ABSTRACT

LIU, ZHIMIN. Thermophilic Anaerobic Co-digestion of Swine Manure with Corn Stover for Biogas Production. (Under the direction of Dr. Jay J. Cheng.)

The transition of anaerobic digestion from mesophilic to thermophilic conditions using a one-step temperature jump from 35 ºC to 50 ºC was first studied in two continuously stirred tank reactors (CSTR) treating swine wastewater and corn stover. Both reactors were operated at constant organic loading rate of 1.074 kg VS/m^3/d and hydraulic retention time (HRT) of 25 days. Although the one-step temperature jump from 35 ºC to 50 ºC resulted in severe fluctuation of daily methane production and volatile fatty acids concentrations, the systems reached stability after 25 days. It can be concluded that the strategy for changing temperature from mesophilic to thermophilic condition using one-step temperature jump is feasible in anaerobic CSTR treating swine wastewater and corn stover. In comparison with mesophilic anaerobic digestion, thermophilic anaerobic digestion performed better in regards to daily gas production, specific gas yield and chemical oxygen demand (COD) removal. The effects of HRT was investigated and it was shown that the system could be operated at an HRT as low as 5 days.

The effect of sodium hydroxide (NaOH), lime ($Ca(OH)_2$) and the combined NaOH and $Ca(OH)_2$ pretreatment on corn stover were investigated in this study. Digestions were performed in 12 L laboratory-scale CSTR operated with an organic loading rate of 1.074 kg VS/m^3/d. HRT was 5 days and the operating temperature was 50 ºC. The results of methane fermentation study on alkaline pretreated corn stover had resulted into significant amount of increase in biogas as well as methane yields. NaOH pretreated corn stover produced 30.95% higher biogas yield and 36.84% higher methane yield compared to the untreated corn stover. Lime pretreated corn stover had resulted into an increase of 32.43% in biogas yield and 37.50% in methane yield compared to that

of untreated corn stover. Combination of NaOH and lime-treated corn stover had resulted into an increase of 38.10% in biogas yield and 44.44% in methane yield compared to that of untreated corn stover.

The effect of total solids (TS) concentration on anaerobic co-digestion of swine manure and corn stover was further investigated in CSTR at solid content of 2.5%, 4% and 5.5%. Digestions were performed in 12 L laboratory-scale CSTR with operating temperature at 50 ^{o}C and HRT of 5 days. The daily loading of swine manure and corn stover was mixed at a mass ratio of 3:1 to achieve a carbon/nitrogen (C/N) ratio of 30 in the mixture and was diluted to desired TS concentration. The Analysis of Variance (ANOVA) showed that the gas yield from the various TS% was significantly different ($p < 0.05$). The results indicated that the gas yield decreased with the increase in the solid content. Higher gas yield achieved at lower TS concentration was attributed to overloading of the substrates. COD and VS reduction were the highest when the reactor was operated at TS of 2.5%.

A mathematical model has been developed to describe the swine manure and corn stover co-digestion process. This model includes multiple-reaction stoichiometry, microbial growth kinetics with acetic acid inhibitory effects and conventional material balances for an ideally mixed reactor. The ordinary differential equations (ODE) of the model were coded in RStudio® software and integrated using the package deSolve. A laboratory batch experiment using swine manure and corn stover as substrates was conducted to calibrate the model. The kinetic parameters used in the model were adjusted for thermophilic conditions and were estimated using least square variation method. Bootstrap is used to estimate the standard error and 95% confidence interval of model kinetic parameters. The applicability of the model was demonstrated by comparing experimental results from literature with the model simulation results.

Thermophilic Anaerobic Co-digestion of Swine Manure with Corn Stover for Biogas Production

by
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DEDICATION

This dissertation is dedicated to my parents and husband for their constant love and support along the way.

BIOGRAPHY

Zhimin Liu was born and grew up in Nanchang, Jiangxi, P.R. China. After finishing high school, she was matriculated to the undergraduate program in Environmental Engineering at Nanchang Hangkong University which was located in her hometown in 2007. Zhimin obtained her B.S. degree and came to the United States for graduate study in the year of 2011. She completed the M.S. program in the department of Biological and Agricultural Engineering at NC state in December 2013. With strong interest in anaerobic digestion technology and the goal of resolving current environmental problems, Zhimin continued her graduate study to purse her Ph.D. degree in the same department under the direction of Dr. Jay J. Cheng. After completing her Ph.D., she will start her career in the biofuels industry.

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CHAPTER 1: THERMOPHILIC ANAEROBIC DIGESTION OF ORGANIC WASTES FOR BIOGAS PRODUCTION: A REVIEW

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Abstract

With the increasing concerns of global climate change caused by the consumption of fossil fuels, much efforts have been spent on the exploration of renewable energy sources in the last couple of decades. Biogas produced from the treatment of organic waste materials is one of the renewables that have attracted a lot of attentions. This chapter provides a summary of the studies on thermophilic anaerobic digestion or co-digestion of various organic waste materials for biogas production. Discussions in this chapter include main advantages of thermophilic anaerobic digestion technology over mesophilic one, factors that critically impact the efficiency of the anaerobic digestion such as temperature, pH, total solids, carbon/nitrogen ratio, and hydraulic retention time, the effect of the pretreatment of lignocellulosic materials on the biogas production from anaerobic co-digestion of animal manure and agricultural residues, and mathematical models describing the anaerobic digestion processes.

Keywords: anaerobic digestion; biogas; organic wastes; thermophilic

1. Introduction

With the rapid development of industry and agriculture, large volume of organic wastes is generated. Organic wastes can be treated using physical, chemical and biological treatment methods. Among different types of biological treatment methods, anaerobic digestion (AD) is regarded as the most cost-effective one because of its environment friendly operations, net high energy recovery and carbon neutral impact (Suryawanshi et al., 2010).

AD involves the breakdown of complex organic wastes and produces biogas by a consortium of anaerobic microorganisms (Cantrell et al., 2008). Biogas is mainly composed of 48-70% methane, 30-49% carbon dioxide, 100-2000 ppm hydrogen sulfide and traces of other gases (Ward et al., 2008). In comparison with other biological treatment methods, AD offers signification advantages including:

- Biogas produced can be used for heat and electricity generation;
- The digestate can be used as fertilizer due to its high nutrients content;
- AD reduces disposed waste volume and weight;
- AD eliminates odor;
- AD is a low cost and low technology system to provide energy for rural areas.

In order to create a stable and optimized AD process, there are many factors that need to be taken into consideration. Among them, temperature is the most important one because it alone can affect the rate of biochemical reactions in the AD process (Suryawanshi et al., 2010). AD can be carried out under psychrophilic (15 – 25 ºC), mesophilic (30 – 40 ºC), and thermophilic (50 – 60 ºC) conditions, of which mesophilic and thermophilic conditions are commonly used in applications. In comparison with mesophilic digestion, thermophilic digestion offers many advantages such as higher specific growth rate for the anaerobic microorganisms, higher kinetic advantage in fermentation, higher percentage destruction of pathogens and weed seeds, improved solid-liquid separation and more stability of organic wastes (Suryawanshi et al., 2010). Thermophilic AD is attracting more and more attentions because of the advantages. In this paper a critical review is presented on themophilic anaerobic digestion of organic wastes including the characteristics of the microorganisms, critical factors, applications, challenges and modeling of the process.

2. Basic scheme of thermophilic anaerobic digestion process

Under thermophilic conditions, the basic scheme of digestion process remains almost the same as that under mesophilic conditions. The thermophilic AD is mainly comprised of four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. A schematic diagram of the AD process is shown in Figure 1.

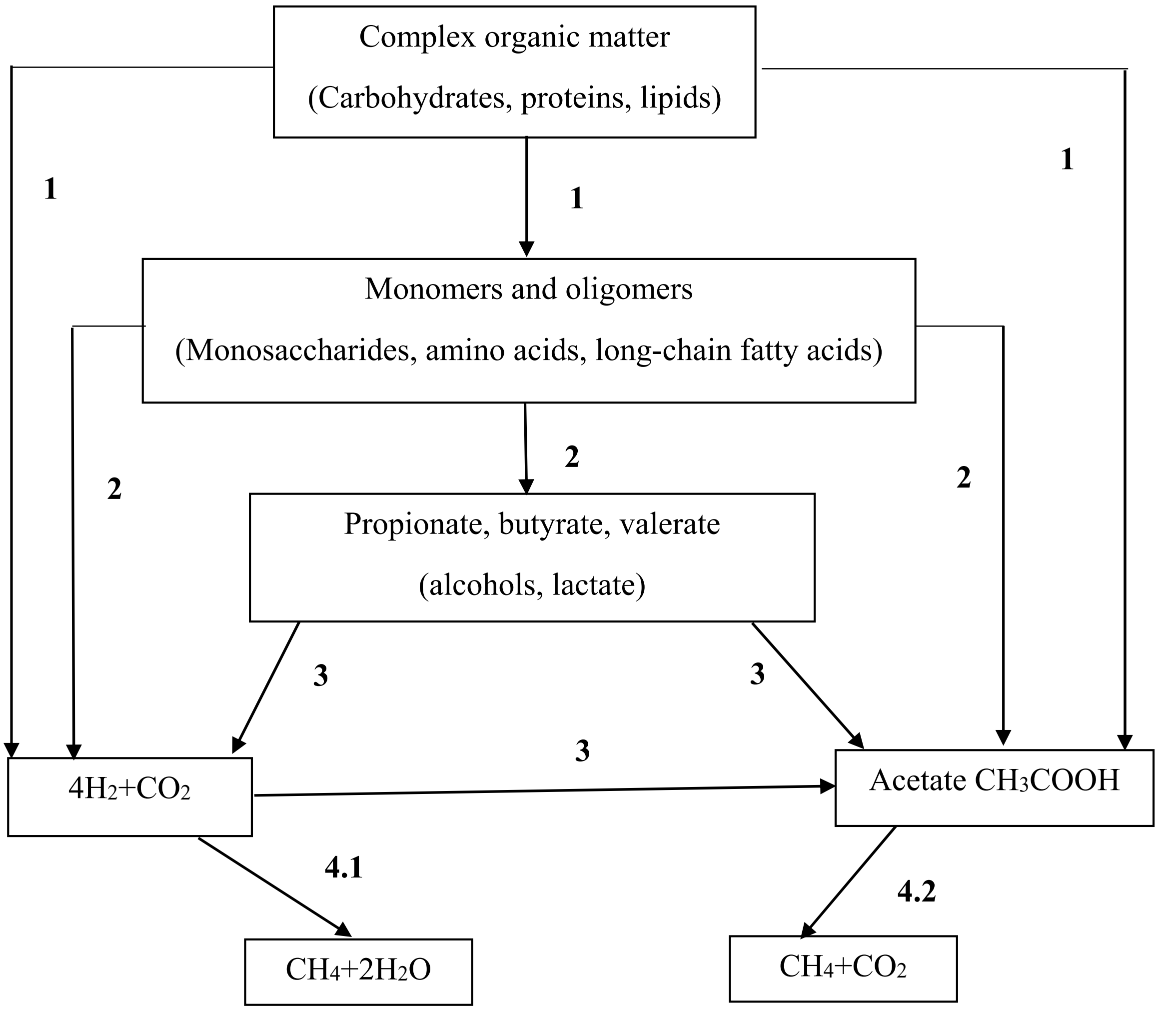


Figure 1. Anaerobic digestion of organic materials into biogas: 1. Hydrolysis; 2. Acidogenesis; 3. Acetogenesis; 4. Methanogenesis; 4.1 Hydrogenotrophic methanogenesis; 4.2 Aceticlastic methanogenesis.

2.1 Hydrolysis

Hydrolysis is undertaken by hydrolytic bacteria to convert the large-molecule compounds (carbohydrates, proteins, lipids and celluloses) into monomers (sugars, amino acids, and peptides) and a small amount of acetic acid, hydrogen and carbon dioxide. The hydrolytic bacteria can be either facultative or obligate anaerobes. They can be found in soil, wood chip piles, sewage, hot springs, ruminant animals, compost sites and biogas plants (Azman et al., 2015; Lynd et al., 2002).

Anaerobic hydrolytic bacteria can be found within the phylum *Firmicutes* (genus: *Clostridium*, *Ruminococcus*, *Caldicellulosiruptor*, *Caldanaerobacter*, *Butyrivibrio*, *Acetivibrio*, *Halocella* and *Eubacterium*), *Bacteroidetes*, *Fibrobacter*, *Spirochaetes* (*Spirochaeta*), and *Thermotogae* (genus: *Fervidobacterium* and *Thermotoga*) (Azman et al., 2015). The optimum temperature range for hydrolytic microorganisms is between 30 ºC and 60 ºC. Most hydrolytic microorganisms have an optimum pH between 5 and 7.

During hydrolysis, complex insoluble substrate such as polysaccharides are hydrolysed into smaller units by hydrolytic bacteria secreting different hydrolyzing enzymes such as cellulase, cellobiase, xylanase, amylase, protease and lipase (Christy et al., 2014, Cirne et al., 2012). An example of hydrolysis reaction where organic waste is degraded into glucose by hydrolytic bacteria is shown in Equation (1):

$$[C_6H_{10}O_4]_n + 2\ n\ H_2O \rightarrow n\ C_6H_{12}O_6 + 2\ n\ H_2 \qquad (1)$$

The hydrolytic reactions have two phases. In the first phase, a bacterial colonization occurs where the hydrolytic bacteria cover the surface of solids (Vavilin et al., 1996). Bacteria secret enzymes and produce monomers which can be utilized by the hydrolytic bacteria and other bacteria (Christy et al., 2014). In the second phase, the surface of solids will be degraded by the bacteria at a constant depth per unit of time (Vavilin et al., 1996).

2.2 Acidogenesis

The mononers produced from the hydrolysis process are then fermented into a wide range of fermentation end products by the acidogenic bacteria in the acidogenesis process. The majority of the products are volatile fatty acids (VFAs). A small amount of acetic acid, hydrogen and carbon dioxide are also produced. Similar to hydrolytic bacteria, the acidogenic bacteria is also either facultative or obligate anaerobes. However, the acidogenic bacteria prefers to live in a slightly acidic environment, with a pH of 4.5 to 5.5 (Christy et al., 2014).

In the acidogenesis process, the acidogenic bacteria utilize the sugars, long chain fatty acids and amino acids as substrates to produce organic acids such as acetic, propionic, butyric and other short-chain fatty acids, alcohols, H_2 and CO_2 (Gujer et al., 1983). Equations (2)-(4) are three typical acidogenesis reactions where the glucose is converted to alcohols, propionic and acetic acid by acidogenic bacteria, respectively.

$$C_6H_{12}O_6 \rightarrow 2CH_3\,CH_2\,OH + 2CO_2 \tag{2}$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \tag{3}$$

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \tag{4}$$

2.3 Acetogenesis

Acetogenesis is a process which converts the volatile fatty acids into acetic acid, hydrogen and carbon dioxide. Acetogenic bacteria play a key role in this process. Acetogenic bacteria are strict anaerobes that produce acetate as the main product of their metabolism. Distinguished from all other bacteria that produce acetate in anaerobic metabolism, acetogenic bacteria utilize a pathway (the acetyl coenzyme A pathway) that contains enzymes, acetyl-CoA synthase (ACS), which is extremely sensitive to O_2 (Christy et al., 2014; Wood et al., 1991).

Acetogenic bacteria grow slowly and are sensitive to fluctuations in organic loadings and environmental changes (Christy et al. 2014). They require a long lag periods to adapt to the new environmental conditions (Müller et al., 2013). The phylogeny and genera of acetogens are diverse. There are 23 bacterial genera that contain more than 100 reported acetogenic species (Müller et al., 2013). Most of the acetogenic species are Gram-positive (Müller et al., 2013).

The function of acetogenic bacteria is to further degrade the products of acidogenesis such as lactate, ethanol, propionate, butyrate, and higher volatile fatty acids into acetate, carbon dioxide and hydrogen which can be consumed directly by the methanogens. Equations (5) - (8) represent some chemical reactions which take place in the acetogenesis by acetogenic bacteria. Equation (8) is referred to as homoacetogenesis since this type of fermentation yields only acetate. This conversion is undertaken by the homoacetogenic bacteria.

$$H_3CH_2COOH^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (5)$$

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (6)$$

$$CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+ \quad (7)$$

$$CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (8)$$

2.4 Methanogenesis

The final step, methanogenesis is to convert the mixture of CO_2 and H_2 and acetic acid into methane by methanogens. Methanogens are very strict anaerobes and they are generally present in nature only in anoxic environments. The optimum pH for methanogenic archaea is between 6.5 and 8.0. If the pH drops below 6, the methanogens can hardly survive (Christy et al., 2014).

The metabolism of methanogens is different from the bacteria since the former utilizes H_2/CO_2, formate, methylated C1 compounds or acetate as energy and carbon source (Christy et al., 2014). Based on the substrate, this group of microorganisms can be categorized into hydrogenotrophic methanogens and aceticlastic methanogens. The hydrogenotrophic methanogens utilize the H_2 and CO_2 to form CH_4. The biochemical reaction is as follows:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (9)$$

The conversion of acetic acid to methane is catalyzed by the aceticlastic methanogens:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (10)$$

During hydrogenotrophic methanogenesis, the CO_2 is gradually reduced to methane by special coenzymes (methanofuran, tetrahydromethanoptein, coenzyme M) through the formyl, methylene and methyl levels (Christy et al., 2014). The most important enzyme is the methyl-coenzyme M reductase which reduces methyl-coenzyme M to methane (Ferry et al., 1997).

During the catabolism of acetate, the methyl and carboxyl groups are converted to CH4 and CO_2, respectively. The reaction involves the initial activation of acetate as acetyl-CoA, then the carbon-carbon bond is then cleaved by the carbon monoxide dehydrogenase system, producing HS-CoA, an enzyme-bound CO and a methyl group (Whitman et al., 2006). The methyl group is then transferred to HS-CoM through H_4MPT and a corrinoid enzyme. Oxidation of the enzyme-bound CO to H_2 provides electrons for the reduction of CH_3-S-CoM to CH_4 (Terlesky et al., 1988).

3. Critcal factors in maintaining a stable thermophilic anaerobic digestion process

3.1 Temperature

The temperature range for thermophilic AD is considered to be between 48 – 60 ºC, with the optimum temperature around 55 ºC (De la Rubia et al., 2013). Compared with mesophilic AD, thermophilic AD has higher rates in biogas production and pathogen destruction. However, thermophilic AD is more sensitive to temperature fluctuations and requires a longer time to adapt to a new environment (Weiland, 2010). Zinder et al. (1984) found that only a few degrees increase in temperature resulted in a completely irreversible deterioration of the thermophilic process. This might be attributed to the temperature limitation of aceticlastic methanogens (Wilson et al., 2008). A fluctuation in temperature results in low biogas production, VFA accumulation and a decrease in pH values (De la Rubia et al., 2013). In order to maintain the stability of the digester performance, keeping the temperature at the optimum level is very important. Willis et al. (2006)

indicated that the reactor temperature fluctuations in modern thermophilic digesters should be 0.1 – 0.2 ºC on a daily basis.

3.2 pH

A pH range of 6.8 to 7.2 is ideal for the AD (Ward et al., 2008). However, the optimum pH of methangenesis and hydrolysis are different. The optimal pH for the methanogenesis is at 6.5-8.2 while the optimum pH for the hydrolysis and acidogenesis is between 5.5 and 6.5 (Kim et al., 2003; Lee et al., 2009). Park et al. (2008) concluded that the appropriate pH range for thermophilic acidogens was between 6 and 7 and the optimum condition was at 6. Liu et al. (2008) found that the maximum biogas yield was achieved when the pH was between 7.2 and 7.3 for the treatment of organic fraction of municipal solid waste under thermophilic condition.

3.3 Total solids (TS)

AD can be categorized into wet, semi-dry and dry digestion based on the TS content in the feed material. The wet digestion processes are operated with TS concentration below 10% which is quite applicable for conventional anaerobic digesters such as continuously stirred tank reactors (CSTRs). The TS content in semi-dry process is usually between 10 and 20%, while the dry digestion is operated with a TS content inside the reactor between 20 and 40% (Weiland, 2010). Compared with the wet AD, dry AD has several advantages such as higher organic loading rate and more efficient energy performance (Forster-Carneiro et al., 2008). Many studies have been conducted by researchers investigating the influence of TS content on the performance of AD. Desai et al. (1994) found that the biogas production was enhanced with the increased TS reaching a maximum at 6% (w/v) in the biomethanation of the mixture of cattle dung, poultry waste and whey. Similar results were also achieved by Igoni et al. (2007) studying the TS concentration on the AD of municipal solid waste (MSW) in batch reactors. With the increase of TS in MSW, the biogas production also increased and the optimum biogas production was obtained at 20% TS concentration. However, contrary result was found when Itodo et al. (1999) tested the effect of different TS concentrations (5%, 10%, 15% and 20% TS) of poultry, cattle and piggery waste slurries on the biogas yield in anaerobic batch digesters. The result indicated that the gas yield increased with the decreasing TS concentration of the slurries. In this case, higher gas yield was obtained from the lower TS. This is because when the TS was too high, the slurry became too thick to digest (Itodo et al., 1999).

