.5 to 60 gCOD/L. The nutrient and mineral media was modified slightly from Chyi and Dague (1994), and each liter of the nutrient contains 345 g of $(NH_4)_2HPO_4$, 10.68 g of $FeCl_2 \cdot 4H_2O$, 625 mg of $ZnCl_2$, 1.215 g of $NiCl_2 \cdot 6H_2O$, 1.212 g of $CoCl_2 \cdot 6H_2O$, 1.081 g of $MnCl_2 \cdot 4H_2O$, 100 mg of $CuSO_4 \cdot 5H_2O$, 100 mg of $AlK(SO_4)_2 \cdot 12H_2O$, 61 g of $CaCl_2 \cdot 2H_2O$, 120 g of $MgSO_4 \cdot 7H_2O$, 100 mg of pyridoxine, 50 mg of thiamine-HCl, 50 mg of riboflavin, 50 mg of nicotinic acid, 50 mg of lipoic acid, 20 mg of biotin and 5 mg of B_{12}. The two-stage system was seeded with digested sludge obtained from the Water Pollution Control Plant in Ames, Iowa. To avoid carryover effects from the previous run and to ensure a random sampling strategy, withdrawing the acclimated-sludge and re-inoculating with the seed sludges were employed for the start-up process for each individual run. The process was registered as the quasi-steady state after a period of more than 2 - 3 turnovers of the second-stage reactor.

Response surface methodology (Box *et al.*, 1978) was used to facilitate a straightforward examination of the dependence of soluble COD production of the first-stage reactor on different HRTs and substrate concentrations.

Methane Converting Capacity (MCC) Test

In this study, MCC tests were performed with 250-mL vials for the microorganisms taken from both the first- and second-stage reactors at 35 °C. Thirty milliliters of mix liquors were individually withdrawn from each reactor serving as the initial inocula when the two-stage process proceeded under a steady-state condition. Acetate, propionate, iso-butyrate, and cellulose were supplemented individually as the substrate at a proper concentration to determine the MCC of the samples. The MCC test procedure used was a slightly modified version of that employed by Owen *et al.* (1979). Nutrient and mineral solutions for the test were the same with those used in the acid-methane process. For each vial, initial pH was adjusted to 7.0 by adding sodium bicarbonate. After displacing

the head space residual air with N_2 gas, it was tightly capped and incubated at a 35 °C orbital shaker. Cumulative biogas production was then measured.

The maximum specific methane production rate ($mL/L_{mix\text{-}liquor}/d$) in each vial experiment using each specific substrate was defined as the MCC consuming the corresponding substrate. To evaluate the MCC, the modified Gompertz equation (Lay *et al.*, 1998b, refers to Eq. (1)) was used to fit the experimental data of each vial test. Subsequently, the MCC was obtained by dividing the maximum methane production rate (R_m, mL/d) by 30 mL, the volume of the initial inocula.

$$M = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \qquad \text{.....(1)}$$

where M is the cumulative methane production (mL), t is the incubation time (day), λ is the lag-phase time (day), P is the methane production potential (mL), and e is the base of natural logarithm.

Data Analysis

The parameters of Eq. (1) were estimated using the "solver" function in Microsoft Excel 97 (Microsoft, Inc., 1995 - 1997). This program uses a Newton algorithm. Up to a hundred iterations were used to converge the sum of square error (SSE) between the experiment and the estimation to a minimum. Starting parameter values were estimated using a built-in visual procedure based on a limited fit algorithm (Wen *et al.*, 1994; Lay *et al.*, 1998b). Among the statistics reported by Wen *et al.* (1994), sum of square error (SSE) and correlation coefficient (r^2) were used to evaluate a fit. Additionally, Window software of Microsoft Excel 97 (Microsoft, Inc., 1995 - 1997) and Igor Pro version 3.12 (WaveMetrics, Inc., 1996) were employed for building-up a quadratic model (refers to Eq. (2)).

Analysis

Suspended solid (SS), volatile suspended solid (VSS), alkalinity, chemical oxygen demand (COD), and titrimetric volatile fatty acid (VFA) were measured according to the standard methods (1995). Soluble COD was measured after filtrating the sample through a 0.45 μm filter paper by

gravity. The pH of the samples was determined using a calibrated pH meter. Methane and carbon dioxide in biogas were separated using a gas chromatograph (Gow-Mac series 350) equipped with a thermal conductivity detector (TCD) and a 1-m stainless column packed with Porapak T (60/80 mesh). The operational temperatures of the injection port, the oven and the detector were maintained at 100, 50, and 100 °C, respectively. Helium was used as the carrier gas at a flow rate of 35 mL/min. A gas chromatograph (Trace Analytical RGA3 Reduction Gas Analyzer) was used to determine low concentration of hydrogen in the biogas, the operational temperatures of the column and detector were maintained at 80 and 264 °C, respectively. Nitrogen gas was used as the carrier gas.

RESULTS AND DISCUSSIONS

Eight experiments were run randomly to systematically quantify the influence/interaction of feed cellulose concentration and hydraulic retention time on the two-stage digestion system mineralizing particulate cellulose. In this study, influent particulate cellulose was supplied with 7.5 – 60 g/L under a system HRT of 15 – 30 days.

Performance of the Acid-methane Digester

Due to the fact that the system reaches a quasi-steady state after operating for more than 2 – 3 turnovers of the second-stage reactor on each individual run, Run 8 served as a typical example of the performance of the two-stage acid-methane digester.

In Run 8, the system was operated with a feed cellulose concentration of 60 g COD/L at a 15-day system HRT, 3 days for the first-stage reactor and 12 days for the second-stage reactor. Figure 2 shows the methane percentage and production, COD, soluble COD, volatile fatty acids, volatile solids and volatile suspended solids changes with the incubation time. Considering the variations in these values, the culture history can be divided into transient period and quasi-steady state, and are outlined as follows.

At the beginning (transient period, days 0 – 32) of Run 8, methane composition in biogas, soluble COD (SCOD) and VFA production of the first-stage reactor were unstable although methane

production and COD were relatively stable. The instability was due to the fact that the microorganisms typically need an adapting period while the cultural condition was changed. During this period, the reactor washed-out some microorganisms and selected appropriate bacteria to take the advantage of their new environment and begin multiplying (Lay *et al.*, 1998a and Grady, 1999). These findings have been claimed for a continuous-mixed reactor while it reaches a quasi-steady state after a period of more than three HRTs (van Hanndel and Lettinga, 1994). Notwithstanding the previous statement, the influent quantity/quality of the second-stage reactor (*i.e.*, the effluent of the first-stage reactor) was still a factor affecting its stability. As shown in Fig. 2, the unstable methane production was found for the second-stage reactor during the transient period. Throughout this study, this phenomenon was seen in other runs (figures not shown).

In the quasi-steady state (days 32 – 53) during Run 8, as shown in Fig. 2, no significant changes in the COD and the methane production rate were observed for both reactors, and the average performance data of COD concentration, methane production rate, including calculated particulate COD, OLR, and COD reduction are summarized in Table 2. Table 2 also lists the quasi-steady state data for Run 1 to 7 in this study. Of these data, it should note that the particulate CODs of the first-stage acid reactor were reduced from 6,833 - 55,379 to 3,760 - 43,173 mg/L, while the particulate CODs of the second-stage reactors were reduced form 3,760 - 43,173 to 1,581 - 7,155 mg/L. This evidenced that hydrolysis of cellulose was not only occurred in the first-stage reactor, but also in the second-stage reactor; thus, ensuring that the methanogenic reactor shared the cellulose hydrolysis/fermentation, as well as mainly converting VFAs into methane.

Further examination of Table 2 shows that there was approximately 4 to 10% COD reduction occurred in the first-stage reactor but no significant methane generated. Low methane production indicated that methanogenesis in this reactor was successfully suppressed. Results in Table 2 indicate that most of the soluble COD reduction and methane formation of the whole system was attained in the second-stage reactor through all eight runs. It is obvious that this reactor possessed a much higher capacity for mineralization of cellulose's metabolites, such as sugars and carboxylic acids towards methane than that of the first-stage reactor. Experimental results obtained from the

eight runs indicate that the first-stage reactor mainly converted particulate cellulose into soluble organics (*e.g.*, sugars and VFAs) with minimal amount of COD reduction, while the second-stage reactor governing the system methane formation and shared in cellulose hydrolysis/fermentation. As a result, the dynamical operation between both reactors was a key to enhance the efficiency of a two-stage digester degrading high-strength cellulose.

Hydrolysis/Fermentation of Cellulose in the First-stage Reactor

To quantitatively describe the relationships among the feed cellulose concentration, operating HRT, and their corresponding effects on cellulose hydrolysis/fermentation, the soluble COD data listed in Table 2 together with previous laboratory results from similar research conducted at Iowa State University by Chyi (Chyi, 1992) were used for the regression analysis and generation of the following equation.

$$SCOD = -5887.23 + 0.807\chi_1 + 2565.787\chi_2 - 7.2\times10^{-6}\chi_1^2 - 216.105\chi_2^2 - 0.074\chi_1\chi_2 \quad .....(2)$$

(degree of freedom = 11; $F = 24$; $R^2 = 0.9159$)

where SCOD is the soluble COD concentration in the acidogenic reactor (mg/L), χ_1 is the feed cellulose concentration (mg COD/L), and χ_2 is the HRT of the first-stage reactor (day). The statistics test, *F*, is defined as *MSR/MSE*, where *MSR* is the mean square of regression, obtained by dividing the sum of squares of regression by the degree of freedom. *MSE* is the mean squares of error from the analysis of variance. If the calculated value of *F* is greater than that in *F* table at a specified probability level (*i.e.*, $F(p\text{-}1, v, 1\text{-}\alpha)$), then a "statistically significant" regression model is obtained, where v is the degree of freedom of error and *p* is number of parameters. $F(p\text{-}1, v, 1\text{-}\alpha)$ is the *F* value at the α probability level. R^2 is defined as *SSR/SST*, whereas *SSR* and *SST* terms respectively represent the sum of squares of regression and sum of squares of total, gives an indication of regression fit. Since the value of R^2 (0.91) is close to 1, the regression model was considered to be an accurate representation of the experimental data.

According to Eq. (2), a series of contour plots was constructed as shown in Fig. 3. Consider the fitted equation [Eq. (2)] graphed in Fig. 3. soluble COD had the shape commonly referred to as a

"ridge". The trend of the ridge confirmed that both chosen values of HRT and feed cellulose concentration were important for the first-stage reactor producing soluble COD. The contour plots depict that soluble COD values, ranging from 3,600 to 13,350 mg/L, depend both on the HRT and cellulose concentration applied in this study. Furthermore, feed cellulose concentration had considerable interaction with HRT, and the optimum condition for soluble COD production was lying around the lower right corner, which means a high feed cellulose concentration at a short HRT.

It is important to note that for a completely stirred tank reactor operated with a constant HRT, the feed cellulose concentration corresponds to the cellulose loading rate. As listed in Table 2, the cellulose loading rate increased from 1,367 to 13,025 mg COD/L-reactor·day with an increase of soluble organics producing rate from 722 to 4,452 mg COD/L-reactor·day. However, soluble organics producing rate dropped 29% when the load was further increased to 18,460 mgCOD/L-reactor·day. Moreover, volatile fatty acid production from the first-stage reactor showed a direct dependency on the loading rate from about 1,400 to 10,000 mgCOD/L-reactor·day until it reached a relative stable stage when the loading was further increased. This implied that volatile fatty acid served as an important intermediate for methane formation on the metabolisms of cellulose mineralization.

An examination of Fig. 4 reveals that when the cellulose loading rate increased from 1.0 to 13 gCOD/L-reactor·day, soluble organics producing efficiency dropped from 50 to 25%; whereas if the loading was above 13 gCOD/L-reactor·day, the efficiency still kept at approximately 20%. Considering the difference between the soluble COD and the VFA production, it increased with an increase in the cellulose loading rate of the first-stage reactor; however, it would start to drop when the cellulose loading rate exceeded 13 gCOD/L-reactor·day. Such a phenomena suggests that 13 gCOD/L-reactor·day might represent a threshold limit for cellulose hydrolysis/fermentation in the first-stage acidogenic reactor.

Methanogenesis in the Second-stage Reactor

For dynamical operation on a two-stage digester, the first-stage acidogenic reactor was mainly responsible for providing maximum, constant, soluble and appropriate substrates that is

preferable for the second-stage reactor; while in this research, the second-stage reactor also shared a portion of the cellulose hydrolysis/fermentation. This phenomenon was evidenced by the particulate COD destruction obtained by this reactor (Table 2). Because hydrolysis/fermentation is essentially the conversion of the biodegradable volatile solids to soluble organics, thus, volatile suspended solid (VSS) is another indication for cellulose solubilization. The VSS reduction versus system organic loading rate was plotted to reveal the relationships between the first- and the second-stage reactors. An examination of Fig. 5 clearly shows an invert trend of VSS reduction obtained between the two reactors. Consequently, the enhancement of the compensation between two reactors can improve the efficiency of the two-stage digester converting cellulose to methane.

In order to investigate whether the second-stage reactor could sufficiently convert the organics transferred from the first-stage reactor into methane, the methane production rates of this unit were plotted against the respective organic loading rates for the second-stage reactor (Fig. 6). In Fig. 6, the methane production rates increased from 84 to 970 mL CH_4/L-reactor·d with the loading increased from 548 to 4,387 mgCOD/L-reactor·d, indicating that the methane production rate was dependent on the organic loading rate resulting from the first-stage reactor.

Methane Converting Capacity

To estimate the methane converting capacity (MCC) of the sludges inside the first- and the second-stage reactors on consuming VFAs and cellulose, a set of MCC experiments were carried out on the sludge anaerobically transferred from the digester while the system proceeded under the quasi-steady state. Equation (1) was used to fit the experimental data of cumulative methane production curve in each individual vial test. In this study, the specific methane production rate ($mL/L_{mixed\text{-}liquor}\cdot d$) for a certain substrate was defined as the MCC of that substrate. The significance of Eq. (1) was estimated using the same statistical approach that has been used for Eq. (2), and all R^2 were larger than 0.91. Average MCC value obtained from Run 5, 7 and 8 shows that the sludge taken from the second-stage reactor possessed a greater MCC of cellulose (274 ± 86 $mL/L_{mixed\text{-}liquor}\cdot d$) than those from the first-stage reactor (170 ± 73 $mL/L_{mixed\text{-}liquor}\cdot d$). This result was expected because

the first-stage reactor was controlled to a low pH condition (5.6 ± 0.2), which was a suitable pH environment for acidogenic/acetogenic bacterial growth, but not for methanogens. Although only small amounts of methane formed in the first-stage reactor throughout this study, the MCC results clearly demonstrated that methanogenic bacteria were existed in this reactor.

In the second-stage methanogenic reactor, the MCC of acetate was significant greater than that of propionate (648 ± 268 $mL/L_{mixed\text{-}liquor}\cdot d$), iso-butyrate (462 ± 131 $mL/L_{mixed\text{-}liquor}\cdot d$) or cellulose (274 ± 86 $mL/L_{mixed\text{-}liquor}\cdot d$). According to the theory of "master reaction", the disappearance of the feed substrate, and therefore the appearance of a product, is controlled by a rate limiting step, which for cellulose mineralization could be hydrolysis, acetogenic conversion of higher fatty acids, methane generation from acetate or some other unidentified reaction step. The results of MCCs for propionic, butyric acids did not show a significant difference with that of acetate, oppositely a big gap with cellulose. This observation suggests that neither acetogenic conversion of higher fatty acids nor methanogenic was the rate limiting step; instead, hydrolysis could be the step that governs the overall process for cellulose conversion to methane in this reactor.

SUMMARY AND CONCLUSIONS

This research demonstrates that the anaerobic acid-methane two-stage digestion had considerable potential for the mineralization of high-strength simulated solid/sludge waste, particulate cellulose. The influent COD, HRT and volumetric organic loading rates for the first- and the second-stage reactors often affect the performance of a two-stage digestion. In this study, eight experiments were performed to investigate the influence of HRT and influent concentration on the two-stage system degrading cellulose. Vial tests were also conducted to evaluate the methane converting capacity by microorganisms developed in the first- and the second- stage reactors. Response surface methodology was introduced to systematically analyze the hydrolysis of the first-stage reactor. Our results showed that:

- The first-stage acidogenic reactor mainly converted the particulate cellulose into soluble organics (*e.g.*, sugars and VFAs). According to response surface plots, both chosen values of HRT and

feed cellulose concentration were important for the first-stage reactor hydrolysing cellulose to soluble COD. The soluble COD concentration in this reactor ranged from 3,611 to 13,355 mg/L.

- The second-stage methanogenic reactor played a more important role in polishing the residual substrates from the first-stage reactor while the system organic loading rate increased because it shared in the hydrolysis of cellulose. The organic loading rate of the second-stage reactor increased from 548 to 4,387 g COD/L·d with an increase in the methane production rate from 84 to 970 mL CH_4/L·d, while a COD to methane yield of 0.235 L CH_4/g COD (R2 = 0.95) was obtained.
- MCC values resulted from the second-stage reactor suggested that neither acetogenic conversion of higher fatty acids nor methanogenesis was the rate limiting step; instead, hydrolysis had been the step that governed the overall process for cellulose conversion to methane in this reactor.

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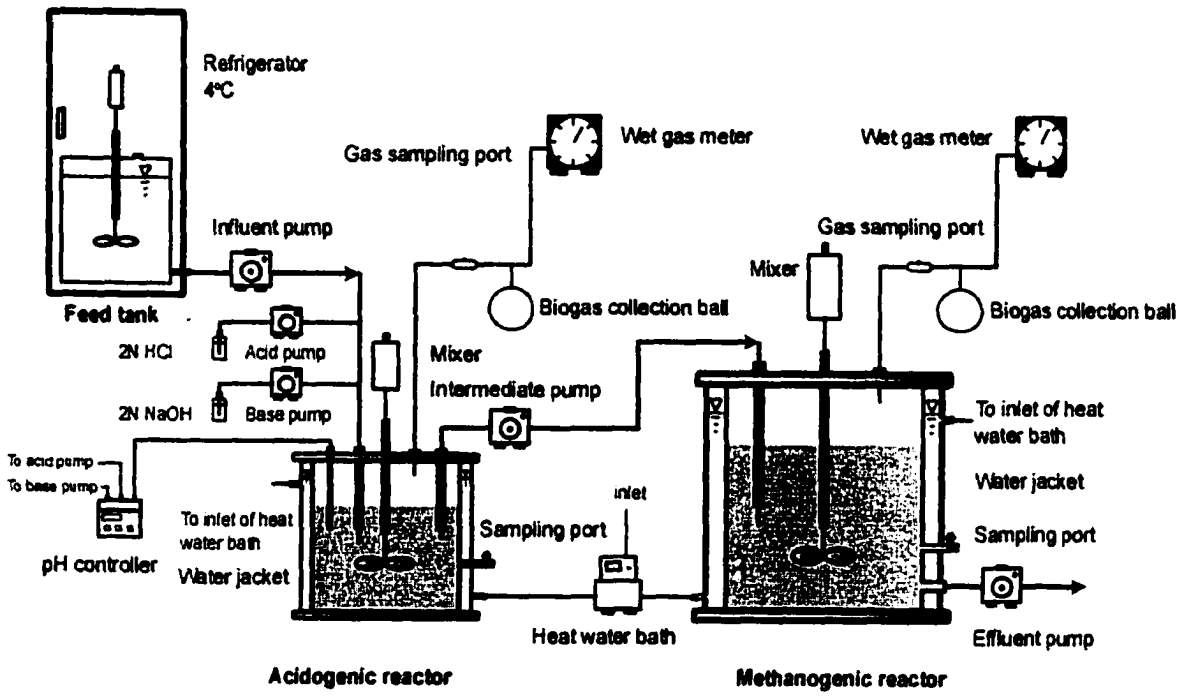

Figure 1. A schematic diagram of the chemostat acid-methane anaerobic digestion system

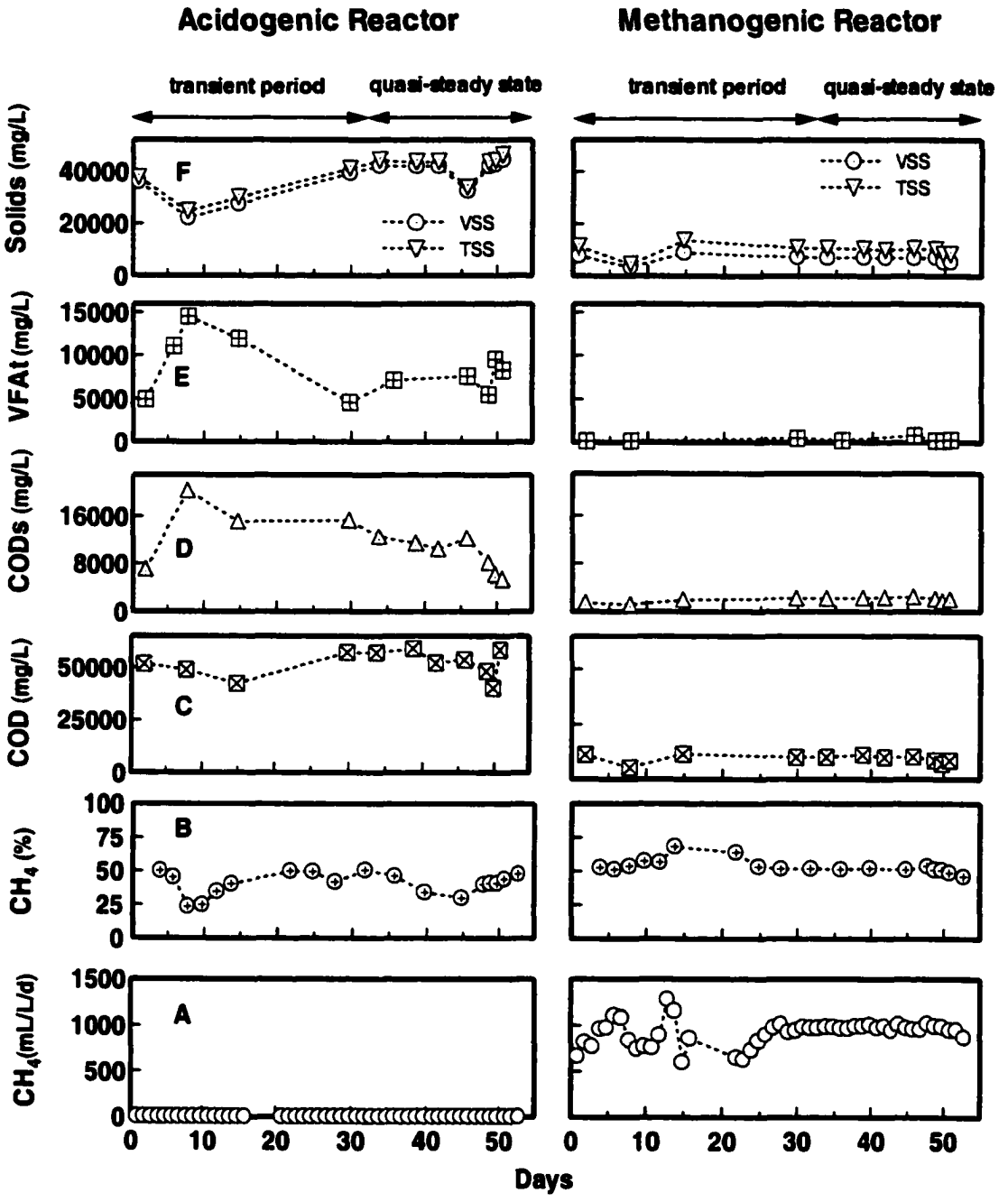

Figure 2. Performance of the acidogenic and the methanogenic reactors as a function of time in Run 8

(A) daily methane production (B) methane content in biogas (C) COD (D) soluble COD (E) total volatile fatty acids (F) suspended and volatile suspended solids

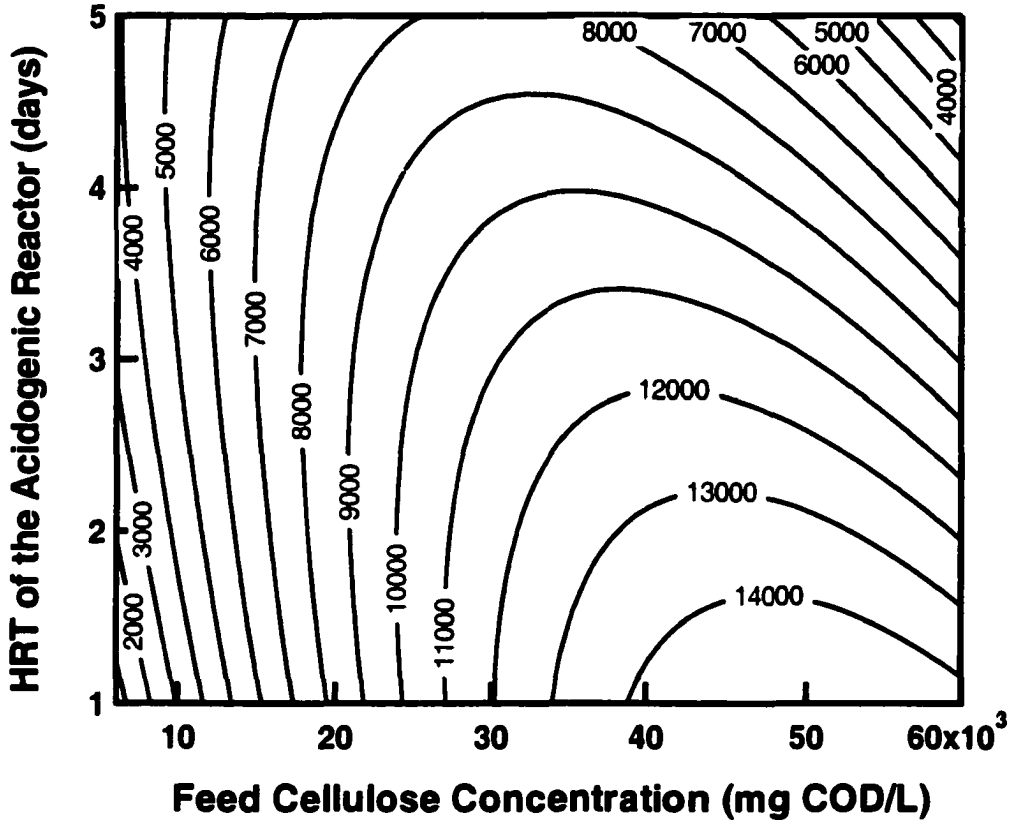

Figure 3. Contour lines of the soluble COD concentration (mg/L) versus feed cellulose concentration and HRT of the acidogenic reactor. Each contour line was estimated using Eq. (2)

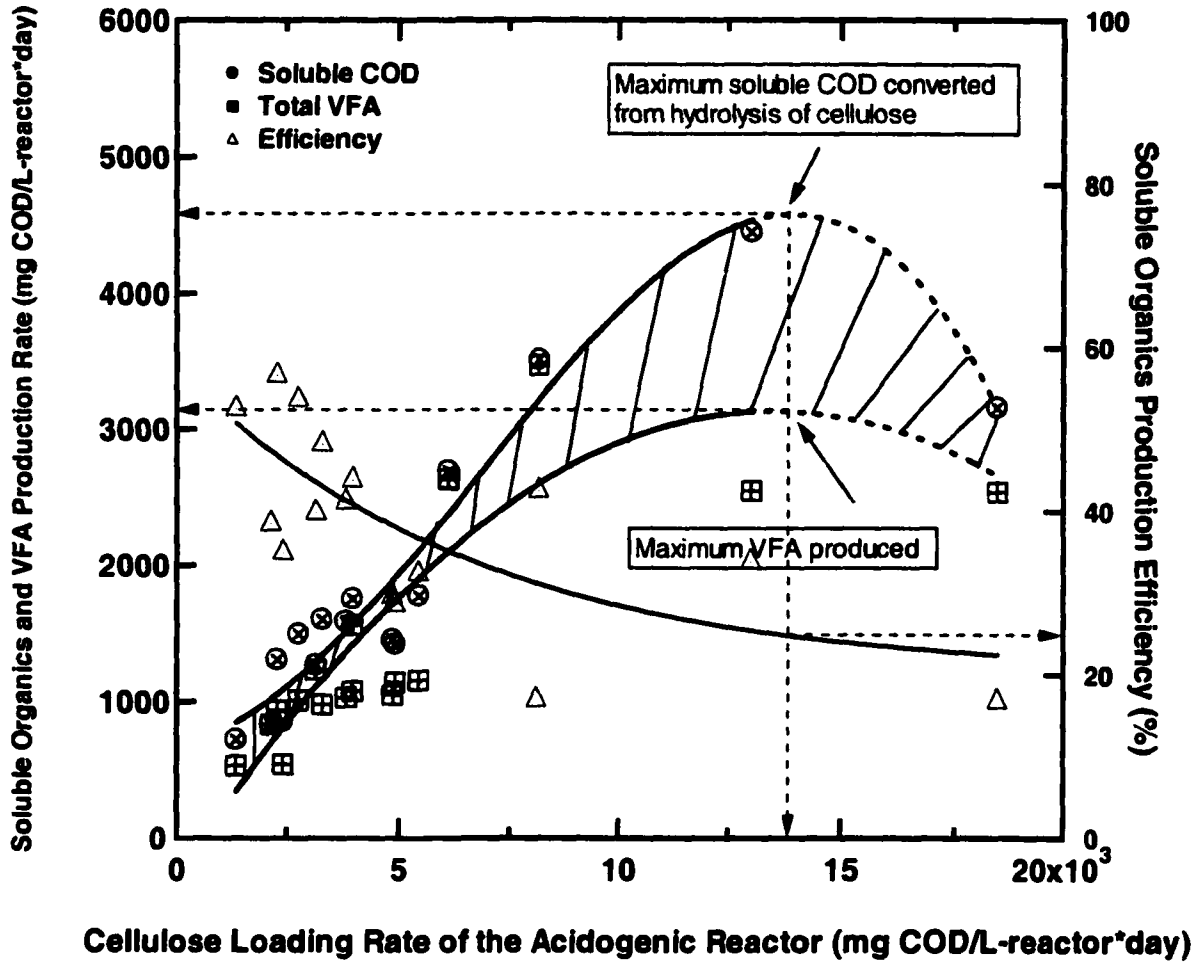

Figure 4. Relationships between soluble organics and VFA production rates, and efficiency of soluble organics production in the acidogenic reactor