3.4 Carbon/nitrogen (C/N) ratio

Methane production would be enhanced if the C/N ratio in the feed to an anaerobic digester was balanced in the optimum range. Hills (1979) investigated the effects of C/N ratio on the AD of dairy manure. The C/N ratio in the study was determined using available carbon (total organic carbon minus the lignin carbon) to total Kjeldahl nitrogen (TKN). Different C/N ratios between 8.0 and 51.7 was studied by combining the dairy manure with glucose. The result showed that the highest methane production was achieved when the C/N of the feed was 25. Sievers et al. (1978) indicated that the C/N (total organic carbon (TOC)/TKN) ratio of 15.5 to 19 was found to be the optimum range when swine waste was digested in anaerobic digesters. Similar results were also obtained by Backus et al. (1988). They tested four C/N ratios of 8.4, 13.9, 22.2 and 27.6 on the performance of AD of raw sweet cheese whey in a semi-batch, fixed film anaerobic digester. The C/N ratio was defined as TOC/TKN in their study. It was observed that the highest percentage of methane and methane production rate occurred when the C/N ratio was between 22 and 28.

3.5 Hydraulic retention time (HRT)

HRT is an important design parameter since it determines the microbe/substrate reaction and further influences the consumption efficiency of the substrate (Aguilar et al., 2013). Typical

HRT for mesophilic anaerobic digestion is 20-25 days, while the HRT for thermophilic anaerobic digestion is 10-15 days.

4. Applications of thermophilic anaerobic digestion/co-digestion

Thermophilic AD has been widely applied in the past decades because of its efficient hygienic treatment and higher biogas production rate. Compared with traditional mesophilic AD process which has lower energy requirements, the operational cost of thermophilic AD is slightly higher. However, the real benefit of thermophilic AD is total (99.9%) reduction of pathogens and thereby reducing the risk of disease transmission (Sahlström, 2003). Substrates that can be used for thermophilic AD are sewage sludge, municipal solid waste, organic fraction of municipal solid waste, animal manure, energy crops and crop residues. Previous studies on thermophilic AD are summarized in Table 1.

Table 1. Summary of previous studies on thermophilic anaerobic digestion (AD) of organic wastes.

Substrates Fed	Operational Conditions	Biogas production (L/d)	References
Cattle manure	4.5L glass reactor, 3.0g VS_{fed}/L/d, 12 days HRT, 55±1 ºC	4.6	Nielsen et al. (2004)
Organic fraction of municipal solid waste	25.0L sequential batch anaerobic composting reactor, 55±1 ºC	6.5	Forster-Carneiro et al. (2004)
Waste milk	0.25L glass reactor, 55±1 ºC	1.2	Onodera et al. (2007)
Fruit and vegetable waste	2.0L anaerobic sequencing batch reactors, 2.5g VS_{fed}/L/d, 10 days HRT, 55±1 ºC	1.7	Bouallagui et al. (2008)

Table 1. Continued

Substrates Fed	Operational Conditions	Biogas production (L/d)	References
Organic fraction of municipal solid waste	5.0L sequential batch anaerobic composting reactor, 55±1 ºC	3.2	Froster-Carneiro et al. (2008)
Organic fraction of municipal solid waste	1.1L batch reactor, 55±1 ºC	4.3	Froster-Carneiro et al. (2007)
Paper mill wastewater	1.13L glass reactor, 12.25g COD_{fed}/L/d, 6 days HRT, 55±1 ºC	3.2	Yilmaz et al. (2008)
Cow Manure	800L continuous stirred tank reactor, 2500g COD_{fed}/L/d, 20 days HRT, 54±1 ºC	628	Kaparaju et al. (2008)

Anaerobic co-digestion (AcoD) is defined as a waste treatment method in which two or more substrates are mixed and treated together, so the biogas production is improved through their joint treatment (Khalid et al., 2011; Mata-Alvarez et al., 2011). The potential benefits of AcoD include dilution of toxic compounds, increased load of biodegradable matter, improved nutrients balance, synergistic effect of microorganisms and better biogas yield (Khalid et al., 2011). AcoD of animal manure with agricultural residues had been extensively investigated in the past decades (Cuetos et al., 2011; Li et al., 2011; Wu et al., 2010; Xie et al., 2011). Early studies using swine manure with corn stover (Fujita et al., 1980), dairy manure and barley straw (Hills, 1980), swine manure and wheat straw (Fischer et al., 1983), beef cattle manure with wheat straw (Hashimoto, 1983) appeared in the early 1980s. Cuetos et al. (2011) studied the AcoD of swine manure with maize, rapseed and sunflower residues under batch and semi-continuous conditions. The results indicated that the AcoD system resulted in a major increase in the amount of daily biogas production. Similar results were achieved by Wu et al. (2010) when they co-digested swine manure with corn stalks, oat straw, and wheat straw. They found the daily maximum biogas production increased by 11.4-fold when the swine manure is co-digested with corn stalks in comparison with the only manure as the substrate. These studies supported that the idea of AcoD of animal manure/wastewater with corn stover could be an effective method in converting organic wastes into biogas.

5. Application of pretreatment on lignocellulosic materials in enhancing biogas production in anaerobic co-digestion

One of the main challenges in converting lignocellulosic materials such as agricultural residues into biogas is the recalcitrant compact structure of the materials. Lignocellulosic materials mainly consist of three components: cellulose, hemicellulose and lignin. Cellulose chains are embedded in a cross-linked matrix of hemicellulose surrounded by lignin. The lignin barrier prevents the enzymes from accessing into the cellulose fraction, making the hydrolysis process difficult. The objective of pretreatment is to break the lignin seal and disrupt the crystalline structure to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier et al., 2005). Pretreatment can be categorized into physical pretreatment, physical-chemical pretreatment, chemical pretreatment and biological pretreatment.

5.1 Physical pretreatment

The objective of physical pretreatment is to reduce the particle size of the influent substrate. Physical pretreatment process can not only increase the available surface area but also decrease the crystallinity and degrees of polymerization of cellulose.

5.2 Physical-chemical pretreatment

Physical-chemical pretreatment combines both the physical and chemical processes. Typical form of physical-chemical pretreatment includes steam explosion, ammonia fiber explosion, carbon dioxide explosion. In comparison with the untreated wheat straw, pretreated wheat straw using wet explosion method can produce 20% more methane production (Bauer et al., 2009).

5.3 Chemical pretreatment

Chemical pretreatment methods include ozonolysis, acid hydrolysis, alkaline hydrolysis and oxidative dilignification. Among all these methods, alkaline pretreatment proves to be a particularly advantageous method in treating agricultural residues in anaerobic digestion due to its low cost and effectiveness. NaOH and lime are commonly used in the alkaline pretreatment. Pretreated corn stover with 2% of NaOH would increase the accessibility and digestibility of cellulose (Chen et al., 2009). Greater than 100% increase of biodegradability is observed when the wheat straw is pretreated with NaOH (Pavlostathis et al., 1985). Pang et al.(2008) showed that NaOH pretreatment can improve the biodegradability of corn stover and improve the biogas yield. When the NaOH dose was 6% and the loading rate was 65g/L, 48.5%, more biogas were produced compared with the untreated ones. Corn stover which is pretreated by NaOH, resulted in 72.9% and 73.4% increase of biogas and methane production, respectively (Zheng et al., 2009).

5.4 Biological pretreatment

Biological pretreatment process uses microorganisms to degrade lignocellulosic materials. The microbes include brown-, white-, and soft-rot fungi. Compared with chemical pretreatment processes, biological pretreatment process needs less energy and no requirement of chemicals. Biological pretreatment can not only degrade the lignin, but also hemicelluloses and cellulose.

Romano et al. (2009) studied the cellulase, hemicellulase and glucosidase effect in the ananerobic digestion process of wheat grass. They found that the solubility of the wheat grass increased; however, the biogas and methane production was similar compared with the control.

6. Mathematical modeling of anaerobic digestion process

Considerable effort has been put into the mathematical modeling of anaerobic digestion in the past decades. There are many publications regarding anaerobic digestion modelling in the last 30 years (Batstone et al., 2006). The development of mathematical models results in a better understanding of the process dynamics, reveals optimization opportunities and is an overall prerequisite for improvement of digester performance (Lauwers et al., 2013). The first anaerobic digestion models were simple kinetic models that describe only the rate-limiting step of the biological process, i.e., the slowest step that limits the rate of the overall process (Andrews et al., 1965, Andrews, 1969; Andrews and Graef, 1971). According to the digester operating conditions and influent characteristics, the limiting step of the anaerobic digestion process is different (Esposito et al., 2011). Hydrolysis, being the first step in overall process, is normally the rate-limiting step of the overall anaerobic digestion process (Angelidaki et al., 2004). Several kinetic equations for hydrolysis are reported in the literature. Vavilin et al. (2001) used traditional first-order kinetics (Equations (11) and (12)) and Contois kinetics (Equation (13)) to describe the hydrolysis of biodegradable solids.

$$\frac{dS}{dt} = -F_S(S) = -kS \tag{11}$$

$$\frac{dS}{dt} = -F_S(S,X) = -\frac{\mu_m}{Y} X \frac{S/X}{K_S^* + S/X} \tag{12}$$

$$\frac{dX}{dt} = F_X(S,X) = YF_S(S,X) - k_d X \tag{13}$$

where $F_S(S,X), F_X(S,X)$ are the substrate consumption and biomass growth rates, respectively, X is the hydrolytic/acidogenic microbe concentration, k is the rate constant, μ_m is the maximum specific growth rate of hydrolytic microorganisms, K_S^* is the half-saturation coefficient for S/X value, and k_d is the microbe decay coefficient. A surface based kinetics (SBK) model had been proposed by some authors to describe the anaerobic hydrolysis process (Hills et al., 1984; Hobson, 1983; Vavilin, 1996). The model assumed that the substrate particles are completely covered with bacteria which secrete the hydrolytic exo-enzymes during digestion (Sanders et al., 2000). The SBK model was represented in Equation (14).

$$\frac{dM}{dt} = -K_{sbk}A \qquad (14)$$

where M is the mass of substrate, t is the time (days), K_{sbk} is the surface based hydrolysis constant (kg/m^2-day), A is the surface available for hydrolysis (m^2). Acetogenesis (Hill, 1982; Bryers, 1985; Mosey, 1983; Siegrist et al., 1993) and methanogenesis (Graef et al.,1974; Hill et al., 1977; Moletta et al., 1986) had been reported as rate-limiting steps as well. The kinetics of these steps is traditionally expressed by Monod type kinetics which consider a single growth-limiting substrate (Equation (15)):

$$\mu = \mu_{max}\frac{[S]}{[S]+K_S} \qquad (15)$$

where μ (d^{-1}) is the specific growth rate, μ_{max} (d^{-1}) the maximum specific growth rate, [S] (g L^{-1}) the substrate concentration and K_S (g L^{-1}) the substrate saturation constant.

It has been found that several chemical compounds at certain concentration are inhibitory to the anaerobic digestion process. These chemical compounds include ammonia, sulfide, metals and some organic compounds such as long-chain fatty acids (Chen et al., 2008). Inhibition usually results in decreased or a complete stop in methane production (Kroeker et al., 1979).

Organic acids accumulation at neutral pH is regarded as the most common product inhibition in the biological model (Costello et al., 1991). Costello et al. (1991) proposed a competitive and a non-competitive inhibition model to account for product inhibition by acetic acid. The concentration of the inhibitor [I] is the millimolar concentration of acetic acid in the reactor. It is assumed that the inhibition of the bacteria by the volatile acid substrates or lactic acid is insignificant. The general equation for the competitive inhibition of the acetogenic bacteria is written as follows:

$$r_S = \frac{k[X][S]}{[S]+K_S(1+\frac{[I]}{K_I})} \quad (16)$$

A noncompetitive model for substrate uptake was used to model product inhibition, the equation is presented in Equation (17):

$$r_S = \frac{k[X][S]}{(K_S+[S])(1+\frac{[I]}{K_I})} \quad (17)$$

In 2002, the International Water Association (IWA) Task Group for Mathematical Modeling of Anaerobic Digestion Processes developed a comprehensive mathematical model known as Anaerobic Digestion Model no.1 (ADM1) (Batstone et al., 2002). The model includes both biochemical and physicochemical processes. The biochemical steps include extracellular disintegration that coverts homogeneous particulate into carbohydrates, proteins and lipids; extracellular hydrolysis that converts these particulate substrates into sugars, amino acids and long chain fatty acids (LCFA); acidogenesis or fermentation that converts sugars and amino acids into volatile fatty acids (VFA) and hydrogen; acetogenesis that converts LCFA and VFA into acetic acid; and acetoclastic and hydrogenotrophic methanogenesis (Hublin et al., 2013; Lauwers et al., 2013). The physicochemical process include ion association and dissociation and gas-liquid transfer (Batstone et al., 2002).

The structured ADM1 model had been successfully applied to simulate the behaviour of a bioreactor for anaerobic digestion of a wide variety of substrates such as municipal waste mixed with activated sludge (Derbal et al., 2009), olive mill wastewater mixed with solid waste (Fezzani et al., 2008), grass silage (Koch et al., 2010), manure mixed with vegetable waste (Galí et al., 2009), blackwater with kitchen refuse (Feng et al., 2006) and microalgae (Mairet et al., 2011).

Further modifications of ADM1 models were proposed by authors to consider sulfate reduction (Fedorovich et al., 2003), $CaCO_3$ precipitation (Batstone et al., 2003) and high concentration of cyanide (Zaher et al., 2006) or sodium (Hierholtzer et al., 2012) for specific situations or subtrates. Recently, some effort has been made to model the solid waste digestion. Esposito et al. (2008), for instance, extended the ADM1 to simulate the organic solid particle disintegration and the effect of LCFA production on pH for a sewage sludge and organic fraction of municipal solid waste co-digestion system. In the future, considerable effort will put into the biological aspect of the modelling that is how to mathematically model the anaerobic digestion performance based on microbial diversity and activity (Lauwers et al., 2013).

REFERENCES

Aguilar, M. A. R., Fdez-Güelfo, L.A., Álvarez-Gallego, C.J., & García, L. I. R. (2013). Effect of HRT on hydrogen production and organic matter solubilization in acidogenic anaerobic digestion of OFMSW. *Chemical Engineering Journal*, *219*, 443–449.

Andrews, J. & Pearson, E.A. (1965). Kinetics and characteristics of volatile fatty acid production in anaerobic fermentation processes. *International Journal of Air Water Pollution*, *9*, 439-461.

Andrews, J. (1969). Dynamic model of the anaerobic digestion process. *Journal of the Sanitary Engineering Division*, *95*(1), 95-116.

Andrews, J. & Graef, S. (1971). Dynamic modeling and simulation of the anaerobic digestion process. *Advances in Chemistry*, *105*,126-162.

Angelidaki, I., & Sanders, W. (2004). Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Bio/Technology*, *3*(2), 117–129.

Azman, S., Khadem, A. F., Van Lier, J. B., Zeeman, G., & Plugge, C. M. (2015). Presence and Role of Anaerobic Hydrolytic Microbes in Conversion of Lignocellulosic Biomass for Biogas Production. *Critical reviews in Environmental Science and Technology*,*45*, 2523-2564.

Backus, B. D., Clanton, C. J., Goodrich, P. R., & Morris, H. A. (1988). Carbon-to-nitrogen ratio and hydraulic retention time effect on the anaerobic digestion of cheese whey. *Transactions of the ASAE*, *31*(4), 1274–1282.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V, Pavlostathis, S. G., & Rozzi, A., et al. (2002). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science & Technology*,*45*(10), 65–73.

Batstone, D. J., & Keller, J. (2003). Industrial applications of the IWA anaerobic digestion model No.1 (ADM1). *Water Science & Technology*, *47*(12), 199–206.

Bauer, A., Bösch, P., Friedl, A., Amon, T. (2009). Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production. *Journal of Biotechnology*, *142*(1), 50-55.

Bouallagui, H., Rachdi, B., Gannoun, H., & Hamdi, M. (2009). Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. *Biodegradation*, *20*(3), 401–409.

Bryers, J. D. (1985). Structured modeling of the anaerobic digestion of biomass particulates. *Biotechnology and Bioengineering*, 27, 638-649.

Cantrell, K. B., Ducey, T., Ro, K. S., & Hunt, P. G. (2008). Livestock waste-to-bioenergy generation opportunities. *Bioresource Technology*, *99*(17), 7941–7953.

Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. *Bioresource Technology*, *99*(10), 4044–4064.

Chen, M., Zhao, J., Xia, L. (2009). Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. *Biomass and Bioenergy*, *33*(10), 1381-1385.

Christy, P. M., Gopinath, L. R., & Divya, D. (2014). A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renewable and Sustainable Energy Reviews*, *34*, 167–173.

Cirne, D.G., Lehtomaki, A., Bjornsson, L., & Blackall L.L. (2012). Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *Journal of Applied Microbiology, 103*(3), 516–527.

Costello, D.J., Greenfield, P.F., & Lee, P.L. (1991). Dynamic modelling of a single-stage high-rate anaerobic reactor - I. Model derivation. *Water Research*, *25*(7), 847–858.

Cuetos, M. J., Fernández, C., Gómez, X., & Morán, A. (2011). Anaerobic co-digestion of swine manure with energy crop residues. *Biotechnology and Bioprocess Engineering*, *16*(5), 1044–1052.

De la Rubia, M.A., Riau, V., Raposo, F., & Borja, R. (2013). Thermophilic anaerobic digestion of sewage sludge: focus on the influence of the start-up. A review. *Critical Reviews in Biotechnology*, *33*(4), 448-460.

Derbal, K., Bencheikh-Lehocine, M., Cecchi, F., Meniai, A-H., & Pavan, P. (2009). Application of the IWA ADM1 model to simulate anaerobic co-digestion of organic waste with waste activated sludge in mesophilic condition. *Bioresource Technology*, *100*(4), 1539–1543.

Desai, M., Patel, V., & Madamwar, D. (1994). Effect of temperature and retention time on biomethanation of cheese whey-poultry waste-cattle dung. *Environmental Pollution*, *83*(3), 311–315.

Esposito, G., Frunzo, L., Panico, A., & d'Antonio, G. (2008). Mathematical modelling of disintegration-limited co-digestion of OFMSW and sewage sludge. *Water Science and Technology*, *58*(7), 1513–1519.

Esposito, G., Frunzo, L., Panico, A, & Pirozzi, F. (2011). Model calibration and validation for OFMSW and sewage sludge co-digestion reactors. *Waste Management*, *31*(12), 2527–2535.

Fedorovich, V., Lens, P., & Kalyuzhnyi, S.V. (2003). Extension of anaerobic digestion model no.1 with process of sulphate reduction. *Applied Biochemistry and Biotechnology*, *109*, 33-45.

Feng, Y., Behrendt, J., Wendland, C., & Otterpohl, R. (2006). Parameter analysis of the IWA Anaerobic Digestion Model No. 1 for the anaerobic digestion of blackwater with kitchen refuse. *Water Science & Technology*, *54*(4), 139-147.

Ferry, J.G. (1997). Enzymology of the fermentation of acetate to methane by Methanosarchia thermophilia. *Biofactor, 6,*25-35.

Fezzani, B., & Cheikh, R. Ben. (2008). Implementation of IWA anaerobic digestion model No. 1 (ADM1) for simulating the thermophilic anaerobic co-digestion of olive mill wastewater with olive mill solid waste in a semi-continuous tubular digester. *Chemical Engineering Journal*, *141*(1-3), 75–88.

Fischer, J. R., Iannotti, E. L., & Fulhage, C. D. (1983). Production of methane gas from combinations of wheat straw and swine manure.*Transactions of the ASAE*, *26*(2), 546-548.

Forster-Carneiro, T., Fernandez, L.A., Pérez, M., Romero, L. I. & Alzarez, C. J. (2004).Optimization of Sebac start up phase of municipal solid waste anaerobic digestion.*Chemical and Biochemical Engineering Quarterly*, *18*(4), 429-439.

Forster-Carneiro, T., Pérez, M., Romero, L. I., & Sales, D. (2007). Dry-thermophilic anaerobic digestion of organic fraction of the municipal solid waste: focusing on the inoculum sources. *Bioresource Technology*, *98*(17), 3195–3203.

Forster-Carneiro, T., Pérez, M., & Romero, L. I. (2008). Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. *Bioresource Technology*, *99*(15), 6994–7002.

Fujita, M., Scharer, J. M., & Moo-Young, M. (1980). Effect of corn stover addition on the anaerobic digestion of swine manure. *Agricultural Wastes*, *2*(3), 177-184.

Galí, A, Benabdallah, T., Astals, S., & Mata-Alvarez, J. (2009). Modified version of ADM1 model for agro-waste application. *Bioresource Technology*, *100*(11), 2783–2790.

Graef, S.P., & Andrews, J.F. (1974). Stability and control of anaerobic digestion. *Journal of Water Pollution Control Federation*, *46*(4), 666-683.

Gujer, W., Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. *Water Science & Technology*, *15*(8-9), 127-167.

Hashimoto, A G. (1983). Conversion of straw-manure mixtures to methane at mesophilic and thermophilic temperatures. *Biotechnology and Bioengineering*, *25*(1), 185–200.

Hierholtzer, A., & Akunna, J. C. (2012). Modelling sodium inhibition on the anaerobic digestion process. *Water Science and Technology*, *66*(7), 1565–1573.

Hill, D. T., & Barth, C. L. (1977). A dynamic model for simulation of animal waste digestion. *Journal of Water Pollution Control Federation*, *10*, 2129-2143.

Hill, D. T. (1982). A comprehensive dynamic model for animal waste methanogenesis. *Transactions of ASAE*, *25*(5), 1374-1380.