(Soluble organics production rate was obtained by dividing the SCOD concentration by HRT of the acidogenic reactor, soluble organics production efficiency was the percentage of the SCOD concentration in the feed cellulose COD concentration)

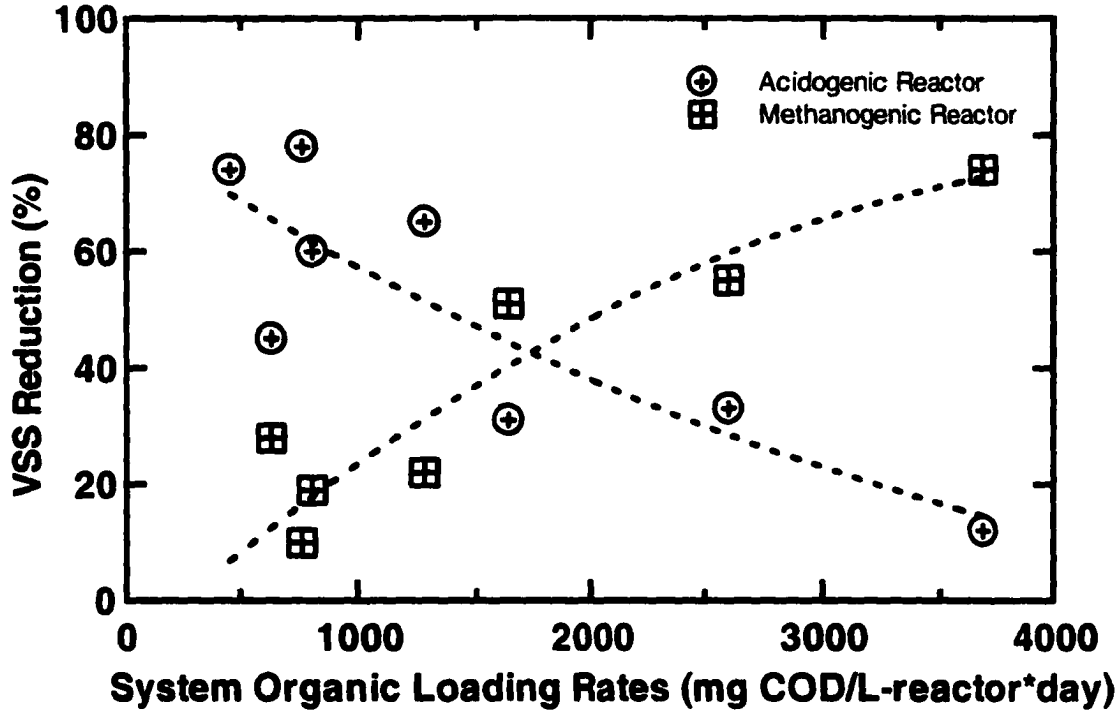

Figure 5. Volatile suspended solids destruction in the acidogenic and methanogenic reactors against system cellulose loading rates

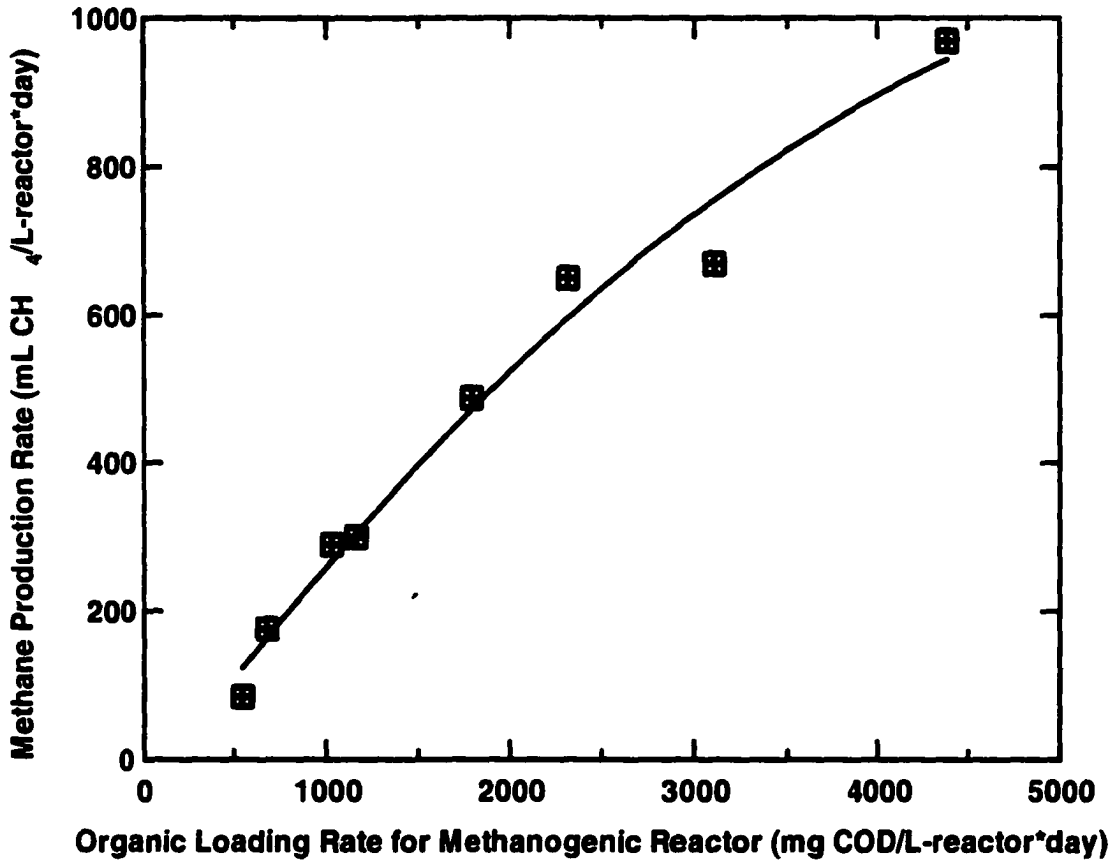

Figure 6. Relationship between methane production rate and organic loading rate of the methanogenic reactor

Table 1. Operating conditions of feed cellulose and HRT for the acid-methane two-stage process

Runs	*Cellulose Con. in Feed (mg COD/L)	HRT/SRT (days)	
		Acidogenic Reactor	Methanogenic Reactor
Run 1	25,000	10	20
Run 2	7,500	5	10
Run 3	15,000	5	10
Run 4	20,000	5	10
Run 5	25,000	5	10
Run 6	15,000	3	20
Run 7	40,000	3	12
Run 8	60,000	3	12

* the feed was made based on 1g cellulose = 1.19 g COD

Table 2. Measured and calculated parameters (± standard deviation) of the acid-methane system under quasi-steady state for each run

Runs		COD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	VFA (mg/L)	VSS (mg/L)	Biogas (%)		OLR (mgCOD/L-reactor/d)		CH_4 Production Rate (mL/L-reactor/d)		COD Reduction (%)
							CH_4	CO_2					
	Feed	24267		24267		20392			*Acid	2427	ND		4
Run 1	*Acid	23188	8561	14627	5415	8066			*Methane	1159	300	± 12	80
	*Methane	4628	1236	3392	329	4133	75	25	*System	809	200	± 8	81±0
	Feed	6833		6833		5742			*Acid*	1367	56	± 31	20
Run 2	*Acid*	5480	3611	1869	2661	1469	55	31	*Methane*	548	84	± 26	51
	Methane	2699	1118	1581	NA	1514	81	20	*System*	456	71	± 19	61±10
	Feed	11479		11479		9646			*Acid*	2296	44	± 16	10
Run 3	*Acid*	10303	6537	3766	4681	2164	40	58	*Methane*	1030	290	± 11	81
	Methane	1957	445	1512	66	1153	69	30	*System*	765	208	± 10	83±1
	Feed	19170		19170		16109			*Acid*	3834	ND		7
Run 4	*Acid*	17920	7952	9968	5164	5582	41	59	*Methane*	1792	488	± 8	79
	Methane	3762	1680	2082	1393	2007	49	51	*System*	1278	325	± 6	80±1
	Feed	24596		24596		20669			*Acid*	4919	ND		6
Run 5	*Acid*	23161	7116	16045	5719	14316	23	56	*Methane*	2316	650	± 14	72
	Methane	6426	4087	2339	2314	3687	51	48	*System*	1640	433	± 9	74±1
	Feed	14588		14588		12259			*Acid*	4863	ND		6
Run 6	*Acid*	13648	4363	9284	3154	6792	37	53	*Methane*	682	176	± 18	82
	Methane	2462	987	1475	227	1752	76	23	*System*	634	153	± 15	83±1
	Feed	39074		39074		32835			*Acid*	13025	13	± 7	5
Run 7	*Acid*	37270	13355	23915	7649	21940	6	84	*Methane*	3106	669	± 20	85
	Methane	5583	718	4866	887	3980	55	44	*System*	2605	538	± 16	86±3
	Feed	55379		55379		46540			*Acid*	18460	ND		5
Run 8	*Acid*	52648	9475	43173	7619	41120	41	45	*Methane*	4387	970	± 31	82
	Methane	9277	2122	7155	313	6460	51	49	*System*	3692	776	± 25	83±2

*Acid = acidogenic reactor, Methane = methanogenic reactor, System = acid-methane system NA: not available; ND not detectable

CHAPTER 4. CELLULOSE MINERALIZATION IN A THERMO-MESO TWO-STAGE ANAEROBIC DIGESTION SYSTEM

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ABSTRACT

A thermo-meso two-stage process, also referred as temperature-phased anaerobic digestion system (TPAD), was operated with a set of feed cellulose concentrations (7.5 to 60 g COD/L) and hydraulic retention times (HRTs, 13 to 30 days) to evaluate their influences on the system performance. The experimental results demonstrated that methane production of the thermophilic reactor were from 337 to 3,354 mL/L·day and of the mesophilic reactor were from 46 to 437 mL/L·day. This suggested that the anaerobic mineralization of cellulose by the thermo-meso process was dominated by the first-stage thermophilic reactor, whereas the second-stage mesophilic reactor played a role in polishing the residual organic matters and volatile acid. The results obtained reveal that organic loading rate (OLR) of 18.3 g cellulose-chemical oxygen demand (COD)/L reactor per day in conjunction with 3-day HRT and 60 g COD/L feed cellulose concentration was a threshold loading limit for the first-stage thermophilic reactor. Seven vial tests were carried out on the mixed liquor from the thermo-meso process under quasi-steady state conditions of each run to measure the methane converting capacity (MCC) of the microorganisms retained in the system. Acetate, propionate, iso-butyrate, and cellulose each served as the substrate for the vial tests. The MCCs of the bacteria from the thermophilic reactor consuming acetate, iso-butyrate, and cellulose were apparently greater than those of the microorganisms retained in the mesophilic reactor. No significant stimulation of the mesophilic maximum methane production rate was obtained when the mesophilic sludge was supplemented with thermophilic sludge.

INTRODUCTION

Due to economic growth, sewage sludge production has increased yearly for both developed and developing countries (Eighmy and Kosson, 1996; Sparling *et al.*, 1997). This increase has led to sewage sludge being considered more of a resource such as for energy production, than as waste (Kosaric and Velayudhan, 1991). In sewage sludge, cellulose is a major component. It also contributes a large proportion of municipal and agricultual wastes due to its abundance on earth (Weimer and Zeikus, 1977; Haug, 1993). The conversion of cellulose to methane by microbial fermentation, therefore, represents a partial solution to organic waste accumulation and depletion of hydrocarbon fuel reserves (Zeikus, 1980).

Anaerobic digestion has been used for stabilizing municipal wastewater treatment plant sludges with simultaneous energy production, but the stabilization is typically archived by a conventional single-stage low-solids (2 to 5% by weight) anaerobic digestion process operating at mesophilic (25 – 40 °C) temperatures (Lay *et al.*, 1998a). Several advantages of anaerobic waste digestion at thermophilic temperatures over mesophilic ones have been reported, such as higher digestion rate, and improved solids settling and sludge dewaterability (Fisher and Greene, 1945; Hashimoto, 1982; Varel *et al.*, 1980). Furthermore, destruction of pathogens is more efficient at higher temperatures (Varel *et al.*, 1980). However, Buhr and Andrews (1977) stated that the thermophilic process has poor process stability and effluent quality (Speece, 1996). While acid-methane two-stage anaerobic digestion (Ghosh, 1984) and semi-dry digestion (Mata-Alvarez, 1993) have improved the efficiency of high-solids anaerobic digestion, however, pathogen destruction is more difficult to accomplish by these processes due to the mesophilic temperatures employed. Dry digestion (Wujcik and Jewell, 1980) needs a relative long hydraulic retention time (HRT) to mineralize organic wastes. The thermo-meso two-stage process (TPAD), consists a thermophilic first stage digster and a mesophilic second stage unit, has all the benefits of thermophilic digestion, *i.e.*, higher volatile solids and nearly complete pathogen destructions and a shorter retention time required, but eliminates its disadvantages (Kaiser *et al.*, 1995; Han and Dague, 1997). It has considerable potential in defining the future of high-solid wastes decomposition.

During anaerobic digestion, organic matter is converted to methane and carbon dioxide by a series of interrelated microbial metabolisms, including hydrolysis/fermentation, acetogenesis, and methanogenesis. The hydrolytic and fermentative bacteria convert organic polymers such as proteins, lipids and carbohydrates to amino acids, long-chain fatty acids and sugars, respectively. These are then fermented to carboxylic acids, alcohols, carbon dioxide, and hydrogen. For a thermo-meso process degrading high-solids, HRT, influent substrate concentration and organic loading rate have been considered to be the important operational factors affecting the efficiency of digesters (Han, *et al.*, 1997; Schmit, 1998). The HRT of a chemostat reactor is extremely important because it has an inverse relation with bacterial growth rate. Moreover, substrate strength and loadings corresponding to the ratio of food-to-microorganisms is one of the major factors affecting microorganisms' metabolisms and kinetic characteristics because the chosen value of this ratio will influence their culture history (Grady *et al.*, 1999).

Designing a high-efficiency thermo-meso process has been hampered by the lack of information for adequate process control by optimization with controlled variables. However, overall process enhancement must be based on an understanding of optimal operation of first-stage thermophilic reactor because it plays a primary role in reducing high-strength substrate (Harris and Dague, 1993). Therefore, the objective of this study was to investigate the influence of HRT, feed cellulose concentration and loading rate on the performance of the thermophilic reactor as well as the thermo-meso process as a whole. For this purpose, a set of HRTs and cellulose concentrations were employed in planning the experimental runs for learning their effects on the first-stage reactor. Moreover, predictive polynomial quadratic equation and response surface methodology were employed to provide a straight forward data analysis for this study (Box *et al.*, 1978). Methane converting capacity (MCC) vial tests using acetate, propionate, iso-butyrate, and cellulose as substrates were also conducted to evaluate the maximum methane production rates by the microorganisms retained in the thermophilic and mesophilic reactors respectively.

METHODS AND MATERIALS

Experimental Setup and Procedure

Thermo-meso Two-stage Process (TPAD)

The experimental setup of the thermo-meso two-stage process is shown in Fig. 1. This process is composed of a completely mixed thermophilic reactor and a following mesophilic reactor. The liquid mixing were carried out with the aid of mechanical mixers. The first-stage reactor was a 4.7-liter plexiglass rectangular tank with 6 inch long (L), 6 inch wide (W) and 8 inch high (H). Its temperature was kept at 55 °C using a heat-water bath. The main mesophilic reactor compartment was an 8"L×8"W×10"H rectangular tank with a total volume of 10.5 liter, while a heat-water jacket was employed to maintain its temperature at 35 °C. The amount of biogas produced from thermo-meso process was recorded daily with two wet gas meters (Precision Scientific). For each reactor, a biogas sampling port was installed between the gas meter and the reactor to allow a direct biogas sampling with a syringe. The headspace pressure of the reactor while decanting was equalized by utilizing an inflatable biogas collection ball. Masterflex positive displacement pumps controlled by timers (Chrontrd) provided semi-continuous (10 times a day) influent and effluent flows for the thermophilic and the mesophilic reactor in order to adjust to its appropriate HRTs as listed in Table 1. The system was fed with a nutrient mineral medium containing particulate cellulose (Sigma, 20μm, S3504) as the sole carbon and energy source with concentrations varied from 7.5 to 60 gCOD/L. The nutrient and mineral media was modified slightly from Chyi and Dague (1994), and each liter of the nutrient contains 345 g of $(NH_4)_2HPO_4$, 10.68 g of $FeCl_2{\cdot}4H_2O$, 625 mg of $ZnCl_2$, 1.215 g of $NiCl_2{\cdot}6H_2O$, 1.212 g of $CoCl_2{\cdot}6H_2O$, 1.081 g of $MnCl_2{\cdot}4H_2O$, 100 mg of $CuSO_4{\cdot}5H_2O$, 100 mg of $AlK(SO_4)_2{\cdot}12H_2O$, 61 g of $CaCl_2{\cdot}2H_2O$, 120 g of $MgSO_4{\cdot}7H_2O$, 100 mg of pyridoxine, 50 mg of thiamine-HCl, 50 mg of riboflavin, 50 mg of nicotinic acid, 50 mg of lipoic acid, 20 mg of biotin and 5 mg of B_{12}. In order to have an optimal methanogenesis rate, appropriate dosage of sodium bicarbonate (2.4 - 18 g/L) was added to the feed to maintain a neutral pH environment for the process. The thermophilic reactor was seeded with a thermophilic sludge taken from a thermo-meso process treating the mixture of

primary sewage sludge and waste activated sludge (Han and Dague, 1997). The mesophilic reactor was inoculated with digested sludge obtained from the Water Pollution Control Plant in Ames, Iowa. To avoid carry over effects from the previous run and ensure random sampling results, withdrawing the acclimated-sludge and re-inoculating with the seed sludges were employed of the start-up process for each individual run in this study. The process was registered as the quasi-steady state after a period of more than two to three mesophilic HRTs, while the averages of chemical oxygen demand (COD), methane production rate as well as other parameters were registered as steady-state values.

The response surface methodology (Box *et al.*, 1978) was used to facilitate straightforward examinations of the dependence of methane production rate on the HRT and feed cellulose concentration.

Methane Converting Capacity (MCC) Test

The rate of methanogenesis, usually incubated in a serum vial, can be readily measured and the effect of organic matter decomposition on this rate can be assessed. The potential of the methanogens in question to degrade an added substrate or their precursors can be determined (Shelton and Tiedie, 1984). In this study, MCC tests were performed with 250-mL-vials for both thermophilic and mesophilic inocula at their corresponding temperatures, 55 and 35 °C. Thirty milliliters of mix liquors were individually withdrawn from the thermophilic and mesophilic reactors serving as the initial inocula when the thermo-meso process proceeded at steady-state conditions. Each of acetate, propionate, iso-butyrate, and cellulose was supplemented as the substrate at an appropriate concentration to determine the MCC of the samples. The MCC test procedure used was a slightly modified version of that employed by Owen *et al.* (1979). Nutrient and mineral solutions for the test were the same with those used in the thermo-meso process. For each vial, after displacing the head space residual air with N_2 gas, it was tightly capped and incubated at 55 °C or 35 °C orbital shakers depending on the thermophilic or mesophilic samples. Accumulated biogas production was then measured.

The maximum specific methane production rate ($mL/L_{mix\text{-}liquor}\cdot d$) in each vial experiment using each specific substrate was defined as the MCC consuming the corresponding substrate. To evaluate the MCC, the modified Gompertz equation (Lay *et al.*, 1998b, refers to Eq. (1)) was used to fit the experimental data from each vial experiment. Subsequently, the MCC was obtained by dividing the maximum methane production rate (R_m, mL/d) by 0.03 L, the volume of initial inocula.

$$M = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \qquad \text{.....(1)}$$

where M is the cumulative methane production (mL), t is the incubation time (day), λ is the lag-phase time, P is the methane production potential (mL), and e is the base of natural logarithm.

Data Analysis

The parameters in Eq. (1) were estimated using the "solver" function in Microsoft Excel 97 (Microsoft, Inc., 1995 - 1997). This program uses a Newton algorithm. Up to a hundred iterations were used to converge the sum of square error (SSE) between the experiment and the estimation to a minimum. Starting parameter values were estimated using a built-in visual procedure based on a limited fit algorithm (Wen *et al.*, 1994; Lay *et al.*, 1998b). Among the statistics reported by Wen *et al.* (1994), sum of square error (SSE) and correlation coefficient (r^2) were used to evaluate a fit. Additionally, Window software of Statistica (StatSoft, 1999) and Igor Pro version 3.12 (WaveMetrics, Inc., 1996) were employed for building-up a quadratic model (refers to Eq. (2)) using multiple regression and response surface contour plots, respectively.

Analysis

Suspended solid (SS), volatile suspended solid (VSS), alkalinity, chemical oxygen demand (COD), and titrimetric volatile fatty acid (VFA) were measured according to American Public Health Association (1995). Soluble COD was measured after filtering the sample through a 0.45 μm filter paper by gravity. Methane and carbon dioxide in biogas were separated using a gas chromatograph (Gow-Mac series 350) equipped with a thermal conductivity detector (TCD) and a 1-m stainless column packed with Porapak T (60/80 mesh). The operational temperatures of the injection port, the oven and the detector were maintained at 100, 50, and 100 ^{0}C, respectively. Helium was used as the

carrier gas at a flow rate of 35 mL/min. The pH of the samples was determined using a calibrated pH meter.

RESULTS AND DISCUSSIONS

Performance of the Thermo-meso System

Experimental results of the thermo-meso process, supplied with 7.5 – 60 g/L of cellulose under various HRTs (13 – 30 days) are presented typically in Fig. 2 (Run 1), including methane percentage and production rate, COD concentration, VFA, VS, VSS, pH, and alkalinity. According to the day-to-day performance variations in these values, the culture history can be divided into transient period and quasi-steady state, which are outlined as follows.

At the beginning operation (transient period, days 0 – 80) in Run 1, methane production and organics (*i.e.*, COD, VFA and volatile solids) of thermophilic reactor showed unstable although methane percentage, pH and alkalinity were maintained at approximately 50%, 7.0, 3000 mg/L, respectively. This was due to the fact that bacteria were transferred from one operational condition to another (here, from start-up condition to this Run 1's operating condition) , the cultures typically need an adapting period. During this period, the reactor washed-out some microorganisms and selected appropriate bacteria to take the advantage of their new environment and began multiplying (Lay *et al.*, 1998a and Grady, 1999). These findings have been claimed for a continuous-mixed reactor while it reaches a quasi-steady state after a period of more than three HRTs (van Hanndel and Lettinga, 1994). Notwithstanding the previous statement, for the mesophilic reactor, the influent quantity/quality (*i.e.*, the effluent of the thermophilic reactor) is still an important factor affecting its stability. As shown in Fig. 2, similar development trends for the methane production and the organics were found between the thermophilic reactor and mesophilic reactor. This phenomenon was repeated in other runs throughout this study (figures not shown).

During Run 1's quasi-steady state (days 81 –102), as shown in Fig. 2, no significant changes in pH, alkalinity, VFA, COD, and methane production were found in two reactors, and the average

performance data of the formers, including OLR, soluble COD, volatile suspended solids and COD reduction are summarized in Table 2. An examination of Table 2 shows that the particulate COD (PCOD) and VSS destructions in the thermophilic reactor were clearly greater than those in the mesophilic reactor. It is obvious that the thermophilic reactor possessed a high capacity on the hydrolysis/fermentation of the cellulose. The methane production rate in the thermophilic reactor was remarkably greater than that in the mesophilic reactor, though the average methane composition in the biogas of the thermophilic reactor (46 ± 4%) was lower than that of the mesophilic reactor (64 ± 7%). Such an experimental result demonstrated that the thermophilic reactor in thermo-meso process not only transferred the particle cellulose into soluble organics (*e.g.*, VFA), but also converted them considerably into methane. This was the reason why the pH value in the thermophilic reactor was maintained in the neutral range for this study as well as our earlier researches (Han, *et al.*, 1997; Schmit, 1998). From Table 2, it is interesting to note that 0 to 31% of the PCOD was hydrolyzed in the mesophilic reactor with simultaneous methane production, thus ensuring that the mesophilic reactor shared in the cellulose hydrolysis/fermentation, as well as converting VFA into methane. This was also evidenced partially by the fact that 10 to 35% COD reduction was attained in the mesophilic reactors. Again, the results in Table 2 indicated that hydrolysis/fermentation and methanogenesis occurred simultaneously both in the thermophilic and the mesophilic reactors. When comparing the amount of COD, VSS destruction and methane production rate of each reactor, the experimental results obtained imply that the thermophilic reactor mineralized high-strength cellulose and the mesophilic reactor provided a buffer capacity for polishing the residual organic matter and volatile acids produced in the former. Therefore, the mesophilic reactor's responses, such as COD reduction, methane production, and bacterial activity, depended highly upon whether or not the thermophilic reactor was controlled optimally.

Effects of OLR on Methane Production in the Thermophilic and Mesophilic Reactors

To learn the capability of thermophilic and mesophilic reactors converting organics to methane in the thermo-meso process, the volumetric methane production rates, were plotted against

the OLRs in Figure 3. An examination of Fig. 3 illustrates that the OLR increased from 1.4 to 18.3 g COD/L·d with a methane production increase from 337 to 3,354 mL CH_4/L·d in the thermophilic reactor. The COD to methane yield was 0.204 L-CH_4/g-COD fed to the reactor (R^2 = 0.95). It is interesting to point out that according to author's laboratory operation experience, when the OLR of the thermophilic reactor was operating at 18.3 g COD/L·d, its methane production was not stable, as indicated by a high standard deviation value shown in Table 2. Therefore, it is hypothesized that an OLR of 18.3 g cellulose-COD/L·d may be the threshold loading limit for the thermophilic sludge to generate methane. Compare the methane production in the mesophilic reactor (46 - 437 mL CH_4/L·d) with that in the thermophilic reactor (337 to 3,354 mL CH_4/L·d, Fig. 3), the value of the mesophilic reactor was significantly lower than that of the thermophilic unit. This phenomenon was not surprising because the OLR of mesophilic reactor was significantly lower than that of the thermophilic reactor. This fact was due to the mesophilic reactor only consuming the organics from the effluent of the thermophilic reactor. As indicated in Fig. 3, the mesophilic methane production increased from 46 to 437 mL CH_4/L·d with an OLR increase from 0.26 to 2.3 g COD/L·d, and a methane yield of 0.151 L-CH_4/g-COD fed to the reactor (R^2 = 0.88) was obtained. The OLR value for this reactor was significantly lower than a mesophilic anaerobic digester treating sewage sludge (Kayhanian and Rich, 1996). With such a low OLR means that the capacity of mesophilic reactor was little used with the HRTs studied. Since the mesophilic reactor received mixed liquor composing more than 40% readily degraded soluble substrate, and mean while, continuous active biomass from the thermophilic reactor, these could suggest when designing or operating a thermo-meso process, a shorter mesophilic HRT could be a better choice for reducing its volume and increase methane generation.

Effects of Feed Cellulose Concentration and HRT on the Thermophilic Reactor

As observed here, methane produced by thermo-meso process was mainly from the thermophilic reactor. The ability of thermophilic bacterial activity dominated the efficiency of thermo-meso process. Four further experiments (Run 10 – 13) were performed to evaluate clearly the effects of cellulose concentration and HRT on thermophilic methane production. The data in Table 2 (Run 1

– 13) was used to construct a series constant relative methane production rate (percentage of the maximum methane production rate obtained, which was 3,354 mL CH_4/L·d) using Eq. (2).

Relative methane production rate(%)

$$= 36.5 + 1.8\times10^{-3}\chi_1 - 10.4\chi_2 + 4.5\times10^{-9}\chi_1^2 + 0.763\chi_2^2 - 1.5\times10^{-4}\chi_1\chi_2 \quad \text{.....(2)}$$

(degree of freedom = 7; F = 125; R^2 = 0.9889)

Where, x_1 is feed cellulose concentration (mg COD/L) and x_2 is the HRT of thermophilic reactor. The statistics test, F, is defined as *MSR/MSE*, where *MSR* is the mean square of regression, obtained by dividing the sum of squares of regression by the degree of freedom. *MSE* is the mean squares of error from the analysis of variance. If the calculated value of F exceeds that in F table at a specified probability level (*i.e.*, $F(P\text{-}1, \nu, 1\text{-}\alpha)$), then a "statistically significant" regression model is obtained, where ν is the degree of freedom of error and P is number of parameters. $F(P\text{-}1, \nu, 1\text{-}\alpha)$ is the F value at the α probability level. Moreover, since the values of R^2 (0.9889) is close to 1.0, the regression model was considered to be an accurate representation of the experimental data (Ang and Tang, 1975). The magnitudes of regression equation coefficients are used as a basis for judging statistical significance and illustrating the relative effects of linear, quadratic and interaction between the variables.

Consider the fitted equation (Eq. 2) graphed in Fig. 4. The constant relative methane production rate curves have the shape commonly referred to a "ridge" system. According to the model obtained, the trend of the ridge confirmed that both values of cellulose concentration and HRT affected methane generation in the thermophilic reactor and that a negative effect of HRT on the methane production was found in the ($x_1 \times x_2$) interaction. At HRT of 5 days, the relative methane production rate increased from 15 to 83% when the feed cellulose increased from 10 to 60 g COD/L. On the other hand, at a feed cellulose concentration of 40 g COD/L, the relative methane production dropped from 73 to 29% as the HRT increased from 3 to 10 days. Also, the relative methane production increased incrementally from 40 to 100% when the HRT decreased from 6 to 3 days and the feed cellulose increased from 35 to 60 g COD/L. It is interesting to note that the thermophilic

reactor was relatively unstable while the HRT was lower than 3 days and feed cellulose exceeded 60 g COD/L. This means that the values were the HRT and feed substrate limits for thermophilic sludge degrading/mineralizing cellulose at 55 ^{0}C.

Methane Converting Capacity

Because generation and consumption of VFAs occur simultaneously in the thermo-meso process, its VFA concentration change was in little use in examining the capacity of the methanogens to convert the VFAs to methane. The maximum specific methane production rate was, therefore, employed to represent the MCC of the sludges inside the thermophilic and the mesophilic reactors on consuming VFAs and cellulose. Equation (1) was used to fit the experimental data of cumulative methane production curve in each individual vial test. In this study, the specific methane production rate ($mL/L_{mix\text{-}liquor}\cdot d$) for a certain substrate was defined as the MCC of that substrate. The significance of Eq. (1) was estimated using the same statistical approach that has been used for Eq. (2), and all R^2 were larger than 0.97. Table 3 summarizes the average MCC values of Run 2, 4, 5, 6, 8, 12 and 13 obtained from the thermo-meso process under quasi-steady state. The MCC of acetate, iso-butyrate and cellulose determined from the thermophilic bacteria were significantly greater than that from the mesophilic reactor. This result was expected because the activity of thermophilic bacteria was usually greater than that of the bacteria proceeded in mesophilic condition (Harris and Dague, 1993) although the MCC of propionate of the thermophilic was lower than the mesophilic microrganisms. In another side-by-side study (Chapter 3), MCC determined from the second-stage reactor in mesophilic acid-methane two-stage digestion was close to that of the thermo-meso process. It was possible to conclude that although the thermophilic bacteria were transferred to the mesophilic reactor, they could not significantly stimulate mesophilic methane production.

CONCLUSIONS

Thermo-meso process has considerable potential for mineralization of high-strength solid wastes. Its influent COD, loading rate and HRT of the thermophilic and mesophilic reactors often

affect the system performance. However, obtaining an appropriate organization on HRT is complicated by the fact that HRT of the mesophilic reactor is a factor dependent on that of the thermophilic reactor. To study this phenomenon, thirteen experiments were carried out to investigate the influence of HRT and influent concentration on the thermo-meso process degrading cellulose. In this study, response surface methodology was used to systematically analyze data set from the thermo-meso process. Further vial experiments were conducted to evaluate the methane converting capacity of the microorganisms in the thermophilic and mesophilic reactors.

Experimental results showed that the methanogenesis occurred simultaneously in both the thermophilic and mesophilic reactors, while the thermophilic reactor had 7 to 27 times higher methane production rate than the mesophilic reactor. Consequently, the mesophilic reactor played a role on polishing the residual COD, including organic solids and volatile acids. When the OLR increased from 1.4 to 18.3 g COD/L·d, the thermophilic methanogenic activity increased from 337 to 3,354 mL CH_4/L·d. An empirical OLR threshold limit of 18.3 g COD/L·d in conjunction with 3-day HRT and 60 g COD/L of feed cellulose was found for the thermophilic reactor. Based on the relationship between the mesophilic reactor's OLR and its methanogenic activity, reducing the HRT of this reactor to less than 10 days was suggested to enhance the mesophilic reactor efficiency in producing methane.

According to the response surface plots, feed cellulose concentration was an important environmental factor affecting thermophilic methane generation, while an interrelation was obtained between cellulose concentration and HRT. At an HRT of 5 days, the relative thermophilic methane production rate increased from 15 to 83% when the feed cellulose increased from 10 to 60 g COD/L. Additionally, the MCC data indicated that the thermophilic sludge carried over to the mesophilic reactor could not stimulate the mesophilic maximum methane production rate.

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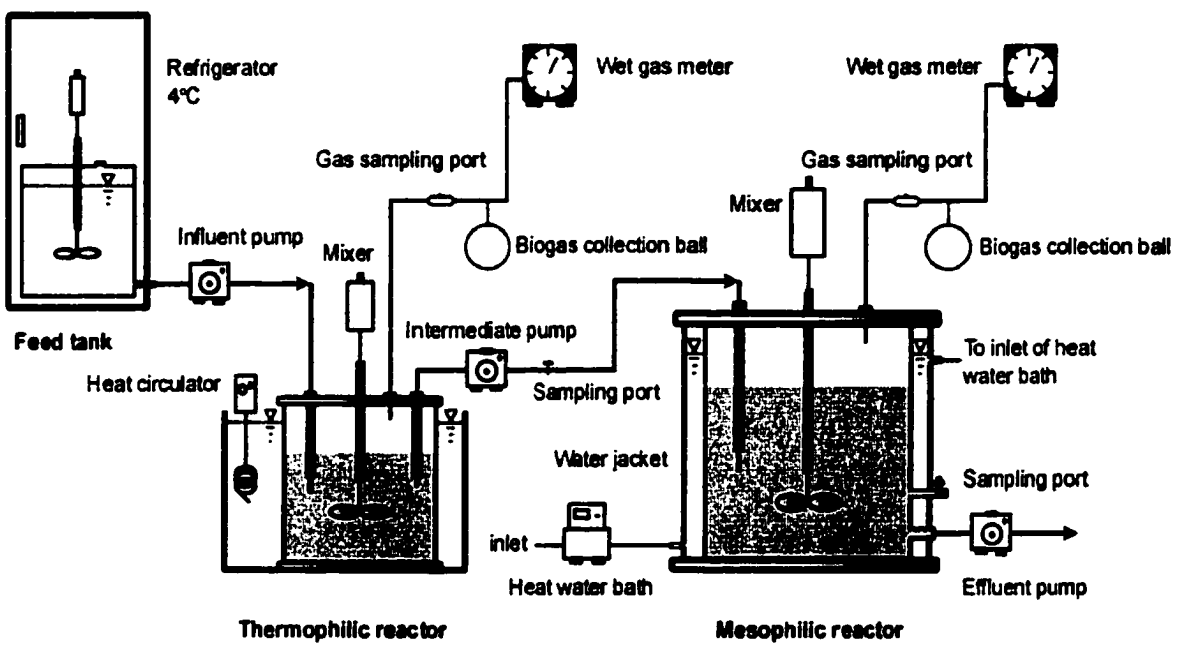

Figure 1. A schematic diagram of the chemostat thermo-meso two-stage anaerobic digestion system

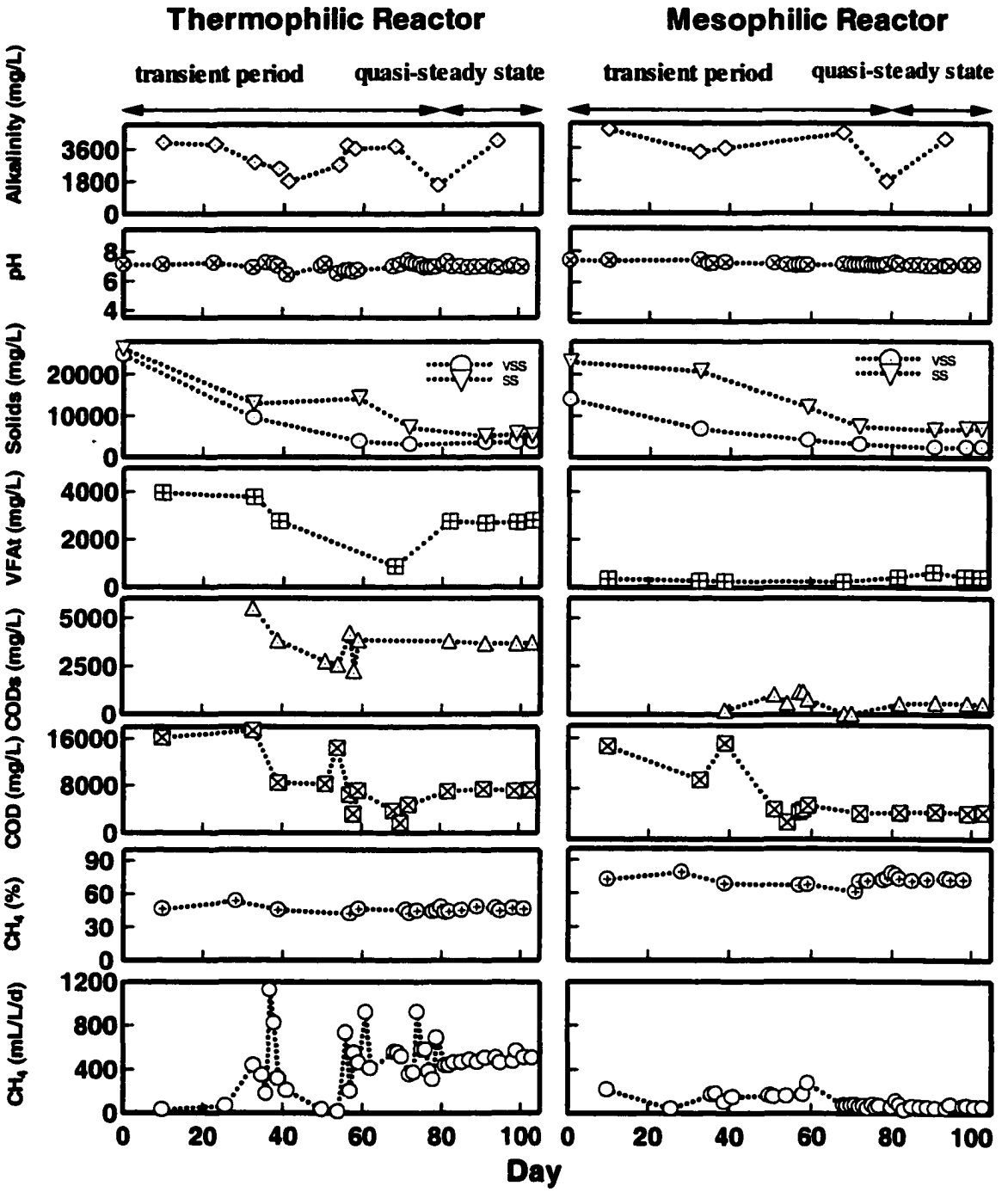

Figure 2. Performance of the thermophilic and the mesophilic reactor as a function of time in Run 1 (A) daily methane production (B) methane content in biogas (C) COD (D) soluble COD (E) total volatile fatty acids (F) suspended and volatile suspended solids (G) pH (H) total alkalinity as calcium carbonate

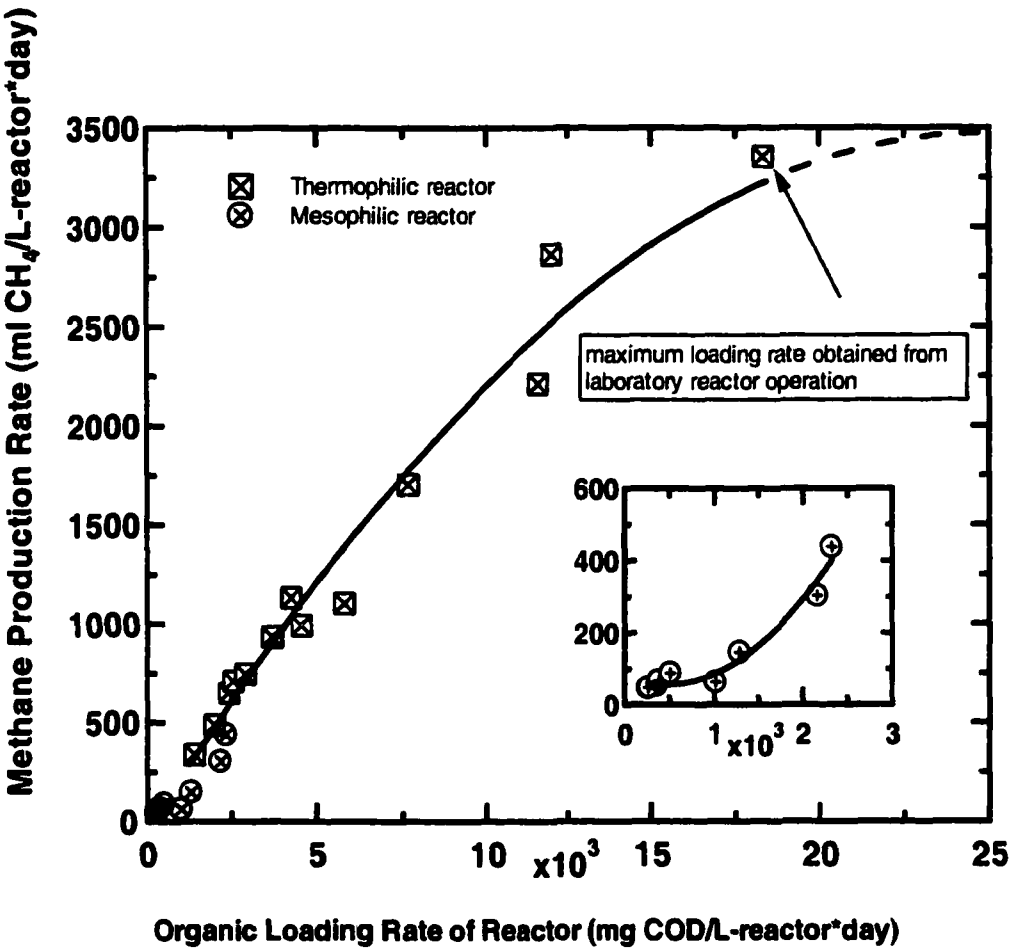

Figure 3. Methane production rate against organic loading rate of the thermophilic and the mesophilic reactors

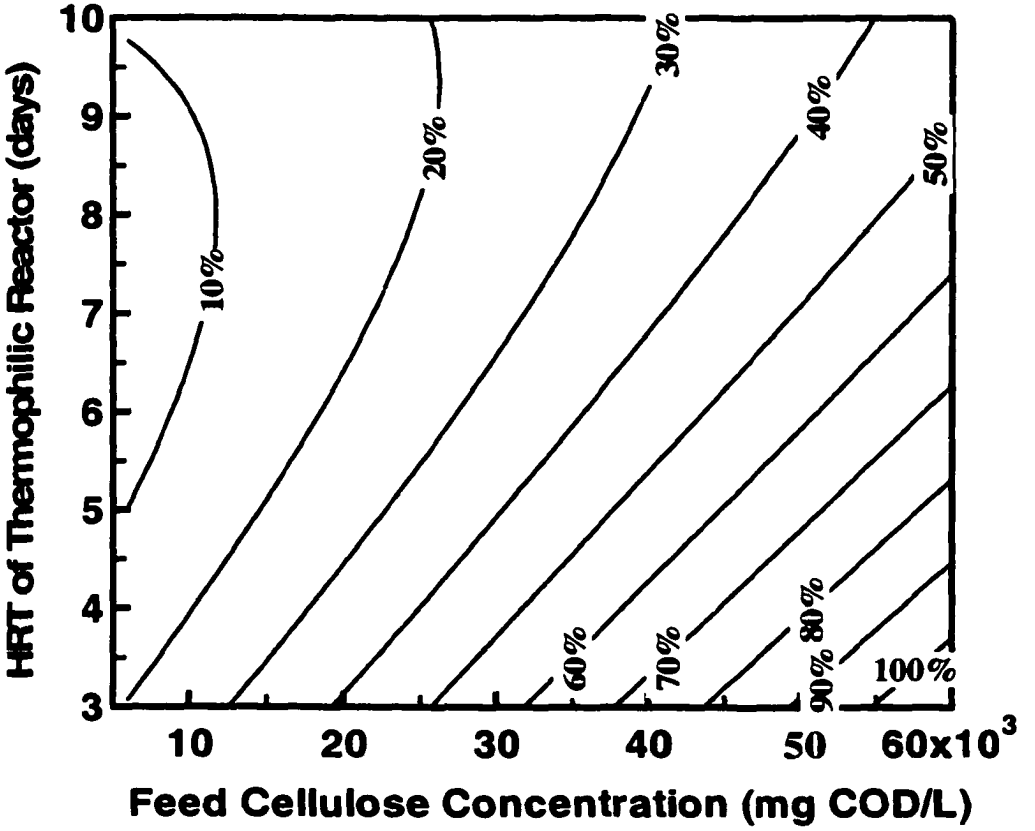

Figure 4. Contour lines of constant relative methane production rate of the thermophilic reactor (%)

(Constant relative methane production rate was obtained using Equation 2)

Table 1. Operating conditions of feed cellulose concentration and HRT for the thermo-meso two-stage process

Runs	*Cellulose Con. in Feed (mg COD/L)	HRT/SRT (days)	
		Thermophilic Reactor	Mesophilic reactor
Run 1	20,000	10	20
Run 2	25,000	10	20
Run 3	7,500	5	10
Run 4	15,000	5	10
Run 5	40,000	5	10
Run 6	60,000	5	10
Run 7	15,000	3	10
Run 8	40,000	3	10
Run 9	60,000	3	10
Run 10	30,000	7.5	**
Run 11	45,000	7.5	**
Run 12	30,000	10	**
Run 13	45,000	10	**

* Feed was made based on 1g cellulose = 1.19 g COD
**Run 10-13 were performed with only the thermophilic reactor

Table 2 Measured and calculated parameters (± standard deviation) of the thermo-meso system under quasi-steady state of each run

Runs		COD (mg/L)	SCOD (mg/L)	PCOD (mg/L)	VFA (mg/L)	VSS (mg/L)	Biogas (%)		OLR (mgCOD/L-reactor/d)		CH_4 Production Rate (ml/L-reactor/d)		COD Reduction (%)
							CH_4	CO_2					
Run 1	*Feed*	19837		19837		16670			*Thermo*	1984	485	± 35	64
	Thermo	7170	3711	3459	2738	3557	46	51	*Meso*	359	54	± 13	42
	Meso	4123	545	3578	466	2397	72	21	*System*	661	198	± 99	79
Run 2	*Feed*	24047		24047		20208			*Thermo*	2405	648	± 28	78
	Thermo	5202	2234	2968	971	3744	48	50	*Meso*	260	46	± 2	46
	Meso	2789	1952	837	NA	3151	72	21	*System*	802	246	± 10	88
Run 3	*Feed*	6894		6894		5792			*Thermo*	1397	337	± 37	61
	Thermo	2707	1034	1673	513	1462	50	47	*Meso*	271	47	± 20	52
	Meso	1305	410	895	172	1280	73	21	*System*	466	148	± 15	81
Run 4	*Feed*	12754		12754		10718			*Thermo*	2551	708	± 63	70
	Thermo	3830	902	2928	130	3241	52	46	*Meso*	383	67	± 33	33
	Meso	2578	266	2312	104	2383	66	30	*System*	850	281	± 28	80
Run 5	*Feed*	38271		38271		32616			*Thermo*	7654	1703	± 48	73
	Thermo	10165	3354	6811	472	6871	47	51	*Meso*	1016	62	± 12	53
	Meso	4733	1330	3403	73	3522	61	57	*System*	2551	609	± 17	88
Run 6	*Feed*	60000		60000		50420			*Thermo*	12000	2861	± 103	64
	Thermo	21642	7452	14190	111	15110	48	51	*Meso*	2164	303	± 17	57
	Meso	9382	1898	7484	77	6946	62	33	*System*	4000	1155	± 34	84
Run 7	*Feed*	13654		13654		11474			*Thermo*	4551	993	± 44	63
	Thermo	5089	1525	3564	891	4235	46	51	*Meso*	509	88	± 14	55
	Meso	2271	306	1965	147	2190	64	34	*System*	1050	297	± 7	83
Run 8	*Feed*	34790		34790		33613			*Thermo*	11597	2210	± 172	63
	Thermo	12844	5479	7365	2426	6698	43	56	*Meso*	1284	147	± 30	43
	Meso	7290	4650	2640	2059	2367	55	42	*System*	2676	623	± 45	79
Run 9	*Feed*	55000		55000		50420			*Thermo*	18333	3354	± 1733	58
	Thermo	23270	6089	17181	5657	11035	38	60	*Meso*	2327	437	± 115	83
	Meso	3892	3495	397	1097	4075	56	42	*System*	4231	1110	± 419	93
*Run 10	*Feed*	27550		27550		23151							
	Thermo	5526	1908	3618	501	2808	48	50	*Thermo*	3673	932	± 40	80
*Run 11	*Feed*	43550		43550		36597							
	Thermo	12223	8001	4222	6615	4486	39	59	*Thermo*	5807	1102	± 355	72
*Run 12	*Feed*	29070		29070		24429							
	Thermo	5920	2166	3754	233	2904	49	49	*Thermo*	2907	745	± 25	80
*Run 13	*Feed*	42495		42495		35710							
	Thermo	9144	4614	4530	2442	3932	49	50	*Thermo*	4249	1131	± 65	78

Thermo = thermophilic reactor; Meso = mesophilic reactor; System = thermo-meso system
*Runs 10 -13 were performed with only the thermophilic reactor

Table 3. Results of the average methane converting capacity (MCC) by microorganisms from the thermophilic and the mesophilic reactor consuming VFAs and cellulose

	MCC (ml CH_4/L-mixed-liquor*day)			
	Acetic Acid	**Propionic Acid**	**Iso-butyric Acid**	**Cellulose**
from Thermophilic Reactor	718 ± 275	183 ± 32	443 ± 204	491 ± 88
from Mesophilic Reactor	525 ± 203	223 ± 64	369 ± 191	277 ± 62

CHAPTER 5. COMPARATIVE PERFORMANCE OF THE TWO-STAGE ANAEROBIC DIGESTION SYSTEMS FOR CELLULOSE MINERALIZATION

A paper to be submitted to Water Environment Research

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ABSTRACT

Feed substrate concentration and hydraulic retention time (HRT) were varied with the aim of delineating similarities and differences between the thermo–meso two-stage and acid-methane two-stage anaerobic digestion system for degradation of synthetic high-strength wastes, particulate cellulose. This research demonstrated the effectiveness of both systems on anaerobic mineralization of particulate cellulose at concentrations up to 60 g COD/L with HRTs of 13 to 30 days. The experimental results showed that in all seven runs, thermo-meso system possessed a higher methane production rate and methane yield than the acid-methane system. The methane production rate and yield of the thermo-meso system was in the range of 148 – 1,110 mL/L_reactor·day and 0.23 – 0.33 L/gCOD_added with the organic loading rates of 466 – 4,000 mgCOD/L_reactor·day. These values obtained with the acid-methane process were 71– 776 mL/L·day and 0.16 – 0.27 L/gCOD when the system loading rates were 456 – 3,692 mgCOD/L·d.

Results from seven continues experimental runs indicated that the first stage thermophilic reactor dominated the methane production in the thermo-meso system. Methane production rate from this reactor was 2 to 4 times higher than that of the methanogenic reactor, which was the primary methane producing reactor in the acid-methane system. Batch methane converting capacity (MCC) test further confirmed that the activities of acetate-utilizing methanogens in the thermophilic reactor was 45 to 50% higher than that of the methanogenic reactor. Microorganisms developed in

the thermophilic reactor also demonstrated 1.3 – 1.9 times higher ability in terms of directly converting cellulose into methane.

Although the acidogenic reactor in acid-methane system was mainly supposed to hydrolyze cellulose, its first-order hydrolysis rate (0.26±0.2 day^{-1}) was much lower than that of the first stage thermophilic reactor (0.79±0.22 day^{-1}) in the thermo-meso system.

A methane generation model regarding to the hydraulic retention time and feed cellulose was established for each system by using predictive polynomial quadratic equation and response surface methodology (Box et al., 1978). With the model obtained, thermo-meso system demonstrates greater methane generation potential for treating high solids with a shorter hydraulic retention time needed than the acid-methane system.

INTRODUCTION

Due to economic growth and human activity, sewage sludge and municipal solid wastes production have increased year-by-year for developed and developing countries (Eighmy and Kosson, 1996; Sparling *et al.*, 1997). The treatment and disposal of these wastes produced have become complicate environmental issues (Li *et al.*, 1996). However, because these wastes are not only biologically degradable, but also a good material for methane generation, anaerobic digestion technology has therefore been widely used as a main process for the stabilization of sludge and the production of biogas (Cecchi and Mata-Alvarez, 1991; Cheremisinoff, 1994). In sewage sludges and municipal solid wastes, cellulose, the most abundant biopolymer on earth, is a major component (Weimer and Zeikus, 1977; Haug, 1993). Therefore, the conversion of cellulose to methane by microbial fermentation represents a partial solution to organic waste accumulation and the depletion of hydrocarbon fuel reserves (Zeikus, 1980).

For anaerobic digestion of wastes, a conventional single stage digester operated at either mesophilic or thermophilic conditions, is a common process employed. Most studies performed at mesophilic conditions reported that a relative long hydraulic retention time (HRT) needed to mineralize organic wastes. Several advantages of anaerobic waste digestion at thermophilic

temperatures have been reported, such as a higher reaction rate, improved sludge dewaterability and pathogens destruction (Fisher and Greene, 1945; Hashimoto, 1982; Varel *et al.*, 1980). However, Buhr and Andrews (1977) stated that a disadvantage of the thermophilic digester is poor process stability due to the difficulty in maintaining a proper population of the acid formers and methane formers in the digester. In order to improve the efficiency and stability of anaerobic digestion, two-stage system, which consists two separate digesters operating in series, has been proposed.

For a two-stage system, pH (*i.e.* acid - neutral) and temperature (*i.e.* thermophilic – mesophilic) are commonly used as the standards for stage separation. Conventional acid-methane process separates acid and methane forming phases based on their different metabolic characteristics and growth rates of these two groups of bacteria, acidogenic and methanogenic organisms. Thus, by using pH or kinetical control, each group of microorganisms will be optimized through this phase separation (Ghosh, 1994). While, thermo-meso two-stage anaerobic digestion systems take the advantages of the thermophilic digestion meanwhile provides a stable system performance by connecting the effluent to a mesophilic digester (Han and Dague 1997). Both digesters in this process are suggested to operate at a neutral environment for methane generation, and thus, syntrophic relationships between different bacterial groups will be maintained.

In thermo-meso systems, in order to maintain a high temperature for the first-stage thermophilic digester, usually an extra energy input is needed. This can be considered as a drawback for this process when compared with the acid-methane system. However, if the increased methane generation could compensate the extra energy requirement, then the thermo-meso system will have an extra benefit of high pathogen destruction, thus the digested sludge can meet EPA 503 CFR for biosolids Class A requirements.

Despite the above mentioned energy requirements and pathogen destructions, the overall system efficiency is an important factor for selecting a two-stage process. The purpose of this research was to compare the performance of thermo-meso two-stage system with the acid-methane system for cellulose mineralization. For a two-stage process degrading high-solids, HRT, influent substrate concentration and digester loading rate have been considered to be the important

operational factors affecting the efficiency of the digester. For this purpose, a set of HRTs and cellulose concentrations were employed in planning the experiments for learning their effects on the hydrolysis/fermentation of cellulose and methanogenic bacterial activities using predictive polynomial quadratic equation and response surface methodology (Box *et al.*, 1978). Methane converting capacity (MCC) vial tests using acetate, propionate, iso-butyrate, and cellulose were then conducted to evaluate the maximum methane production rates of the microorganisms developed in each reactor of a two-stage system.