Hills, D. J. (1979). Effects of carbon:nitrogen ratio on anaerobic digestion of dairy manure. *Agricultural Wastes*, *1*(4), 267–278.

Hills, D. J. (1980). Biogas from a high solids combination of dairy manure and barley straw. *Transactions of the ASAE*, *23*(6), 1500–1504.

Hills, D. J., & Nakano, K. (1984). Effects of particle size on anaerobic digestion of tomato solid wastes. *Agricultural Wastes*, *10*(4), 285–295.

Hobson, P. (1983). The kinetics of anaerobic digestion of farm wastes. *Journal of Chemical Technology and Biotechnology*, *33*(1),1-20.

Hublin, A., & Zelic, B. (2013). Modelling of the whey and cow manure co-digestion process. *Waste management & research, 31*(4), 353–360.

Igoni, A. H., Abowei, M. F. N., & Ayotamuno, J. M. (2007). Effect of total solids concentration of municipal solid waste in anaerobic batch digestion on the biogas produced. *Journal of Food, Agriculture & Environment*, *5*(2), 333–337.

Itodo, I. N., & Awulu J. O. (1999). Effects of total solids concentrations of poultry, cattle, and piggery waste slurries on biogas yield. *Transactions of the ASAE*, *42*(6),1853-1855.

Kaparaju, P., & Angelidaki, I. (2008). Effect of temperature and active biogas process on passive separation of digested manure. *Bioresource Technology*, *99*(5), 1345–1352.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Management*, *31*(8), 1737–1744.

Kim, J., Park, C., Kim, T. H., Lee, M., Kim, S., Kim, S.W., & Lee, J. (2003). Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. *Journal of Bioscience and Bioengineering*, *95*(3), 271–275.

Koch, K., Lübken, M., Gehring, T., Wichern, M., & Horn, H. (2010). Biogas from grass silage - Measurements and modeling with ADM1. *Bioresource Technology*, *101*(21), 8158–8165.

Kroeker, E. J., Schulte, D. D., Sparling, A. B., & Lapp, H. M. (1979). Anaerobic treatment process stability. *Journal of Water Pollution Control Federation*, *51*, 718-727.

Lauwers, J., Appels, L., Thompson, I. P., Degrève, J., Van Impe, J. F., & Dewil, R. (2013). Mathematical modelling of anaerobic digestion of biomass and waste: Power and limitations. *Progress in Energy and Combustion Science*, *39*(4), 383–402.

Lee, D. H., Behera, S. K., Kim, J. W., & Park, H.S. (2009). Methane production potential of leachate generated from Korean food waste recycling facilities: a lab-scale study. *Waste Management*, *29*(2), 876–882.

Li, Y., Yan, X. L., Fan, J. P., Zhu, J. H., & Zhou, W. B. (2011). Feasibility of biogas production from anaerobic co-digestion of herbal-extraction residues with swine manure. *Bioresource Technology*, *102*(11), 6458–6463.

Liu, C. F., Yuan, X. Z., Zeng, G. M., Li, W.W., & Li, J. (2008). Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource Technology*, *99*(4), 882–888.

Lynd, L. R., Weimer, P. J., Zyl, W. H. Van, & Pretorius, I. S. (2002). Microbial Cellulose Utilization : Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews, 66*(3), 506–577.

Mairet, F., Bernard, O., Ras, M., Lardon, L., & Steyer, J.P. (2011). Modeling anaerobic digestion of microalgae using ADM1. *Bioresource Technology*, *102*(13), 6823–6829.

Mata-Alvarez, J., Dosta, J., Macé, S., & Astals, S. (2011). Co-digestion of solid wastes: A review of its uses and perspectives including modeling. *Critical Reviews in Biotechnology*, *31*(2), 99–111.

Moletta, R., Verrier, D., & Albagnac, G. (1986). Dynamic modelling of anaerobic digestion. *Water Research*, *20*(4), 427-434.

Mosey, F.E. (1983). Mathematical modelling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile fatty acids from glucose. *Water Science and Technology*, *15*(8-9), 209-232.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*,*96*(6), 673–686.

Müller, V., & Frerichs, J. (2013). Acetogenic bacteria.In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0020086.pub2.

Nielsen, H. B., Mladenovska, Z., Westermann, P., & Ahring, B. K. (2004). Comparison of two-stage thermophilic (68 ºC /55 ºC) anaerobic digestion with one-stage thermophilic (55 ºC) digestion of cattle manure. *Biotechnology and Bioengineering*, *86*(3), 291–300.

Onodera, M., Ootsu, T., Sato, E., Kusakabe, M., Takesono, S., Harashima, I., & Shigeno, T. (2007). Biogas production from waste milk by thermophilic anaerobic digestion. *Journal of Biotechnology*, *131*(2), S176.

Pang, Y.Z., Liu, Y.P., Li, X.J., Wang, K.S., Yuan H.R. (2008). Improving Biodegradability and Biogas Production of Corn Stover through Sodium Hydroxide Solid State Pretreatment. *Energy& Fuels*, *22*(4), 2761-2766.

Park, Y. J., Tsuno, H., Hidaka, T., & Cheon, J. H. (2008). Evaluation of operational parameters in thermophilic acid fermentation of kitchen waste. *Journal of Material Cycles and Waste Management*, *10*(1), 46–52.

Pavlostathis, S. G., & Gossett, J. M. (1985). Alkaline treatment of wheat straw for increasing anaerobic biodegradability. *Biotechnology and Bioengineering*, *27*(3), 334–344.

Romano, R. T., Zhang, R., Teter, S., & Mcgarvey, J. A. (2009). The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. *Bioresource Technology*, *100*(20), 4564–4571.

Sahlström, L. (2003). A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*, *87*(2), 161–166.

Sanders, W.T.M., Geerink, M., Zeeman, G., & Lettinga, G. (2000). Anaerobic hydrolysis kinetics of particulate substrates. *Water Science and Technology*, *41*(3), 17-24.

Siegrist, H., Renggli, D., & Gujer, W. (1993). Mathematical modelling of anaerobic mesophilic sewage sludge treatment. *Water Science and Technology*, *27*(2), 25–36.

Sievers, D. M., & Brune, D. E. (1978). Carbon / nitrogen ratio and anaerobic digestion of swine waste. *Transactions of the ASAE*, *21*(3), 537–541.

Suryawanshi, P. C., Chaudhari, A. B., & Kothari, R. M. (2010). Thermophilic anaerobic digestion: the best option for waste treatment. *Critical Reviews in Biotechnology*, *30*(1), 31–40.

Terlesky, K.C., & Ferry, J.G. (1988). Ferredoxin requirement for electron transport from the carbon monoxide dehydrogenase complex to a membrane-bound hydrogenase in acetate-grown Methanosarcina thermophila. *The Journal of Biological Chemistry*, *263*, 4075-4079.

Vavilin, V. A., Rytov, S.V., & Lokshina, L. Y. A. (1996). A decription of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresource Technology, 56*(2), 229–237.

Vavilin, V. A., Rytov, S. V, Lokshina, L. Y. A., Rintala, J. A., & Lyberatos, G. (2001). Simplified hydrolysis models for the optimal design of two-stage anaerobic digestion. Water Research, *35*(17), 4247–4251.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928–7940.

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology*, *85*(4), 849–860.

Whitman, W. B., Bowen, T. L., & Boone, D. R. (2006). *The Prokaryotes*. (M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt, Eds.) (pp. 165–207). New York, NY: Springer New York. doi:10.1007/0-387-30743-5.

Willis, J., Schafer, P. (2006). Advances in thermophilic anaerobic digestion. Brown and Caldwell Technical Papers. *Water Environment Foundation*. 5378-5392.

Wilson, C. A., Murthy, S. M., Fang, Y., & Novak, J. T. (2008). The effect of temperature on the performance and stability of thermophilic anaerobic digestion. *Water Science and Technology*, *57*(2), 297–304.

Wood, H.G., & Ljungdahl L. (1991). Autotrophic character of acetogenic bacteria. In J.M. Shively, L.L. Barton (Eds.), *Variations in autotrophic life* (pp. 201-250). San Diego, CA: Academic Press. d

Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology*, *101*(11), 4042–4047.

Xie, S., Lawlor, P. G., Frost, J. P., Hu, Z., & Zhan, X. (2011). Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technology*, *102*(10), 5728–5733.

Yilmaz, T., Yuceer, A., & Basibuyuk, M. (2008). A comparison of the performance of mesophilic and thermophilic anaerobic filters treating papermill wastewater. *Bioresource Technology*, *99*(1), 156–163.

Zaher, U., Li, R., Jeppsson, U., Steyer, J.-P., & Chen, S. (2009). GISCOD: general integrated solid waste co-digestion model. *Water Research*, *43*(10), 2717–2727.

Zheng, M., Li, X., Li, L., Yang, X., & He, Y. (2009). Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment. *Bioresource Technology*, *100*(21), 5140–5145.

Zinder, S.H., Anguish, T. & Cardwell, S.C. (1984). Effects of temperature on methanogenesis in a thermophilic (58 oC) anaerobic digestor. *Applied and Environmental Microbiology*, *47*(4), 808-813.

CHAPTER 2: TRANSITION OF ANAEROBIC DIGESTION FROM MESOPHILIC TO THERMOPHILIC CONDITIONS FOR THE TREATMENT OF SWINE WASTEWASTER AND CORN STOVER

Abstract

The transition of anaerobic digestion from mesophilic to thermophilic conditions using a one-step temperature jump from 35 ºC to 50 ºC was studied in two continuously stirred tank reactors (CSTRs) treating swine wastewater and corn stover. Both reactors were operated at constant organic loading rate of 1.074 kg VS/m^3/d for a hydraulic retention time (HRT) of 25 days. Although the one-step temperature jump from 35 ºC to 50 ºC resulted in severe fluctuation of daily methane production and volatile fatty acids concentrations, the systems reached stability after 25 days. It can be concluded that the strategy for changing temperature from mesophilic to thermophilic condition using one-step temperature jump is feasible in anaerobic CSTR treating swine wastewater and corn stover. A comparison of process stability and efficiency of reactors at steady state was also evaluated under mesophilic and thermophilic conditions. The results indicated that thermophilic anaerobic digestion performed better in regards to daily gas production, specific gas yield and chemical oxygen demand (COD) removal. The effects of HRT on thermophilic anaerobic digestion process was investigated and it was shown that the system could be operated at an HRT as low as 5 days.

Keywords: Anaerobic digestion; Corn stover; Hydraulic retention time; Mesophilic; Swine wastewater; Thermophilic; Volatile fatty acid

1. Introduction

Rapid expansion of swine industry has led to large volumes of manure and wastewater which contain organic matter and inorganic nutrients (Ahn et al., 2006). Anaerobic digestion has been proven to be a cost-effective solution for the treatment of swine manure and wastewater due to its methane formation and low sludge production (Yang et al., 2004). Swine manure is considered as an excellent feedstock for anaerobic digestion, because of its strong buffer capacity and high concentration of macro- and micro-nutrients (Weiland, 2000). The methane potential of swine manure is varied and influenced by factors such as type, growth stage, feed amount, and bedding materials (Møller et al. 2004). However, ammonia inhibition had been observed in some studies when treating swine manure alone (Hansen et al., 1998; Kaparaju et al., 2005; Strik et al., 2006). This is attributed to its low carbon/nitrogen (C/N) ratio which is typically in the range of 2-8 for swine manure (Hansen et al., 1998).

Substantial quantities of unused corn stover, wheat straw and other agricultural residues are left-overs of a variety of crops, and can be used for energy generation (Kalra et al., 1986). Utilizing agricultural residues as substrates in anaerobic digestion is an environmentally-friendly method since the traditional method of burning residues brings potential environmental problems such as air pollution (Li et al., 2008). Agricultural residues are not commonly used as the sole substrate in the anaerobic digestion process due to their high C/N ratio (60-90). Unbalanced carbon and nitrogen in the agricultural residues may cause nutrient deficiency in anaerobic digestion. The idea of combining animal manure with crop residues is to create a suitable C/N ratio for the anaerobic digestion process.

Anaerobic co-digestion of swine manure and agricultural residues has been extensively investigated in the past decades (Cuetos et al., 2011; Li et al., 2011; Wu et al., 2010; Xie et al., 2011). Early studies on co-digestion include swine manure with corn stover (Fujita et al., 1980), dairy manure and barley straw (Hills, 1980), swine manure and wheat straw (Fischer et al., 1983), and beef cattle manure with wheat straw (Hashimoto, 1983). Cuetos et al. (2011) investigated the anaerobic co-digestion of swine manure with maize, rapeseed and sunflower residues under batch and semi-continuous conditions. The results indicated that the anaerobic co-digestion system resulted in a major increase in the amount of biogas produced daily. Similar results were also observed by Wu et al. (2010) when they co-digested swine manure with corn stalks, oat straw and wheat straw. They found the daily maximum biogas volume increased by 11.4-fold when the swine manure was co-digested with corn stalks in comparison with the control. The C/N ratio, concentrations of macro- and micro-nutrients and buffer capacity are balanced by mixing the substrates together, thus results in an increase in biogas production (Zhang et al., 2011).

Anaerobic digestion process can be carried out under mesophilic (30-40 ºC) and thermophilic (50-60 ºC) conditions (El-Mashad et al., 2004), with mesophilic condition being commonly applied in industry. Recently, more and more attention is paid to thermophilic anaerobic digestion. This is because in comparison with mesophilic digestion, thermophilic anaerobic digestion offers many advantages such as higher specific growth rate of anaerobic microbes, higher biochemical reaction rates in fermentation, higher percentage of destruction of pathogens and weed seeds, improved solid-liquid separation, and greater stability of digestate (van Lier, 1995; Duran and Speece, 1997; Suryawanshi et al., 2010). However, one of the major challenges for thermophilic anaerobic digestion is the longer start-up time, which is attributed to the low growth yields of thermophilic microorganisms (Suryawanshi et al., 2010). The longer start-up time make the thermophilic anaerobic digestion process more susceptible to toxicity and results in the failure subsequently. To address the challeng, the transition of anaerobic stabilization process from mesophilic to thermophilic conditions has been investigated by many researchers.

Generally, there are two strategies for changing the temperature from mesophilic to thermophilic conditions: one is one-step temperature jump, and the other is step-wise gradual temperature increase. These two strategies have been applied at both laboratory- and full-scale levels (Fang and Lau, 1996; Iranpour et al., 2002; Palatsi et al., 2009; Syutsubo et al., 1997; van Lier et al., 1992; Zábranská et al., 2002). Boušková et al. (2005) evaluated these two strategies for the transtion from mesophlic to thermophilic digestion of sewage sludge in a CSTR with a hydraulic retention time (HRT) of 20 days and organic loading rate of 1.38g VS/L/day. A one-step temperature jump from 37 to 55 ºC took about 30 days to reach a stable operation under thermophilic conditions, compared to that of 70 days by applying step-wise temperature increase (37, 42, 47, 51, and 55 ºC). Palatsi et al. (2009) evaluated a single direct temperature change, from 35 ºC to 55 ºC, and a multi-step temperature change (35 ºC -43 ºC -50 ºC -55 ºC) for the transition from mesophilic to thermophilic condition in a CSTR treating sewage sludge. The results also indicated the one-step strategy is better than the multi-step strategy in terms of time spent. Ghosh (2000) concluded that the best strategy for temperature changes (mesophilic to thermophilic condition) is to raise the temperature of mesophilic inoculum to 55 ºC in one step for anaerobic digestion of sewage sludge. . However, information relative to the temperature transition from mesophilic to thermophilic digestion for the anaerobic co-digestion process of animal manure with agricultural residues is limited. The purpose of this study was to investigate the adaption of stable mesophilic (35 ºC) reactors to a thermophilic (50 ºC) condition by applying a one-step increase of temperature in anaerobic CSTRs treating swine wastewater and corn stover. Further evaluation of the effect of HRT on thermophilic anaerobic co-digestion was also investigated in the study.

2. Materials and Methods

2.1 Swine wastewater

Raw swine wastewater was obtained from the Swine Unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University, Raleigh, NC, USA. The swine wastewater was delivered to the laboratory and stored in the refrigerator at 4 ºC until use. The chemical characteristics of the swine wastewater are shown in Table 1.

Table 1. Characteristics of the swine wastewater from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University, Raleigh, NC, USA.

Parameter	Unit	Value
Total Solids (TS)	%	0.25
Volatile Solids (VS)	% of TS	51.26
Chemical Oxygen Demand (COD)	mg/L	5,710
Total Organic Carbon (TOC)	mg/L	1,137
Total Kjeldahl Nitrogen (TKN)	mg/L	825
C/N Ratio	-	1.38
pH	-	6.90

2.2 Corn stover

Corn stover is low-cost and the most abundant agricultural residue in the United States (Kadam et al., 2003). The estimated availability of corn stover in the US is 153 million t/yr on dry basis (Glassner et al., 1998). The composition of corn stover is typically 35-40% of cellulose, 17-35% of hemicellulose and 7-18% of lignin (Garrote et al., 1999). In this study, air-dried corn stover was collected from the Central Crops Research Station in Clayton, North Carolina, USA. After the corn stover was collected, it was ground using a Thomas Wiley Laboratory Mill (Model No. 4) to reduce its particle size into 2 mm. The corn stover was stored in sealed plastic bags at room temperature. The chemical characteristics of the corn stover are shown in Table 2.

Table 2. Characteristic of the corn stover from the Central Crops Research Station in Clayton, North Carolina, USA.

Parameter	Unit	Value
TS	%	92.6
VS (%TS)	% of TS	94.68
Total Carbon	%	41.43
Total Nitrogen	%	0.46
COD	mg/g	2,031
C/N Ratio	-	90.07
pH	-	6.65

2.3 Experimental setup and operation

The experiment was carried out in two CSTRs (R1 and R2) (model number: MGF-214, New Brunswick Scientific Company, Enfield, Connecticut, USA). Each reactor had a working volume of 12 L. The temperature was controlled by wrapping heating tape (model number: BS0051080L, BriskHeat Corporation, Columbus, Ohio, USA) around the vessel of the reactor. Before the experiment was started, both reactors were operated at steady-state mesophilic operation at 35 ^{o}C with an HRT of 25 days and organic loading rate (OLR) of 1.074 kg VS/m^3/d. The system was determined as steady-state when the fluctuations in biogas production, methane content and pH were below 10% in a week (Boušková et al., 2005). Once stabilization of these parameters were achieved, the temperature in both reactors was increased in one-step from 35 ºC directly to 50 ºC on Day 1. During the experiment, the reactors were operated at constant feed rate of 480 mL/day and hydraulic retention time of 25 days.

2.4 Analytical methods

TS, VS, COD, pH, TKN, TOC, total carbon and total nitrogen were determined according to Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998). Biogas production was recorded daily by a wet-tip gas meter (Archae Press, Nashville, Tennessee, USA) and biogas composition was determined by gas chromatograph (GC) (model number: GC-17A, Shimadzu Corporation, Kyoto, Japan) equipped with a thermal conductivity detector and 100/120 carbosieve SII column (dimensions: 3.0 m length × 3.00 mm inner diameter). The temperature of detector and column was set at 250.0 ºC and 50.0 ºC, respectively. Helium was used as the carrier gas at a flow rate of 30.0 mL/min. The GC detected relative percentages of nitrogen, methane and carbon dioxide. The GC was calibrated using three custom-made gas standards ($N_2/He/CH_4/CO_2$= 5/5/70/20; $N_2/He/CH_4/CO_2$= 20/5/5/70; $N_2/He/CH_4/CO_2$= 70/5/20/5). VFAs concentration was determined using high-performance liquid chromatography (HPLC) (model number: Dionex UltiMate 3000, Dionex Corporation, Sunnyvale, California, USA) equipped with a refractive index detector and an Aminex HPX-87H column.

3. Results and discussion

3.1 Mesophilic to thermophilic transition

Daily methane production (ml/day) and methane content (%) for R1 and R2 during transition period are shown in Figure 1 and Figure 2, respectively. As shown in Figure 1, the temperature of R1 was increased in one-step from 35 ºC directly to 50 ºC on Day 1. An immediate drop of methane production was observed after the temperature change. However, the methane production increased by 38.15% in R1 from Day 3 to Day 4. The increase in methane production was probably the result of the degradation of VFAs by the thermophiles which already existed under the mesophilic condition. Chachkhiani et al. (2004) concluded that the dominant species taking part in the start-up of thermophilic anaerobic digestion were the thermophilic microorganisms already present in the mesophilic inoculum at a sub-dominant level but became dominant under thermophilic anaerobic conditions. Thereafter, a continuous decrease in methane production was observed. The phenomenon could be explained by two possibilities: one is the instable development of culture (Iranpour et al., 2002) and the other is the accumulation of VFAs which is unfavorable for the growth of methanogens. Considering the continuous drop in methane production, the feeding was suspended in R1 from Day 12. The methane production stopped on Day 13 and it remained the same for another 2days, and then a sudden increase in methane production was observed on Day 16. The sudden increase could be explained by a degradation of accumulated VFAs in R1. Daily feeding was resumed from Day 17. R1 was operated at constant feed rate of 480 mL/day swine wastewater and 14g/d corn stover. R2 performed similar to R1 except that methane production started to increase two days later.

The methane content of R1 responded to the one-step temperature jump by an initial decrease reaching the minimum level of 19% on Day 10. Methane production increased subsequently and the methane content increased to a peak of 56% on Day 18. By Day 25, the methane content for the reactor was stabilized, slightly fluctuating around the value of 41% in the biogas. The methane content of R2 performed similar to R1 except that methane content started to increase two days later in R2. The steady-state thermophilic operation were achieved in both reactors in 25 days. The results were similar to that obtained from Boušková et al. (2005) who reported the required time for adaptation of the reactor to thermophilic temperature in one-step increase for anaerobic digestion of sewage sludge was 30 days.

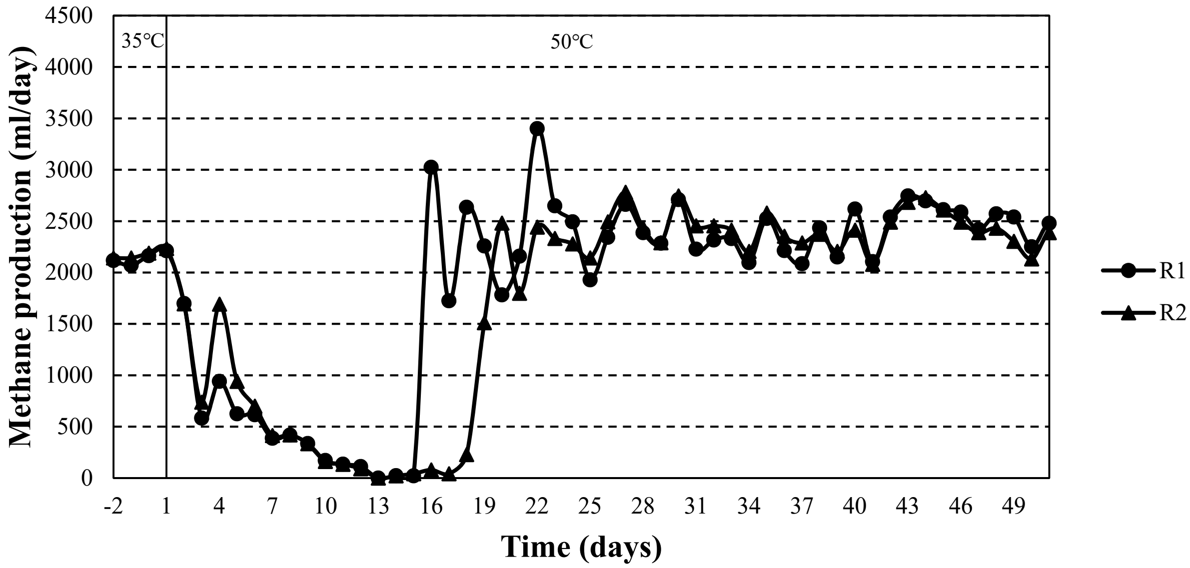


Figure 1. Daily methane production in anaerobic reactors treating swine wastewater and corn stover during the transition from mesophilic (35 ºC) to thermophilic conditions (50 ºC).

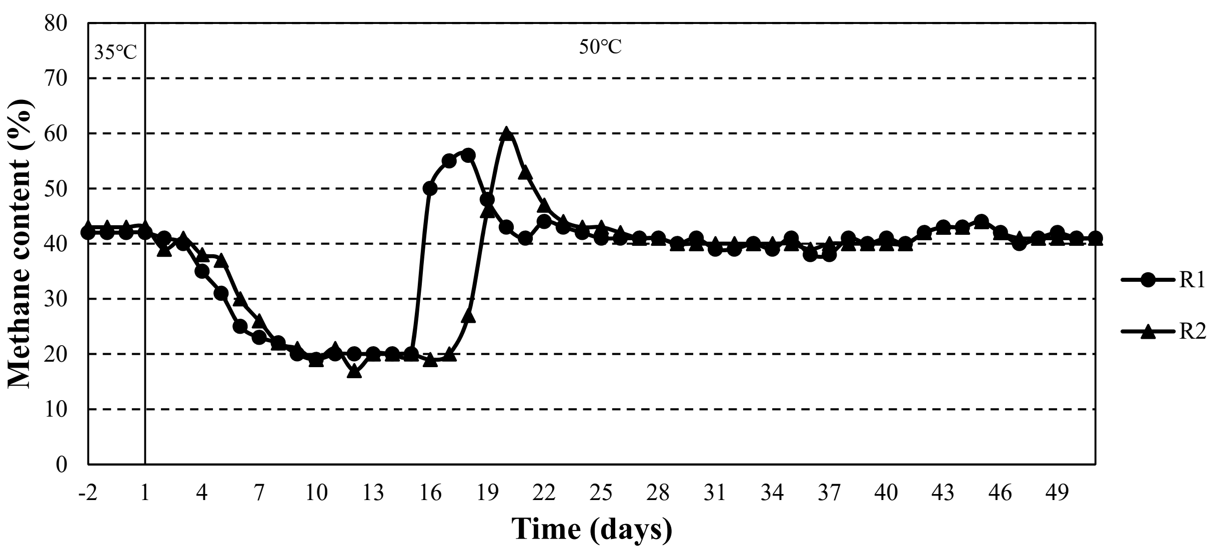


Figure 2. Methane content in the biogas from anaerobic reactors treating swine wastewater and corn stover during the transition from mesophilic (35 ºC) to thermophilic conditions (50 ºC).

3.2 VFAs concentration during transition period

VFAs, namely acetic acid, propionic acid and butyric acid are important intermediates in the anaerobic digestion process. High VFAs concentration has an inhibitory effect on the methanogenesis process (Ward et al., 2008). The toxicity of VFAs concentration in the anaerobic digestion process has been studied by many researchers. Siegert et al. (2005) studied the effect of VFAs additions on the anaerobic digestion of cellulose and glucose and found the fermentation of cellulose and glucose were inhibited at VFAs concentration above 2 g l^{-1} and 4 g l^{-1}, respectively. Gourdon et al. (1987) investigated the effects of propionic acid concentration on anaerobic digestion of pig manure and it was concluded that the increase in propionic acid concentration could be a sign of digestion instability and measures must be taken to avoid digester failure. Ahring et al. (1995) discussed the use of VFAs as indicators of process imbalance in thermophilic anaerobic digestion using manure as substrate. Their results was testified that the changes in VFAs was a good parameter to indicate process stability. In this study, only acetic acid was detected during transition period and the results are shown in Figure 3.

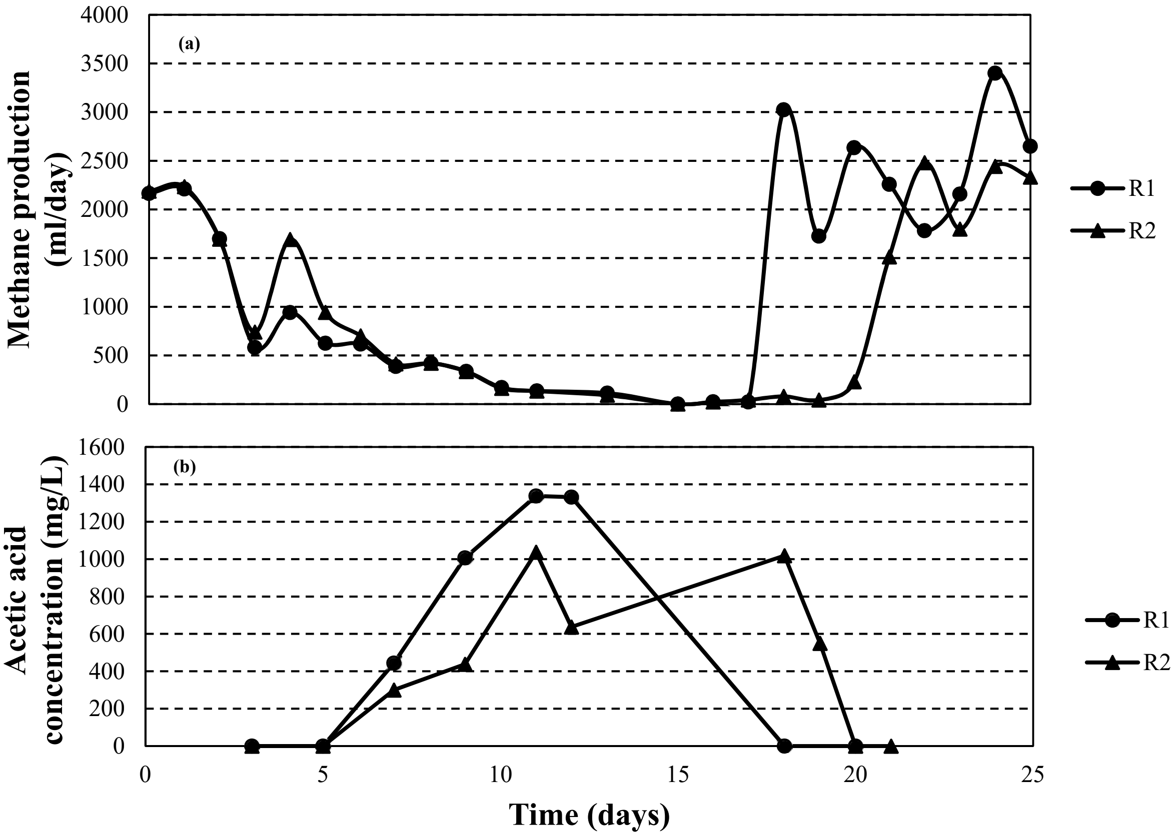


Figure 3. Acetic acid concentration in anaerobic reactors treating swine wastewater and corn stover during the transition from mesophilic (35 ºC) to thermophilic conditions (50 ºC).

It can be seen from Figure 3 that with the increase in acetic acid concentration in an anaerobic reactor, the methane production decreased. For R1, when the acetate concentration accumulated to 1,338 mg/l, the corresponding methane production dropped to a low level of 134 ml/day. The accumulated acetic acid resulted in an acidic environment that inhibited the growth of the methanogens. Methane production started to increase when the acetic acid was consumed by thermophilic microorganisms. Acetic acid accumulation and low methane production were also observed in previous studies using the one step temperature jump from mesophilic to thermophilic condition (Bouškova ́et al., 2005; Fang and Lau, 1996; Palatsi et al., 2009). Bouškova ́et al. (2005)

reported the acetic acid accumulated for 11 days after the temperature was increased in one-step from mesophilic to thermophilic condition for anaerobic digestion of sewage sludge. The acetic acid reached a highest concentration of 2,232 mg/L which was higher than the value observed in this study. This is attributed to the differences in the substrates used.

3.3 Comparison of mesophilic and thermophilic process efficiencies

The comparative process stability and efficiency of mesophilic (35 ºC) and thermophilic (50 ºC) anaerobic digestion were evaluated and the results are shown in Table 3. The data were obtained from the analyses of the steady state conditions in reactors under mesophilic and thermophilic operation, respectively. The results are expressed as an average and a standard deviation.

Table 3. Performances of anaerobic co-digestion at stable operation under mesophilic (35 ºC) and thermophilic (50 ºC) conditions.

	Mesophilic	Thermophilic
HRT (days)	25	25
Temperature (ºC)	35	50
OLR (kg VS/m^3/d)	1.074	1.074
Methane content (%)	42.5±0.7	41±0.1
Biogas production (mL/d)	5,064±34	5,894±14
Methane production (mL/d)	2,152±50	2,408±11
Specific biogas yield (m^3/kg VS_{added})	0.39±0.02	0.46±0.03
Specific methane yield (m^3/kg VS_{added})	0.17±0.01	0.19±0.02
COD removed (%)	77.47	80.99
TS removed (%)	65.16	65.51

Table 3 Continued

	Mesophilic	Thermophilic
VS removed (%)	70.50	70.50
pH	6.64±0.06	6.68±0.13

Table 3 shows that the average daily gas production and specific gas yield are higher at thermophilic conditions compared with those at mesophilic conditions. This is attributed to the higher substrate utilization and growth rates of the thermophilic microorganisms (Duran et al., 1997; Mladenovska et al., 2000). The COD removal rate increased by 4.35% when the reactor was operated under thermophilic condition than that under mesophilic conditions. There are no noticeable changes with regards to TS and VS reductions. The pHs of both thermal conditions are within the optimum pH range of 6.5-7.5.

3.4 Effect of HRT on thermophilic anaerobic digestion

The effect of HRT on the performance and treatment efficiency of the thermophilic anaerobic co-digestion was also nvestigated in this study. HRT is an important design parameter since it determines the microbial/substrate reaction time and further influences the removal efficiency of the substrate (Aguilar et al., 2013). A number of previous studies on the effect of HRT on anaerobic digestion are available (Feng et al., 2008; Rizvi et al., 2015; Xing et al., 1992). In this study, the HRT of R1 was decreased step-wise: 25d→20d→15d→10d→5d. The reactor was operated for a period of at least one retention time at each level. The average gas production and specific gas yield at different HRTs are summarized in Table 4. The results indicated that the gas production and specific gas yield were higher at longer HRTs. This is because a higher HRT increases the contact time between the substrate and microbial community and lowers the washout of thermophilic microorganisms (Rizvi et al., 2015).

Table 4. Average biogas production and gas yield at different hydraulic retention times (HRTs) in a thermophilic anaerobic reactor treating swine wastewater and corn stover.

	HRT (days)				
	25	20	15	10	5
Biogas production (mL/d)	5,936	5,398	5,003	5,047	4,756
Methane production (mL/d)	2,420	2,189	1,987	1,976	1,869
Specific biogas yield ($m^3/kg\ VS_{added}$)	0.46	0.41	0.38	0.37	0.31
Specific methane yield ($m^3/kg\ VS_{added}$)	0.19	0.17	0.15	0.14	0.12

COD and VS removals are good indicators of the performance of anaerobic digestion processes (De la Rubia et al., 2002). Figure 4 illustrates the COD and VS removal efficiency at each HRT studied under thermophilic conditions. It can be seen from Figure 4 that the COD removal rate and VS removal rate were the highest when the reactor was operated at an HRT of 25 days. Further research work is needed to investigate methods (e.g. the application of pretreatment) to enhance the process efficiency at low HRT levels.

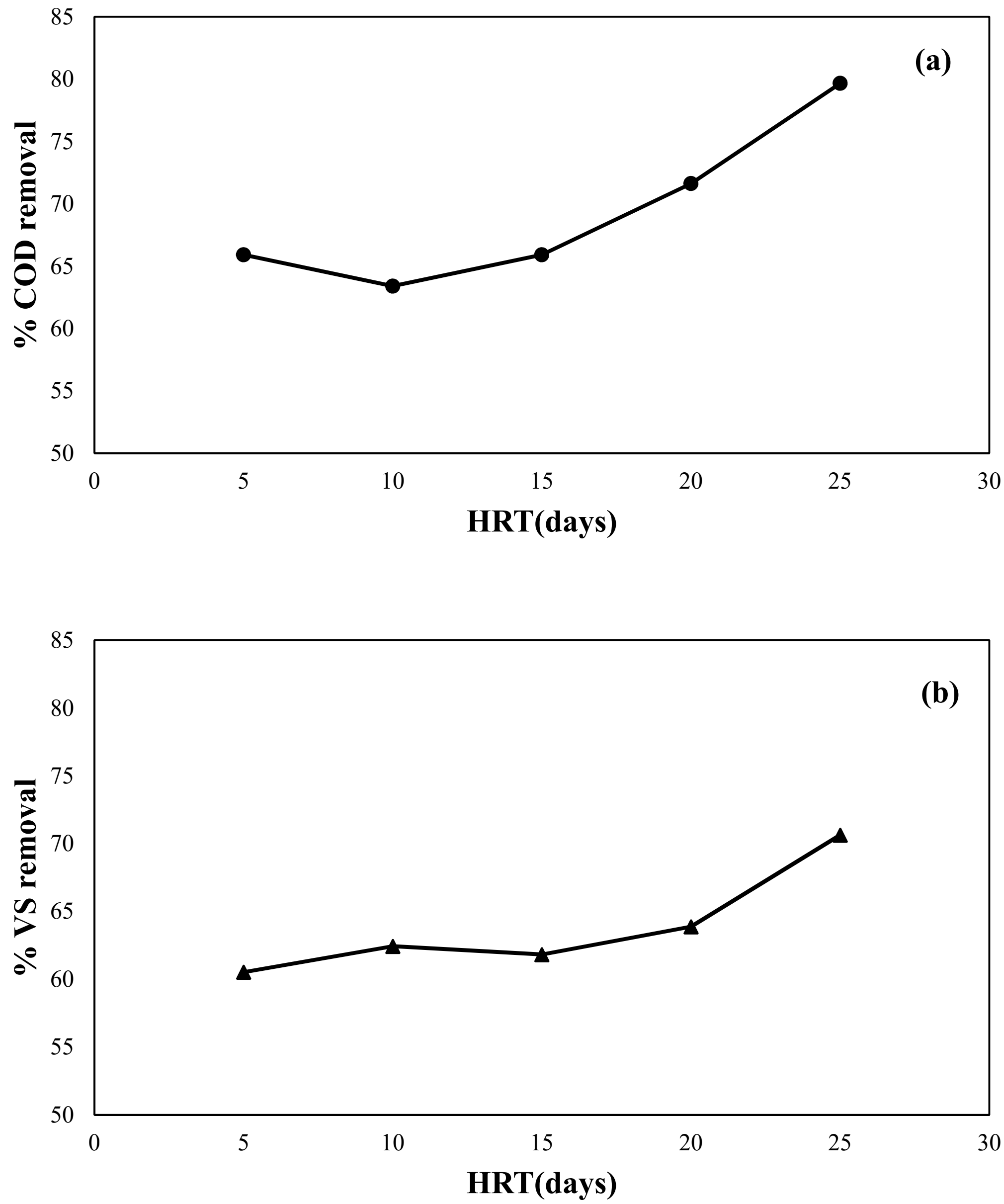


Figure 4. a) COD removal and b) VS reduction at different hydraulic retention times (HRTs) in a thermophilic anaerobic reactor treating swine wastewater and corn stover.

4. Conclusions

Based on the results and discussion, the following conclusions can be made from this study:

(a) One-step temperature jump has been proved to be an effective way to change from mesophilic (35 ºC) to thermophilic (50 ºC) culture in anaerobic CSTRs treating swine wastewater and corn stover. It took 25 days in total to get stabilized thermophilic culture in duplicate reactors.

(b) The accumulated acetate inhibited the growth of methanogens and resulted in low methane production during the transition period. The methane production increased back to normal levels when the acetate was consumed.

(c) The comparative results of the reactor performance at stable mesophilic and thermophilic conditions clearly showed the superior performance of thermophilic anaerobic digestion with respect to higher daily biogas production, specific gas yield and COD removal.

(d) The thermophilic anaerobic co-digestion system could be stably operated at an HRT as low as 5 days. However, a longer HRT resulted in higher gas yield and improved COD and VS removal efficiencies.

REFERENCES

Aguilar, M. A. R., Fdez-Güelfo, L.A., Álvarez-Gallego, C.J., & García, L. I. R. (2013). Effect of HRT on hydrogen production and organic matter solubilization in acidogenic anaerobic digestion of OFMSW. *Chemical Engineering Journal*, *219*, 443–449.

Ahn, J., Hoan, T., Kim, S. D., & Hwang, S. (2006). The effect of calcium on the anaerobic digestion treating swine wastewater. *Biochemical Engineering Journal*, *30*(1), 33–38.

Ahring, B. K., Sandberg, M., & Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Applied Microbiology Biotechnology*, *43*(3), 559-565.

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed). New York, USA: American Public Health Association.

Boušková, A., Dohányos, M., Schmidt, J.E., & Angelidaki, I. (2005). Strategies for changing temperature from mesophilic to thermophilic conditions in anaerobic CSTR reactors treating sewage sludge. *Water Research*, *39*(8), 1481–1488.

Chachkhiani, M., Dabert, P., Abzianidze, T., Partskhaladze, G., Tsiklauri, L., Dudauri, T., & Gordon, J.J. (2004). 16S rDNA characterisation of bacterial and archaeal communities during start-up of anaerobic thermophilic digestion of cattle manure. *Bioresource Technology*, *93*(3), 227–232.

Cuetos, M. J., Fernández, C., Gómez, X., & Morán, A. (2011). Anaerobic co-digestion of swine manure with energy crop residues. *Biotechnology and Bioprocess Engineering*, *16*(5), 1044–1052.

De la Rubia, M.A., Perez, M., Romero, L. I., & Sales, D. (2002). Anaerobic Mesophilic and Thermophilic Municipal Sludge Digestion. *Chemical & Biochemical Engineering Quarterly, 16*(3), 119–124.

De la Rubia, M. A., Romero, L. I., Sales, D., & Perez, M. (2005). Temperature Conversion (Mesophilic to Thermophilic) of Municipal Sludge Digestion. *American Institute of Chemical Engineers Journal*, *51*(9), 2581–2586.

Duran, M., & Speece, R.E. (1997). Temperature staged anaerobic processes. *Environmental Technology*, *18*(7), 747-754.

El-Mashad, H. M., Zeeman, G., van Loon, W. K. P., Bot, G. P. A, & Lettinga, G. (2004). Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Bioresource Technology*, *95*(2), 191–201.

Fang, H.H.P., & Lau, I.W.C. (1996). Startup of thermophilic (55℃) UASB reactors using different mesophilic seed sludges. *Water Science and Technology*, *34*(5-6), 445-452.

Feng, H. J., Hu, L. F., Shan, D., Fang, C. R., & Shen, D. S. (2008). Effects of temperature and hydraulic residence time (HRT) on treatment of dilute wastewater in a carrier anaerobic baffled reactor. *Biomedical and Environmental Sciences*, *21*(6), 460–466.