METHODS AND MATERIALS

Experimental Setup and Procedure

Thermo-meso Process and Acid-methane Processes

The experimental setup of the thermo-meso and acid-methane systems is shown in Fig. 1. The thermo-meso process is composed of a thermophilic and a mesophilic completely-mixed reactor (Fig.1 (A)). The thermophilic reactor was a 4.7-liter plexiglass rectangular tank with 6 inch long (L), 6 inch wide (W) and 8 inch high (H). Its temperature was kept at 55 °C using a heat-water bath. The main mesophilic reactor compartment was an 8"L×8"W×10"H rectangular tank with a total volume of 10.5 liter, while a heat-water jacket was employed to maintain its temperature at 35 °C. Experimental setup of the acid-methane system is shown in Fig. 1 (B). This system is composed of a 4.7-liter acidogenic and a 10.5-liter methanogenic completely mixed reactors with both temperatures maintained at 35 °C by using heat-water jackets. By addition of 2 N HCL or NaOH charged by an automatic pH controller, the pH of the acidogenic reactor was maintained at 5.6±0.2, a reported optimal value for cellulose fermentation (Chyi, 1992).

For each reactor, the liquid mixing were carried out with the aid of mechanical mixers. The amount of biogas produced was recorded daily with wet gas meters (Precision Scientific). A biogas sampling port was installed between the gas meter and the reactor to allow a direct biogas sampling with a syringe. The headspace pressure of the reactor while decanting was equalized using an

inflatable biogas collection ball. Masterflex positive displacement pumps controlled by timers (Chrontrd) provided semi-continuous (10 times a day) influent and effluent flows to each reactor in order to adjust to appropriate HRTs as listed in Table 1.

Each of the acid-methane and thermo-meso system was supplied with a nutrient mineral medium containing particulate cellulose (Sigma, 20μm, S3504) as the sole carbon and energy source with concentrations varied from 7.5 to 60 gCOD/L. The nutrient and mineral media was modified slightly from Chyi and Dague (1994), and each liter of the nutrient contains 345 g of $(NH_4)_2HPO_4$, 10.68 g of $FeCl_2 \cdot 4H_2O$, 625 mg of $ZnCl_2$, 1.215 g of $NiCl_2 \cdot 6H_2O$, 1.212 g of $CoCl_2 \cdot 6H_2O$, 1.081 g of $MnCl_2 \cdot 4H_2O$, 100 mg of $CuSO_4 \cdot 5H_2O$, 100 mg of $AlK(SO_4)_2 \cdot 12H_2O$, 61 g of $CaCl_2 \cdot 2H_2O$, 120 g of $MgSO_4 \cdot 7H_2O$, 100 mg of pyridoxine, 50 mg of thiamine-HCl, 50 mg of riboflavin, 50 mg of nicotinic acid, 50 mg of lipoic acid, 20 mg of biotin and 5 mg of B_{12}. In order to have an optimal methanogenesis rate, appropriate dosage of sodium bicarbonate (2.4 - 18 g/L) was supplemented to the feed for thermo-meso process to maintain a neutral pH environment for the system.

The thermophilic reactor was seeded with a thermophilic sludge taken from a thermo-meso process treating the mixture of primary sewage sludge and waste activated sludge (Han and Dague, 1997). The mesophilic reactor, acid and methane reactor were inoculated with digested sludge obtained from the Water Pollution Control Plant in Ames, Iowa. To avoid any carry over effects from previous runs and ensure random sampling results, withdrawing the acclimated-sludge and re-inoculating with the seed sludges were employed in the start-up process of each individual run in this study. For each run, the process was registered as the quasi-steady state after a period of more than two to three HRTs of the second stage reactor, while the averages of chemical oxygen demand (COD), methane production rate as well as other parameters were registered as steady-state values.

It needs to be pointed out that for each run, an appropriate amount of cellulose was used to make the target feed concentration as listed in Table 1 (Part 1). However, due to the nature of cellulose's insolubility in water and thus easily settling in the feed tubing, the actual feed COD concentration for each system was measured several times during the operation and the average of them was then recorded as the feed concentration and tabulated in Table 1 (Part 1).

The response surface methodology (Box *et al.*, 1978) was used to facilitate straightforward examinations of the dependence of methane production rate on the HRT and feed cellulose concentration.

First-order Hydrolysis Rate

A more comprehensive approach to represent hydrolysis occurred, can be derived by combining the first-order hydrolysis with the mass balance equations for a continues-mix reactor at steady states. The following is the equation used to calculate the first-order hydrolysis rate with respect to the particulate substrate concentration only:

$$K_h = \frac{PCOD_{in} - PCOD_{out}}{PCOD_{out} * HRT} \qquad \text{.....(1)}$$

Where $PCOD_{in}$ and $PCOD_{out}$ are the particulate chemical oxygen demand in the influent and effluent of the reactor (mg/L). It is represented by the difference between the total COD and soluble COD. K_h is the first-order hydrolysis rate (day^{-1}), and HRT is the hydraulic retention time of the reactor (day).

Methane Converting Capacity (MCC) Test

In this study, MCC tests were performed with 250-mL-vials for both systems. Thirty milliliters of mix liquors were individually withdrawn from the reactor interested and served as the initial inocula when the process was proceeded at its steady state conditions. Each of acetate, propionate, iso-butyrate, and cellulose was supplemented as the substrate to determine the MCC of the samples. The MCC test procedure used was a slightly modified version of that employed by Owen *et al.* (1979). Nutrient and mineral solutions for these batch tests were the same with those used for the continues experimental runs as described earlier. For each vial, initial pH was adjusted to 6.85-6.95 by adding sodium bicarbonate. After displacing the head space residual air with N_2 gas, it was tightly capped and incubated at 35 °C for the inocula withdraw from the acidogenic, methanogenic reactor and mesophilic reactor, or a 55 °C orbital shaker for the inocula from the thermophilic reactor. Accumulated biogas production and methane concentration was measured then.

The maximum specific methane production rate ($mL/L_{mix\text{-}liquor}/d$) in each vial test using each specific substrate was defined as the MCC consuming the corresponding substrate. To evaluate the MCC, the modified Gompertz equation (Lay *et al.*, 1998b, refers to Eq. (2)) was used to fit the experimental data (mean of the duplicates) from each vial experiment. Subsequently, the MCC was obtained by dividing the maximum methane production rate (R_m, mL/d) by 0.03 L, the volume of the initial inocula.

$$M = P \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\right\} \qquad \text{.....(2)}$$

where M is the cumulative methane production (mL), t is the incubation time (day), λ is the lag-phase time (day), P is the methane production potential (mL), and e is the base of natural logarithm.

Data Analysis

The parameters of Eq. (2) were estimated using the function of "solver" in Microsoft Excel 97 (Microsoft, Inc., 1995 - 1997). This program uses a Newton algorithm. Up to a hundred iterations were used to converge the sum of square error (SSE) between the experiment and the estimation to a minimum. Starting parameter values were estimated using a built-in visual procedure based on a limited fit algorithm (Wen *et al.*, 1994; Lay *et al.*, 1998b). Among the statistics reported by Wen *et al.* (1994), sum of square error (SSE) and correlation coefficient (r^2) were used to evaluate a fit. Additionally, Window software of Statistica (StatSoft, 1999) and Igor Pro version 3.12 (WaveMetrics, Inc., 1996) were employed for building-up a quadratic model (refers to Eq. (3)) using multiple regression and response surface contour plots, respectively.

Analysis

Suspended solid (SS), volatile suspended solid (VSS), alkalinity, chemical oxygen demand (COD), and titrimetric volatile fatty acid (VFA) were measured according to American Public Health Association (1995). Soluble COD was measured after filtrating the sample through a 0.45 μm filter paper by gravity. pH of the samples was determined using a calibrated pH meter. Methane and carbon dioxide in biogas were separated using a gas chromatograph (Gow-Mac series 350) equipped

with a thermal conductivity detector (TCD) and a 1-m stainless column packed with Porapak T (60/80 mesh). The operational temperatures of the injection port, oven and detector were maintained at 100, 50, and 100 ^{0}C, respectively. Helium was used as the carrier gas at a flow rate of 35 mL/min.

RESULTS

Five experiments runs were conducted to directly compare the hydrolysis and methanization of cellulose in the thermo-meso and acid-methane process under a same substrate concentration and system HRT. Additionally, two more runs, Run 7 and Run 8 were performed in thermo-meso process with a same feed cellulose as that applied for acid-methane process but a shorter system HRT.

Performances of the Thermo-meso and Acid-methane System

The performance of the thermo-meso and acid-methane system, supplied with 40 gCOD/L under 15 days system HRT (Run 4) is presented in Fig. 2 and serves as an example for other runs in this research. In this run, for acid-methane process, acidogenic reactor was operated with a 3-day HRT, while 12 days for the methanogenic reactor. The HRT of the thermophilic reactor was 5 days and 10 days for the mesophilic reactor in thermo-meso system.

Graph A in Fig. 2 shows the methane production rate from each reactor changes with the experimental time after finishing the start-up process for this run. As shown in this figure, methane production from the thermophilic reactor and mesophilic reactor were not stable in the beginning operation although methane content in the biogas were maintained at approximately 46% and 60% (Fig. 2(B)). After running the reactors for about 30 days, a stable methane production of 1,703 and 62 mL/Lreactor·day were obtained from the thermophilic and mesophilic reactors, respectively. Similar development trends of the methane production from the acidogenic and methanogenic reactors in acid-methane process were observed in graph A. According to this day-to-day variations in methane production, the cultural history for each system was divided into transient period and quasi-steady state. At the beginning operation (transient period), the unstable methane production was due to the fact that the cultures typically need an adapting period when the operating condition

was changed. During this period, the reactor washed-out some microorganisms and selected appropriate bacteria to take the advantage of their new environment and begin multiplying (Lay *et al.*, 1998a and Grady, 1999). These findings have been claimed for a continuous-mixed reactor while it reaches a quasi-steady state after a period of more than three HRTs (van Hanndel and Lettinga, 1994). Notwithstanding the previous statement, for a two-stage system, the influent quantity/quality (*i.e.*, the effluent of the first-stage reactor) is still an important factor affecting the stability of the second-stage reactor. When the system achieved and operated in the quasi-steady state, no significant changes in methane production rate were observed for all reactors as shown in Fig. 2 (A).

Figure 2 (C) shows the COD level in each reactor from the operation of both thermo-meso and acid-methane systems. As shown in this graph, for the acid-methane process, COD level in the acidogenic reactor was flocculated around 36 g/L, which is about the same level as that of the feed. However, this high COD level was dramatically reduced to 6 g/L by the second stage methanogenic reactor. Contrary to the acid-methane process, large part of the substrate COD was reduced by the first-stage thermophilic reactor in thermo-meso process. As can be seen in this run, the initial feed COD level was 38.3 g/L, it was reduced to 10.2 g/L after the thermophilic reactor and this level was further decreased to 4.7 g/L by the mesophilic reactor (Fig. 2 (C)). It is not surprising to note that a similar pattern was observed for the volatile suspended solids destruction in the thermo-meso processes as illustrated in Fig. 2 (E). The feed VSS level was reduced to 6.9 g/L and 3.5 g/L by the thermophilic and mesophilic reactor, respectively.

Soluble COD and volatile fatty acids levels in each reactor were monitored as indications for a stable reactor performance because they are important intermediates in anaerobic cellulose degradation. As presented in Fig. 2 (D) and the data in Table 1, both reactors in thermo-meso process maintained a relatively stable SCOD and VFA concentrations at approximately 3,354 mg/L and 472 mg/L, and 1,330 mg/L, 73 mg/L. However, unstable SCOD and VFA concentrations were observed for acid-methane process even during the defined quasi-steady state period in this experimental run.

In order to better represent each experimental run, the average performance data of methane production and methane content in biogas, together with chemical oxygen demand (COD), soluble COD, volatile fatty acid (VFA) and volatile suspended solids (VSS) are registered as the steady state value and tabulated in Table 1. This table also includes the calculated organic loading rate for each system.

Methane Converting Capacity (MCC)

In Run 4 and Run 5, batch tests on methane converting characteristics of sludge consuming acetate, propoinate, iso-butyric acids and cellulose were performed according to the method described previously. The results from these tests are summarized in Table 2.

Hydrolysis of Cellulose in the Thermo-meso and Acid-methane Process

Hydrolysis of cellulose in each system was characterized by looking at the hydrolysis rate of the particulate COD in each reactor. As shown in Table 3, average of the first-order hydrolysis rates for the first stage thermophilic reactor was 0.79 day^{-1} and 0.26 day^{-1} for the acidogenic reactor in the acid-methane process.

DISCUSSIONS

In anaerobic wastes stabilization, COD destruction is directly related to methane evolution, only minimum COD reduction occurs without methane production. This allows to use methane production as a parameter for system performance. In this research, the thermo-meso system had higher methane yields (0.23 to 0.33 L/gCOD) than those of the acid-methane system (0.16 to 0.27 L/gCOD) in all runs, even for Run 7 and Run 8, where a shorter system HRT (13 day) was used for thermo-meso system. An examination of the system methane production rate also showed that thermo-meso was superior to the acid-methane system under a similar organic loading rate.

For a two-stage system, hydraulic retention time and feed solids play important roles for converting cellulose to methane efficiently. To evaluate their influences on each system, the methane production data listed in Table 1 together with some previous data obtained from this study (data

shown in Chapter 3 and 4) were used to construct a serial contour lines of constant methane production rate (mLCH_4/L-reactor/day) from both systems using Eq. (3-A) and (3-B), respectively.

Acid-methane System:

$$\text{Methane Production Rate} = -212 + 0.00455\chi_1 + 37.41\chi_2 - 9.9\times10^{-8}\chi_1^{\,2} - 1.66\chi_2^{\,2} + 0.001\chi_1\chi_2 \quad \text{.....(3-A)}$$

(degree of freedom = 2; F = 26; R^2 = 0.985)

Thermo-meso System:

$$\text{Methane Production Rate} = -734 + 0.011\chi_1 - 60.21\chi_2 + 1.91\times10^{-7}\chi_1^{\,2} - 1.33\chi_2^{\,2} - 0.00036\chi_1\chi_2 \quad \text{.....(3-B)}$$

(degree of freedom = 3; F = 196; R^2 = 0.997)

Where, x_1 is feed cellulose concentration (mg COD/L) and x_2 is the system HRT. The statistics test, F, is defined as *MSR/MSE*, where *MSR* is the mean square of regression, obtained by dividing the sum of squares of regression by the degree of freedom. *MSE* is the mean squares of error from the analysis of variance. If the calculated value of F exceeds that in F table at a specified probability level (*i.e.*, $F(P\text{-}1,\ v,\ 1\text{-}\alpha)$), then a "statistically significant" regression model is obtained, where v is the degree of freedom of error and P is number of parameters. $F(P\text{-}1,\ v,\ 1\text{-}\alpha)$ is the F value at the α probability level. Moreover, since the values of R^2 is close to 1.0, the regression model was considered to be an accurate representation of the experimental data (Ang and Tang, 1975). The magnitudes of regression equation coefficients are used as a basis for judging statistical significance and illustrate the relative effects of linear, quadratic and interaction between the variables.

Consider the fitted equations (Eq. 3-A and 3-B) graphed in Fig. 3, the constant methane production rate curves have the shape commonly referred to a "ridge" for both systems. According to the model obtained, the trend of the ridge confirmed that both values of cellulose concentration and HRT were important for each system generating methane. As shown in Fig. 4, at the HRT of 15 days, methane production rate increased from 120 to 800 mL/L·d for acid-methane process and from 180 to 1,155 mL/L/d for thermo-meso system when the feed cellulose increased from 7.5 to 60 g

COD/L. On the other hand, under the feed of 55 g COD/L of cellulose, the methane production increased from 755 to 1,025 mL/L·d as the acid-methane system HRT increased from 15 to 30 days. Contrary to this feature of the acid-methane process, methane production from TAPD system dropped from 1,103 to 713 mL/L/d when the HRT increased from 13 to 30 days.

To further reveal the influences of the system HRT and feed cellulose concentration on cellulose mineralization, 55 g/L feed cellulose and 15-day HRT were each selected as a constant to calculate system methane production rate by using Eq. 3, and these results are presented in Fig. 4. Fig. 4(A) shows the system methane production changes with HRT when the feed cellulose was maintained constantly at 55 g COD/L, while figure 4(B) presents the system methane production changes with the feed cellulose if the system operated with a constant HRT of 15 days. An examination of Fig. 4(A) clearly shows that with an 18.5-day HRT, both acid-methane and thermo-meso systems will possess a same methane converting rate of 890 mL/L·d. This value is decreasing for the acid-methane process when a shorter HRT is applied. However, methane production rate of thermo-meso system is increasing with the decrease of the system HRT. This clearly demonstrates that thermo-meso process has a greater methane production over the acid-methane process in at a shorter HRT. Another advantage of thermo-meso process is the higher methane conversion rate when cellulose concentrations were above 30 gCOD/L as illustrated in Fig. 4(B). In summary, thermo-meso process demonstrates high potential for cellulose degradation at high feed solids but low HRT. Low HRT allows reduction of reactor volume and consequently the associated capital costs for the application of this process.

While the system converting cellulose, the steady states data summarized in Table 1 indicate that in thermo-meso system, methane production from the thermophilic reactor was 7 – 27 times of that from the mesophilic reactor. Methane yields of this reactor (0.18 to 0.28 L/gCOD) were also higher than those of the mesophilic reactor (0.06 to 0.19 L/gCOD) except for Run 7. This suggests that anaerobic mineralization of cellulose by thermo-meso process was overruled mainly by the ability of thermophilic reactor, whereas the mesophilic reactor played a role on polishing the residual organic matters. On the other hand, for acid-methane process, methane production of the acidogenic reactor

was from not measurable to 56 ml/L-reactor per day, but 84 to 970 ml/L-reactor per day for the methanogenic reactor. Obviously, the second stage methanogenic reactor governs the system methane generation in acid-methane system. Compare the methane production from these two dominating methane generation reactors in each system, methane produced from the thermophilic reactor was 2 to 4 times more than the methanogenic reactor. Batch methane converting capacity (MCC) test further confirmed that the activity of acetate-utilizing methanogens in the thermophilic reactor were 45 - 50% higher than those in the methanogenic reactor. The acitivity of aceticlastic methanogens is important for achieving a significant waste stabilization because it counts approximately two third of the methane production in anaerobic digesters. Microorganisms developed in the thermophilic reactor also demonstrated a 1.3 – 1.9 times higher ability in terms of directly converting cellulose into methane (Table 2).

It has been widely reported that hydrolysis of particulate metters is the rate limiting step in mesophilic anaerobic sludge digestion. This research showed the agreement with this finding for the acid-methane two-stage process. The methane converting capacity vial test results clearly showed that the MCC value of acetate, propionate and iso-butyrate in the methanogenic reactor was all greater than that of cellulose. According to the theory of "master reaction", the disappearance of the feed substrate, and therefore the appearance of a product, is controlled by a rate limiting step, which for cellulose mineralization could be hydrolysis, acetogenic conversion of higher fatty acids, methane generation from acetate or some other unidentified reaction step. Here, the lowest MCC for cellulose utilization suggests that hydrolysis of cellulose could be the rate limiting step that governs the cellulose conversion to methane if cellulose is fed directly to this reactor. Therefore, in some extent, the performance of this reactor is dependent on the hydrolysis ability of the first stage reactor, *i.e.* acidogenic reactor.

In the acid-methane two-stage process, hydrolysis/acidogenisis is expected to be optimized through phase separation from methanogenesis in two reactors. The hydrolysis of particulate organics is encouraged in the first-stage acidogenic in order to obtain maximum destruction of organics. However this research showed that hydrolysis coupled with methanogenesis enabled

greater destruction of organics. At all tested conditions, the first stage of the thermo-meso system achieved higher hydrolysis of particulate cellulose (average 0.79 day^{-1}) than the first stage of the acid-methane system (average 0.26 day^{-1}), where most of the hydrolysis suppose to occur. These results obtained are in agreement with those from a previous study by Schmit (1998). In the first stage (thermophilic reactor) of the thermo-meso system, concomitant production of methane allowed for continued hydrolysis and acidification of incoming particulate organics. But, in the first stage of the acid-methane system, hydrolysis rate was inhibited by the accumulation of hydrolysis intermediates that were not utilized for methane formation because methanogenesis was operational supressed in this reactor.

Higher temperature could be another factor contribute to the high hydrolysis rate in the thermophilic reactor. The hydrolysis rate obtained here is apparent higher than some literature values obtained at mesophilic conditions. Preffer (1974) reported a hydrolysis rate of 0.15 day^{-1} for municipal solid waste, and Vavilin's research showed that the hydrolysis rate for sludge was 0.25 at 35 ^{0}C, and 0.15 day^{-1} for cellulose at 28 ^{0}C (Vavilin *et al.*, 1997).

SUMMARY AND CONCLUSIONS

Two-stage anaerobic digestion systems possess high potential for wastes stabilization with a simultaneous energy production. A good understanding of the similarities and differences between each system is extremely important for selecting, designing and operating these two-stage digestion biosystems. Unfortunately, limited information is available based on a direct comparison of these systems and no systematical analysis has been provided. For this purpose, seven experimental runs were carried out in a mesophilic acid-methane system and thermo-meso system to determine their performances on cellulose degradation. A set of HRTs and influent substrate concentrations were employed in planning these experimental runs. In addition, a model of the methane generation capacity using predictive polynomial quadratic equation and response surface methodology (Box *et al.*, 1978) was established for each system. The effects of the HRT and feed cellulose concentration on the methane production were evaluated using the model obtained. Further batch methane

converting capacity (MCC) tests using acetate, propionate, iso-butyrate, and cellulose were conducted to evaluate the maximum methane production rates of the microorganisms developed in each reactor of a two-stage system. First-order hydrolysis rate was also calculated for the comparison of cellulose hydrolysis in these two systems. Based on the results obtained and discussions, our results showed that:

- Both thermo-meso (TPAD) and acid-methane systems were successfully used for the anaerobic degradation of cellulose at concentrations up to 60 g/L with system HRT of 13 to 30 days.
- Methane production rate and methane yield of the thermo-meso system was in the range of 148 – 1,100 mL/L reactor/d and 0.23 – 0.33 L/g COD fed when the organic loading rates were 466 to 4,000 mg/L reactor/d. These values obtained were higher than the acid-methane system under similar loading rates. Methane production rate and methane yield of the acid-methane system were 71 – 776 mL/L/d and 0.16 – 0.27 L/g COD, respectively.
- Thermophilic reactor, the dominate unit for solids destruction and methane production in the thermo-meso system, possessed 2 – 4 times higher methane production rate than the dominating reactor (methanogenic reactor) in acid-methane system. Batch methane converting capacity (MCC) test further confirmed that the activity of acetate-utilizing methanogens in the thermophilic reactor were 45 - 50% higher than those in the methanogenic reactor. Microorganisms developed in the thermophilic reactor also demonstrated 1.3 – 1.9 times higher capability in terms of directly converting cellulose into methane.
- First-order hydrolysis rate of the thermophilic reactor was 0.79±0.22 day^{-1}. It was greater than the hydrolysis rate obtained in the acidogenic reactor (0.26±0.2 day^{-1}), where most cellulose hydrolysis was supposed to occur in acid-methane system.
- With the methane generation model obtained, thermo-meso system demonstrates higher methane generation potential in treating high solids with a shorter hydraulic retention time than the acid-methane system.

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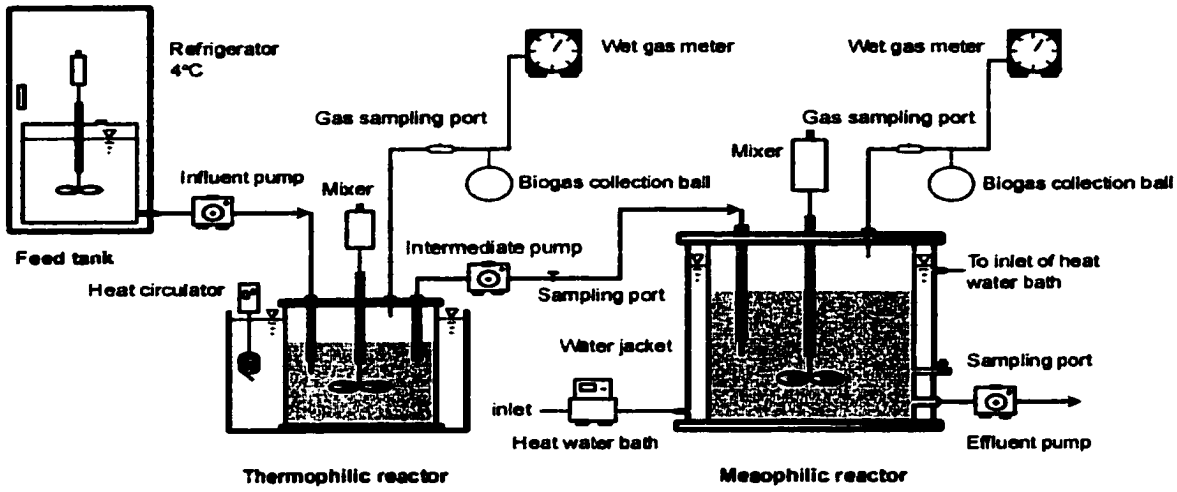

Fig. 1 (A). Thermo-meso Two-stage Process

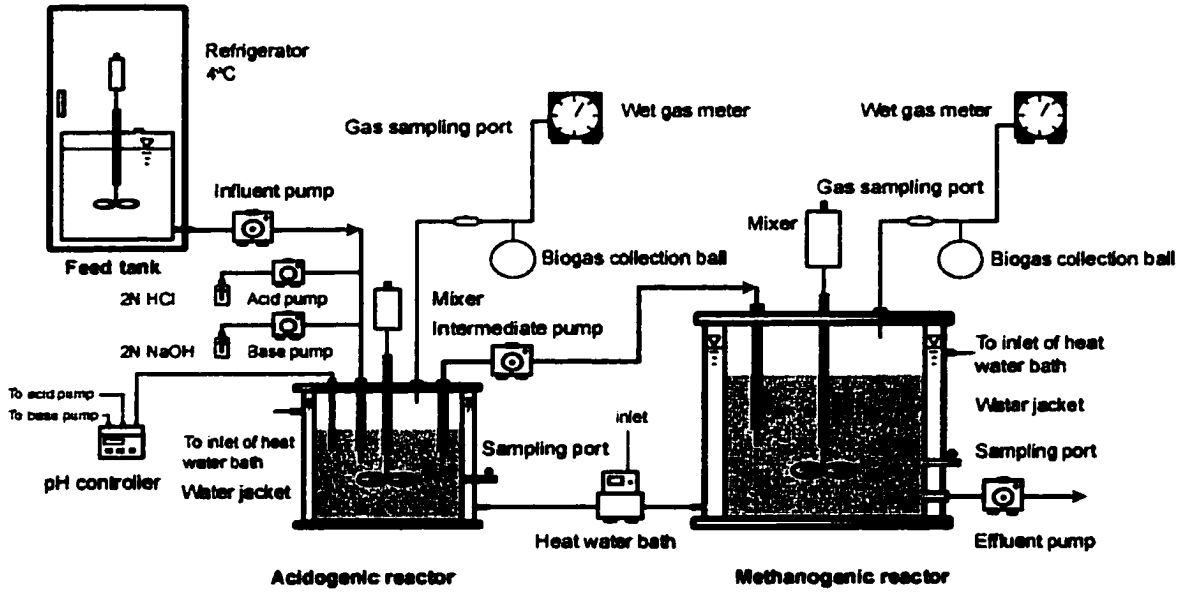

Fig. 1 (B). Acid-methane Two-stage Process

Figure 1. A schematic diagram of the chemostat thermo-meso and acid-methane anaerobic digestion systems