Fischer, J. R., Iannotti, E. L., & Fulhage, C. D. (1983). Production of methane gas from combinations of wheat straw and swine manure.*Transactions of the ASAE*, *26*(2), 546-548.

Fujita, M., Scharer, J. M., & Moo-Young, M. (1980). Effect of corn stover addition on the anaerobic digestion of swine manure. *Agricultural Wastes*, *2*(3), 177-184.

Garrote, G., Dom_ınguez, H., Paraj_o, J.C. (1999). Hydrothermal processing of lignocellulosic materials. Holz Roh Werkst 57, 191–202.

Ghoso, S., (2000). Comparative evaluation of thermophilic and mesophilic anaerobic digestion of municipal sludge. WEFTEC, 905-923.

Glassner, D., Hettenhaus, J., & Schechinger, T. (1998). Corn stover collection project—a pilot for establishing infrastructure for agricultural residue and other crop collection for biomass processing to ethanol. In: Proc. Bioenergy 1998 Conference, 4–8 October, 1998, Madison, WI, 1100–1110.

Gourdon R., & Vermande P. (1987). Effects of Propionic Acid Concentration on Anaerobic Digestion of Pig Manure. *Biomass*, *13*(1), 1-12.

Hansen, K. H., Angelidaki, I., & Ahring, B. K. (1998). Anaerobic digestion of swine manure: inhibition by ammonia. *Water Research*, *32*(1), 5–12.

Hashimoto, A G. (1983). Conversion of straw-manure mixtures to methane at mesophilic and thermophilic temperatures. *Biotechnology and Bioengineering*, *25*(1), 185–200.

Hills, D. J. (1980). Biogas from a high solids combination of dairy manure and barley straw. *Transactions of the ASAE*, *23*(6), 1500–1504.

Iranpour, R., Oh, S., Cox, H. H. J., Shao, Y. J., Moghaddam, O., Kearney, R. J., Deshusses, M.A., Stenstorm, M.K., & Ahring, B. K. (2002). Changing Mesophilic Wastewater Sludge Digestion into Thermophilic Operation at Terminal Island Treatment Plant. *Water Environment Research, 74*(5), 494-507.

Kadam, K. L., & McMillan, J. D. (2003). Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresource Technology, 88(1), 17–25.

Kalra, M. S., & Panwar, J. S. (1986). Anaerobic digestion of rice crop residues. *Agricultural Wastes*, *17*(4), 263–269.

Kaparaju, P., & Rintala, J. (2005). Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. *Resources, Conservation and Recycling*, *43*(2), 175–188.

Li, L., Wang, Y., Zhang, Q., Li, J., Yang, X., & Jin, J. (2008). Wheat straw burning and its associated impacts on Beijing air quality. Science in China Series D: Earth Sciences, 51(3), 403–414.

Li, Y., Yan, X.-L., Fan, J.-P., Zhu, J.-H., & Zhou, W.-B. (2011). Feasibility of biogas production from anaerobic co-digestion of herbal-extraction residues with swine manure. *Bioresource technology*, *102*(11), 6458–6463.

Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. Biomass and Bioenergy, 26(5), 485–495.

Mladenovska, Z., & Ahring, B. K. (2000). Growth kinetics of thermophilic Methanosarcina spp. isolated from full-scale biogas plants treating animal manures. *Fems Microbiology Ecology*, *31*(3), 225–229.

Palatsi, J., Gimenez-Lorang, A., Ferrer, I., & Flotats, X. (2009). Start-up strategies of thermophilic anaerobic digestion of sewage sludge. *Water Science and Technology*, *59*(9), 1777–1784.

Rizvi, H., Ahmad, N., Abbas, F., Bukhari, I. H., Yasar, A., Ali, S., … Riaz, M. (2015). Start-up of UASB reactors treating municipal wastewater and effect of temperature / sludge age and hydraulic retention time (HRT) on its performance. *Arabian Journal of Chemistry*, *8*(6), 780–786.

Siegert, I., & Banks, C. (2005). The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochemistry*, *40*, 3412–3418.

Strik, D. P. B. T. B., Domnanovich, A. M., & Holubar, P. (2006). A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. *Process Biochemistry*, *41*(6), 1235–1238.

Suryawanshi, P. C., Chaudhari, A. B., & Kothari, R. M. (2010). Thermophilic anaerobic digestion: the best option for waste treatment. *Critical Reviews in Biotechnology*, *30*(1), 31–40.

Syutsubo, K., Harada, H., Ohashi, A., & Suzuki, H. (1997). An effective strart-up of thermophilic UASB reactor by seeding mesophilically-grown granular sludge. *Water Science and Technology, 36*(6-7), 391-398.

van Lier, J. B., Grolle, K. C., Stams, A. J. M., de Maccario, E. C., & Lettinga, G. (1992). Start-up of a thermophilic upflow anaerobic sludge bed (UASB) reactor with mesophilic granular sludge. *Applied Microbiology Biotechnology*, *37*(1), 130–135.

van Lier, J.B. (1995). Thermophilic anaerobic wastewater treatment: temperature aspects and process stability. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928–7940.

Weiland, P. (2000). Anaerobic waste digestion in Germany-status and recent developments. *Biodegradation*, *11*(6), 415–421.

Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH4 productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresource technology, 101(11), 4042–4047.

Xie, S., Lawlor, P. G., Frost, J. P., Hu, Z., & Zhan, X. (2011). Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technology*, *102*(10), 5728–5733.

Xing, J., & Tilche, A. (1992). The effect of hydraulic retention time on the hybrid anaerobic baffled reactor performance at constant loading. *Biomass and Bioenergy*, *3*(1), 25–29.

Yang, K., Oh, C., & Hwang, S. (2004). Optimizing volatile fatty acid production in partial acidogenesis of swine wastewater. *Water Science and Technology*, *50*(8), 169-176.

Zábranská, J., Dohányos, M., Jenícek, P., Zaplatílková, P., & Kutil, J. (2002). The contribution of thermophilic anaerobic digestion to the stable operation of wastewater sludge treatment. *Water Science and Technology, 46*(4-5), 447–453.

Zhang, L., Lee, Y. W., & Jahng, D. (2011). Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. *Bioresource Technology*, *102*(8), 5048–5059.

CHAPTER 3: IMPROVING BIODEGRADABILITY AND BIOGAS PRODUCTION OF CORN STOVER USING ALKALINE PRETREATMENT

Abstract

Agricultural residues such as corn stover is an abundant, promising material for biogas production. The biodegradability of corn stover is, however, limited because of the recalcitrant nature of the lignin it contains. Thus, a pretreatment process is necessary to disrupt recalcitrant lignocellulosic matrix and enhance the enzymatic hydrolysis of cellulose and hemicelluloses. The objective of the research was to study the sodium hydroxide (NaOH), lime ($Ca(OH)_2$) and the combination of both pretreated corn stover in co-digestion with swine wastewater for biogas production in comparison with that without pretreatment. Digestions were performed in 12 L laboratory-scale completely stirred tank reactors (CSTR) operated with an organic loading rate of 1.074 kg VS/m^3/d. The hydraulic retention time (HRT) was 5 days and the operating temperature was 50 ºC. The pretreatment conditions were set as: (a) residence time of 12 h at 50 ºC, NaOH loading of 0.10 g/g raw biomass; (b) residence time of 24 h at 50 ºC, $Ca(OH)_2$ loading of 0.10 g/g raw biomass; (c) residence time of 6 h at ambient temperature, NaOH loading of 0.10g/g raw biomass, NaOH addition at the beginning, $Ca(OH)_2$ loading of 0.02 g/g raw biomass. The results of methane fermentation study on alkaline pretreated corn stover had resulted into significant amount of increase in biogas as well as methane yields. NaOH pretreated corn stover produced 30.95% higher biogas yield and 36.84% higher methane yield compared to the untreated corn stover. Lime pretreated corn stover had resulted into an increase of 32.43% in biogas yield and 37.50% in methane yield compared to that of untreated corn stover. Combination of NaOH and lime-treated corn stover had resulted into an increase of 38.10% in biogas yield and 44.44% in methane yield compared to that of untreated corn stover.

Keywords: Anaerobic digestion; Biogas; Lignocellulose; Pretreatment; Sodium hydroxide; Lime

1. Introduction

A variety of agricultural residues such as straws, nut shells, fruit shells, fruit seeds, plant stovers, green leaves and molasses can be potentially used for renewable energy production (Zhong et al., 2011). Among them, corn stover is the most abundant agricultural residue in the United States (Kadam et al., 2003). According to the USDA (2002), the estimated availability of corn stover is 153 million dry t/yr. Conversion of corn stover to biogas through anaerobic digestion process is a cost-effective technology for corn stover disposal and utilization (Zhong et al., 2011). However, one of the challenges in converting corn stover to biogas is attributed to its lignocellulosic structure. Corn stover is mainly composed of 35-40% cellulose, 17-35% hemicellulose and 7-18% lignin (Garrote et al., 1999). Cellulose chains are embedded in a cross-linked matrix of hemicellulose surrounded by lignin. The lignin is poorly degraded under anaerobic condition, thus it prevents the enzyme from accessing into the cellulose fraction, making hydrolysis difficult. To solve this problem, pretreatmenthas been applied to improve the biodegradability of lignocellulosic materials by breaking the lignin seal and disrupt the crystalline structure of cellulose and making cellulose and hemicellulose more accessible to bacteria (Mosier et al., 2005).

A number of studies have been investigated for pretreating lignocellulosic material to enhance the enzyme accessibility. The most common pretreatment methods include physical such as size reduction (Hartmann et al. 2010) and thermal treatment (Chandra et al., 2012; Li et al.,2013), chemical such as acids, alkaline and oxidants (Taherzadeh et al., 2008) treatment, a combination of both (Talebnia et al., 2010; Geddes et al., 2010, Wang et al., 2009) and biological treatment (Yang et al., 2011; Zhong et al., 2011). Alkaline pretreatment has been proven to be more effective and compatible with subsequent anaerobic digestion when compared to other pretreatment methods (Pavlostathis et al., 1985; Sharmas et al., 2002; Soto et al., 1994). The positive effects of alkaline pretreatment include the solubilization of lignin and neutralization of various acidic products degraded from the lignocellulosic complex and the residual alkaline in treated solids is very useful in preventing the drop of pH during subsequent acidogenesis process (Pavlostathis et al., 1985). Sodium hydroxide, potassium hydroxide, ammonia and calcium hydroxide (lime) are chemicals that can be used for alkaline pretreatment (Taherzadeh et al., 2008). Pang et al. (2008) proved the sodium hydroxide pretreatment is an efficient method to improve the biodegradability and enhance biogas production of corn stover at mesophilic temperature (35 ºC). Their results indicated that when the corn stover was pretreated using 6% sodium hydroxide, 48.5% more biogas production and 71.0% more bioenergy gain were achieved when compared with untreated one. You et al. (2014) studied the effect of sodium hydroxide pretreatment on corn stover for anaerobic co-digestion of corn stover with swine manure at mesophilic condition. The optimum pretreatment condition was achieved when the corn stover was pretreated with 6% NaOH at 35 ºC for 3h, the pretreated corn stover resulted in 34.59% higher bigoas production compared with untreated one..In contrast with sodium hydroxide, lime is safer, much less expensive and easier to recover (Chang et al., 1997). Calcium ions can crosslink lignin molecules extensively under alkaline conditions (Xu et al., 2010b). Song et al. (2012) indicated the improved biodegradability and biogas production of rice straw using lime pretreatment. Xu et al. (2010a, b) and Xu et al. (2011) proved that the NaOH, lime and combination of NaOH and lime preatment of switchgrass could improve the enzymatic hydrolysis for sugar production. However, the effects of these alkaline pretreatment methods on biogas production of lignocellulosic materials in thermophilic anaerobic digestion have not been determined yet. The objective of this study is tostudy the sodium hydroxide (NaOH), lime ($Ca(OH)_2$) and combination of NaOH and $Ca(OH)_2$ pretreated corn stover in co-digestion with swine wastewater for biogas production in comparison with that without pretreatment under thermophilic condition.

2. Materials and methods

2.1 Substrates

Raw swine wastewater was taken from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University. The swine wastewater was delivered to the laboratory and stored in the refrigerator at 4 ºC until use. The air-dried corn stover was obtained from the Central Crops Research Station in Clayton, North Carolina, USA. After the corn stover was collected, it was grinded using a Thomas Wiley Laboratory Mill (Model No. 4) to reduce its particle size into 2 mm. The corn stover was stored in sealed plastic bags at room temperature.

2.2 Alkaline pretreatment of corn stover

The pretreatment conditions were determined based on the works of Xu et al. (2010a, b) and Xu et al. (2011). For NaOH pretreatment, a total of 50g of corn stover sample and 500 mL of 1% NaOH solution were placed in a storage bottle and mixed using a glass rod, forming a slurry at a solid concentration of 0.1g/mL. The bottle was purged with N_2 for 2-3 minutes to remove O_2 and sealed. Thereafter, the bottle was placed in a thermostatic water bath maintained at thermophilic temperature (50 ± 1 ºC) for 12 hours. For $Ca(OH)_2$ pretreatment, 50g of corn stover sample and 500 mL of deionized water and 5g of $Ca(OH)_2$ were mixed in a storage bottle, forming a slurry of biomass and lime milk. The bottle was purged with N_2 for 2-3 minutes to remove O_2 and sealed. Thereafter, the bottle was placed in a thermostatic water bath maintained at thermophilic temperature (50 ± 1 ºC) for 24 hours. For the combination of NaOH and $Ca(OH)_2$ pretreatment, NaOH and $Ca(OH)_2$ were mixed with corn stover at loadings of 0.1g NaOH/g raw biomass and 0.02g $Ca(OH)_2$/g raw biomass at the beginning of the experiment for residence time of 6 hours at ambient temperature.

2.3 Experimental setup and operation

The experiment was carried out in two continuously stirred tank reactor (CSTRs, R1 and R2) (model number: MGF-214, New Brunswick Scientific Company, Enfield, Connecticut, USA). Each reactor had a working volume of 12 L. The reactor was heated to desired temperature by wrapping a heating tape (model number: BS0051080L, BriskHeat Corporation, Columbus, Ohio, USA) around the vessel of the reactor. Before the experiment was started, both reactors were operated at steady-state thermophilic operation at 50 ºC with a hydraulic retention time (HRT) of 5 days and an organic loading rate of 1.279 kg VS/m^3/d. During the experiment, both reactors were still operated under HRT of 5 days at thermophilic condition (50 ºC). R1 was set as the control which was fed with swine wastewater and untreated corn stover while R2 was set as the experiment unit which was fed with swine wastewater and alkaline pretreated corn stover. When both reactors reached steady-state, they were operated for a period of 15 days (3 HRTs).

2.4 Analytical methods

Total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) content of biomass were determined in accordance with American Public Health Association (APHA) standard methods (APHA, 1998). Biogas production was recorded daily by a wet-tip gas meter (Archae Press, Nashville, Tennessee, USA) and biogas composition was determined by gas chromatograph (GC) (model number: GC-17A, Shimadzu Corporation, Kyoto, Japan) equipped with a thermal conductivity detector and 100/120 carbosieve SII column (dimensions 3.0 m length× 3.00 mm inner diameter). The temperature of detector and column was set at 250.0 ºC and

50.0 ºC, respectively. Helium was used as the carrier gas at a flow rate of 30.0 mL/min. The GC detected relative percentages of nitrogen, methane and carbon dioxide. The GC was calibrated using three custom-made gas standards ($N_2/He/CH_4/CO_2$= 5/5/70/20; $N_2/He/CH_4/CO_2$= 20/5/5/70; $N_2/He/CH_4/CO_2$= 70/5/20/5).

2.5 Statistical analysis

Analysis of variance (ANOVA) using 95% confidence level was performed to evaluate the difference in averages of biogas production between the control (R1) and experiment unit (R2). All statistical tests were performed using the SAS software version 9.4 (SAS institute, Cary, NC).

3. Results and discussion

3.1 Chemical characteristics of swine wastewater and corn stover

The chemical characteristics of swine wastewater and corn stover were shown in Table 1 and Table 2, respectively. The low C/N ratio of swine wastewater and high C/N ratio of corn stover indicate that these two substrates are suitable for anaerobic co-digestion. The C/N ratio of swine wastewater in this study was higher than the value which was 0.3 reported by Han et al. (2012). The C/N ratio of corn stover was higher than the values which were 70.9 and 26.66 reported by Zhong et al. (2012) and Wu et al. (2010), respectively.

Table 1. Characteristics of the swine wastewater from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University, Raleigh, NC, USA.

Parameter	Unit	Value
Total Solids (TS)	%	0.25
Volatile Solids (VS)	% of TS	51.26
Chemical Oxygen Demand (COD)	mg/L	5710
Total Organic Carbon (TOC)	mg/L	1137

Table 1 Continued

Parameter	Unit	Value
Total Kjeldahl Nitrogen (TKN)	mg/L	825
C/N Ratio	-	1.38
pH	-	6.90

Table 2. Characteristic of the corn stover from the Central Crops Research Station in Clayton, North Carolina, USA.

Parameter	Unit	Value
TS	%	92.6
VS (%TS)	% of TS	94.68
Total Carbon	%	41.43
Total Nitrogen	%	0.46
COD	mg/g	2031
C/N Ratio	-	90.07
pH	-	6.65

3.2 Effect of sodium hydroxide pretreatment

In this study, the daily biogas production for the NaOH-treated corn stover and untreated corn stover at steady-state was shown in Figure 1. It can be observed from Figure 1 that there was significant increase ($p<0.05$) in biogas production when the corn stover was pretreated using sodium hydroxide. Performances of two digesters are summarized in Table 3. The average biogas production was 4444 ± 384 ml in R1 and 6444 ± 184 ml in R2, respectively. 31.04% more biogas was produced when the sodium hydroxide pretreated corn stover was added compared with the untreated one. Gas analysis results indicated that the average methane content in R1 was 40% while the average methane content in R2 was 45%. Daily methane production was calculated by timing daily biogas production with corresponding methane content. Biogas and methane production based on VS loading was calculated to analyze the effects of NaOH-treatment on the gas yield of co-digestion. The biogas yield of NaOH-pretreated corn stover in this study was 0.42 $m^3/kg\ VS_{added}$ which was higher than the value (0.372 $m^3/kg\ VS_{added}$) reported by Zhu et al. (2010) for solid-state anaerobic digestion of 5% NaOH-pretreated corn stover. The difference is attributed to different substrates used and experimental procedures. The methane yield in R2 was 0.19 $m^3/kg\ VS_{added}$, the value increased 36.84% compared with the methane yield in R1 which was 0.12 $m^3/kg\ VS_{added}$.

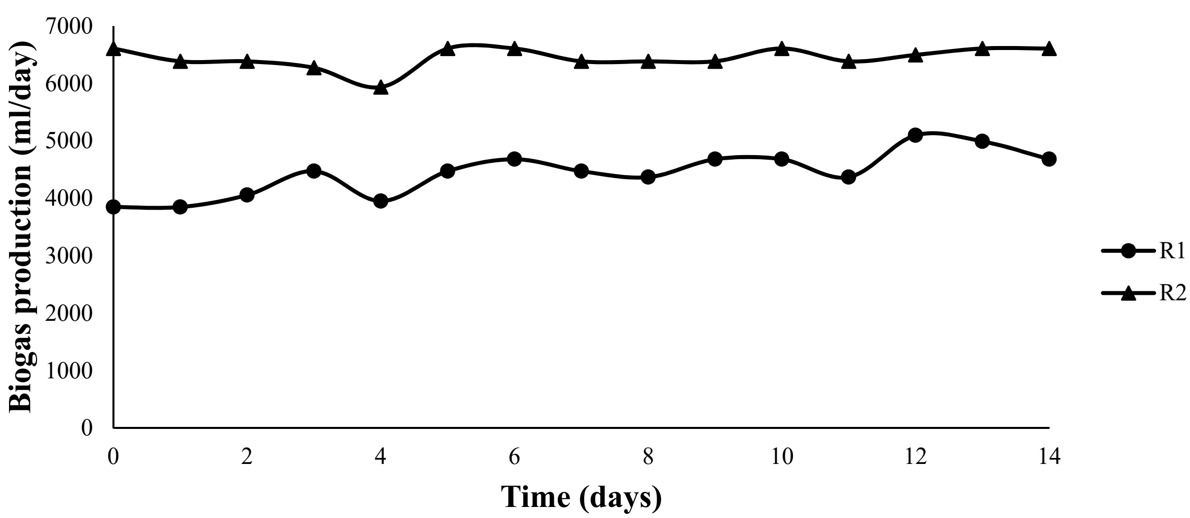


Figure 1. Daily biogas production in anaerobic reactors treating swine wastewater with untreated and NaOH-treated corn stover

Table 3. Comparison of anaerobic digesters performance using untreated corn stover and NaOH-treated corn stover

	R1	R2	Increase (%)
Biogas production (mL/d)	4444 ± 384	6444 ± 184	31.04
Methane production (mL/d)	1778 ± 154	2895 ± 100	38.58
Specific biogas yield (m^3/kg VS_{added})	0.29	0.42	30.95
Specific methane yield (m^3/kg VS_{added})	0.12	0.19	36.84

Increased biogas and methane production can be attributed to improved biodegradability of NaOH-treated corn stover that resulted from the complex physical and chemical roles of NaOH-treatment (Pang et al., 2008; Zhang et al., 2013; Li et al., 2009). It was calculated that with NaOH-treated corn stover, the TS reduction and VS reduction in the co-digestion systems was 4.96% and 13.26% higher, respectively, than that of control. The results proved that the biodegradability of NaOH-treated corn stover was improved and more components converted to biogas by anaerobic microbial activity (Zhang et al., 2013). The results were in agreement with reports of Zhang et al. (2013) who observed increases in TS and VS reduction when the NaOH-treated banana stem was co-digested with swine manure. The pH of R2 was 6.91±0.09 which was 5.8% higher than R1, the higher pH was attributed to some residual NaOH left in the pretreated substrates.