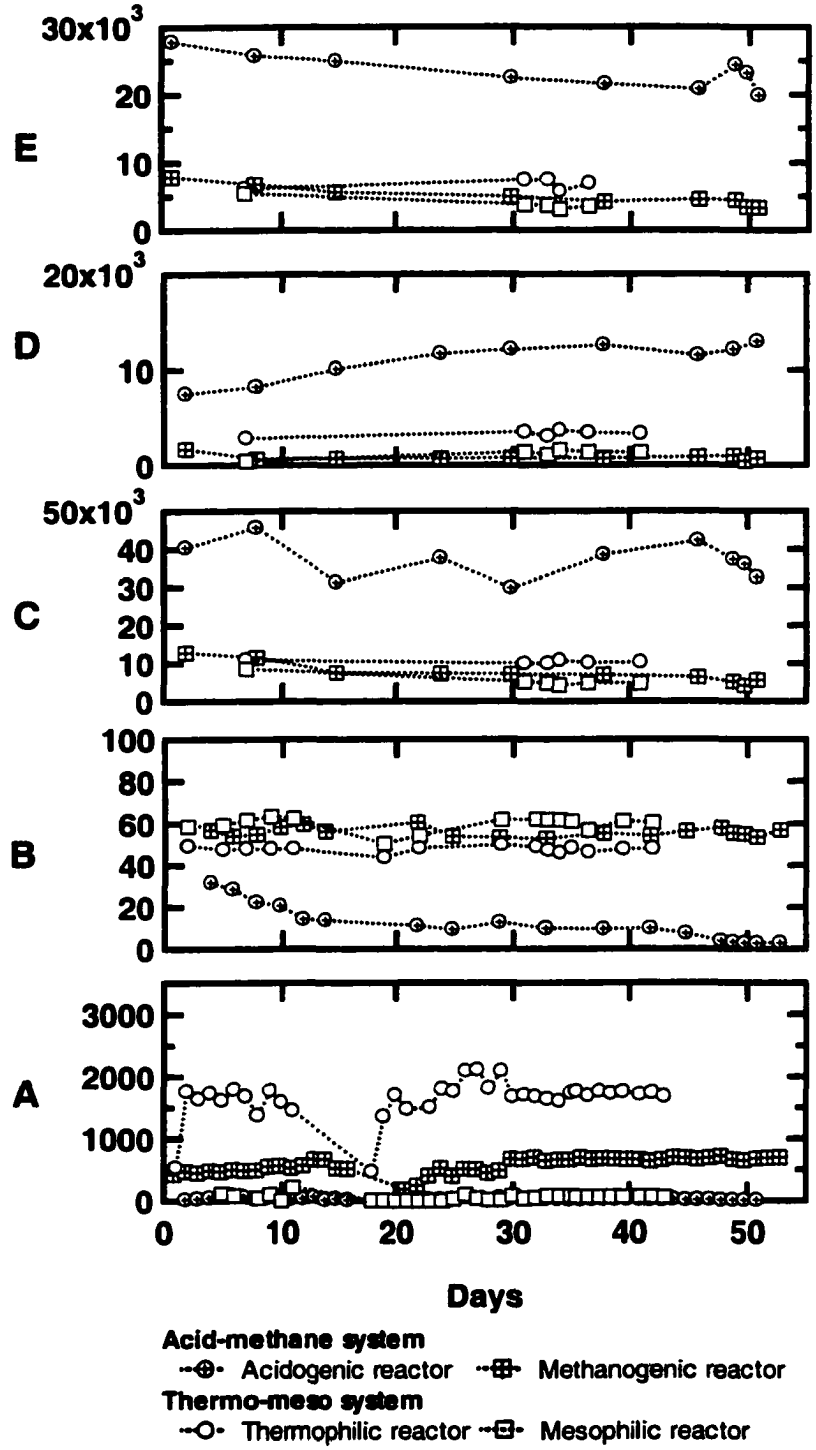

Figure 2. Performance of acid-methane and thermo-meso systems as a function of time in Run 4 (A) daily methane production (mL/L-reactor·d) (B) methane content in biogas (%) (C) COD (mg/L) (D) soluble COD (mg/L) (E) volatile suspended solids (mg/L)

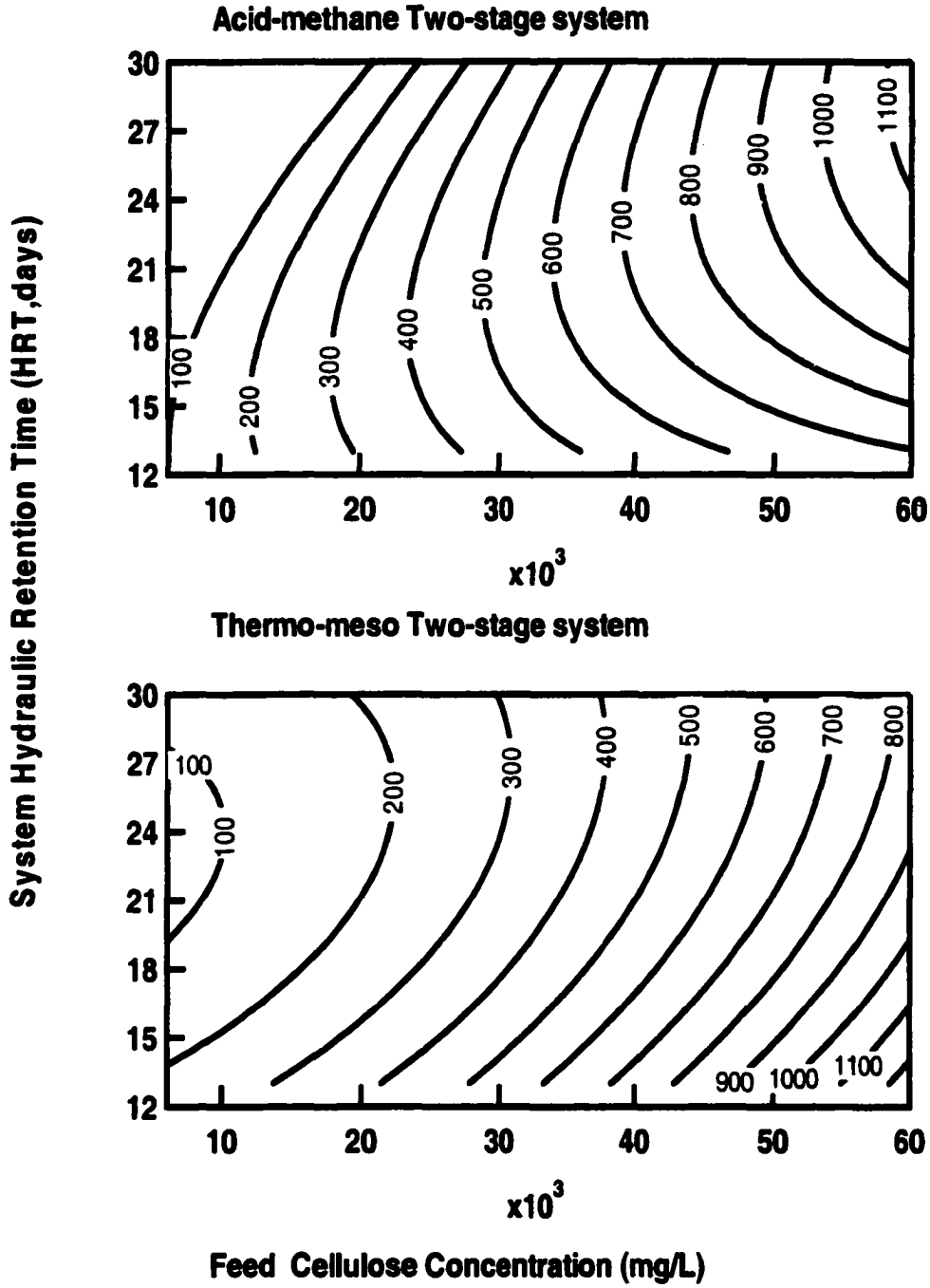

Figure 3. Methane production rate (mL CH_4/L-reactor·d) as a function of feed cellulose concentration and hydraulic retention time for acid-methane and thermo-meso systems

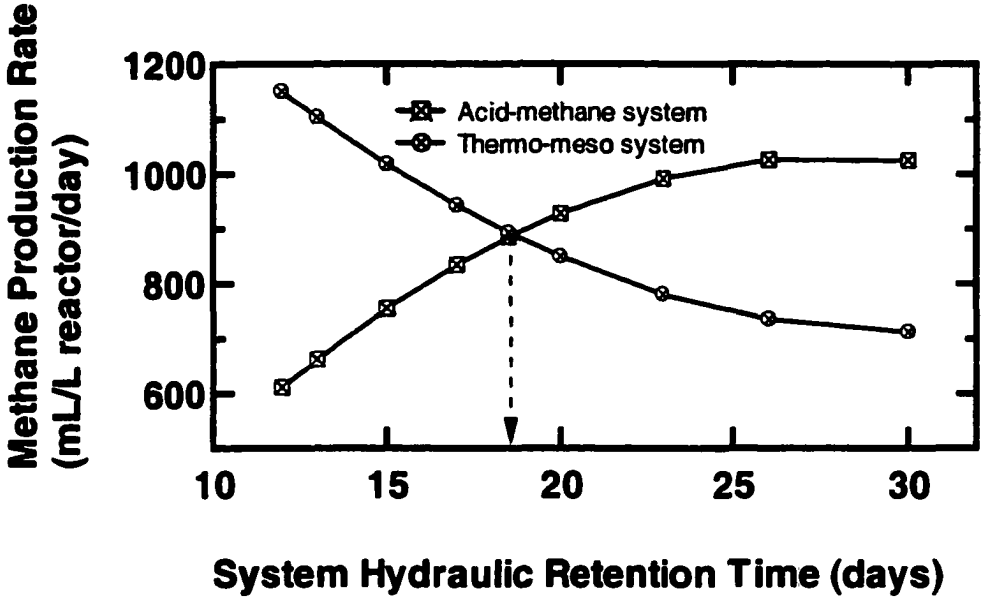

Fig. 4 - A

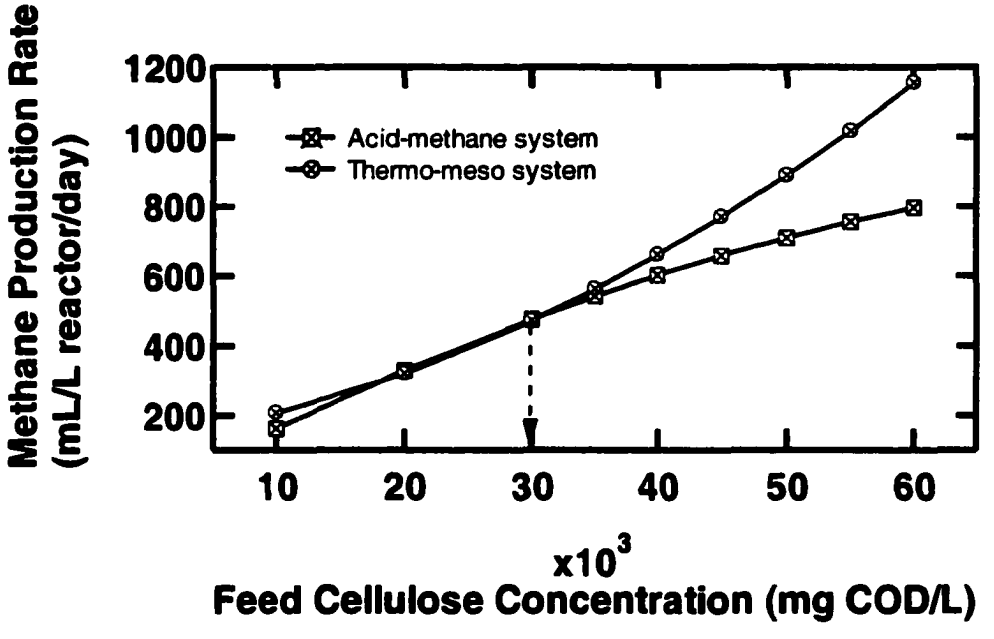

Fig. 4 - B

Figure 4. Predicted methane production rate of the acid-methane and thermo-meso systems changes with: (A) HRT at a constant feed cellulose concentration of 55 g/L (B) with feed cellulose concentration at a fixed HRT of 15 days by using Equation 3

Table 1. (Part I). Operating conditions (HRT and feed cellulose concentration) and summary of COD, soluble COD and VFA of the acid-methane system and thermo-meso systems in quasi-steady states

Runs	[1]Cellulose Con. in Feed (mg COD/L)	System HRT (days)	HRT/SRT (days)				COD Concentration (mg/L)						Soluble COD (mg/L)				VFA (mg/L)			
			Acid-methane		Thermo-meso		Acid-methane			Thermo-meso			Acid-methane		Thermo-meso		Acid-methane		Thermo-meso	
			Acid	Meth	Thermo	Meso	[2]Feed	[3]Acid	[4]Meth	Feed	[5]Thermo	[6]Meso	Acid	Meth	Thermo	Meso	Acid	Methane	Thermo	Meso
Run 1	25,000	30	10	20	10	20	24267	23188	4628	24047	5202	2789	8561	1236	2234	1952	5415	329	971	NA
Run 2	7,500	15	5	10	5	10	6833	5480	2699	6894	2707	1305	3611	1118	1034	410	2661	NA	513	172
Run 3	15,000	15	5	10	5	10	11479	10303	1957	12754	3830	2578	6537	445	902	266	4681	66	130	104
Run 4	40,000	15	3	12	5	10	39074	37270	5583	38271	10165	4733	13355	718	3354	1330	7649	887	472	73
Run 5	60,000	15	3	12	5	10	55379	52648	9277	60000	21642	9382	9475	2122	7452	1898	7619	313	111	77
Run 6	40,000	13			3	10				34790	12844	7290			5479	4650			2426	2059
Run 7	60,000	13			3	10				55000	23270	3892			6089	3495			5657	1097

(1) The feed was made based on 1g cellulose = 1.19 g COD (2) Measured COD fed to the system (3) Acidogenic reactor (4) Methanogenic reactor (5) Thermophilic reactor (6) Mesophilic reactor

Table 1. (Part II). VSS, organic loading rate(OLR), methane content, production rate and methane yield of the acid-methane and thermo-meso systems in quasi-steady states

Runs	VSS (mg/L)				Methane Content (%)				Organic Loading Rate (mg COD/L-reactor/day)						Methane Production Rate (mL/L-reactor/day)						Methane Yield (L/g COD fed)	
	Acid-methane		Thermo-meso		Acid-methane		Thermo-meso		Acid-methane			Thermo-meso			Acid-methane			Thermo-meso			Acid-methane	Thermo-meso
	Acid	Meth	Thermo	Meso	Acid	Meth	Thermo	Meso	Acid	Meth	[5]Sys	Thermo	Meso	Sys	Acid	Meth	System	Thermo	Meso	System	System	System
Run 1	8066	4133	3744	3151	NA	75	48	72	2427	1159	809	2405	260	802	ND	300±12	200±8	648±28	46±2	246±10	0.25±0.01	0.31±0.01
Run 2	1469	1514	1462	1280	55	81	50	73	1367	548	456	1397	271	466	56±31	84±26	71±19	337±37	47±20	148±15	0.16±0.04	0.32±0.03
Run 3	2164	1153	3241	2383	40	69	52	66	2296	1030	765	2551	383	850	44±16	290±11	208±10	708±63	67±33	281±28	0.27±0.01	0.33±0.03
Run 4	21940	3980	6871	3522	6	55	47	61	13025	3106	2605	7654	1016	2551	13±7	669±20	538±16	1703±48	62±12	609±17	0.21±0.01	0.24±0.01
Run 5	41120	6460	15110	6946	41	51	48	62	18460	4387	3692	12000	2164	4000	ND	970±31	776±25	2861± 103	303±17	1155±34	0.21±0.01	0.29±0.01
Run 6			6698	2367			43	55				11597	1284	2676				2210± 172	147±30	623±45		0.23±0.02
Run 7			11035	4075			38	56				18333	2327	4231				3354±1733	437±115	1110±419		0.26±0.10

(5) Sys = system ND: not detectable ±: standard deviation

Table 2. Methane converting capacity (MCC) of the microorganisms in the acid-methane and the thermo-meso process (mL CH4/L-mixed liquor-day)

Reactor	Run 4				Run 5			
	Acetate	Propionate	Iso-butyrate	Cellulose	Acetate	Propionate	Iso-butyrate	Cellulose
Acidogenic	83			87	33			
Methanogenic	617	340	573	222	707	830	317	227
Thermophilic	893	160	349	507	1060	161	363	647
Mesophilic	603	187	231	327	630	297	587	295

Table 3. First-order hydrolysis rate of each reactor in the acid-methane and thermo-meso systems (day^{-1})

	Reactor	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Average
Acid-methane system	Acidogenic	0.07	0.53	0.41	0.21	0.09			0.26±0.20
	Methanogenic	0.17	0.02	0.15	0.33	0.42			0.22±0.16
Thermo-meso system	Thermophilic	0.71	0.63	0.67	0.92	0.65	1.24	0.73	0.79±0.22
	Mesophilic	0.13	0.09	0.03	0.1	0.09	0.18		0.10±0.05

CHAPTER 6. THERMO-MESO TWO-STAGE ANAEROBIC DIGESTION SYSTEM APPLICATION

Laboratory-, Pilot- and Full-scale Operations of Thermo-meso Two-stage System for Anaerobic Digestion of Primary, Secondary Sewage Sludge and Organic Fraction of Municipal Solid Wastes

A paper to be submitted to Waste Management

Yuyun Shang

INTRODUCTION

Thermo-meso process, also known as temperature-phased anaerobic digestion system (TPAD), is a two-stage anaerobic digestion process with the first stage unit operated at the thermophilic temperatures (55 – 60 ^{0}C) and the following second stage unit maintained at mesophilic temperature (35 – 37 ^{0}C). The combination of the thermophilic with mesophilic temperatures allows this process take the advantages of thermophilic anaerobic digestion but eliminate its disadvantages (Kaiser *et al.*, 1995; Han and Dague, 1997). Several studies on comparing the thermo-meso systems with single-stage or mesophilic acid-methane processes have reported higher volatile solids destruction, enhanced methane production rate and shorter hydraulic retention time, thus less reactor volume was required for waste organics degradation (Han and Dague, 1995; Han and Dague, 1997; Schmit, 1998). Furthermore, nearly complete pathogen destruction achieved by the thermo-meso process has offered this process an ability for producing 40 CFR Part 503 Class A biosolids (Han and Dague, 1997; Vik and Olsen, 1997; Streeter, 1997; Vandenburgh, 1998; Chao, 1998). Class A biosolids requires that the digested sludge contains less than 1,000 MPN fecal coliform per gram of total solids (TS) or less than 3 MPN Salmonella sp. bacteria per four grams TS . Unlike Class B biosolids, Class A bisolids produced can be utilized in agricultural, landscape activities, land reclamation and other fields which allow the public come into direct contact with the biosolids. Thus, the production of Class A biosolids converts the wastes into the beneficial material.

Recently, interested in upgrading the existing digesters for Class A biosolids production or alleviating the digester overload problem has turned the thermo-meso process more attractive. It was estimated that in the United States, 52% of the municipal wastewater treatment plants which utilize anaerobic digestion for sludge stabilization was approved for only class B biosolids production (USEPA-AMSA 1997). On the other hand, converting the existing digestion system into the thermo-meso process is a practical and cheap solution because most of the treatment plants own more than one digesters. Meanwhile, the extra heating requirement for maintaining the thermophilic temperatures can be minimized by the installation of a heat-water exchanger between the thermophilic and mesophilic unit, and by the utilization of produced methane for heating the raw wastes.

Although many bench-scale and full-scale thermo-meso systems have been successfully used for anaerobic sludge stabilization, a lack of uniform data presentation as well as limited information on operational parameters provides the opportunity for this work. Therefore, the objective of this paper was to summarize the results from the previous operation of thermo-meso systems in a consistent and universal form and the focus was on the volatile solids destructions and methane productions. In addition, the collected data will be analyzed to provide important guidelines on operating parameters such as feed strength, hydraulic retention time and organic loading rates for digester upgrading or new thermo-meso process designing.

OPERATION RESULTS AND DISCUSSIONS

Performance of the Thermo-meso Process

The available results of the bench-, pilot- or full-scale operation of the two-stage thermophilic /mesophilic system have been collected and summarized in Table 1. The original data has been transformed to a consistent and universal way for data analysis. Volatile solids (VS) reduction was calculated by the percentage of the difference between the influent and the effluent VS concentration divided by the influent VS concentration.

Performance of the Thermo-meso System Under Various Hydraulic Retention Times

To investigate the effect of the operating hydraulic retention time on the system performance and methane production, volatile solids destruction and methane yield were plotted against the system HRT in Figure 1. System HRT was the sum of the HRTs for the thermophilic and mesophilic reactors. As shown in this figure, except in one experiment, where 34% VS reduction was obtained, under all the operation conditions where these data were collected, thermo-meso process obtained more than 38% volatile solid reduction. Methane yield per gram of the volatile solids applied to the system was in the range of 0.16 to 0.88 with 72% of the data points fall between 0.2 to 0.4 L CH_4/g VS applied. An examination of this graph shows that an average of 51 ± 10% volatile solids destruction and 0.28 ± 0.08 L CH_4/g VS methane yield were obtained with the system HRT around 15 to 30 days. Although these values were higher when the thermo-meso process was operated at longer HRTs, increased volatile solids reductions may not worth the cost for the extended system HRT. This is because in sludge digestion, HRT directly corresponds to the reactor volume needed. Therefore, the thermo-meso system is suggested to operate with 10 to 30 days HRT and still be able to achieve high VS reduction.

Performance of the Thermo-meso System under Various Feed Concentrations

Feed strength plays an important role in anaerobic sludge digestion because the thickened sludge increases the digester capacity and reduces the volume needed. Therefore, pre-thickening sludge to 5% or greater prior to digestion is a common process employed in the wastewater treatment plants throughout the United States. However, the increase of the feed volatile solids concentration could be limited by considering the possible deteriorated digester performance due to substrate transport limitations and accumulations of toxic byproducts such as hydrogen sulfide or ammonia in the digester. Here, the effects of feed solids concentration on the thermo-meso process is evaluated and presented in Figure 2.

An examination of Figure 2 shows that in the tested feed solids range of 8 to 28 g VS/L, the thermo-meso process obtained more than 38% volatile solids reduction and the average methane

yield was 0.33 ± 0.15 L CH_4/g VS. This figure illustrates that a higher VS reduction averaged at about 60% was obtained when the feed volatile solids was around 32 to 42 g VS/L.

Capacity of the Thermo-meso System Degrading Organic Wastes

In order to evaluate the capacity of the thermo-meso process for waste degradation, volatile solids destruction and volumetric methane production rate were plotted against the volatile solids loading rates and presented in Figure 3.

An examination of Fig. 3 illustrates that the thermo-meso system can handle the solids load up to 4.5 g VS/L_reactor·day and still obtain more than 38% volatile solids destruction with the methane yield of 0.16 to 0.88 L CH_4/gVS. The fitted line shows that the VS destruction decreases as the system loading rate increased. If 38% VS reduction is a set goal, then the volatile solids loading rate of 4.5 g VS/L_reactor·day was the threshold limit for this process.

Effect of Hydraulic Retention Times on the Thermophilic Reactor

As mentioned previously, thermo-meso process achieved almost complete destruction of fecal coliform due to the high temperatures employed. Therefore, in thermo-meso system, the first-stage thermophilic reactor plays an important role in assuring this process meeting the pathogen destruction requirements for Class A biosolids production. In anaerobic sludge stabilization, destruction of pathogen is usually assumed as a first order reaction with respects to its concentration and reaction time. Thus, the hydraulic retention time of the thermophilic reactor is not only important in defining the system volatile solids reduction, but also critical for pathogen destruction. For this reason, volatile solids destruction of this reactor was plotted against its HRT in Figure 4. It can be used as a reference for selecting the HRT of the thermophilic reactor in conjunction with pathogen destruction if the data is available.

Figure 4 shows that both the volatile solids destruction and methane yield increased as the reactor HRT was increased. A maximum of more than 60% VS reduction was obtained if operating the reactor with 25 days HRT. However, selection of HRT for this reactor should take the consideration of energy requirement and also the combination with the subsequent mesophilic

digester to ensure an optimal overall system performance. Generally, less than 10 days HRT for this thermophilic reactor is suggested.

SUMMARY AND RECOMMENDATIONS

The thermo-meso process has considerable potential for the stabilization of various wastes especially domestic sewage sludges and municipal solid wastes. This paper summarizes the results from laboratory experiments and full scale operations of the thermo-meso systems for treating primary, secondary and municipal solid wastes. The collected data of volatile solids destructions and methane productions was analyzed to provide important guidelines on thermo-meso operational parameters such as feed strength, hydraulic retention time and organic loading rate for the digester upgrading or designing new thermo-meso systems. Based on the results from this study, a ten to thirty days system HRT and solid loading rate of 4.5 g VS/L_reactor·day are suggested as the optimal operational parameters. The thermo-meso system can stabilize the solids with a feed concentration of 8 to 58 g VS/L, but VS reduction was higher when the feed was maintained in the range of 32 to 42 g VS/L. Less than 10 days HRT was suggested for the first-stage thermophilic reactor.

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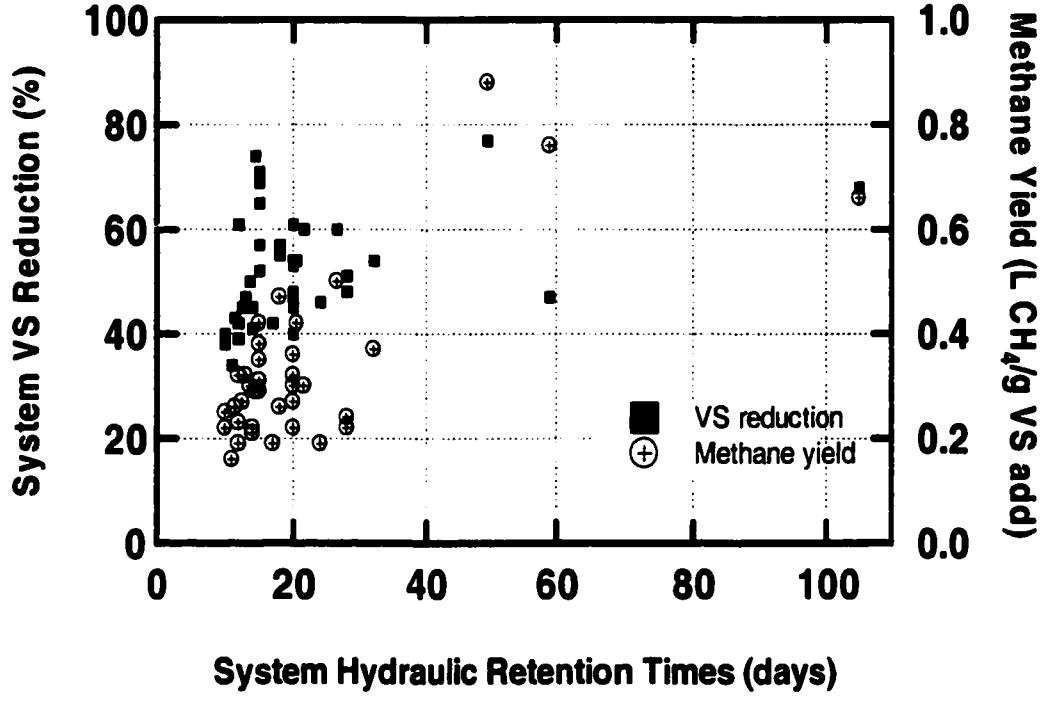

Figure 1. System volatile solids destruction and methane yield of the thermo-meso two-stage process under various system hydraulic retention times

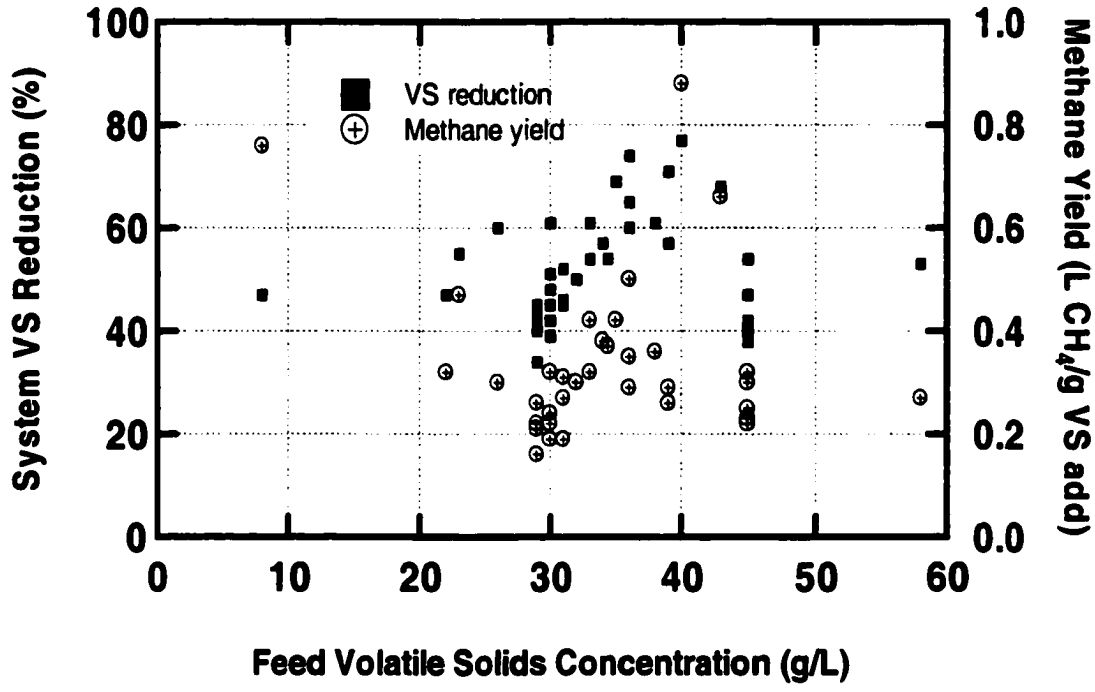

Figure 2. System volatile solids destruction and methane yield of the thermo-meso process under various system feed volatile solids concentrations