3.3 Effect of lime pretreatment

In this study, the daily biogas production for the lime-treated corn stover and untreated corn stover at steady-state was shown in Figure 2. It can be observed from Figure 2 that there was significant increase ($p<0.05$) in biogas production when the corn stover was pretreated using lime.

Performances of two digesters are summarized in Table 4. The average biogas production was 3786 ± 129 ml in R1 and 5652 ± 140 ml in R2, respectively. 33% more biogas was produced when the lime pretreated corn stover was added compared with the untreated one. Gas analysis results indicated that the average methane content in R1 was 40% while the average methane content in R2 was 43%. Daily methane production was calculated by timing daily biogas production with corresponding methane content. Biogas and methane production based on VS loading was calculated to analyze the effects of lime-treatment on the gas yield of co-digestion. The methane yield of lime-pretreated corn stover in this study was 0.16 $m^3/kg\ VS_{added}$ which increased 37.50% compared with that of the control. Similar study was conducted by Bruni et al. (2010) to investigate the effect of lime pretreatment on biogas potential of biofibers in digested manure. They reported that the lime pretreatment resulted in an increase in methane production and the highest methane yield (0.239 $m^3/kg\ VS_{added}$) was achieved when the biofibers was pretreated with 6% CaO.

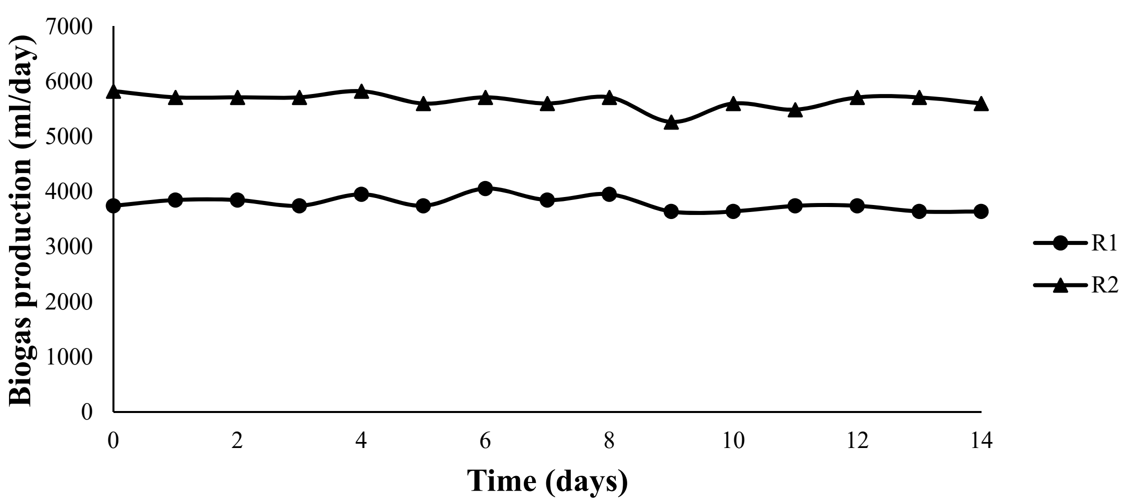


Figure 2. Daily biogas production in anaerobic reactors treating swine wastewater with untreated and lime-treated corn stover

Table 4. Comparison of anaerobic digesters performance using untreated corn stover and lime-treated corn stover

	R1	R2	Increase (%)
Biogas production (mL/d)	3786 ± 129	5652 ± 140	33
Methane production (mL/d)	1514 ± 52	2397 ± 63	36.84
Specific biogas yield (m^3/kg VS_{added})	0.25	0.37	32.43
Specific methane yield (m^3/kg VS_{added})	0.1	0.16	37.50

Increased biogas and methane production can be attributed to improved biodegradability of lime-treated corn stover that resulted from the complex physical and chemical roles of lime-treatment (Chang et al., 1997; Bruni et al., 2010; Song et al., 2012). It was calculated that the TS removal rate and VS removal rate of lime-treated corn stover was 1.02 and 1.10 times of the control. Song et al. (2012) reported the VS removal rate of (6%, 8%, 10%) lime pretreated rice straw was between 1.38 and 1.76 times of the control. pH of R2 was 6.72± 0.12 which was 3.27% higher than that of R1. Higher pH was attributed to some residual lime left in the pretreated substrates.

3.4 Effect of combination of sodium hydroxide and lime pretreatment

Daily biogas production for the combination of NaOH and lime-treated corn stover and untreated corn stover at steady-state was shown in Figure 3. It can be observed from Figure 3 that there was significant increase ($p<0.05$) in biogas production when the corn stover was pretreated using the combination of NaOH and lime. Performances of two digesters are summarized in Table 5. The average biogas production was 4028 ± 298 ml in R1 and 6466 ± 318 ml in R2, respectively. 37.70% more biogas was produced when the combination of NaOH and lime-treated corn stover was added compared with the untreated one. Gas analysis results indicated that the average methane content in R1 was 40% while the average methane content in R2 was 43%. Daily methane production was calculated by timing daily biogas production with corresponding methane content. Biogas and methane production based on VS loading was calculated to analyze the effects of combination of NaOH and lime pretreatment on the gas yield of co-digestion. The methane yield of combination of NaOH and lime-pretreated corn stover in this study was $0.18m^3/kg\ VS_{added}$ which increased 44.44% compared with that of the control.

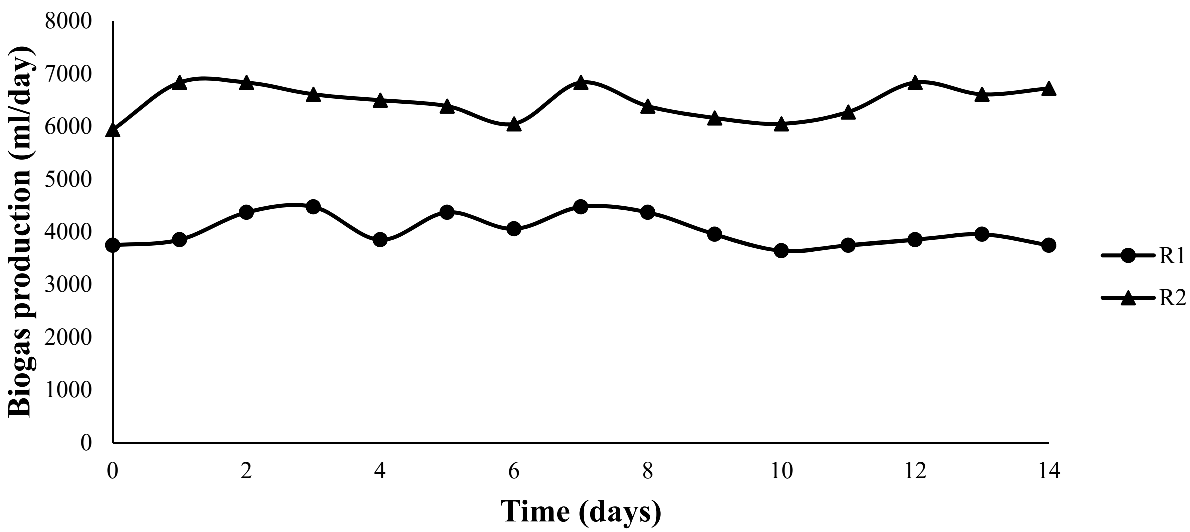


Figure 3. Daily biogas production in anaerobic reactors treating swine wastewater with untreated and combined NaOH and lime-treated corn stover

Table 5. Comparison of anaerobic digesters performance using untreated corn stover and combined NaOH and lime-treated corn stover

	R1	R2	Increase (%)
Biogas production (mL/d)	4028 ± 298	6466 ± 318	37.70
Methane production (mL/d)	1611 ± 119	2768 ± 161	41.79
Specific biogas yield (m^3/kg VS_{added})	0.26	0.42	38.10
Specific methane yield (m^3/kg VS_{added})	0.1	0.18	44.44

Increased biogas and methane production can be attributed to improved biodegradability of the combination of NaOH and lime-treated corn stover that resulted from the complex physical and chemical roles of combination of NaOH and lime treatment (Xu et al., 2011). It was calculated that with the combination of NaOH and lime-treated corn stover, the TS reduction and VS reduction in the co-digestion systems was 4.81% and 12.39% higher, respectively, than that of control. pH of R2 was 6.72±0.08 which was 3.27% higher than that of R1. Higher pH was attributed to some residual alkaline left in the pretreated substrates.

4. Conclusions

Based on the results and discussion, the following conclusions can be made from our research:

(a) The pretreatments of corn stover with NaOH, lime, and combination of NaOH and lime improved the digestibility of CS and biogas production. Among these pretreatments, the combination of NaOH and lime was the best because it resulted in the highest increase of methane yield.

(b) NaOH pretreated corn stover substrate had resulted into specific methane and biogas production yields of 0.19 $m^3/kg\ VS_{added}$ and 0.42 $m^3/kg\ VS_{added}$, respectively. Compared to the untreated corn stover, the methane yield and biogas yield increased by 36.84% and 30.95%, respectively. The improved biodegradability was indicated by the increased TS and VS reduction of NaOH pretreated corn stover.

(c) The specific methane and biogas production yield of lime pretreated corn stover substrate had resulted into 0.16 $m^3/kg\ VS_{added}$ and 0.37 $m^3/kg\ VS_{added}$, respectively. The methane yield and biogas yield increased 32.43% and 37.50%, respectively. The TS removal rate and VS removal rate of lime-treated corn stover was 1.02 and 1.10 times of the control.

(d) The combination of NaOH and lime pretreated corn stover substrate had resulted to yield specific methane and biogas production of 0.18 $m^3/kg\ VS_{added}$ and 0.42 $m^3/kg\ VS_{added}$, respectively. The methane yield and biogas yield increased 38.10% and 44.44%, respectively. The VS reduction of combination of NaOH and lime-treated corn stover was 12.39% higher than that of untreated corn stover.

REFERENCES

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed). New York, USA: American Public Health Association.

Bruni, E., Jensen, A. P., & Angelidaki, I. (2010). Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. *Bioresource Technology*, *101*(22), 8713–8717. doi:10.1016/j.biortech.2010.06.108

Chandra, R., Takeuchi, H., Hasegawa, T., & Kumar, R. (2012). Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy*, *43*(1), 273–282. doi:10.1016/j.energy.2012.04.029

Chang, V. S., Burr, B., & Holtzapple, M. T. (1997). Lime pretreatment of switchgrass. *Applied Biochemistry and Biotechnology*, *63*(4), 3-19.

Garrote, G., Domínguez, H., & Parajó, J. C. (1999). Hydrothermal processing of lignocellulosic materials. *Holz als Roh- und Werkstoff* , *57*(3), 191-202.

Geddes, C. C., Peterson, J. J., Roslander, C., Zacchi, G., Mullinnix, M. T., Shanmugam, K. T., & Ingram, L. O. (2010). Optimizing the saccharification of sugar cane bagasse using dilute phosphoric acid followed by fungal cellulases. *Bioresource Technology*, *101*(6), 1851–1857. doi:10.1016/j.biortech.2009.09.070

Han, M. J., Behera, S. K., & Park, H.S. (2012). Anaerobic co-digestion of food waste leachate and piggery wastewater for methane production: statistical optimization of key process parameters. *Journal of Chemical Technology & Biotechnology*, *87*(11), 1541–1550. doi:10.1002/jctb.3786

Hartmann, H., Angelidaki, I., & Ahring, B. K. (2000). Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration. *Water Science and Technology*, *41*(3), 145–153.

Kadam, K. L., & Mcmillan, J. D. (2003). Availability of corn stover as a sustainable feedstock for bioethanol production. *Bioresource Technology*, *88*(1), 17–25.

Li, X., Li, L., Zheng, M., Fu, G., & Lar, J. S. (2009). Anaerobic co-digestion of cattle manure with corn stover pretreated by sodium hydroxide for efficient biogas production. *Energy & Fuels*, *23*(9), 4635–4639. doi:10.1021/ef900384p

Li, C., Champagne, P., & Anderson, B. C. (2013). Effects of ultrasonic and thermo-chemical pre-treatments on methane production from fat, oil and grease (FOG) and synthetic kitchen waste (KW) in anaerobic co-digestion. *Bioresource technology*, *130*, 187–197. doi:10.1016/j.biortech.2012.11.053

Pang, Y.Z., Liu, Y.P., Li, X.J., Wang, K.S., Yuan H.R. (2008). Improving Biodegradability and Biogas Production of Corn Stover through Sodium Hydroxide Solid State Pretreatment. *Energy& Fuels*, *22*, 2761-2766.

Pavlostathis, S. G., & Gossett, J. M. (1985). Alkaline treatment of wheat straw for increasing anaerobic biodegradability. *Biotechnology and Bioengineering*, *27*(3), 334–344.

Sharma, S. K., Kalra, K. L., & Grewal, H. S. (2002). Enzymatic sacchariÿcation of pretreated sunflower stalks. *Biomass and Bioenergy*, *23*(3), 237–243.

Song, Z., Yang, G., Zhang, T., Feng, Y., Ren, G., & Han X. (2012). Effect of $Ca(OH)_2$ pretreatment on biogas production of rice straw fermentation. *Transactios of the Chinese Society of Agricultural Engineering*, *28*(19), 207-213.

Soto, M. L., Domfnguez, H., Ntifiez, M. J., & Lema, J. M. (1994). Enzymatic saccharification of alkali-treated sunflower stalks. *Bioresource Technology*, *49*(1), 53–59.

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International journal of molecular sciences*, *9*(9), 1621-1651. doi:10.3390/ijms9091621

Talebnia, F., Karakashev, D., & Angelidaki, I. (2010). Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. *Bioresource Technology*, *101*(13), 4744–4753. doi:10.1016/j.biortech.2009.11.080

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*,*96*, 673–686.

USDA. (2002). Crop production-annual summary (PCP-BB). National Agricultural Statistical Service, US Department of Agriculture (USDA), Washington, DC.

Wang, H., Wang, H., Lu, W., & Zhao, Y. (2009). Digestibility improvement of sorted waste with alkaline hydrothermal pretreatment. *Tsinghua Science and Technology*, *14*(3), 378-382.

Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource technology*, *101*(11), 4042–4047.

Yang, X., Ma, F., Yu, H., Zhang, X., & Chen, S. (2011). Effects of biopretreatment of corn stover with white-rot fungus on low-temperature pyrolysis products. *Bioresource Technology*, *102*(3), 3498–3503. doi:10.1016/j.biortech.2010.11.021

Xu, J., Cheng, J. J., Sharma-Shivappa, R. R., & Burns, J. C. (2010a). Sodium Hydroxide Pretreatment of Switchgrass for Ethanol Production. *Energy & Fuels*, *24*(3), 2113–2119. doi:10.1021/ef9014718

Xu, J., Cheng, J. J., Sharma-Shivappa, R. R., & Burns, J. C. (2010b). Lime pretreatment of switchgrass at mild temperatures for ethanol production. *Bioresource Technology*, *101*(8), 2900–2903. doi:10.1016/j.biortech.2009.12.015

Xu, J., & Cheng, J. J. (2011). Pretreatment of switchgrass for sugar production with the combination of sodium hydroxide and lime. *Bioresource Technology*, *102*(4), 3861–3868. doi:10.1016/j.biortech.2010.12.038

You, Z., Wei, T., & Cheng, J. J. (2014). Improving anaerobic codigestion of corn stover using sodium hydroxide pretreatment. *Energy & Fuels*, *28*, 549-554.

Zhang, C., Li, J., Liu, C., Liu, X., Wang, J., Li, S., Fan, G., et al. (2013). Alkaline pretreatment for enhancement of biogas production from banana stem and swine manure by anaerobic codigestion. *Bioresource Technology*, *149*, 353–358. doi:10.1016/j.biortech.2013.09.070

Zheng, M., Li, X., Li, L., Yang, X., & He, Y. (2009). Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment. *Bioresource Technology*, *100*(21), 5140–5145. doi:10.1016/j.biortech.2009.05.045

Zhong, W., Zhang, Z., Luo, Y., Sun, S., Qiao, W., & Xiao, M. (2011). Effect of biological pretreatments in enhancing corn straw biogas production. *Bioresource Technology*, *102*(24), 11177–11182.

Zhong, W., Zhang, Z., Luo, Y., Qiao, W., Xiao, M., & Zhang, M. (2012). Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresource Technology*, *114*, 281–286.

Zhu, J., Wan, C., & Li, Y. (2010). Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresource Technology*, *101*(19), 7523–7528. doi:10.1016/j.biortech.2010.04.060

CHAPTER 4: EFFECT OF TOTAL SOLIDS CONCENTRATION ON THERMOPHILIC ANAEROBIC CO-DIGESTION OF SWINE MANURE AND CORN STOVER

Abstract

The effect of total solids (TS) concentration on anaerobic co-digestion of swine manure and corn stover was investigated in continuously stirred tank reactors (CSTRs) at solid content of 2.5%, 4% and 5.5%. Digestions were performed in 12 L laboratory-scale CSTR with operating temperature at 50 ºC and hydraulic retention time (HRT) of 5 days. The daily loading of swine manure and corn stover was mixed at a mass ratio of 3:1 to achieve a carbon/nitrogen (C/N) ratio of 30 in the mixture and was diluted to desired TS concentration. The reactor was operated for a period of 3 HRTs. Daily biogas production was recorded by a wet-tip gas meter and biogas composition was measured with a gas chromatograph (GC). Analysis of variance (ANOVA) was used to determine the significant difference in gas yield at $p < 0.05$. The ANOVA showed that the gas yield from the various TS% was significantly different ($p < 0.05$). The results indicated that the gas yield decreased with the increase in the solid content. Higher gas yield achieved at lower TS concentration was attributed to overloading of the substrates. It was calculated that chemical oxygen demand (COD) reduction ranged from 46.20% - 52.26% and volatile solids (VS) reduction ranged from 50.18% - 53.29% at the TS concentrations investigated. COD and VS reduction were the highest when the reactor was operated at TS of 2.5%.

Keywords: Anaerobic digestion; Biogas; Corn stover; Swine manure; Total solids content

1. Introduction

The anaerobic digestion process is characterized by a series of biochemical reactions brought on by different consortia of microorganisms that convert the organic matter into methane, carbon dioxide, ammonia and traces of other gases and volatile fatty acids in the absence of oxygen (Fernández, et al., 2010; Motte et al., 2013). This technology has been successfully employed throughout the United States and many European countries to treat organic wastes in the past decades (Khalid et al., 2011; Ward et al., 2008). As a good replacement of natural gas, the biogas produced from anaerobic digestion can be used for heat and electricity generation and gaseous vehicle fuel (Ward et al., 2008).

Animal manure is considered as an excellent feedstock for anaerobic digestion because of its strong buffer capacity and high concentration of macro- and micro-nutrients (Weiland, 2000). Many studies had been reported to use animal manure as a monosubstrate in anaerobic digestion for biogas production (Møller et al., 2004; Nielsen et al., 2007; Labatut et al., 2011). However, ammonia inhibition problems had occurred in several studies when treating animal manure alone (Hansen et al., 1998; Kaparaju et al., 2005; Strik et al., 2006). This was attributed to the inherent deficiency of carbon in animal manure (Hansen et al., 1998). Co-digestion of animal manure with agricultural residues had been successfully developed by many researchers in recent years (Angelidaki et al., 2003; Esposito et al., 2012). The biogas production improved in the co-digestion of swine manure with agricultural residues because the carbon to nitrogen (C/N) ratio, concentrations of macro and micronutrients and buffer capacity were well balanced by mixing these substrates together (Zhang et al., 2011). Cuetos et al. (2011) investigated the anaerobic co-digestion of swine manure with maize, rapseed and sunflower residues under batch and semi-continuous conditions. The results indicated that the anaerobic co-digestion system resulted in a major increase in the amount of biogas produced daily. Similar results were also observed by Wu et al. (2010) who co-digested swine manure with corn stalks, oat straw and wheat straw. They found the daily maximum biogas volume increased by 11.4-fold when the swine manure is co-digested with corn stalks in comparison with swine manure added as a single substrate.

One of the most important factors affecting anaerobic digestion of organic solid waste is total solids (TS) content (Motte et al., 2013; Fernández et al., 2010). Based on the TS content of solid waste, the anaerobic digestion can be categorized into three types: wet (≤ 10% TS), semi-dry (10-20% TS) and dry (≥20% TS) processes (Abbassi-Guendouz et al., 2012). Wet anaerobic digestion is usually undertaken in conventional anaerobic digesters such as continuously stirred tank reactors (CSTR) (Forster-Carneiro et al., 2008). Semi-dry and dry anaerobic digestion produces an inert bio-solid product with higher methane productivity (Mata-Alverez et al., 2000; De Baere, 2000). The solids content is closely related with the substrate loading rate and will have a great influence on the cost, performance and stability of the digestion process (Lissens et al., 2001). The TS effect on anaerobic digestion of different kinds of substrates such as food waste, slaughterhouse waste and municipal solid waste have been investigated. Forster-Carneiro et al. (2008) studied the influence of three different TS percentages (20%, 25% and 30%) on the biomethanization process of food waste in dry thermophilic anaerobic digestion. The results indicated that the specific methane yield reduced with increasing TS content. This was attributed to the accumulation of acids at start-up phase which inhibited the process at higher TS content. The reactor with 20% TS and 30% of inoculum source was the most effective operation condition, showing a methane yield of 0.49 L CH_4/g VS. The results were in agreement with Mshandete et al. (2004) who found the specific methane yield reduced with increasing solids content. Wu et al.

(2009) investigated the effect of solid content on anaerobic digestion of meat and bone meal at TS contents of 1%, 2%, 5% and 10%. The highest methane yield (0.482 L CH_4/g $VS_{removed}$) was achieved at the TS content of 5% while the methane yields were between 0.384 and 0.448 L CH_4/g $VS_{removed}$ at other TS content. Less methane was produced at solid content of 10% than that at 5% TS was attributed to the overloading or insufficient buffering capacity (Alvarez et al., 2008). Abbassi-Guendouz et al. (2012) suggested that 30% TS could be considered as a threshold concentration for an inhibitory effect in high solids anaerobic digestion. However, few reports can be found on the study of thermophilic anaerobic co-digestion of swine manure and corn stover at various solid contents.

In this study, thermophilic anaerobic co-digestion of swine manure and corn stover at three different solid contents (2.5%, 4%, 5.5%) was investigated in CSTR. The aim of this study was to examine the methane production potential and the effect of the solid content on anaerobic co-digestion of swine manure and corn stover.

2. Materials and methods

2.1 Swine manure and corn stover

Raw swine manure used in this study was taken from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University. The swine manure was stored at 4 ºC to avoid chemical composition modification before it was used for experiment. Air-dried corn stover was obtained from the Central Crops Research Station in Clayton, North Carolina, USA. After the corn stover was collected, it was ground using a Thomas Wiley Laboratory Mill (Model No. 4) to reduce its particle size into 2 mm. The corn stover was stored in sealed plastic bags at room temperature.

2.2 Experimental setup and procedure

The experiments were carried out in CSTR (model number: MGF-214, New Brunswick Scientific Company, Enfield, Connecticut, USA). Each reactor had a working volume of 12 L. The reactor was heated to desired temperature (50 ºC) by wrapping a heating tape (model number: BS0051080L, BriskHeat Corporation, Columbus, Ohio, USA) around the vessel of the reactor. Before the experiment was started, each reactor was operated at steady-state thermophilic operation at 50 ºC with a hydraulic retention time (HRT) of 5 days and an organic loading rate of 1.279 kg VS/m^3/d. During the experiment, reactors were operated at HRT of 5 days at thermophilic condition (50 ºC). The daily loading of swine manure and corn stover was mixed at a mass ratio of 3:1 to achieve a C/N ratio of 30 in the mixture. The mixture was diluted to desired TS concentration and was fed from the upper part of the reactor. When the reactor reached steady-state, it was operated for a period of 15 days (3 HRTs). The system was determined as steady-state when the fluctuations in biogas production, methane content and pH were below 10% in a week (Boušková et al., 2005).

2.3 Analytical methods

TS, volatile solids (VS), pH, total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) content of biomass were determined in accordance with American Public Health Association (APHA) standard methods (APHA, 1998). TS content was determined by drying the sample in an isotemp oven at 105 °C for 24h. After 24h, the dried sample was put in a muffle furnace (Model 48000, Barnstead/Thermolyne Inc., Dubuque, IA) at 550 °C for 1 h to determine the VS content. The value of pH was determined using a portable pH meter (FG2, Mettler-Toledo International Inc., Schwerzenbach, Switzerland). TKN analysis was conducted using a Bran+Luebbe® digital autoanalyzer Ⅲ system. COD was determined using the potassium dichromate-sulfuric acid digestion and colorimetric analysis. The colorimetric analysis was conducted using a spectrophotometer (DR/2010, Hach Co. Loveland, CO). TC and TOC content were determined using a Teledyne Tekmar Apollo 9000 combustion TOC analyzer with auto sampler. For solid biomass samples, TC and TN were determined using a Leco Carbon/Nitrogen 2000 analyzer with autoloader. Organic carbon was determined using the potassium dichromate-sulfuric acid digestion and colorimetric analysis.

Biogas production was recorded by a wet-tip gas meter (Archae Press, Nashville, Tennessee, USA) and biogas composition was determined with a gas chromatograph (GC) (model number: GC-17A, Shimadzu Corporation, Kyoto, Japan) equipped with a thermal conductivity detector and 100/120 carbosieve SII column (dimensions 3.0 m length× 3.00 mm inner diameter). The temperature of detector and column was set at 250.0 ºC and 50.0 ºC, respectively. Helium was used as the carrier gas at a flow rate of 30.0 mL/min. The GC detected relative percentages of nitrogen, methane and carbon dioxide. The GC was calibrated using three custom-made gas standards ($N_2/He/CH_4/CO_2$= 5/5/70/20; $N_2/He/CH_4/CO_2$= 20/5/5/70; $N_2/He/CH_4/CO_2$= 70/5/20/5).

2.4 Statistical analysis

All statistical analyses were performed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. SAS software version 9.4 (SAS institute, Cary, NC) was used to conduct the statistical analysis. A value of p less than or equal to 0.05 was considered to indicate statistically significant differences.

3. Results and discussion

3.1 Chemical characteristics of swine manure and corn stover

The chemical characteristics of swine manure and corn stover are shown in Table 1. The low C/N ratio of swine manure and high C/N ratio of corn stover indicate that these two substrates are suitable for co-digestion. The C/N ratio of swine manure was similar to the values which were 14.4 and 10.2 reported by Li et al. (2011) and Cuetos et al. (2011), respectively. The C/N ratio of corn stover was higher than the values which were 70.9 and 26.66 reported by Zhong et al. (2012) and Wu et al. (2010), respectively.

Table 1. Characteristics of swine manure and corn stover used in the experiment

Parameter	Swine Manure	Corn Stover
C/N Ratio	11.51	90.07
COD (mg/g)	372	2031
TOC (mg/L)	3.59×10^4	-
TKN (mg/L)	1.805×10^4	-
pH	6.77	6.65
TS (%)	38.1	92.60
VS (% of TS)	84.5	94.68
TC (%)	38.2	41.43
TN (%)	3.32	0.46

3.2 Daily biogas and methane production at different TS concentration

The daily biogas production for different TS concentration at steady state are shown in Figure 1. Statistical analysis results indicated that there was significant difference ($p<0.05$) in the daily biogas production at different TS content. The average daily biogas production was 16 ± 1, 25 ± 1 and 21 ± 1 L for TS content of 2.5%, 4% and 5.5%, respectively. The highest biogas production was obtained at the solid content of 4%.

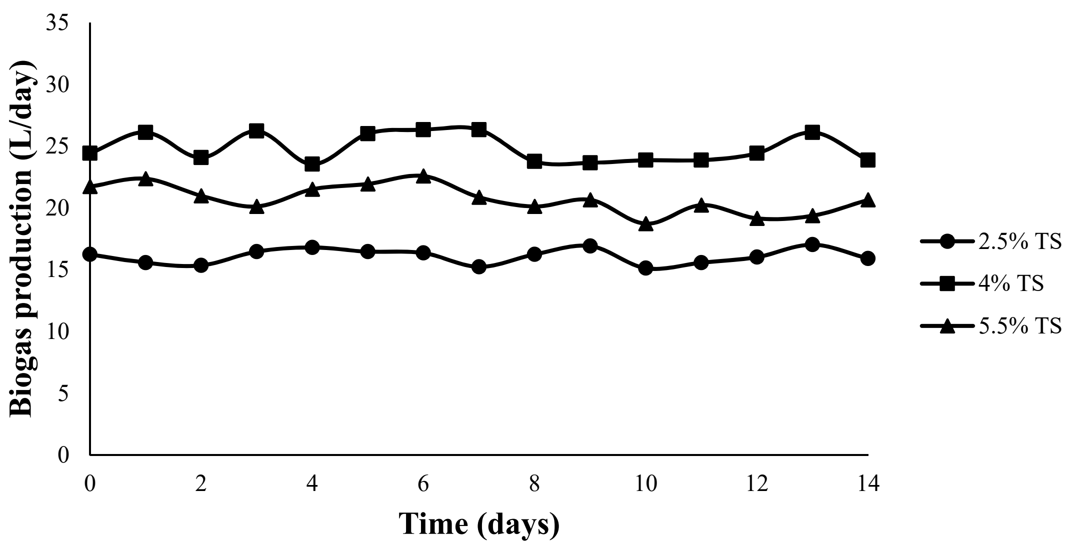


Figure 1. Daily biogas production in anaerobic reactors treating swine manure and corn stover at different solid contents

The daily methane production for different TS concentration at steady state are shown in Figure 2. Daily methane production was calculated by timing daily biogas production with corresponding methane content. Statistical analysis results indicated that there was significant difference ($p<0.05$) in the daily methane production at different TS content. The average daily methane production was 7.6 ± 0.3, 11.7 ± 0.5 and 8.5 ± 0.5 L for TS content of 2.5%, 4% and 5.5%, respectively. The highest methane production was obtained at solid content of 4%.

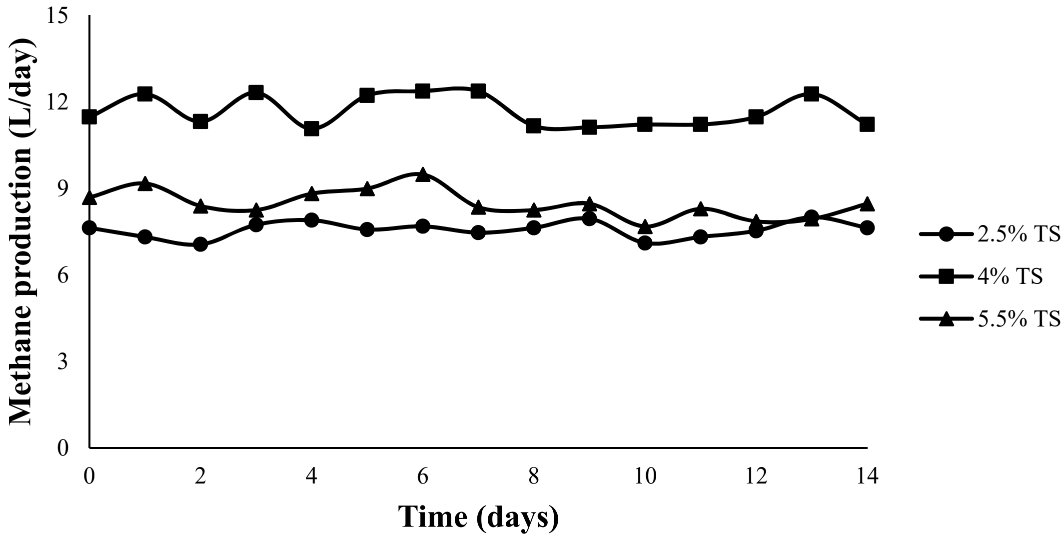


Figure 2. Daily methane production in anaerobic reactors treating swine manure and corn stover at different solid contents

3.3 Specific biogas and methane yield at different TS concentration

The influence of TS content on the specific biogas yield is given in Figure 3(a). Statistical analysis results indicated that there was no significant difference ($p=0.0836$) in the specific biogas yield with respect to the VS added at TS content of 2.5% and 4.0%. However, the specific biogas yield was significantly different ($p<0.05$) at TS content of 5.5%. The average specific biogas yield was 0.301, 0.290, 0.176 m^3/kg VS_{added} for TS content of 2.5%, 4% and 5.5%, respectively. The highest specific biogas yield was obtained at solid content of 2.5%.

The influence of TS content on the specific methane yield is given in Figure 3(b). Statistical analysis results indicated that there was significant difference ($p<0.05$) in the specific methane yield at different TS content. The average specific methane yield was 0.142, 0.137, 0.072 m^3/kg VS_{added} for TS content of 2.5%, 4% and 5.5%, respectively. The highest specific methane yield was obtained at solid content of 2.5%.

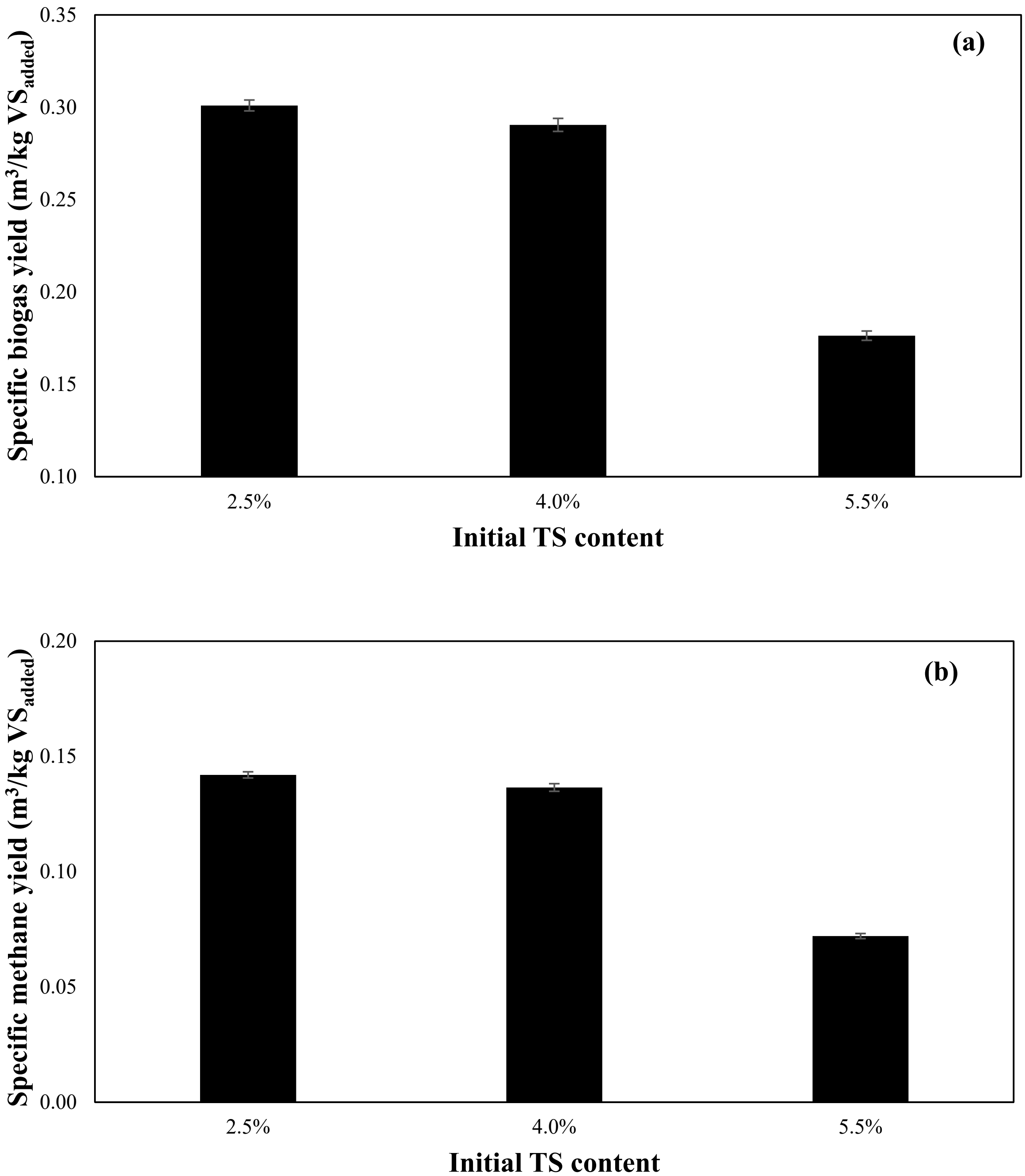


Figure 3. Influence of TS content on specific gas yield of anaerobic co-digestion of swine manure and corn stover. (a) Specific biogas yield; (b) Specific methane yield.

3.4 COD and solid reduction analysis

The COD and the VS reduction at different solids content are given in Figure 4. The COD reduction was 52.26% at 2.5%, 46.25% at 4.0%, and 46.20% at 5.5% solid contents. The VS reduction was 53.29%, 52.28%, 50.18% at solid contents of 2.5%, 4.0% and 5.5%, respectively. It can be observed from Figure 4 that the COD and VS reduction were the highest when the reactor was operated at TS of 2.5%.

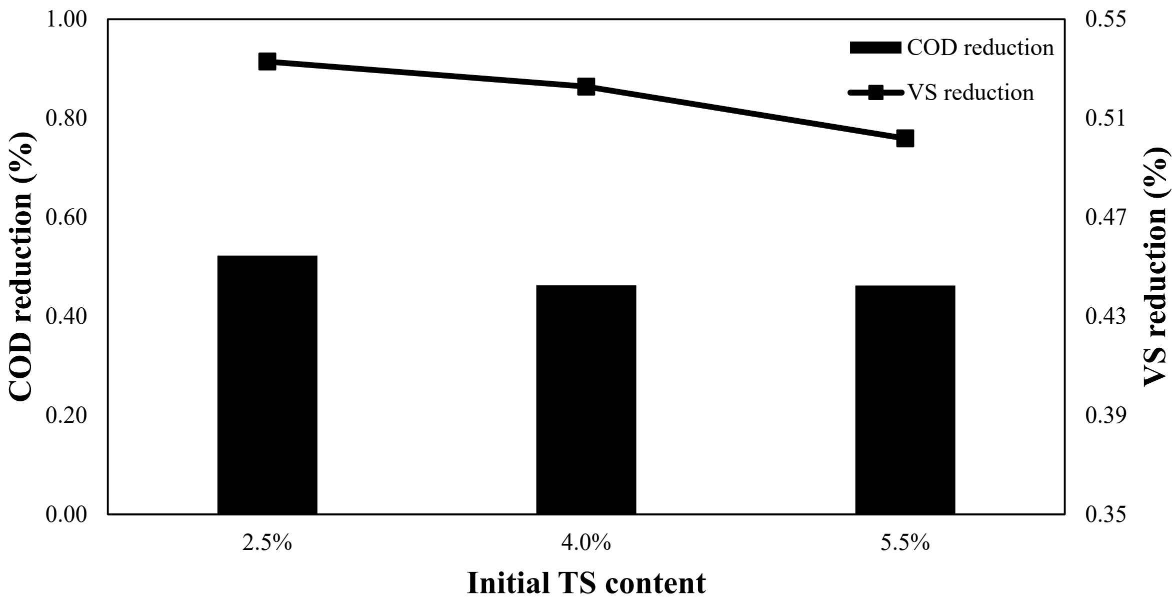


Figure 4. COD and VS reduction in anaerobic reactors treating swine manure and corn stover at various solid contents

3.5 Discussion

This study indicates that thermophilic anaerobic co-digestion of swine manure and corn stover is feasible at solid contents ranging from 2.5% to 5.5% in CSTR. The specific methane yield was 0.072 – 0.142 m^3/kg VS_{added} in this study which is lower than that of 0.269 m^3/kg VS_{added} obtained by Fujita et al. (1980) in anaerobic co-digestion of swine manure and corn stover under thermophilic condition. Ahn et al. (2010) reported a specific methane yield of 0.337±0.065 m^3/kg VS_{added} for thermophilic anaerobic co-digestion of swine manure with switchgrass. The methane yield in this study were lower in comparison with that. The highest biogas yield (0.301 m^3/kg VS_{added}) was obtained at the solid content of 2.5% in the present study. This is similar to the results obtained by Itodo et al. (1999) who found that the specific biogas yield reduced with increasing the solid content of poultry, cattle and piggery waste. The reason for higher gas yield achieved at lower TS concentration could be partially due to overloading of the substrates. The slurry became thick at higher TS content, the gas produced may have found it difficult to bubble through the slurry and out of the digester for measurement (Itodo et al., 1999).

A VS reduction of 50.18% - 53.29% was obtained in this study. It is similar to that of 40.3% - 52.3% in anaerobic digestion of energy crop (maize, rapeseed and sunflower) residues with swine manure (Cuetos et al., 2011). This indicates that anaerobic co-digestion of swine manure and corn stover is a potential technology for both energy-recovery and waste disposal.

4. Conclusions

Thermophilic anaerobic co-digestion of swine manure and corn stover was investigated at solid contents of 2.5%, 4% and 5.5% in CSTR. The highest average daily biogas and methane production were obtained at solid content of 4%. Statistical analysis results indicated that there was significant difference ($p<0.05$) in the specific methane yield at different TS content. It was observed that the methane yield (m^3/kg VS_{added}) decreased with increasing TS concentration for the substrates investigated. The average specific methane yield was 0.142, 0.137, 0.072 m3/kg VS_{added} for TS content of 2.5%, 4% and 5.5%, respectively. The reason for higher gas yield achieved at lower TS concentration could be partially attributed to overloading of the substrates. It was calculated that COD reduction ranged from 46.20% - 52.26% and VS reduction ranged from

50.18% - 53.29% at the solids content studied. Further studies on feasibility of anaerobic co-digestion of swine manure and corn stover in large-scale reactors and digestion kinetics are required.