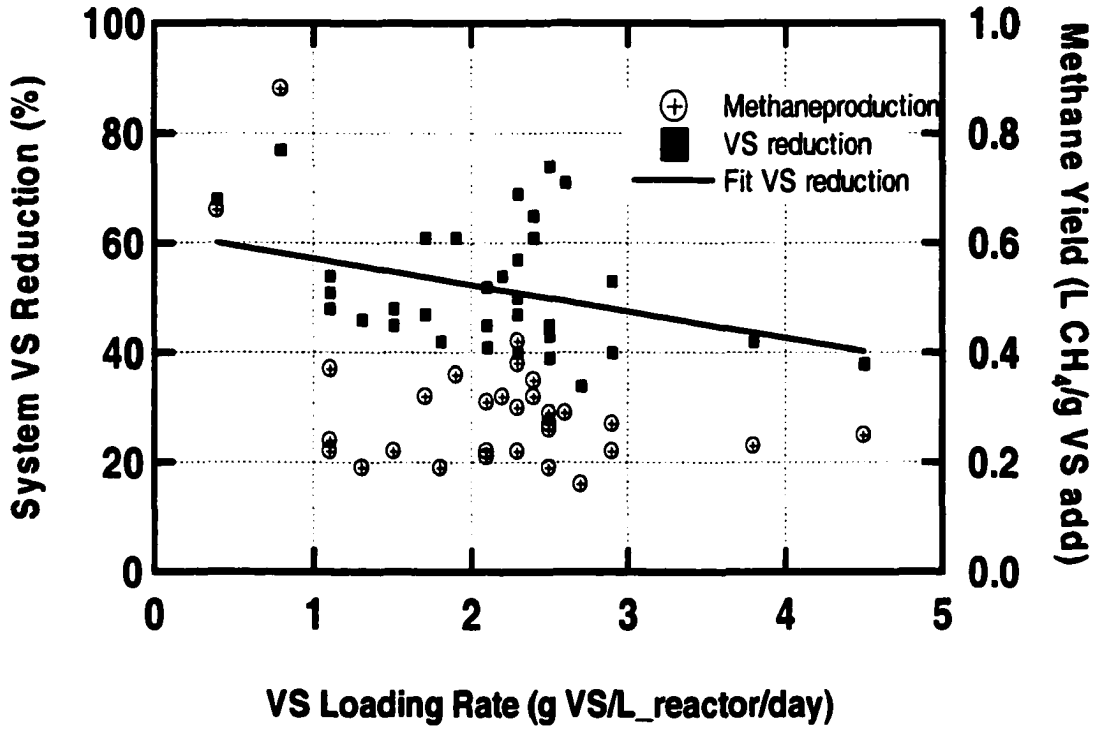

Figure 3. System volatile solids destruction and methane yield of the thermo-meso process under different system volatile solids loading rates

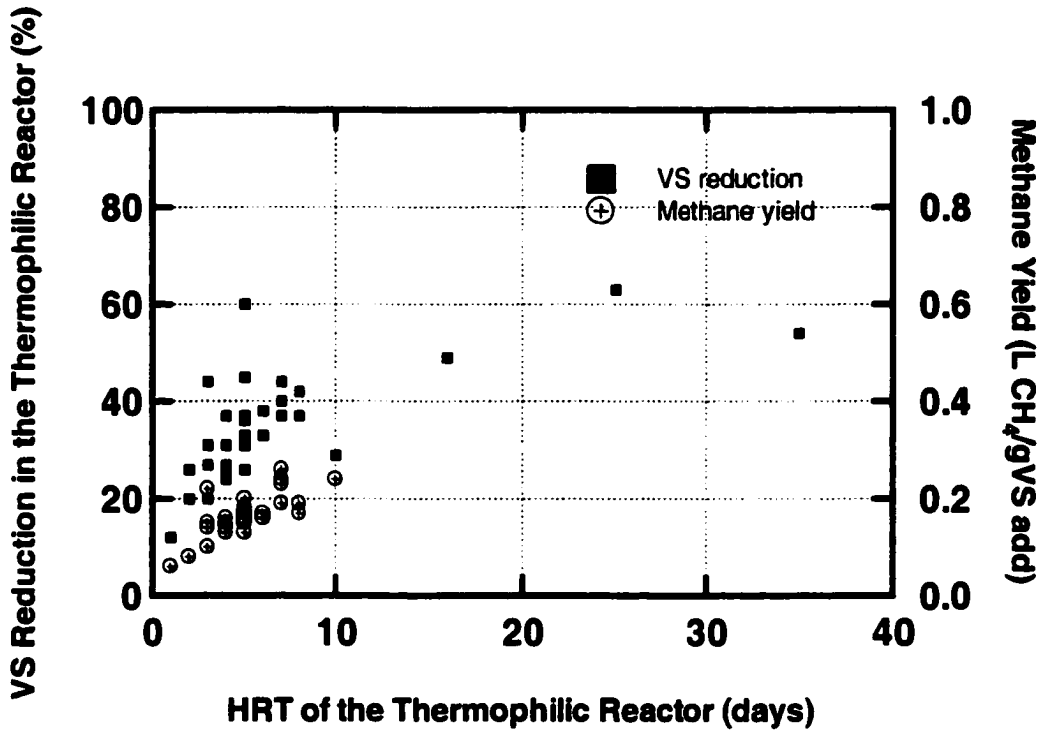

Figure 4. Effects of HRT on the volatile solids destruction and methane yield of the thermophilic reactor

Table 1. Operation results of the thermo-meso process for sludge and municipal solid wastes stabilization

Feed Composition (%)			Feed (g/L)	HRT (days)		VS Load (g VS/L/d)	VS Reduction (%)			Methane Production (L-CH4/L-reactor/d)		Methane Yield (L-CH4/g VS)	Methane Content (%)		VFA (mg/L)		References	
PS	WAS	OFMSW	TVS	Therm	Meso	Sys	Therm	Meso	Sys	Therm	Meso	System	Therm	Meso	Therm	Meso		
100		0	22	3	10	1.7	44	6	47	1.6	0.1	0.32	66	70	529	147	Healy (1998)	Lab-scale
80		20	34	5	10	2.3	17	63	57	0.9	0.7	0.38	50	65	3158	167		
60		40	35	5	10	2.3	37	51	69	1.1	0.6	0.42	49	68	3697	175		
40		60	36	5	10	2.4	36	46	65	1.3	0.4	0.35	46	60	3394	1398		
20		80	39	5	10	2.6	45	48	71	1.6	0.2	0.29	51	67	1683	159		
	100		45	10	10	2.3	29	16	40	1.1	0.3	0.30	64	67	680	430	Chao (1998)	Pilot-scale
	100		45	5	15	2.3	33	20	47	1.4	0.2	0.22	58	64	1060	330		
	100		45	3	9	3.8	31	16	42	2.2	0.4	0.23	56	63	1620	460		
	100		45	3	6	4.5	27	15	38	2.5	0.7	0.25	59	59	2180	530		
100			31	5	10	2.1	26	34	52	1.0	0.5	0.31	65	69	1565	243	Han (1995)	Lab-scale
100			32	5	9	2.3	31	27	50	1.3	0.4	0.30	67	71	1723	267		
100			31	4	8	2.5	26	26	45	1.2	0.4	0.27	67	70	1915	248		
100			29	4	8	2.5	24	26	43	1.1	0.4	0.26	66	70	2118	205		
100			29	3	7	2.9	20	25	40	0.9	0.5	0.22	66	69	2050	284		
50	50		29	4	10	2.1	31	20	45	1.0	0.2	0.22	65	70	1360	210		
50	50		30	6	14	1.5	38	16	48	0.9	0.1	0.22	69	71	1010	200		
50	50		30	8	20	1.1	42	15	51	0.7	0.0	0.24	68	71	800	190		
50	50		29	1	10	2.7	12	25	34	1.7	0.3	0.16	58	68	2150	230		
50	50		30	2	10	2.5	20	23	39	1.3	0.3	0.19	63	67	1730	180		
50	50		30	3	14	1.8	31	16	42	1.5	0.1	0.19	64	70	1350	190	Han (1995)	Lab-scale
50	50		31	4	20	1.3	37	14	46	1.2	0.0	0.19	67	71	1080	160		
33	67		29	4	10	2.1	27	20	41	1.0	0.2	0.21	66	70	1200	190		
33	67		30	6	14	1.5	33	19	45	0.8	0.1	0.22	69	71	910	170		
33	67		30	8	20	1.1	37	16	48	0.7	0.0	0.22	70	72	700	150		
50	50		33	7	13	1.7	40	35	61	1.1	0.3	0.32			599	219	Vandenburgh (1998)	Lab-scale
50	50		38	7	13	1.9	44	31	61	1.4	0.3	0.36			689	185		
50	50		45	7	13	2.2	40	24	54	1.4	0.3	0.32			1479	450		
50	50		58	7	13	2.9	37	25	53	1.5	0.4	0.27			3067	567		
			23	4	14				55			0.47					Osterode*	
			8	4	55				47			0.76					Geseke*	
			39	3	15				57			0.26					Auenheim*	
			26	3	19				60			0.30					Erkelenz*	
			33	4	17				54			0.42					Altenmarkt*	
			36	6	21				60			0.50					Koln-Stammheim*	
60	40		30	2	10	2.4	26	47	61	0.0	0.9	0.32		61	2803	140	Belmont, IN (Huyard, 1998) PILOT	
50	50		40	25	25	0.8	63	38	77			0.88					Sturgeon Bay, WI (Vik et al, 1997) FULL	
			43	35	70	0.4	54	30	68			0.66			250	180	Newton, IA Full (Streeter et al, 1997) FULL	
55	45		36	5	10	2.5	60	34	74			0.29			464	152	Papillion Creek WWTF FULL	
40	60			16	16	1.1	49	10	54			0.37	60	60	1300	850	Neenah-Menasha Sewage Commision FULL	

* Full-scale experience of Anaerobic Stabilization Thermophilic/Mesophilic (ASTM) process referred to Oles *et al.*, 1997

CHAPTER 7. GENERAL CONCLUSIONS

This research demonstrates that both the thermo-meso two-stage process and mesophilic acid-methane two-stage process have considerable potential on mineralization of the high-strength simulated solid/sludge waste, namely particulate cellulose. The hydrolysis and methanization of cellulose in these two systems were studied under the different feed substrate concentrations, hydraulic retention times and organic loading rates. Furthermore, a direct comparison study was performed to characterize the similarities and differences between the thermo-meso process and the acid-methane system.

Predictive polynomial quadratic equation and response surface methodology were adopted to provide a systematical and straightforward experimental data analysis. Moreover, methane converting capacity vial tests using each of acetate, propionate, iso-butyrate, and cellulose as substrate were also conducted to evaluate the characteristics of the microorganism developed in each reactor of the two-stage systems and their maximum methane production rates were calculated.

Acid-methane process used in this study was consisted of a first-stage acidogenic reactor followed by a second-stage methanogenic reactor. The pH in the acidogenic reactor was controlled at 5.6. Both of the reactors were operated at the mesophilic temperature (35 ^{0}C). The thermo-meso process was consisted of a thermophilic first-stage reactor followed by a mesophilic second-stage unit. The temperatures for the first- and second- stage reactors were controlled at 55 ^{0}C and 35 ^{0}C, respectively. Both reactors were operated as methane generation units, therefore, sodium bicarbonate was added to the feed to maintain a neutral environment for this process. In this research, all the reactors used were continues stirred tank reactors (CSTRs).

The conclusions from this research were broken into four parts as discussed individually in the corresponding four papers presented in Chapter 3 through Chapter 6.

7.1. Acid-methane Two-stage Process

Eight experiments were performed to investigate the influence of HRT (15 to 30 days) and influent concentration (7.5 to 60 g COD/L) on the mesophilic acid-methane process degrading cellulose. Based on the results and discussion, our results showed that:

- The first-stage acidogenic reactor mainly converted the particulate cellulose into soluble organics (*e.g.*, sugars and VFAs). According to response surface plots, both chosen values of HRT and feed cellulose concentration were important for the acidogenic reactor producing soluble COD. Soluble COD produced from this reator was ranged between 3,611 and 13,355 mg/L. The results obtained with the acidogenic reactor indicated that the cellulose loading rate increased from 1,367 to 13,025 mgCOD/L-reactor/day with an increase in soluble COD production rate from 722 to 4,452 mgCOD/L-reactor/day, and a yield of 0.352 g soluble COD/g cellulose COD added.
- The second-stage methanogenic reactor played a more important role on polishing the residual organics from the first-stage reactor while the system organic loading rate increased. It also shared the hydrolysis of cellulose. The organic loading rate of the second-stage reactor increased from 548 to 4,387 g COD/L/d with an increase in the methane production rate from 84 to 970 mL CH_4/L/d, while a COD to methane yield of 0.235 L CH_4/g COD (R^2 = 0.95) was obtained.
- MCC values resulted from the methanogenic reactor suggest that neither acetogenesis of higher fatty acids nor methanogenesis was the rate limiting step; instead, hydrolysis could be the step that governs the overall process for cellulose conversion to methane in this reactor.

7.2. Thermo-meso Two-stage Process

Thirteen experimental results with varying hydraulic retention times (HRTs, 13 to 30 days) and cellulose concentrations (7.5 to 60 g COD/L) demonstrate that:

- The methanogenesis occurred simultaneously in both the thermophilic and mesophilic reactors. Methane production of the thermophilic and mesophilic reactors were from 337 to 3,354 and from 46 to 437 mL/L reactor per day, respectively. The thermophilic reactor had 7 to 27 times higher methane production rate than the mesophilic reactor. This suggests that anaerobic mineralization of cellulose by thermo-meso process was overruled mainly by the ability of thermophilic reactor, whereas the mesophilic reactor played a role on polishing the residual organic matters and volatile acid.

- Organic loading rate (OLR) of 18.3 g cellulose-chemical oxygen demand (COD)/L reactor per day in conjunction with 3-day HRT and 60 g COD/L feed cellulose was a threshold loading limit for the first-stage thermophilic reactor.
- Vial tests on methane converting capacity showed that the MCCs resulted from the thermophilic bacteria consuming acetate, iso-butyrate, and cellulose were apparently greater than those of the microorganisms retained in the mesophilic reactor. No significant stimulation on the mesophilic maximum methane production rate was obtained when the mesophilic sludges supplemented with the thermophilic sludge.

7.3. Comparison of the Thermo-meso with Acid-methane Processes

Feed cellulose concentration and hydraulic retention time were varied with the aim of delineating similarities and differences between the thermo-meso (TPAD) and mesophilic acid-methane anaerobic digestion systems (two-phase) for cellulose degradation. Based on the results obtained and discussions, our results showed that:

- Both thermo-meso (TPAD) and acid-methane systems (two-phase system) were successfully for the anaerobic degradation of cellulose at concentrations up to 60 g/L with system HRT of 13 to 30 days.
- Methane production rate and methane yield of the thermo-meso system was in the range of 148 – 1,100 mL/L reactor/d and 0.23 – 0.33 L/g COD fed when the organic loading rates were 466 to 4,000 mg/L reactor/d. These values obtained were higher than the acid-methane system with similar loading rates, which were 71 – 776 mL/L/d and 0.16 – 0.27 L/g COD, respectively.
- Thermophilic reactor, the dominate unit for solids destruction and methane production in the thermo-meso system, possessed 2 – 4 times higher methane production rate than the dominating reactor (methanogenic reactor) in acid-methane system. Batch methane converting capacity (MCC) test further confirmed that the activity of acetate-utilizing methanogens in the thermophilic reactor were 45 - 50% higher than those in the methanogenic reactor. Microorganisms developed in the thermophilic reactor also demonstrated a 1.3 – 1.9 times higher ability in terms of directly converting cellulose into methane.

- First-order hydrolysis rate of the thermophilic reactor was 0.79±0.22 day^{-1}. It was greater than the hydrolysis rate obtained in the acidogenic reactor (0.26±0.2 day^{-1}), where most cellulose hydrolysis was supposed to occur in acid-methane system.
- With the methane generation model obtained, thermo-meso system demonstrates higher methane generation potential in treating high solids at a shorter hydraulic retention time than the acid-methane system.

7.4. Application of the Thermo-meso Two-stage Process

The last Chapter is a paper that summarized the results from laboratory and full scale operation of the thermo-meso system for treating primary, secondary and municipal solid wastes. The collected data of volatile solids destructions and methane productions was analyzed to provide important guidelines on thermo-meso operating parameters such as feed strength, hydraulic retention time and organic loading rates for digester upgrading or new thermo-meso process designing. Based on the results, a ten to thirty days system HRT and solid loading rate of 4.5 g VS/L_reactor/day were suggested. Thermo-meso system achieved more than 38% VS destruction when the feed solids concentration was 8 to 58 g VS/L. Higher VS reduction can be obtained if the process was fed with a volatile solid concentration of 32 to 42 g VS/L. Less than 10 days HRT was suggested for the thermophilic reactor.

7.5. Summary

This research demonstrated that both the thermo-meso two-stage process and mesophilic acid-methane two-stage process have considerable potentials on mineralization of particulate cellulose at concentrations up to 60 gCOD/L with system hydraulic retention time of 13 to 30 days. Thermo-meso two-stage system possessed higher methaneproduction rate if treating high solids wastes with a shorter hydraulic retention time when compared with the acid-methane two-stage system.

APPENDIX (EXPERIMENTAL DATA)

Acid-methane Two-stage Anaerobic Digestion System

(Chapter 3 Table 2)

Run 1

Methane Production (mL/L/d)

Time	Acidogenic	Methanogenic
9	0	177
10	0	219
11	0	179
12	0	131
14	0	184
15	0	155
16	0	247
24	0	333
25	0	200
26	0	150
27	0	374
28	0	365
29	0	523
33	0	262
34	0	294
35	0	326
37	0	300
38	0	278
39	0	266
40	0	294
41	0	318
42	0	307
43	0	294
45	0	262
46	0	247
48	0	237
49	0	258
50	0	252
50	0	271
51	0	267
52	0	278
53	0	299
55	0	309
56	0	326
56	0	339
58	0	310
58	0	304
59	0	279
60	0	291
61	0	297
63	0	295
64	0	305
65	0	296
66	0	307
66	0	313
68	0	303
69	0	289
71	0	309
72	0	305
73	0	277
73	0	263
75	0	290
76	0	302
76	0	300
77	0	304
78	0	305
79	0	302
80	0	311
81	0	321
82	0	303
84	0	309

COD (mg/L)

Time	Acidogenic		Methanogenic	
(days)	COD	SCOD	COD	SCOD
24	---	700	---	---
37	---	659	4537	1998
51	---	3558	4326	---
56	---	---	4216	---
60	---	---	4133	---
76	22981	8695	4531	1124
77	23143	8788	4682	1341
80	23021	8433	4753	892
82	22436	8564	4567	1367

VFA (mg/L)

Time	Acidogenic	Methanogenic
0	2314	---
7	2357	---
18	3411	---
24	343	---
27	917	---
30	429	---
35	626	---
41	1763	---
51	3066	---
76	5371	343
77	5601	366
80	5550	330
82	5208	320

Solids (mg/L)

Time	Acidogenic		Methanogenic	
(days)	SS	VSS	SS	VSS
24	16520	16070	14120	4130
37	16260	---	5740	---
76	9120	8420	6900	4012
77	10200	8080	6870	4217
80	9650	8240	7400	4156
82	9550	7560	7310	4098

Biogas Composition (%)

Time	Methanogenic	
(days)	CH_4	CO_2
9	79.72	20.28
10	58.40	41.60
18	45.22	54.78
24	48.97	51.03
27	56.13	43.87
35	56.11	43.89
37	56.28	43.72
40	65.04	34.96
43	65.46	34.54
48	69.58	30.42
51	71.33	28.67
56	76.31	23.69
60	75.68	24.32
66	76.16	23.84
72	75.65	24.35
76	74.91	25.09
77	75.17	24.83
80	75.50	24.50
82	75.33	24.67

Run 2

VFA (mg/L)

Time	Acidogenic
0	2712.9
3	2371.4
24	1680.0
38	3154.3
50	2168.6

Biogas (%)

time	Acidogenic		Methanogenic	
(days)	CH_4	CO_2	CH_4	CO_2
0	91.55	2.08		
2	79.30	13.96		
3	71.54	21.22		
5	47.75	47.21		
7	57.51	36.86		
8	58.94	31.82		
10	54.38	40.16		
11	58.21	31.72		
14	51.05	42.94	81.83	20.51
17	54.46	38.42	81.81	21.23
19	50.90	42.94	82.06	20.71
21	50.27	43.48	81.27	20.49
23	12.28	22.40	82.01	25.23
26	84.25	9.16	80.94	19.48
27	75.59	18.50	81.03	20.57
29	14.74	7.56	80.56	21.06
31	79.92	10.28	81.88	20.26
32	77.12	11.95	81.12	19.33
34	65.48	21.48	81.07	20.96
36	67.18	18.36	81.11	20.53
38	64.41	19.26	82.20	20.47
40	49.14	39.96	82.22	17.25
42	48.29	36.88	81.80	21.00
44	49.28	35.11	80.46	19.10
46	53.35	27.34	79.83	19.06
53	50.97	38.13		

Solids (mg/L)

time	Acidogenic		Methanogenic	
(days)	SS	VSS	SS	VSS
8	2330	1900		
11	3010	---		
19	2410	---	10990	---
32	1880	1540	5400	2580
36	1924	1692	3392	1760
38	1630	1492	2972	1736
39	1536	1448	3456	1680
42	1556	1476	2752	1392
43	1384	1312	2260	1296
45	1396	1384	2424	1268
46	1664	1476	3072	1468

Methane Production (mL/L/d)

Time	Acidogenic	Time	Methanogenic
11	88		
14	104	14	283
17	97	17	199
19	112	18	167
21	79	19	176
27	61	21	129
32	48	23	147
34	71	24	137
35	40	26	91
36	52	27	127
37	41	28	87
38	39	29	122
41	41	30	116
42	53	31	104
43	14	32	102
44	31	34	126
45	44	35	121
46	52	36	123
49	126	37	116
50	98	38	112
53	77	39	63
		40	58
		41	89
		42	95
		43	92
		44	103
		45	56
		46	56
		49	48
		50	53
		53	99
		54	99

COD (mg/L)

Time	Acidogenic		Time	Methaogenic	
	COD	SCOD		COD	SCOD
5	8095.8	3099.4	19	8288.4	1243.9
8	5575.1	3610.8	32	3478.4	1404.5
11	6117.9	2759.3	36	2801.2	872.3
19	5330.2	2930.2	38	1327.4	418.9
25	---	1866.3	39	2462.2	446.7
32	7152.4	3305.3	42	2961.1	1307.3
36	5243.6	3691.0	43	3006.2	1503.1
38	5622.7	3856.2	45	3043.5	1627.3
39	5623.3	3552.6	46	3289.4	1649.7
42	5907.7	3777.1			
43	5443.7	3896.9			
45	5132.9	3557.8			
46	5386.3	3639.8			
53		2913.8			

Run 3

Solids (mg/L)

Time	Acidogenic		Methanogenic	
	SS	VSS	SS	VSS
16	2790	2640		
17	2940	2685		
18	2200	2120		
34	---	---	3480	1693
36	2590	2495	1875	1250
37	2385	2280	1915	1250
38	2300	2210	1745	1120
39	2060	1947	1715	1080
53	2340	2130	1870	1160
56	2540	2240	1920	1090
59	2410	2180	1990	1220

VFA (mg/L)

Time	Acidogenic	Methanogenic
12	3634.3	---
35	12977.1	---
36	4474.3	77.1
37	4761.4	51.4
38	---	30.0
39	---	30.0
53	4644.3	91.4
56	4508.6	82.9
59	4807.7	112.9

Methane Production (ml/L/d)

time	Acidogenic	time	Methanogenic
2	106	1	120
4	94	2	131
6	82	3	160
16	226	4	183
17	187	6	196
18	170	7	221
20	198	8	233
21	168	9	263
23	228	13	252
24	70	14	233
25	171	15	320
27	227	16	285
29	253	17	266
30	291	18	272
31	223	20	304
32	154	21	317
34	272	23	310
35	95	24	282
36	56	25	301
37	60	27	296
38	54	28	306
39	46	29	288
40	32	30	291
41	80	31	291
43	47	32	286
44	52	34	246
45	64	35	267
46	63	36	305
49	39	37	298
51	18	38	307
52	38	39	307
53	36	40	301
54	23	41	285
55	29	43	305
56	37	44	292
57	41	45	282
58	31	46	291
59	42	49	273
		51	277
		52	269
		53	286
		54	282
		55	297
		56	298
		57	290
		58	283
		59	291

Biogas (%)

time	Acidogenic		Methanogenic	
(days)	CH_4	CO_2	CH_4	CO_2
0	44.04	51.72	59.36	38.07
2	50.17	40.78	58.85	36.95
7	45.89	49.02	66.91	29.69
9	49.05	46.88	69.05	27.91
11	40.39	58.66	71.47	25.30
14	43.50	52.90	71.50	25.90
18	43.57	52.56	68.07	29.31
21	40.50	56.80	70.30	26.60
24	38.80	59.00	68.13	29.67
29	38.50	59.05	67.35	30.17
32	39.15	58.17	69.14	28.72
37	38.81	59.01	69.71	27.87
40	39.43	58.48	69.79	28.20
44	38.78	58.77	68.27	29.08
49	39.02	58.74	68.33	29.75
53	39.40	58.02	66.86	31.45
56	40.24	57.36	69.02	29.45
59	40.95	56.47	67.64	30.78

COD (mg/L)

Time	Acidogenic		Methanogenic	
	COD	SCOD	COD	SCOD
8	8734.5	5765.0		
16	8376.4	5779.9		
17	8674.8	2825.3		
18	8615.1	407.9		
25	12276.1	5690.4		
36	9769.1	5923.6	1974.7	509.8
37	9619.9	6605.3	2079.2	383.0
38	9858.7	6675.8	1885.2	442.7
39	10067.6	6581.8	1721.0	375.5
53	11023.0	6423.0	2045.0	457.0
56	10674.0	6347.0	1996.0	534.0
59	10575.3	6588.0	2014.0	478.0

Run 4

COD (mg/L)

Time	Acidogenic		Methanogenic	
	COD	SCOD	COD	SCOD
41	16895.6	7236.5	3566.5	1647.0
44	18693.5	7964.2	3786.0	1578.0
48	17896.3	7976.5	3804.0	1469.0
53	17469.6	8568.7	3889.3	1842.5
56	18645.4	8016.4	3764.0	1865.6

Methane Production (mL/L/d)

Time	Acidogenic	Methanogenic
0	0.0	172.9
1	0.0	336.8
2	0.0	417.1
3	0.0	225.4
5	0.0	175.7
5	0.0	164.7
6	0.0	207.9
7	0.0	315.6
9	0.0	422.6
9	0.0	372.5
10	0.0	516.1
11	0.0	886.0
12	0.0	823.3
13	0.0	857.5
15	0.0	835.8
16	0.0	696.5
17	0.0	470.8
18	0.0	485.1
19	0.0	478.0
20	0.0	488.3
20	0.0	452.0
21	0.0	505.9
22	0.0	490.4
24	0.0	451.4
25	0.0	423.1
25	0.0	459.4
26	0.0	480.1
28	0.0	497.8
28	0.0	489.7
29	0.0	476.0
30	0.0	477.4
31	0.0	490.0
32	0.0	486.0
33	0.0	480.8
35	0.0	477.5
36	0.0	486.6
36	0.0	491.2
37	0.0	484.9
38	0.0	512.6
39	0.0	477.1
40	0.0	497.0
41	0.0	488.6
43	0.0	497.4
44	0.0	489.7
44	0.0	481.7
45	0.0	491.4
46	0.0	498.1
47	0.0	485.3
48	0.0	496.2
49	0.0	491.9
50	0.0	484.9
51	0.0	470.4
52	0.0	488.6
53	0.0	483.6
54	0.0	488.3
55	0.0	491.1
56	0.0	488.4

Biogas (%)

Time	Acidogenic		Methanogenic	
(days)	CH_4	CO_2	CH_4	CO_2
9	98.62	1.38	78.96	21.04
10	---	---	60.67	39.33
11	97.23	2.77	57.67	42.33
12	92.95	7.05	52.83	47.17
13	81.81	18.19	52.43	47.57
15	89.27	10.73	54.92	45.08
18	49.95	50.05	50.16	49.84
20	---	---	45.79	54.21
20	46.38	53.62	45.86	54.14
21	---	---	52.46	47.54
25	44.09	55.91	48.18	51.82
28	42.56	57.44	49.84	50.16
29	41.48	58.52	49.34	50.66
32	43.06	56.94	50.03	49.97
36	41.11	58.89	49.58	50.42
38	41.27	58.73	51.06	48.94
41	42.49	57.51	49.76	50.24
44	42.00	58.00	49.02	50.98
48	40.79	59.21	49.13	50.87
50	41.59	58.41	48.67	51.33
52	39.79	60.21	48.74	51.26
54	38.96	61.04	49.07	50.93
56	38.38	61.62	48.94	51.06

Solids (mg/L)

Time	Acidogenic		Methanogenic	
	TSS	TVSS	TSS	TVSS
41	5694	5456	3485	2084
44	5904	5674	3541	2108
48	5817	5568	3171	1902
53	5720	5469	3234	1930
56	6004	5745	3368	2013

VFA (mg/L)

Time	Acidogenic	Time	Methanogenic
0	12661.4		
1	11142.9		
2	10444.9		
5	7837.1		
7	4521.4		
13	151.9		
21	2620.4		
25	9224.3	22	1469.4
41	4887.1	41	1464.3
44	5608.6	44	1406.4
48	5537.1	48	1225.1
53	4978.6	53	1407.7
56	4810.0	56	1459.0

Run 5

Biogas (%)

Time	Acidogenic		Methanogenic	
	CH_4	CO_2	CH_4	CO_2
32	21.53	61.70	67.91	30.01
36	16.90	51.43	66.75	32.00
39	18.25	72.15	67.18	30.99
41	17.92	69.86	62.00	33.10
44	17.79	69.22	58.62	39.63
45	19.80	62.26	59.44	38.58
48	22.57	61.58	55.96	42.95
49	24.17	52.55	50.37	48.59
51	21.74	57.95	50.55	48.58
52	22.71	59.96	51.42	47.85
53	---	---	51.67	47.36
55	23.40	56.23	51.10	48.41
58	22.50	58.05	52.20	46.81
61	24.93	55.90	51.69	47.47
64	22.34	56.37	51.26	47.97
67	24.09	52.69	50.65	48.50
70	24.39	54.08	49.92	49.69
75	21.33	55.88	52.14	47.50

COD (mg/L)

Time	Acidogenic		Methanogenic	
	COD	SCOD	COD	SCOD
31		>8000		
39			3939.5	1910.05
40	15056.9	10883.1	5097.8	2521.61
42	18750.0	11288.9	6133.3	3437.04
52	23932.4	7109.0	6425.3	4137.18
58	23046.5	7084.5	6154.6	4285.30
61	22859.6	7193.3	6321.0	4036.90
64	22631.2	7068.5	6647.2	4138.65
67	23147.8	7021.0	6608.0	4032.00
70	22245.1	7210.3	6578.3	3967.50
75	24266.6	7125.4	6247.5	4012.94

VFA (mg/L)

Time	Acidogenic	Methanogenic
36	19482.9	1328.6
42	8871.4	2751.4
52	5657.1	2142.9
58	5732.9	2392.9
61	5565.7	2214.3
64	5685.7	2492.9
67	5885.7	2314.3
70	5758.6	2235.7
75	5745.7	2407.1

Solids (mg/L)

Time	Acidogenic		Methanogenic	
	TSS	TVSS	TSS	TVSS
52	14680	14330	6290	3620
58	14870	14320	6540	3750
61	15020	14540	6330	3870
64	14840	14030	6870	3900
67	15100	14570	6540	3660
70	14890	14300	6800	3540
75	14570	14120	6650	3470

Methane Production (mL/L/day)

Time	Acidogenic	Time	Methanogenic
0.0	0.0	1.0	442.1
1.0	0.0	2.0	696.4
2.0	0.0	4.0	639.4
4.0	0.0	5.0	335.0
5.0	0.0	6.1	456.0
6.0	0.0	8.0	939.1
8.0	0.0	9.0	870.0
9.0	0.0	10.1	873.7
10.1	0.0	11.0	788.7
11.0	0.0	12.2	744.7
12.2	0.0	14.4	667.4
13.0	0.0	15.0	683.0
14.4	0.0	15.9	666.1
15.0	0.0	19.1	610.9
15.9	0.0	21.1	609.2
19.1	0.0	23.4	606.1
21.1	0.0	24.4	377.3
23.4	0.0	25.9	542.4
24.4	0.0	26.9	639.5
25.9	0.0	32.1	503.8
26.9	0.0	34.0	573.5
28.0	0.0	35.0	527.5
31.1	0.0	36.0	466.7
32.1	36.9	37.0	453.3
33.1	0.0	38.0	430.1
34.0	0.0	39.0	297.7
35.0	0.0	40.0	333.8
36.0	23.9	41.0	300.7
37.0	0.0	42.0	348.1
38.0	0.0	43.0	345.6
39.0	34.6	44.0	384.7
40.0	0.0	45.0	415.6
41.0	31.2	46.0	456.2
42.0	0.0	46.9	518.3
43.0	0.0	48.0	558.9
44.0	4.3	48.9	574.9
45.0	2.4	50.0	671.4
46.0	0.0	51.0	649.0
46.9	0.0	52.0	645.6
48.0	0.0	53.0	687.3
48.9	0.0	54.0	661.9
50.0	0.0	55.0	649.7
51.0	0.0	56.3	652.6
52.0	0.0	57.2	648.6
53.0	0.0	58.2	655.9
54.0	0.0	59.3	648.8
55.0	0.0	60.2	678.0
56.3	0.0	61.2	654.9
57.2	0.0	62.3	639.9
58.2	0.0	63.2	650.8
59.3	0.0	64.2	643.1
60.2	0.0	65.2	649.1
61.2	0.0	66.4	639.8
62.3	0.0	67.4	631.0
63.2	0.0	68.5	640.1
64.2	0.0	69.5	636.5
65.2	0.0	70.1	626.4
66.4	0.0	72.1	657.0
67.4	0.0	74.5	654.9
68.5	0.0		
69.5	0.0		
70.1	0.0		
72.1	0.0		
74.5	0.0		

Run 6

COD (mg/L)

Time	Acidogenic		Methanogenic	
	COD	SCOD	COD	SCOD
10	11800.3	---	12313.0	---
24	10253.2	1821.1	7907.7	---
27	9018.6	670.8	5664.6	---
30	12000.0	1259.0	6518.5	1037.0
39	12192.7	---	7421.7	554.2
51	14387.3	4483.2	8089.6	1177.6
57	12099.2	4495.6	2504.1	570.3
59	15390.5	4228.2	2781.0	---
68	11853.0	---	---	---
76	12741.5	4125.6	2357.4	957.0
70	12470.6	---	---	---
72	11422.7	4334.0	2340.4	1064.5
74	13020.2	4536.2	2268.9	1123.0
78	11956.3	4457.8	2415.2	1006.0
80	12874.5	4365.5	2567.4	1201.0

VFA (mg/L)

Time	Acidogenic	Methanogenic
68	3194.3	308.6
71	3082.9	262.9
74	2837.1	231.4
76	2877.1	214.3
78	3392.9	148.6
80	3540.0	194.3

Methane Production (mL/L/d)

Time	Acidogenic	Methanogenic
5	0.0	117.6
7	0.0	360.3
8	0.0	257.0
9	0.0	205.0
10	0.0	188.2
24	0.0	171.2
25	0.0	119.8
26	0.0	158.8
27	0.0	145.9
30	0.0	83.3
34	0.0	73.0
36	0.0	108.4
37	0.0	208.4
38	0.0	188.3
39	0.0	100.0
43	0.0	---
44	0.0	66.7
50	0.0	96.2
51	0.0	141.0
53	0.0	179.1
54	0.0	169.0
56	0.0	203.6
57	0.0	213.6
58	0.0	188.3
59	0.0	168.2
60	0.0	187.9
61	0.0	194.3
63	0.0	175.0
67	0.0	180.3
68	0.0	159.5
69	0.0	162.9
70	0.0	211.2
71	0.0	171.3
72	0.0	159.4
73	0.0	184.2
74	0.0	159.4
75	0.0	173.3
76	0.0	154.2
77	0.0	173.8
78	0.0	160.9
79	0.0	159.0
80	0.0	158.2

Biogas (%)

Time	Acidogenic		Methanogenic	
	CH_4	CO_2	CH_4	CO_2
10	35.30	50.45	77.03	21.69
26	43.24	48.01	73.79	24.41
37	---	---	74.03	24.28
50	34.30	52.62	74.44	23.96
57	39.10	50.55	75.87	22.77
60	38.66	52.17	76.02	22.68
68	36.86	52.37	73.55	25.19
71	36.05	53.73	75.65	22.78
74	35.41	54.88	77.33	21.65
76	36.16	53.46	77.69	21.17
78	35.89	54.48	77.05	21.55
80	36.56	53.88	76.23	22.22

Solids (mg/L)

Time	Acidogenic		Methanogenic	
	SS	VSS	SS	VSS
0	8920	---	21380	---
10	11280	11250	15190	8530
27	9420	9330	10830	5450
59	7220	6950	5290	---
72	6970	6480	2780	1710
74	6550	6230	2940	1820
76	6710	6320	3020	1780
78	6900	6540	2890	1670
80	6400	6120	2950	1780

Run 7

COD (mg/L)

	Acidogenic		Methanogenic	
Time	COD	SCOD	COD	SCOD
2	40291.1	7463.3	12786.4	1681.2
8	45603.0	8224.3	11514.0	635.5
15	31209.2	10072.3	7542.2	689.8
24	37564.0	11623.2	7356.4	701.5
30	29766.1	12074.3	7170.3	761.6
38	38456.3	12540.2	6937.1	689.3
46	42150.3	11461.8	6324.2	850.2
49	37193.3	12087.1	5073.6	970.0
50	36034.6	17768.8	4077.1	413.8
51	32513.9	12917.5	5505.3	665.7

Methane Production (mL/L/d)

Time	Acidogenic	Methanogenic
1	---	429
2	14	470
3	26	448
4	44	481
5	56	467
6	84	500
7	62	486
8	23	495
9	52	551
10	31	560
11	29	529
12	33	565
13	60	667
14	8	664
15	32	521
16	8	515
21	12	175
22	17	241
23	13	403
24	14	521
25	13	399
26	---	512
27	21	504
28	---	444
29	46	486
30	28	668
31	22	670
32	24	696
33	24	642
34	19	657
35	17	663
36	16	694
37	20	669
38	20	677
39	17	669
40	18	666
41	17	656
42	13	633
43	12	662
44	17	697
45	8	686
46	11	668
47	14	688
48	5	713
49	3	661
50	5	647
51	5	676
52	---	688
53	---	694

Solids (mg/L)

	Acidogenic		Methanogenic	
Time	SS	VSS	SS	VSS
1	29622	27820	13416	7844
8	26500	25730	10260	6800
15	25840	24920	8770	5590
30	23045	22420	7330	4960
38	22340	21540	7190	4240
46	21265	20780	7320	4550
49	24900	24380	6980	4420
50	23645	23175	5960	3390
51	20205	19810	6050	3320

VFA (mg/L)

Time	Acidogenic	Methanogenic
1	7071.43	4542.86
6	5794.29	---
8	6574.29	77.14
12	7542.86	171.43
15	128.57	154.29
30	9368.57	145.71
33	9077.43	224.29
38	8177.14	177.14
46	8640.00	162.86
49	9197.14	85.71
50	4500.00	942.86
51	6300.00	3728.57

Biogas (%)

Time	Acidogenic		Methanogenic	
	CH_4	CO_2	CH_4	CO_2
4	31.73	48.25	56.77	42.06
6	28.56	58.58	54.05	45.32
8	22.31	64.80	54.71	44.74
10	20.60	70.57	58.37	40.91
12	14.09	81.23	59.62	40.38
14	13.66	79.41	56.21	43.79
22	10.99	75.68	60.57	37.78
25	9.30	79.17	53.80	45.86
29	12.72	71.05	53.31	46.69
33	9.81	81.82	52.80	46.35
38	9.45	81.58	55.29	44.30
42	9.69	80.75	54.12	45.31
45	7.40	82.26	56.29	42.67
48	3.57	88.71	57.84	41.21
49	3.13	88.81	55.33	44.16
50	2.69	89.08	54.85	45.15
51	2.62	88.38	53.43	45.62
53	2.80	70.57	56.87	42.22

Biogas (%)

Time	Acidogenic		Methanogenic	
	CH_4	CO_2	CH_4	CO_2
4	50.13	22.56	52.46	46.67
6	45.12	32.56	51.02	48.25
8	23.43	47.53	53.61	45.77
10	24.52	54.70	57.33	41.96
12	34.55	53.79	56.46	43.54
14	40.13	53.31	68.05	31.95
22	49.78	41.96	63.83	36.17
25	49.40	27.42	53.19	45.98
28	41.55	47.93	51.69	47.80
32	50.56	28.89	51.73	48.27
36	46.25	43.46	51.19	48.81
40	33.98	58.56	52.06	47.73
45	29.61	66.61	51.10	47.85
48	39.67	48.40	53.61	45.64
49	40.53	42.04	50.85	48.62
50	40.52	36.82	50.75	48.56
51	43.79	29.51	48.65	50.50
53	47.57	46.24	45.83	53.24

Run 8

COD (mg/L)

Time	Acidogenic		Methanogenic	
	COD	SCOD	COD	SCOD
2	51630.0	7024.3	11140.1	1462.0
8	48868.4	20299.1	5084.1	1158.9
15	42313.8	15060.2	11409.6	1927.7
30	57182.3	15306.5	9956.7	2210.5
34	56987.2	12543.2	10024.6	2154.7
39	59031.4	11457.6	11052.1	2253.6
42	52143.5	10472.1	9875.5	2312.3
46	53645.8	12269.1	10140.7	2446.5
49	48205.3	8132.6	8431.1	2070.5
50	40213.6	6121.7	6998.0	1673.4
51	58308.2	5325.3	8419.8	1943.0

Solids (mg/L)

Time	Acidogenic		Methanogenic	
	SS	VSS	SS	VSS
1	37612	35988	11175	7650
8	24630	22050	4290	3170
15	29990	27430	13590	8900
30	41060	39400	10700	7140
34	44210	42180	10560	6940
39	43500	42010	10320	7010
42	43650	41970	9760	6950
46	33815	32585	10410	6770
49	43235	41990	10020	6940
50	43975	42715	8290	5330
51	46180	44410	8170	5260

Methane Production (mL/L/d)

Time	Acidogenic	Methanogenic
1	0.0	662.1
2	3.0	814.9
3	0.0	771.3
4	0.0	958.3
5	0.0	970.2
6	0.0	1099.1
7	0.0	1076.9
8	0.0	833.7
9	0.0	742.2
10	0.0	768.8
11	0.0	756.7
12	0.0	891.6
13	0.0	1275.2
14	0.0	1154.8
15	0.0	596.6
16	0.0	858.6
21	0.0	109.1
22	0.0	646.6
23	0.0	625.7
24	0.0	727.6
25	0.0	827.8
26	0.0	898.4
27	0.0	974.9
28	0.0	1011.1
29	0.0	925.4
30	0.0	940.8
31	0.0	979.2
32	0.0	969.2
33	0.0	969.1
34	0.0	981.4
35	0.0	979.5
36	0.0	966.7
37	0.0	962.2
38	0.0	986.0
39	0.0	988.5
40	0.0	1005.0
41	0.0	970.6
42	0.0	986.0
43	0.0	943.0
44	0.0	1012.7
45	0.0	972.0
46	0.0	955.0
47	0.0	953.7
48	0.0	1017.2
49	0.0	988.6
50	0.0	982.7
51	0.0	947.6
52	0.0	947.4
53	0.0	865.4

VFA (mg/L)

Time	Acidogenic	Methanogenic
2	4920.0	120.0
6	11048.6	---
8	14502.9	128.6
15	11922.9	8734.3
30	4560.0	462.9
36	7177.1	210.0
46	7620.0	771.4
49	5485.7	154.3
50	9514.3	171.4
51	8297.1	257.1

Chyi Data (Chyi, 1992)

Feed (mg/L)	HRT (days)	Cellulose OLR (mg/Lreactor/day)	SCOD (mg/L)	VFA (mg/L)
14588	3	4863	4363	3154
24267	10	2427	8561	5415
6833	5	1367	3611	2661
11479	5	2296	6537	4681
19170	5	3834	7952	5164
24596	5	4919	7116	5719
39074	3	13025	13355	7649
55379	3	18460	9475	7619
7946	2	3973	3506	2153
4316	2	2158	1674	1671
6324	2	3162	2538	2468
7946	2	3973	3506	3122
12230	2	6115	5390	5267
16382	2	8191	7022	6939
8125	1	8125	1406	1561
8159	1.5	5439	2669	1740
8260	2.5	3304	4010	2451
8316	3	2772	4491	3037

Thermo-meso Two—stage Anaerobic Digestion System

(Chapter 4 Table 2)

Run 1

Methane Production (mL/L/d)

Time	Thermophilic	Time	Mesophilic
10	25.8	10	214.6
26	62.3	26	39.5
33	433.1	36	167.6
35	343.7	37	178.7
36	174.2	39	97.6
37	1121.5	41	145.9
38	817.5	50	162.7
39	313.6	51	149.8
41	204.5	54	156.0
50	29.7	58	170.3
54	9.0	59	272.6
56	730.0	68	69.1
57	199.3	69	63.2
58	548.5	70	68.7
59	458.5	71	70.3
61	917.7	72	54.7
62	406.7	73	64.5
68	557.0	74	42.4
69	550.7	75	74.6
70	516.3	76	48.0
72	350.1	77	64.0
73	368.0	80	52.8
74	921.1	81	109.3
75	580.2	82	75.9
76	579.7	83	27.2
77	383.0	85	55.9
78	306.0	87	52.1
79	684.5	89	48.7
81	433.8	91	42.5
82	435.2	94	43.0
83	460.8	95	73.6
85	464.6	98	57.1
87	485.5	99	63.4
89	462.7	101	53.8
91	503.1	103	54.6
94	504.4		
95	458.2		
98	468.9		
99	567.4		
101	507.3		
103	502.9		

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
10	46.06	36.90	72.23	24.76
28	53.40	21.99	78.50	10.12
39	45.20	49.68	68.39	25.62
57	42.00	57.60	66.70	32.50
59	46.31	46.72	67.23	30.48
71	45.38	51.48	60.84	20.14
72	42.23	51.69	69.89	20.13
74	44.45	52.91	70.55	20.05
78	43.86	52.74	71.34	13.53
79	44.86	54.69	73.07	13.94
80	48.10	48.70	77.60	17.00
81	43.25	53.22	75.79	19.56
82	43.97	51.00	72.45	20.41
85	45.23	52.32	70.38	22.34
89	48.14	50.71	71.65	22.07
94	47.17	49.18	72.45	21.92
95	44.37	52.43	71.66	21.60
98	46.87	51.16	71.64	20.69
101	46.02	52.87		

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	SS	VSS	SS	VSS
0	26190	24710	22930	14030
33	12970	9480	20730	6830
59	14250	3790	12050	4130
72	7120	3000	7340	3090
91	4980	3540	6570	2340
99	5650	3520	6900	2400
103	5102	3610	6847	2450

VFA (mg/L)

Time	Thermophilic	Mesophilic
10	3955.14	342.86
33	3765.29	269.39
39	2755.14	244.90
68	857.14	220.41
82	2760.57	409.14
91	2679.47	621.71
99	2720.43	425.09
103	2791.86	408.06

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
10	16188.82	---	14698.14	---
33	17407.00	5481.50	9333.00	3851.70
39	8433.70	3807.21	15156.55	192.77
51	8231.40	2727.27	4661.16	1016.53
54	14387.21	2560.01	2636.81	563.25
57	6446.28	4214.88	4289.26	1165.29
58	3161.91	2247.62	4647.62	1142.86
59	7123.81	3847.62	5257.14	761.90
68	3647.06	---	---	---
70	1611.76	94.12	---	---
72	4694.85	---	4008.25	---
82	7023.12	3796.20	4103.50	541.23
91	7346.80	3659.24	4231.74	580.42
99	7104.70	3673.12	4023.47	550.22
103	7206.80	3714.20	4132.65	509.87

pH

Time	Thermophilic	Time	Mesophilic
0	7.11	0	7.41
10	7.11	10	7.40
23	7.23	33	7.46
33	6.90	35	7.18
36	7.22	36	7.22
38	7.16	39	7.28
39	7.00	51	7.24
41	6.39	54	7.12
50	7.00	56	7.08
51	7.17	57	7.14
54	6.48	58	7.12
56	6.68	59	7.11
57	6.70	68	7.16
58	6.61	70	7.14
59	6.73	71	7.10
68	6.97	72	7.12
70	7.10	73	7.11
72	7.37	74	7.17
73	7.18	75	7.08
74	7.12	76	7.07
75	7.10	77	7.06
76	6.90	78	7.08
77	6.95	79	7.15
78	6.92	81	7.26
79	6.94	82	7.14
81	7.13	85	7.08
82	7.34	87	7.10
83	6.95	89	7.05
85	6.99	91	7.04
87	6.89	94	7.06
89	6.92	95	7.06
91	6.98	99	7.13
94	6.94	101	7.13
95	6.86		
98	6.95		
99	7.04		
101	6.92		

Run 2

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
16	24.54	70.35	59.20	38.20
24	59.20	39.81	63.98	32.65
25	65.03	34.34	65.54	30.70
28	62.01	38.40	66.20	29.80
33	49.00	50.60	68.30	30.50
36	47.96	48.36	69.01	28.67
39	45.42	50.01	70.77	25.52
42	48.84	49.57	73.16	21.24
48	48.67	51.02	70.20	24.60
52	44.28	52.82	75.83	14.61
56	43.20	56.03	71.30	19.80
58	49.60	50.00	71.00	20.60
61	48.56	47.60	73.50	21.40
63	48.36	49.54	71.90	22.00
64	48.01	49.30	71.01	20.79
66	46.51	51.20	72.41	21.52
69	47.69	50.50	71.87	22.67
71	48.25	49.56	72.31	20.74
73	47.55	50.74	70.96	21.20

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
39	4430	3160	7870	6330
52	3520	2810	4320	1700
60	4620	3340	3980	2670
61	4010	3690	3200	2780
62	5020	4120	4310	3070
63	4870	3560	4020	3680
68	5102	3725	4125	3125
72	5021	3625	4031	3099

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
16	485.5	160.0
21	423.0	240.3
22	196.9	114.4
23	498.8	149.1
24	287.0	42.1
25	708.0	61.5
26	920.1	78.9
27	636.2	68.2
28	151.2	94.7
29	67.6	114.8
30	1103.6	149.4
31	125.2	123.3
32	171.6	115.8
33	1155.4	167.8
34	404.5	195.2
35	421.1	152.5
36	408.0	143.6
37	990.3	128.7
39	602.0	112.2
40	152.5	67.6
41	987.0	51.8
42	566.8	106.7
43	522.9	42.4
44	418.5	34.9
48	600.2	32.5
49	884.3	43.6
50	584.5	43.8
52	363.9	49.9
54	576.7	43.5
56	772.2	46.2
57	696.1	46.4
58	758.1	44.3
59	598.6	44.0
60	662.9	47.1
61	702.7	50.8
62	683.6	46.0
63	589.4	45.7
64	635.6	45.0
65	632.4	47.4
66	650.4	40.7
67	612.3	48.9
68	658.5	46.4
69	668.3	42.5
70	652.3	44.8
71	647.5	45.3
72	632.1	44.1
73	647.6	45.9
74	662.9	44.4

pH

Time	Thermophilic	Time	Mesophilic
16	5.47		
21	6.77	21	6.89
22	6.66	24	6.94
23	6.89	25	6.97
24	6.81	27	7.39
25	7.23	32	6.93
27	7.87	33	7.06
28	7.26	40	7.23
29	7.26	42	7.25
30	6.43	43	7.21
31	7.00	44	7.18
32	7.05	52	7.16
33	6.94	54	7.23
34	6.84	62	7.18
37	6.76	67	7.21
39	6.72	72	7.20
40	6.71		
42	6.66		
43	6.60		
44	6.35		
48	6.87		
49	7.04		
50	6.80		
52	6.77		
54	6.96		
56	6.88		
62	6.85		
67	6.97		
72	6.93		

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
52		4456.1	2612.2	922.0
60	4908.8	2028.8	2702.7	1936.6
61	5103.6	2352.3	2823.3	2014.6
62	5230.5	2259.7	2956.4	1864.8
63	5249.6	2169.1	2576.4	1998.5
68	5306.7	2241.3	2781.3	1935.7
72	5119.8	2145.6	2806.8	1945.2

VFA (mg/L)

Time	Thermophilic
60	885.71
61	985.71
62	940.00
63	1014.29
68	952.86
72	961.43

Run 3

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH4	CO2	CH4	CO2
0	59.55	36.15	73.99	19.86
2	54.74	42.40	77.06	15.17
3	55.11	40.74	75.65	17.59
6	54.04	41.98	76.34	16.44
7	58.94	31.82	---	---
9	56.45	38.75	78.95	11.32
11	55.08	40.46	77.32	13.18
13	54.87	40.74	77.09	14.82
15	51.86	43.70	75.35	14.52
16	55.11	40.30	80.10	7.42
19	54.56	40.93	74.64	7.06
20	51.64	45.47	77.82	8.95
22	53.02	43.87	77.14	12.79
24	53.23	42.48	75.52	15.79
26	54.44	41.83	76.07	12.26
27	53.98	42.19	76.23	11.15
29	52.45	43.39	73.76	15.55
31	51.26	43.00	84.22	6.67
48	50.82	44.78	75.11	15.38
51	50.65	45.67	74.92	15.93
53	47.61	49.06	71.80	23.48
65	47.44	49.02	70.85	24.25
67	41.27	56.29	72.14	22.86
72	54.16	42.13	75.83	17.87
74	52.50	43.06	75.13	17.18
77	52.13	44.47	75.18	17.80
79	55.84	42.37	70.60	23.90
89	48.00	48.50	73.13	21.78

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
3	4605.1	1165.5	1779.2	694.6
11	2455.8	837.2	3781.4	600.0
20	2876.7	981.9	3977.4	839.2
27	2894.0	1146.1	2425.1	762.4
31	2896.0	1051.7	2203.1	573.2
52	2559.2	1208.7	1447.5	470.1
54	2865.1	1238.5	1425.1	440.2
65	2716.0	1145.6	1334.6	438.7
74	2904.9	1128.9	1323.1	452.9
86	2684.2	1034.7	1314.5	441.2
96	2367.7	621.8	1129.1	278.5

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
3	4590	3450	1880	1200
11	2395	1725	6320	2900
27	2665	2310	6380	1720
31	2820	1736	3600	1460
52	2416	1448	2796	996
54	2144	1344	2112	972
74	2216	1712	2440	2435
86	2348	1437	2390	1013
92	2120	1570	2217	976
95	1747	1247	2101	1005

VFA (mg/L)

Time	Thermophilic	Mesophilic
18	128.6	42.9
52	128.6	171.6
55	435.1	168.5
66	807.4	---
77	514.3	171.4
89	439.7	169.6
96	368.6	176.9

Methane Production (mL/L/d)

Time	Thermophilic	Time	Mesophilic
0	334.5	0	41.7
1	376.6	1	33.3
2	355.2	2	34.7
3	437.0	3	51.1
5	373.3	5	28.2
6	371.7	6	22.9
8	372.4	8	20.0
9	334.0	9	23.7
10	368.4	10	17.3
11	312.7	11	40.6
12	303.3	12	17.3
13	308.2	13	34.7
15	319.3	15	45.3
16	305.2	21	11.6
17	386.9	22	29.3
18	334.1	24	58.6
19	210.9	25	58.2
20	412.6	27	8.6
21	482.3	29	46.9
22	482.8	30	50.8
24	241.4	31	12.6
25	380.5	45	22.5
26	378.8	48	22.6
27	365.6	49	42.2
28	---	50	41.6
29	286.7	51	26.3
30	312.4	52	43.3
31	287.9	53	27.0
32	257.3	63	18.1
45	274.2	69	35.4
48	221.6	71	30.9
49	327.7	72	32.9
50	317.9	73	44.5
51	315.2	74	50.1
52	316.4	75	50.0
53	316.0	77	50.0
54	313.4	78	63.9
55	307.6	86	90.6
56	328.5	88	69.3
63	449.8		
69	312.8		
71	328.9		
72	406.4		
73	328.0		
74	353.3		
75	346.7		
77	341.8		
78	376.3		
86	326.8		
88	296.8		
89	324.4		
92	315.0		
93	298.4		
95	320.1		
96	346.0		

pH

Time	Thermophilic	Time	Mesophilic
0	7.05	0	7.06
1	7.04	2	7.13
2	6.93	3	7.09
3	6.78	5	7.16
5	6.87	6	7.09
6	6.62	8	7.10
7	6.73	11	7.10
8	6.70	12	7.02
9	6.82	15	7.07
10	6.78	18	7.20
11	6.79	19	7.44
12	6.88	20	7.10
13	6.97	21	7.07
15	6.82	22	7.08
16	6.87	24	7.09
18	6.93	25	7.05
19	6.80	27	7.19
20	6.82	31	7.29
21	6.96	32	7.19
22	7.00	71	7.20
24	7.00	77	7.19
25	6.93	86	7.20
26	7.05	88	7.18
27	6.94	95	7.12
28	6.93		
31	7.04		
32	7.09		
45	7.05		
48	7.00		
51	6.90		
52	6.89		
55	7.05		
63	7.00		
66	7.05		
67	6.88		
71	7.02		
77	7.01		
86	6.99		
88	7.00		
95	6.98		

Run 4

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
2	677.8	48.8
5	819.3	37.4
8	784.7	50.8
11	730.9	59.1
17	738.2	50.3
21	682.6	67.7
27	932.4	70.0
28	838.2	134.3
33	224.9	123.4
34	523.2	78.1
35	679.4	102.5
36	696.6	102.5
37	835.4	107.9
38	706.3	97.6
39	797.6	94.8
40	779.4	96.6
42	726.7	87.0
43	765.8	85.3
44	594.1	67.0
46	733.6	80.1
47	726.7	79.7
48	753.0	122.0
49	780.9	11.3
50	743.4	56.5
51	735.8	64.7
54	795.6	45.8
57	757.0	40.4
59	696.6	39.4
61	660.7	47.7
62	605.1	35.9
66	684.4	67.0
67	673.2	65.4
68	627.9	63.5
69	615.5	65.6
70	676.2	64.3
71	699.9	92.1
72	676.1	160.8
73	667.2	97.6
74	669.9	79.8
75	882.3	58.6
76	606.0	37.8
77	730.4	44.2
78	756.7	56.8
79	728.4	50.8
80	725.0	47.8
81	716.0	48.7
82	711.2	49.2
83	671.6	45.1

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
8	53.12	45.42	68.90	30.20
11	54.81	44.30	67.46	31.52
14	53.79	45.67	68.34	31.45
17	57.21	41.63	71.54	28.75
21	55.52	43.76	72.42	26.57
27	53.12	44.41	70.31	28.15
31	54.74	43.25	68.79	30.47
34	51.64	44.15	63.17	33.48
37	52.37	45.76	64.59	34.71
39	45.61	50.94	60.16	36.53
42	50.49	47.64	62.48	37.14
44	52.61	47.08	67.89	30.12
48	51.97	46.10	64.17	34.54
51	52.31	44.57	65.03	32.86
57	51.63	44.90	66.07	31.90
62	52.54	45.69	65.38	32.46
66	52.31	45.10	64.72	33.12
70	55.29	41.55	69.06	25.73
74	49.68	49.08	62.53	34.31
77	52.63	45.95	65.39	30.20
79	49.87	48.58	65.52	30.02
82	53.57	43.23	65.46	29.44
83	52.00	45.67	66.75	27.51

VFA (mg/L)

Time	Thermophilic	Mesophilic
70	120.0	145.7
74	68.6	214.3
75	128.6	34.3
81	131.4	102.9
82	127.1	85.7
83	205.7	42.9

pH

Time	Thermophilic	Mesophilic
5	6.99	7.08
34	7.00	6.97
64	7.02	6.97
73	7.02	7.01
81	7.03	7.09

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
70	4050	3525	4475	2820
74	3460	3360	---	2417
75	3450	3201	4040	2357
78	4480	3190	3930	2060
80	3550	3050	3570	2080
83	3675	3121	3714	2563

Run 5

pH

Time	Thermophilic	Time	Mesophilic
0	7.11		
2	7.00		
4	6.89	2	7.63
5	6.88	5	7.41
6	7.21	7	7.54
7	6.90	8	7.52
8	6.88	9	7.52
9	7.00	10	7.43
10	7.00	11	7.42
11	6.98	20	7.65
18	6.98	23	7.19
19	6.88	24	7.68
20	6.99	25	7.09
22	7.04	34	7.31
23	7.04	35	7.20
24	7.06	36	7.29
25	7.06	38	7.25
26	7.08	42	7.18
27	7.08		
29	7.20		
31	7.12		
32	7.34		
33	7.09		
34	7.22		
35	7.24		
36	7.16		
38	7.09		
42	7.14		

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
2	49.20	49.62	58.61	37.32
5	47.47	51.59	59.15	34.38
7	47.80	50.66	61.70	19.62
9	47.88	52.12	63.33	29.79
11	47.94	52.06	62.45	32.92
19	43.61	56.39	50.33	3.51
22	48.25	---	54.28	45.72
29	49.78	49.13	61.77	21.00
32	48.83	50.32	62.08	26.10
33	47.15	52.03	61.74	30.38
34	45.78	52.77	61.62	29.15
35	48.31	45.74	60.94	33.03
36	46.26	52.15	56.77	8.82
39	47.62	50.40	61.23	31.65
42	47.90	51.34	60.40	30.50

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
7	10881.0	2816.7	8527.3	424.4
31	9919.5	3467.5	5164.1	1362.2
33	9886.0	3009.3	4775.1	1069.4
34	10685.6	3643.0	4223.1	1549.7
36	10119.0	3403.0	4801.4	1362.3
41	10216.9	3249.6	4703.5	1307.8

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
7	7680	6130	8520	5470
31	9730	7390	5710	3770
33	9260	7440	5320	3660
34	7710	5720	4840	3110
36	9014	6934	5203	3548

VFA (mg/L)

Time	Thermophilic	Mesophilic
7	1268.6	77.1
31	432.7	71.1
33	408.6	60.0
34	600.0	85.7
36	446.1	74.9

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
1	530.0	—
2	1759.3	—
3	1635.6	—
4	1725.4	—
5	1614.1	111.5
6	1787.5	82.0
7	1677.5	—
8	1371.0	42.8
9	1767.2	98.4
10	1580.6	2.7
11	1449.5	214.7
18	461.1	4.7
19	1353.6	2.2
20	1696.2	0.0
21	1468.3	0.0
22	3321.6	0.0
23	1500.2	0.0
24	1796.6	0.0
25	1756.3	24.9
26	2085.7	94.0
27	2100.1	39.6
28	1806.6	13.2
29	2088.1	12.4
30	1668.3	75.5
31	1702.1	33.3
32	1673.0	46.4
33	1634.4	77.3
34	1601.3	71.8
35	1733.1	71.0
35	1759.1	66.3
36	1687.9	65.6
37	1756.6	63.2
38	1721.4	60.8
39	1749.2	65.2
41	1707.7	64.3
42	1734.7	66.6
43	1678.7	55.5

Run 6

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
1	49.51	49.40		
2	47.52	51.90	61.09	34.29
5	47.42	51.79	62.18	34.66
7	48.09	51.15	63.81	31.78
9	46.82	53.18	63.11	32.33
11	49.32	50.68	63.12	32.65
14	48.94	50.58	62.89	32.99
17	49.22	50.01	62.21	34.22
20	46.65	52.76	62.78	34.13
24	48.41	51.08	62.58	33.24
27	49.56	50.44	63.27	32.55
30	48.53	51.27	60.58	33.66
33	49.13	50.00	61.95	33.01
37	47.27	51.78	61.11	33.81
43	35.87	61.79	62.36	32.21
46	44.53	54.55	60.23	36.23
53	47.70	46.13	53.18	44.94
56	40.72	50.26	60.71	36.96
57	19.44	77.39	58.14	41.86
58	49.16	46.32	60.39	38.84
59	44.51	49.57	62.72	35.89
61	40.00	58.23	63.86	33.14

VFA (mg/L)

Time	Thermophilic	Mesophilic
6	1320.0	68.6
37	111.7	76.4
39	110.9	77.7
41	109.3	76.7
57	11177.1	188.6
59	5571.4	85.7
60	8400.0	171.4

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
6	9810	7480	10720	6720
37	18256	15213	10230	7004
39	19001	15147	11011	6976
41	18463	14970	10645	6858
57	18570	15040	12350	8170
58	23340	18710	10220	6410
59	14350	11580	10080	6260

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
1	3035.1	---
2	2905.3	---
3	2893.5	---
4	2630.5	---
5	2712.5	277.2
6	2840.7	263.1
7	2871.8	257.0
8	2756.9	251.9
9	2834.1	278.7
10	2855.6	239.2
11	2585.2	242.4
12	3023.7	316.3
13	2865.8	336.2
14	2714.9	332.4
15	2887.2	308.2
16	3039.2	286.9
17	2886.1	301.1
18	2757.6	290.6
19	2795.5	336.6
20	2823.4	329.8
21	2930.4	310.0
22	2837.4	317.6
23	2729.7	299.3
24	3003.7	303.5
25	2747.4	294.1
26	2885.2	328.8
27	2929.5	332.6
28	2993.1	299.4
29	2867.9	304.4
30	2998.1	275.2
31	2857.0	286.0
32	2869.8	278.8
33	2887.8	325.9
34	2839.3	298.4
35	2892.9	326.7
36	3100.0	295.2
37	2917.8	303.8
38	2732.3	322.5
39	2863.9	297.9
40	2707.1	308.6
41	2805.6	284.8
42	133.3	129.0
43	1080.5	146.2
44	2640.9	493.1
45	3663.6	294.3
46	2498.6	482.6
47	2865.1	406.5
48	2806.6	389.2
49	1443.7	378.0
50	511.1	207.1
51	596.3	373.7
52	1594.1	573.1
54	2251.0	762.6
55	728.6	606.7
56	842.2	933.4
57	824.3	969.2
58	1011.1	1092.8
59	1919.5	959.6
60	1785.9	---
61	1808.4	547.5

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
6	12964.6	4656.0	10186.5	1176.9
37	22564.3	7364.0	9117.7	2005.7
39	21002.5	7536.0	9458.5	1875.3
41	21359.2	7456.1	9569.8	1813.0
57	---	---	10147.2	2074.2
58	21665.1	10064.5	8428.0	1718.5
59	21642.2	10158.4	9571.5	1899.9

pH

Time	Thermophilic	Time	Mesophilic
0	7.00	2	7.32
2	7.20	4	7.40
4	7.28	5	7.47
5	7.18	6	7.24
6	7.08	8	7.22
7	7.11	9	7.36
8	6.94	10	7.31
9	7.03	11	7.54
10	7.00	15	7.24
11	6.98	19	7.56
12	6.95	22	7.47
14	7.04	25	7.52
15	7.12	28	7.34
16	7.09	31	7.35
17	7.06	33	7.47
18	7.10	36	7.43
19	7.06	38	7.24
20	6.99	40	7.35
21	7.03	44	7.57
22	7.04	48	7.70
23	7.05	49	7.27
24	7.15	58	7.34
25	7.08	59	7.29
26	7.04		
27	7.12		
28	7.20		
29	6.98		
30	6.97		
31	7.06		
32	7.03		
33	7.32		
34	7.47		
36	7.28		
38	7.31		
40	7.01		
43	6.43		
44	6.70		
46	6.75		
47	7.10		
48	7.08		
49	7.22		
50	7.35		
51	6.98		
53	7.17		
55	5.13		
56	6.36		
57	6.28		
58	7.30		
59	6.79		
60	6.80		

Run 7

pH

Time	Thermophilic	Time	Mesophilic
23	6.57	23	7.05
24	7.05	45	7.32
29	6.62	47	7.21
30	6.45	50	7.27
32	6.23	60	7.24
35	7.09		
40	7.34		
44	7.21		
45	7.05		
46	7.03		
47	6.97		
48	6.84		
49	7.08		
50	7.11		
52	7.04		
54	7.00		
56	6.95		
60	6.89		

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
5	41.14	57.63	70.74	24.84
9	42.15	56.47	71.24	26.02
14	41.96	54.21	70.41	25.36
21	42.70	50.96	69.60	26.32
23	44.54	50.21	65.74	32.10
29	45.72	51.45	62.64	34.51
35	46.12	50.40	65.64	35.10
40	46.55	45.81	63.07	33.30
47	45.54	53.21	64.21	34.24
51	46.34	51.24	63.78	35.12
53	46.87	52.64	64.15	34.57
55	47.13	51.09	63.29	33.61
58	46.52	50.84	62.57	35.14

VFA (mg/L)

Time	Thermophilic	Mesophilic
51	832.6	111.4
53	785.1	123.5
55	1002.0	184.2
58	945.6	167.6

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
1	475.1	338.0
2	2432.8	299.2
4	316.1	306.1
5	718.3	367.6
7	563.8	246.4
9	1832.2	299.3
13	910.6	226.4
14	886.0	317.4
15	962.2	277.0
18	693.0	250.9
19	583.7	260.5
20	451.1	193.0
21	558.7	138.7
22	692.8	109.5
23	---	124.2
24	874.0	94.8
25	908.3	99.5
29	802.3	81.2
30	444.5	317.2
32	620.9	164.8
35	803.0	154.8
40	905.3	120.6
44	914.8	114.2
45	984.6	90.9
46	1045.6	80.3
47	966.9	84.5
48	973.5	81.7
49	1010.7	79.1
50	998.7	85.3
51	1030.7	80.8
52	982.2	83.8
53	1077.5	58.9
54	968.8	79.3
55	1024.3	95.9
56	1016.1	86.1
58	982.6	84.6
60	1001.0	96.5

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
51	5136.3	1456.1	2406.8	285.7
53	4935.6	1562.5	2245.6	301.4
55	5236.4	1478.9	2346.7	324.1
58	5047.3	1602.1	2084.6	315.6

Volatile Suspended Solids (mg/L)

Time	Thermophilic	Mesophilic
51	4471.2	2257.6
53	4020.0	2312.3
55	4321.3	2134.5
58	4126.0	2054.4

Run 8

Biogas (%)

Tiime	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
9	55.36	42.19	66.04	19.72
11	50.47	48.58	51.43	44.38
14	36.44	62.31	56.25	41.66
16	34.41	63.22	58.74	39.67
17	32.55	65.45	62.34	35.33
18	25.25	73.67	63.31	34.14
19	29.47	68.82	64.84	32.67
20	31.47	67.21	67.59	30.35
22	42.93	56.38	68.44	29.21
24	44.15	55.43	69.63	28.85
26	44.46	54.94	65.73	32.41
29	46.99	52.08	61.25	37.05
47	46.04	53.45	57.21	38.78
51	42.40	56.79	55.45	41.84
52	41.96	57.29	53.59	43.80
53	42.53	56.30	54.52	43.08
54	42.31	56.88	54.92	42.45

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
51	12876.3	5794.6	7452.3	4758.6
52	12901.1	6159.7	7323.2	4825.1
53	12833.0	5437.2	7733.6	5200.8
54	12765.5	4525.3	6652.9	3816.1

VFA (mg/L)

Time	Thermophilic	Mesophilic
51	2734.3	1952.9
52	2768.6	1500.0
53	2751.4	3000.0
54	1448.6	1782.9

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
1	1111.3	85.4
2	1442.7	116.8
3	1677.4	92.3
4	2080.1	142.4
5	1944.9	113.9
6	1851.1	140.1
7	2016.2	113.9
8	2882.8	108.2
9	1093.2	---
10	702.4	92.7
11	2424.0	289.0
12	996.8	350.3
13	1704.0	328.9
14	1282.8	441.2
15	769.3	432.4
16	525.7	715.0
17	613.7	511.7
18	1100.0	519.6
19	1155.3	540.8
20	980.8	681.4
21	1133.2	---
22	1855.4	539.3
23	2767.8	489.1
24	2330.8	493.0
25	2466.4	406.6
26	2443.1	351.0
27	2337.0	280.6
28	2294.0	275.9
29	1570.5	247.4
30	348.4	185.2
31	2883.4	---
32	1602.0	369.5
34	2359.3	190.6
35	2286.4	147.8
36	2182.4	134.1
37	2295.6	149.6
38	2506.7	144.4
39	2268.4	134.2
40	2074.1	129.0
41	2090.3	113.4
42	2153.6	---
43	2167.1	127.5
44	1956.2	117.5
45	2136.5	116.3
46	2283.8	114.7
47	2588.4	126.4
48	2339.2	138.5
50	2010.6	138.6
51	2200.7	168.8
52	2289.3	181.6
53	2131.4	201.2
54	1882.8	209.4

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	SS	VSS	SS	VSS
51	7840	6357	3391	2547
52	7693	6293	3500	2470
53	7987	6850	3230	2170
54	8340	7290	3370	2280

pH

Time	Thermophilic	Time	Mesophilic
1	7.20	1	7.25
2	7.20	10	7.30
3	7.07	11	7.40
4	7.13	12	7.30
5	7.10	17	7.26
8	7.27	18	7.22
9	7.28	22	7.64
10	7.17	32	7.26
11	7.11	44	7.58
12	7.33	45	7.29
13	6.04	52	7.39
14	6.14	53	7.11
15	6.27	54	7.17
16	6.21		
17	6.49		
18	5.83		
19	6.38		
20	6.68		
21	6.93		
22	7.22		
23	7.41		
24	7.37		
25	7.58		
26	7.60		
27	7.50		
29	7.60		
30	7.77		
31	7.00		
32	6.88		
34	7.21		
36	7.31		
38	7.42		
39	7.42		
40	7.47		
41	7.57		
42	7.41		
43	7.53		
44	7.56		
45	7.29		
47	7.23		
50	6.90		
52	7.08		
53	7.15		
54	7.17		

Run 9

pH

Time	Thermophilic	Time	Mesophilic
1	5.90	1	7.17
2	6.15	8	7.47
4	6.30	10	7.43
5	6.36	11	7.23
6	6.68	12	7.55
7	7.25	17	7.36
8	7.35	18	7.19
9	7.20	22	7.61
10	7.63	32	7.46
11	6.97	44	7.75
12	7.53	45	7.29
13	7.34	54	7.20
14	6.05		
15	6.43		
16	6.36		
17	6.21		
18	6.35		
19	6.87		
20	7.26		
21	7.50		
22	7.80		
23	7.48		
24	7.64		
25	7.63		
26	7.63		
27	7.23		
29	7.03		
30	7.38		
31	7.32		
32	7.34		
34	7.25		
36	7.37		
38	7.55		
39	7.42		
40	7.34		
41	7.75		
42	6.87		
43	7.19		
44	7.31		
45	7.27		
46	7.31		
47	6.93		
48	7.20		
50	6.80		
51	7.12		
52	6.21		
54	6.91		

Methane Production (mL/L/d)

Time	Thermophilic	Mesophilic
0	2148.1	284.8
1	1539.8	284.8
2	1941.3	301.8
4	972.5	379.7
5	972.5	370.2
6	1612.0	389.1
7	3838.1	379.7
8	3046.5	354.2
9	3925.2	56.5
10	785.6	558.2
11	2039.3	465.5
12	1539.8	567.7
13	648.3	149.5
14	2557.0	656.8
15	827.8	775.2
16	743.5	906.4
17	1419.6	874.4
18	1654.3	1181.0
19	2472.5	1065.8
20	2675.2	---
21	2176.4	624.8
22	1826.9	659.3
23	692.6	811.9
24	5136.9	831.9
25	1148.6	894.7
26	860.8	933.0
27	3120.0	920.1
28	5736.0	883.3
29	3733.2	689.7
30	3191.6	490.0
31	5610.6	404.1
32	3833.9	401.4
34	3214.3	402.2
35	3923.0	320.5
36	3512.4	351.0
37	3879.4	340.6
38	1460.6	345.6
39	683.1	324.8
40	6478.1	521.0
42	2603.3	529.1
43	5105.9	529.2
44	4452.9	472.7
45	2045.6	386.6
46	357.1	293.7
47	4611.8	387.8
48	1464.3	608.5
50	4977.4	678.6
51	1079.8	561.8
52	2401.3	481.2
53	5410.9	255.8
54	3319.2	585.1

Biogas (%)

Time	Thermophilic		Mesophilic	
	CH_4	CO_2	CH_4	CO_2
9	48.52	50.61	65.44	31.15
11	40.09	59.20	56.65	41.71
14	---	---	52.50	46.47
16	34.55	63.38	55.37	43.49
17	36.16	62.58	53.96	45.18
18	36.10	59.34	54.42	44.08
19	41.07	55.53	61.99	36.69
20	48.53	48.27	65.13	33.25
22	56.03	39.56	52.43	46.36
24	61.26	37.26	51.03	48.55
29	---	---	56.59	42.37
31	53.44	45.57	55.32	43.23
39	31.26	67.51	54.45	43.70
47	33.26	66.74	61.47	36.79
52	23.98	71.98	57.74	40.41
53	46.85	51.70	53.20	45.53
54	42.14	56.31		

VFA (mg/L)

Time	Thermophilic	Mesophilic
52	7431.4	685.7
53	3882.9	1508.6

COD (mg/L)

Time	Thermophilic		Mesophilic	
	COD	SCOD	COD	SCOD
52	26570.3	6707.2	3798.5	3288.5
53	19970.4	5470.9	3985.0	3701.6

Solids (mg/L)

Time	Thermophilic		Mesophilic	
	TSS	TVSS	TSS	TVSS
52	14770	12700	5640	4100
53	12200	9370	5810	4050

Run 10

Methane Production (mL/L/d)

Time	Thermophilic
1	723
2	411
3	922
4	954
5	901
6	983
7	1010
8	1109
10	985
11	933
12	925
13	931
14	932
15	888
17	806
18	970
19	791
20	754
21	665
24	701
25	691
26	923
27	781
29	697
30	1193
31	904
32	642
33	760
34	946
35	976
36	873
38	971
40	866
41	906
42	863
44	1037
45	873
46	922
47	940
48	930
49	973
50	932
51	952
52	927
53	908
54	936
55	879
56	913
57	962
58	959
59	952
60	945

pH

Time	Thermophilic
1	7.64
3	6.91
4	7.00
5	7.27
6	7.17
7	7.30
8	7.21
17	6.74
18	6.77
19	6.80
20	6.70
21	7.12
24	7.03
25	7.12
26	7.00
27	6.95
32	7.13
33	7.13
34	7.00
36	7.32
38	7.32
40	7.16
46	7.12
49	7.08
52	7.06
55	7.11
58	7.06

VFA (mg/L)

Time	Thermophilic
41	317.1
46	308.6
47	411.4
48	1131.4
55	434.3
57	401.4

Biogas (%)

Time	Thermophilic Reactor	
	CH_4	CO_2
1	56.73	29.67
3	48.37	49.55
5	46.29	51.99
17	44.18	53.98
19	48.43	50.27
24	52.38	44.50
26	49.76	47.94
29	48.24	50.02
31	48.98	48.98
35	46.91	51.38
38	46.00	52.50
41	48.20	50.00
44	48.40	49.50
46	47.20	51.10
48	48.23	50.13
49	48.75	49.67
52	48.42	50.05
55	47.36	50.91
57	49.59	48.77
59	48.69	49.79

COD (mg/L)

Time	COD	SCOD
18	4500.00	2178.65
19	4615.00	2521.87
34	4041.45	1554.40
36	4265.29	1386.53
38	4377.20	1274.61
40	4414.51	1442.49
41	4961.47	1717.43
46	5401.84	1790.83
47	5754.13	2011.01
48	5577.98	2077.06
55	5641.97	1845.08
57	5817.67	2008.40

Solids (mg/L)

Time	TSS	VSS
41	4030	2930
44	3980	2490
46	4100	---
47	4000	2920
48	3790	2770
55	3870	2840
57	3910	2900

Run 11

pH

Time	Thermophilic
51	5.99
52	7.17
53	6.35
55	6.30
57	6.89
60	6.67
64	6.56
67	7.22
83	7.95
85	7.84
86	6.76
87	6.77
88	6.84
89	6.71
90	6.76
91	6.56
92	6.54
94	6.24
95	6.36
96	6.52
97	6.19
99	6.60
100	6.70
101	6.91
102	7.05

COD (mg/L)

Time	Thermophilic	
	COD	SCOD
90	11552.3	8259.6
94	12893.5	7286.9
95	13079.6	8150.6
96	11107.7	5590.3
100	9432.5	7775.4
101	14822.6	10622.6
102	12673.8	8321.7

VFA (mg/L)

Time	Thermophilic
92	4329
95	15086
96	4440
101	5443
102	3780

Methane Production (mL/L/d)

Time	Thermophilic
2	1297.0
3	1717.8
4	1571.7
5	1531.1
6	1473.4
7	1462.8
9	1427.4
11	1362.6
12	1395.2
13	1564.2
14	1449.3
17	1439.5
19	1354.1
22	1359.7
44	1019.3
45	993.6
49	594.9
50	181.2
51	192.1
52	1039.4
53	997.5
54	1042.4
55	481.9
59	521.4
60	1032.8
61	1006.7
63	752.3
64	1076.0
65	1140.2
66	1182.2
67	1128.5
68	1016.1
69	1098.4
70	1651.6
76	740.7
78	1296.2
79	1254.6
81	1098.9
82	879.8
83	685.6
86	1863.1
87	1964.4
88	1485.7
89	1212.2
90	1199.9
91	884.7
92	946.1
93	958.6
94	730.8
95	937.3
96	678.1
99	596.1
100	599.7
101	1295.8
102	1758.0

Biogas (%)

Time	Thermophilic	
	CH_4	CO_2
87	45.65	53.36
91	40.82	58.44
94	37.89	61.25
96	40.78	55.11
99	33.47	62.77
100	34.54	62.41

Solids (mg/L)

Time	Thermophilic	
	TSS	TVSS
94	6100	4470
95	6050	4510
96	7690	5590
100	5380	3660
102	6220	4200

Run 12

Solids (mg/L)

Time	Thermphilic	
	TSS	TVSS
41	4590	3050
44	3867	2533
46	4050	2900
47	4120	2980
48	3990	2850
52	4020	3110

VFA (mg/L)

Time	Thermophilic
41	111.4
46	265.7
47	300.0
48	197.1
52	291.4

Methane Production (mL/L/d)

Time	Thermophilic
1	357.4
2	706.2
3	857.7
4	781.0
5	737.7
6	770.2
7	796.0
8	839.7
10	830.4
11	789.1
12	756.2
13	755.0
14	1119.9
15	392.8
17	664.6
18	770.6
19	641.8
20	586.6
21	506.8
22	471.9
23	362.9
24	459.8
25	552.3
26	702.4
27	622.1
29	547.6
30	664.7
31	808.2
32	540.1
33	500.4
34	793.0
35	742.8
36	619.6
38	668.9
40	739.0
41	706.1
42	742.0
44	735.3
45	732.7
46	729.7
47	734.0
48	797.1
49	797.3
50	734.8
51	766.3
52	714.4
53	732.9
54	713.3
55	757.0
56	734.1
57	748.6
58	758.2
59	761.8
60	755.1

pH

Time	Thermophilic
1	7.41
3	7.30
4	7.45
5	7.33
6	7.26
7	7.32
8	7.18
15	6.74
17	7.05
18	7.03
19	6.98
20	6.85
21	6.97
24	7.32
25	6.96
26	7.00
27	6.97
32	7.13
33	7.17
34	7.18
36	7.23
38	7.18
40	7.12
46	7.30
50	7.27
54	7.19
58	7.18

COD (mg/L)

Time	COD	SCOD
18		2312.95
19		2462.18
35	6018.65	1780.73
40	5720.21	2109.02
41	5272.54	2049.33
46	5812.84	2011.01
47	6282.57	2216.51
48	6047.71	2385.32
52	6185.21	2165.74

Biogas (%)

Time	Thermophilic	
	CH_4	CO_2
4	48.54	48.96
6	48.21	50.10
18	47.41	50.81
20	50.22	48.16
25	47.96	49.11
27	49.79	47.42
30	49.35	48.44
32	47.60	50.60
36	45.44	52.14
40	46.20	51.50
42	49.05	48.65
45	48.20	49.40
47	49.50	48.00
49	48.25	49.65
52	47.68	50.02
55	48.21	49.48
58	49.11	48.88
40	46.20	51.50
42	49.05	48.65
45	48.20	49.40
47	49.50	48.00
49	48.25	49.65
52	47.68	50.02
55	48.21	49.48
58	49.11	48.88

Run 13

Biogas (%)

Time	Thermophilic	
	CH_4	CO_2
87	41.41	57.36
91	47.99	51.12
94	49.15	50.00
96	50.99	47.75
99	52.07	47.16
100	48.88	49.82
102	49.34	49.86
104	49.44	49.66
106	49.12	49.88

COD (mg/L)

Time	Thermophilic	
	COD	SCOD
93	9500	3078
94	9063	4600
96	7837	3569
100	9848	5215
101	9294	5812
104	9325	5413

VFA (mg/L)

Time	Thermophilic
92	3814.3
93	5125.7
94	1962.9
95	1440.0
96	145.7
100	2571.4
101	2014.3
104	2464.9

pH

Time	Thermophilic
51	6.31
52	6.76
53	7.05
55	6.87
63	6.65
64	7.00
67	7.28
82	7.15
85	7.42
88	6.76
89	6.54
90	6.69
91	6.70
92	7.01
94	7.16
95	7.11
96	7.29
97	6.98
99	7.00
100	6.98
102	7.14
104	7.04
106	7.05

Methane Production (mL/L/d)

Time	Thermophilic
2	952.5
3	1307.6
4	1307.1
5	1239.7
6	1277.4
7	1314.3
9	1530.2
11	1183.8
12	1269.7
13	1308.8
14	1246.3
17	1078.5
19	1381.3
22	1213.4
42	1717.5
44	1670.8
45	1621.6
49	2603.8
50	1512.7
51	1501.3
52	1143.9
53	1150.4
54	970.9
55	1056.0
56	1009.4
57	984.9
59	968.9
60	994.2
61	988.2
63	1136.0
64	1273.5
65	1210.4
66	1241.0
67	1146.1
68	1273.2
69	1242.7
70	1055.4
71	1006.8
74	1110.4
76	1037.4
78	1054.2
79	1027.6
81	1124.1
82	1096.2
83	1074.9
84	1161.8
85	1119.9
86	1176.5
87	990.5
88	1203.9
89	1129.9
90	1175.7
91	1152.0
92	1188.3
93	1273.0
94	1168.9
95	1116.6
96	1194.3
97	1124.4
98	1179.3
99	1253.0
100	1158.5
101	1095.7
102	1137.4
103	1136.0
104	1142.6
105	1131.8
106	1131.3

Solids (mg/L)

Time	Thermophilic	
	TSS	TVSS
93	5255	3585
94	5880	3680
95	5000	3375
96	5970	4230
100	8540	4910
101	6050	3810

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BIOGRAPHICAL SKETCH

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