REFERENCES

Abbassi-Guendouz, A., Brockmann, D., Trably, E., Dumas, C., Delgenès, J.-P., Steyer, J.-P., & Escudié, R. (2012). Total solids content drives high solid anaerobic digestion via mass transfer limitation. *Bioresource technology*, *111*, 55–61. doi:10.1016/j.biortech.2012.01.174

Ahn, H. K., Smith, M. C., Kondrad, S. L., & White, J. W. (2010). Evaluation of biogas production potential by dry anaerobic digestion of switchgrass-animal manure mixtures. *Applied Biochemistry and Biotechnology*, *160*(4), 965–975.

Alvarez, R., & Lidén, G. (2008). Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. *Renewable Energy*, *33*(4), 726–734. doi:10.1016/j.renene.2007.05.001

Angelidaki, I., & Ellegaard, L. (2003). Codigestion of manure and organic wastes in centralized biogas plants: status and future trends. *Applied Biochemistry and Biotechnology*, *109*(1), 95-105.

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed). New York, USA: American Public Health Association.

Boušková, A., Dohányos, M., Schmidt, J.E., & Angelidaki, I. (2005). Strategies for changing temperature from mesophilic to thermophilic conditions in anaerobic CSTR reactors treating sewage sludge. *Water Research*, *39*(8), 1481–1488.

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

Cuetos, M. J., Fernández, C., Gómez, X., & Morán, A. (2011). Anaerobic co-digestion of swine manure with energy crop residues. *Biotechnology and Bioprocess Engineering*, *16*(5), 1044–1052.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A., & Pirozzi, F. (2012). Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Bio/Technology*, 325–341.

Fernández, J., Pérez, M., & Romero, L. I. (2010). Kinetics of mesophilic anaerobic digestion of the organic fraction of municipal solid waste: Influence of initial total solid concentration. *Bioresource Technology*, *101*(16), 6322–6328. doi:10.1016/j.biortech.2010.03.046

Forster-Carneiro, T., Pérez, M., & Romero, L. I. (2008). Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste. *Bioresource Technology*, *99*(15), 6994–7002. doi:10.1016/j.biortech.2008.01.018

Fujita, M., Scharer, J. M., & Moo-Young, M. (1980). Effect of corn stover addition on the anaerobic digestion of swine manure. *Agricultural Wastes*, *2*(3), 177-184.

Hansen, K. H., Angelidaki, I., & Ahring, B. K. (1998). Anaerobic digestion of swine manure: inhibition by ammonia. *Water Research*, *32*(1), 5–12.

Itodo, I. N., & Awulu J. O. (1999). Effects of total solids concentrations of poultry, cattle, and piggery waste slurries on biogas yield. *Transactions of the ASAE*, *42*(6),1853-1855.

Kaparaju, P., & Rintala, J. (2005). Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. *Resources, Conservation and Recycling*, *43*(2), 175–188.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., & Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Management*, *31*(8), 1737–1744.

Labatut, R. A., Angenent, L. T., & Scott, N. R. (2011). Biochemical methane potential and biodegradability of complex organic substrates. *Bioresource Technology*, *102*(3), 2255–2264.

Li, Y., Yan, X.-L., Fan, J.-P., Zhu, J.-H., & Zhou, W.-B. (2011). Feasibility of biogas production from anaerobic co-digestion of herbal-extraction residues with swine manure. *Bioresource technology*, *102*(11), 6458–6463.

Lissens, G., Vandevivere, P., Baere, L. De, Biey, E. M., & Verstraete, W. (2001). Solid waste digestors : process performance and practice for municipal solid waste digestion. *Water Science and Technology*, *44*(8), 91–102.

Mata-Alvarez, J., Macé, S., & Llabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresouce Technology*, *74*(1), 3-16.

Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy*, *26*(5), 485–495.

Motte, J., Trably, E., Escudié, R., Hamelin, J., Steyer, J., Bernet, N., Delgenes, J., et al. (2013). Total solids content : a key parameter of metabolic pathways in dry anaerobic digestion. *Biotechnology for Biofuels*, 6, 164.

Mshandete, A., Kivaisi, A., Rubindamayugi, M., & Mattiasson, B. (2004). Anaerobic batch co-digestion of sisal pulp and fish wastes. *Bioresource Technology*, *95*(1), 19–24. doi:10.1016/j.biortech.2004.01.011

Nielsen, H. B., Mladenovska, Z., & Ahring, B. K. (2007). Bioaugmentation of a two-stage thermophilic (68 °C / 55 °C) anaerobic digestion concept for improvement of the methane yield from cattle manure. *Biotechnology and Bioengineering*, *97*(6), 1638–1643.

Strik, D. P. B. T. B., Domnanovich, A. M., & Holubar, P. (2006). A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. *Process Biochemistry*, *41*(6), 1235–1238.

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*, *99*(17), 7928–7940.

Weiland, P. (2000). Anaerobic waste digestion in Germany-status and recent developments. *Biodegradation*, *11*(6), 415–421.

Wu, G., Healy, M. G., & Zhan, X. (2009). Effect of the solid content on anaerobic digestion of meat and bone meal. *Bioresource Technology*, *100*(19), 4326–4331. doi:10.1016/j.biortech.2009.04.007

Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. Bioresource technology, 101(11), 4042–4047.

Zhang, L., Lee, Y. W., & Jahng, D. (2011). Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. *Bioresource Technology*, *102*(8), 5048–5059.

Zhong, W., Zhang, Z., Luo, Y., Qiao, W., Xiao, M., & Zhang, M. (2012). Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresource Technology*, *114*, 281–286.

CHAPTER 5: MATHEMATICAL MODELING OF ANAEROBIC CO-DIGESTION OF SWINE MANURE AND CORN STOVER

Abstract

Biogas as a renewable energy source plays an important role in reducing greenhouse gases. Anaerobic co-digestion of swine manure with agricultural residues has been widely applied for biogas production. A mathematical model has been developed to describe the swine manure and corn stover co-digestion process. This model includes multiple-reaction stoichiometry, microbial growth kinetics with acetic acid inhibitory effects and conventional material balances for an ideally mixed reactor. The ordinary differential equations (ODE) of the model were coded in RStudio® software and integrated using the package deSolve. A laboratory batch experiment using swine manure and corn stover as substrates was conducted to calibrate the model. The kinetic parameters used in the model were adjusted for thermophilic conditions and were estimated using least square variation method. Bootstrap is used to estimate the standard error and 95% confidence interval of model kinetic parameters. The applicability of the model was demonstrated by comparing experimental results from literature with the model simulation results.

Keywords: Anaerobic; Co-digestion; Corn stover; Methane; Modeling; Swine manure

1. Introduction

The dependency of the global economy on fossil fuels causes CO_2 emissions and associated global warming problems. In this regard, developing renewable forms of energy is of great interest to a sustainable global development. Energy from biomass or bioenergy is considered as one of the most promising renewable energy sources (Lauwers et al., 2013). Biogas,as a renewable energy source,is usually generated through the anaerobic digestion (AD) process. AD is defined as a process in which the organic matter is decomposed into methane and carbon dioxide through a consortium of anaerobic microorganisms in an oxygen-free environment (Keshtkar et al., 2001). Swine manure is an excellent feedstock for AD because of its strong buffer capacity and high concentration of macro and micronutrients (Weiland, 2000). However, ammonia inhibition had been observed in some studies when treating swine manure as a single substrate in the AD (Hansen et al., 1998; Kaparaju et al., 2005; Strik et al., 2006). This was attributed to the low carbon to nitrogen (C/N) ratio which is typically in the range of 2 to 8 for swine manure (Hansen et al., 1998). To address this problem, anaerobic co-digestion of swine manure with other high C/N ratio substrates such as agricultural residues has been investigated by many researchers (Cuetos et al., 2011; Li et al., 2011; Wu et al., 2010; Xie et al., 2011). Li et al. (2011) indicated that the specific methane yield increased 45.7% when 50% herbal extraction residues was added compared to that obtained from swine manure alone. Wu et al. (2010) also observed an increase in 11 fold in cumulative total biogas production and 16 fold in cumulative net methane volume when the swine manure was co-digested with corn stalks, as compared to that achieved from swine manure alone. Biogas production was improved in the co-digestion of swine manure with agricultural residues because the C/N ratio, concentrations of macro and micronutrients, and buffer capacity were well balanced by mixing these two substrates together (Zhang et al., 2011).

Given the importance of anaerobic co-digestion, kinetic modeling of methane production from anaerobic co-digestion of swine manure with agricultural residues is of interest. Various models had been developed to simulate AD processes in the last few years. Some models were developed based on the kinetics (De Gioannis et al., 2009; Hublin et al., 2013; Lo et al., 2010; Rao et al., 2004; Sosnowski et al., 2008), while others were based on the Anaerobic Digestion Model No. 1 (ADM 1) which was developed by the International Water Association Task Group for mathematical modeling of anaerobic digestion processes (Batstone et al., 2002; Boubaker et al., 2008; Derbal et al., 2009; Esposito et al., 2011; Koch et al., 2010). Kinetic models, especially the first order kinetics were commonly applied to simulate the anaerobic degradation which is attributed to the microbial role in the anaerobic process (Lo et al., 2010).

The aim of this study was to develop a kinetic model which was modified from the model developed by Gelegenis et al. (2007) and Hublin et al. (2013) to describe swine manure and corn stover co-digestion process and optimize the kinetic parameters using experimental results.

2. Materials and methods

2.1 Swine manure and corn stover

Raw swine manure used in this study was taken from the swine unit at the Lake Wheeler Road Field Laboratory (LWRFL) of North Carolina State University. The swine manure was stored at 4 ºC before it was used for experiment. Air-dried corn stover was obtained from the Central Crops Research Station in Clayton, North Carolina, USA. After the corn stover was collected, it was ground using a Thomas Wiley Laboratory Mill (Model No. 4) to reduce its particle size to 2 mm. The corn stover was stored in sealed plastic bags at room temperature.

2.2 Experimental setup and procedure

The experiment was carried out in a continuously stirred tank reactor (CSTR) (model number: MGF-214, New Brunswick Scientific Company, Enfield, Connecticut, USA) with a working volume of 12 L. The temperature of the reactor was maintained at 50 °C. The reactor was heated to desired temperature by wrapping a heating tape (model number: BS0051080L, BriskHeat Corporation, Columbus, Ohio, USA) around the vessel of the reactor. The inoculum for the batch experiment was taken from a steady-state CSTR which was operated at 50 °C for treating the mixture of swine manure and corn stover. The swine manure and corn stover were mixed at a mass ratio of 3:1 to achieve a C/N ratio of 30 in the mixture. The mixture was diluted to 4% total solids (TS) concentration and was fed from the upper part of the reactor. Before the experiment was started, the reactor was purged with N_2 for 2-3 minutes to remove O_2. Biogas production from the batch CSTR was recorded daily until the cessation of the biogas production.

2.3 Analytical methods

Total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) contents of the mixed biomass were determined in accordance with American Public Health Association (APHA) standard methods (APHA, 1998). Biogas production was recorded by a wet-tip gas meter (Archae Press, Nashville, Tennessee, USA) and biogas composition was determined with a gas chromatograph (GC) (model number: GC-17A, Shimadzu Corporation, Kyoto, Japan) equipped with a thermal conductivity detector and 100/120 carbosieve SII column (dimensions 3.0 m length× 3.00 mm inner diameter). The temperature of detector and column was set at 250.0 °C and 50.0 °C, respectively. Helium was used as the carrier gas at a flow rate of 30.0 mL/min. The GC detected relative percentages of nitrogen, methane and carbon dioxide. The GC was calibrated using three custom-made gas standards ($N_2/He/CH_4/CO_2$= 5/5/70/20; $N_2/He/CH_4/CO_2$= 20/5/5/70; $N_2/He/CH_4/CO_2$= 70/5/20/5).

2.4 Kinetic modeling

The kinetic model based on the works of Gelegenis et al. (2007) and Hublin et al. (2013) was modified to simulate the methane production. The model includes five biological processes namely hydrolysis (of cellulose and proteins), fermentation (of sugar and amino acids), acetogenesis, acetoclastic methanogenesis, and hydrogenotrophic methanogenesis. The proposed reaction scheme is shown in Figure 1.

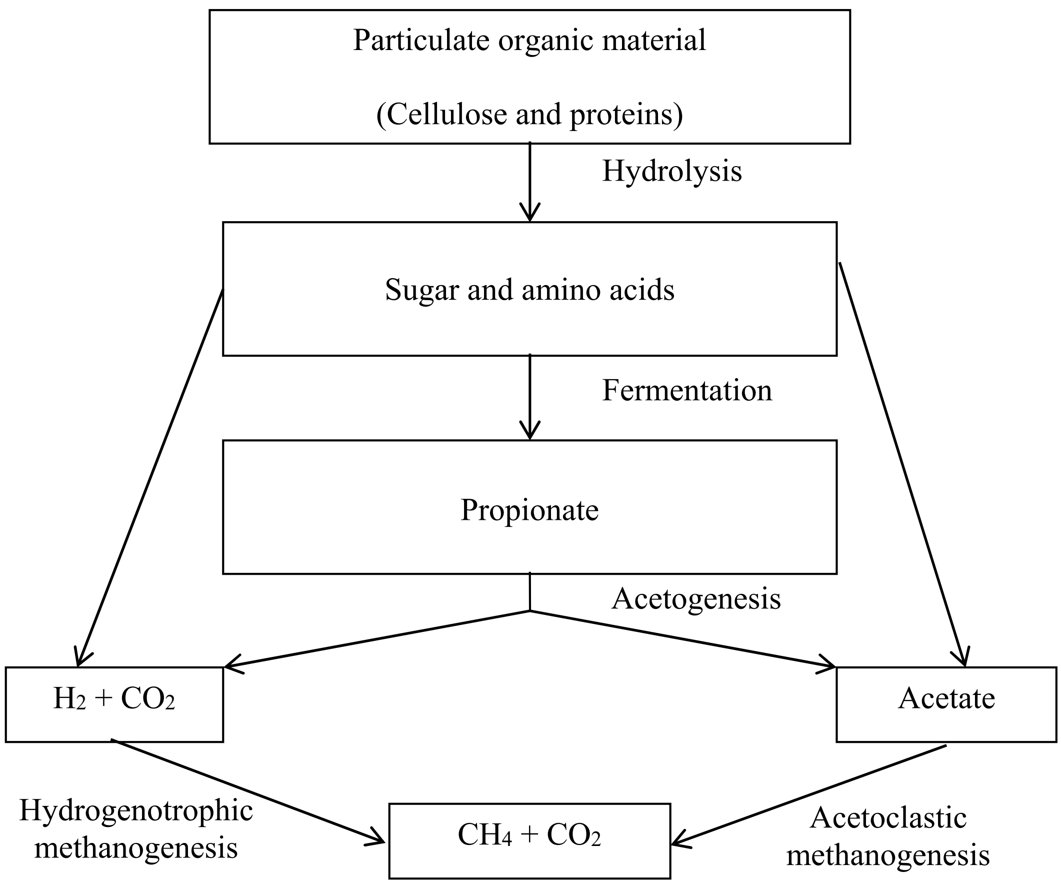


Figure 1. Proposed reaction scheme for the anaerobic co-digestion of swine manure and corn stover

Hydrolysis reactions were assumed to follow first-order kinetics (Equations (1) to (2)), whereas the Monod equation was applied to all other reactions (Equations (3) to (7)). A competitive acetic acid inhibition term was included for the fermentation reaction (Equation (3) and (4)), while a non-competitive acetic acid inhibition term was included for the acetogenesis (Equation (5)) (Costello et al., 1991). The kinetic models (rate equations) for key components of the reaction system are shown in Equations (1) to (7).

$$r_C = k_{H_C} \cdot c_C \qquad (1)$$

r_C is the reaction rate of hydrolysis of cellulose (mg/L·d); k_{H_C}, hydrolysis constant of cellulose (d^{-1}); c_C, concentration of cellulose (mg/L).

$$r_P = k_{H_P} \cdot c_P \qquad (2)$$

r_p is the reaction rate of hydrolysis of protein (mg/L·d); k_{H_P}, hydrolysis constant of protein (d^{-1}); c_p, concentration of protein (mg/L).

$$\mu_S = \mu_{max_f} \cdot \frac{c_S}{K_{S_f} \cdot \left(1 + {c_A}/{K_{i,f}^A}\right) + c_S} \qquad (3)$$

μ_S is the reaction rate of fermentation of sugar (d^{-1}); μ_{max_f}, maximum reaction velocity of fermentation process (d^{-1}); K_{S_f}, half-velocity constant of fermentation process ($gCOD/m^3$); $K_{i,f}^A$, inhibition constant of acetic acid in fermentation process ($gCOD/m^3$); c_A, concentration of acetic acid (mg/L); c_S, concentration of sugar (mg/L).

$$\mu_{AA} = \mu_{max_f} \cdot \frac{c_{AA}}{K_{S_f} \cdot \left(1 + {c_A}/{K_{i,f}^A}\right) + c_{AA}} \qquad (4)$$

μ_{AA} is the reaction rate of fermentation of amino acids (d^{-1}); c_{AA}, concentration of amino acids (mg/L).

$$\mu_{PP} = \mu_{max_{PP}} \cdot \frac{c_{PP}}{(K_{S_{PP}}+c_{PP})\cdot\left(1+{c_A}/{K_{i,a}^A}\right)} \quad (5)$$

μ_{PP} is the reaction rate of acetogenesis of propionate (d^{-1}); $\mu_{max_{PP}}$, maximum reaction velocity of acetogenesis of propionate (d^{-1}); $K_{S_{PP}}$, half-velocity constant of acetogenesis of propionate ($gCOD/m^3$); $K_{i,a}^A$, inhibition constant of acetic acid in acetogenesis of propionate ($gCOD/m^3$); c_{PP}, concentration of propionate (mg/L).

$$\mu_A = \mu_{max_A} \cdot \frac{c_A}{K_{S_A}+c_A} \quad (6)$$

μ_A is the reaction rate of aceticlastic methanogenesis (d^{-1}); μ_{max_A}, maximum reaction velocity of aceticlastic methanogenesis (d^{-1}); K_{S_A}, half-velocity constant of aceticlastic methanogenesis ($gCOD/m^3$).

$$\mu_{H_2} = \mu_{max_{H_2}} \cdot \frac{c_{H_2}}{K_{S_{H_2}}+c_{H_2}} \quad (7)$$

μ_{H_2} is the reaction rate of hydrogenotrophic methanogenesis (d^{-1}); $\mu_{max_{H_2}}$, maximum reaction velocity of hydrogenotrophic methanogenesis (d^{-1}); $K_{S_{H_2}}$, half-velocity constant of hydrogenotrophic methanogenesis ($gCOD/m^3$), c_{H_2}, concentration of hydrogen (mg/L).

A mass balance over the reactor was conducted in order to describe reactor concept and macro-kinetic behavior. Mass balances for key components of the reaction system are shown in Equations (8) to (15).

$$\frac{dc_C}{dt} = -r_C \quad (8)$$

$$\frac{dc_S}{dt} = r_C - \mu_S \cdot c_S \quad (9)$$

$$\frac{dc_P}{dt} = -r_P \quad (10)$$

$$\frac{d_{c_{AA}}}{dt} = r_P - \mu_{AA} \cdot c_{AA} \quad (11)$$

$$\frac{d_{c_{PP}}}{dt} = \mu_S \cdot c_S \cdot Y_f + \mu_{AA} \cdot c_{AA} \cdot Y_f - \mu_{PP} \cdot c_{PP} \quad (12)$$

$$\frac{d_{c_A}}{dt} = \mu_S \cdot c_S \cdot Y_f + \mu_{AA} \cdot c_{AA} \cdot Y_f + \mu_{PP} \cdot c_{PP} \cdot Y_{PP} - \mu_A \cdot c_A \quad (13)$$

$$\frac{d_{c_{H_2}}}{dt} = \mu_S \cdot c_S \cdot Y_f + \mu_{AA} \cdot c_{AA} \cdot Y_f + \mu_{PP} \cdot c_{PP} \cdot Y_{PP} - \mu_{H_2} \cdot c_{H_2} \quad (14)$$

$$\frac{d_{c_{CH_4}}}{dt} = \mu_A \cdot c_A \cdot Y_A + \mu_{H_2} \cdot c_{H_2} \cdot Y_{H_2} \quad (15)$$

$\frac{d_{c_C}}{dt}, \frac{d_{c_P}}{dt}, \frac{d_{c_S}}{dt}, \frac{d_{c_{AA}}}{dt}, \frac{d_{c_{PP}}}{dt}, \frac{d_{c_A}}{dt}, \frac{d_{c_{H_2}}}{dt}, \frac{d_{c_{CH_4}}}{dt}$ are the variation rate of the concentration of cellulose, protein, sugar, amino acids, propionate, acetic acids, hydrogen and methane (mg/L·d), respectively; Y_f, biomass yield coefficient for fermentation (of sugar and amino acids) (gVSS/gCOD) ; Y_{PP}, biomass yield coefficient for acetogenesis (gVSS/gCOD); Y_A, biomass yield coefficient for aceticlastic methanogenesis (gVSS/gCOD); Y_{H_2}, biomass yield coefficient for hydrogenotrophic methanogenesis (gVSS/gCOD).

2.5 Curve fitting for the estimation of kinetic parameters

A computer program called RStudio® (RStudio incorporation, Boston, Massachusetts, USA) was used to integrate the rate equations and mass balances to simulate the process. Kinetic parameters were estimated using curve fitting techniques with experimental data. Figure 2 describes the methodology used in fitting the model to the set of experimental results.

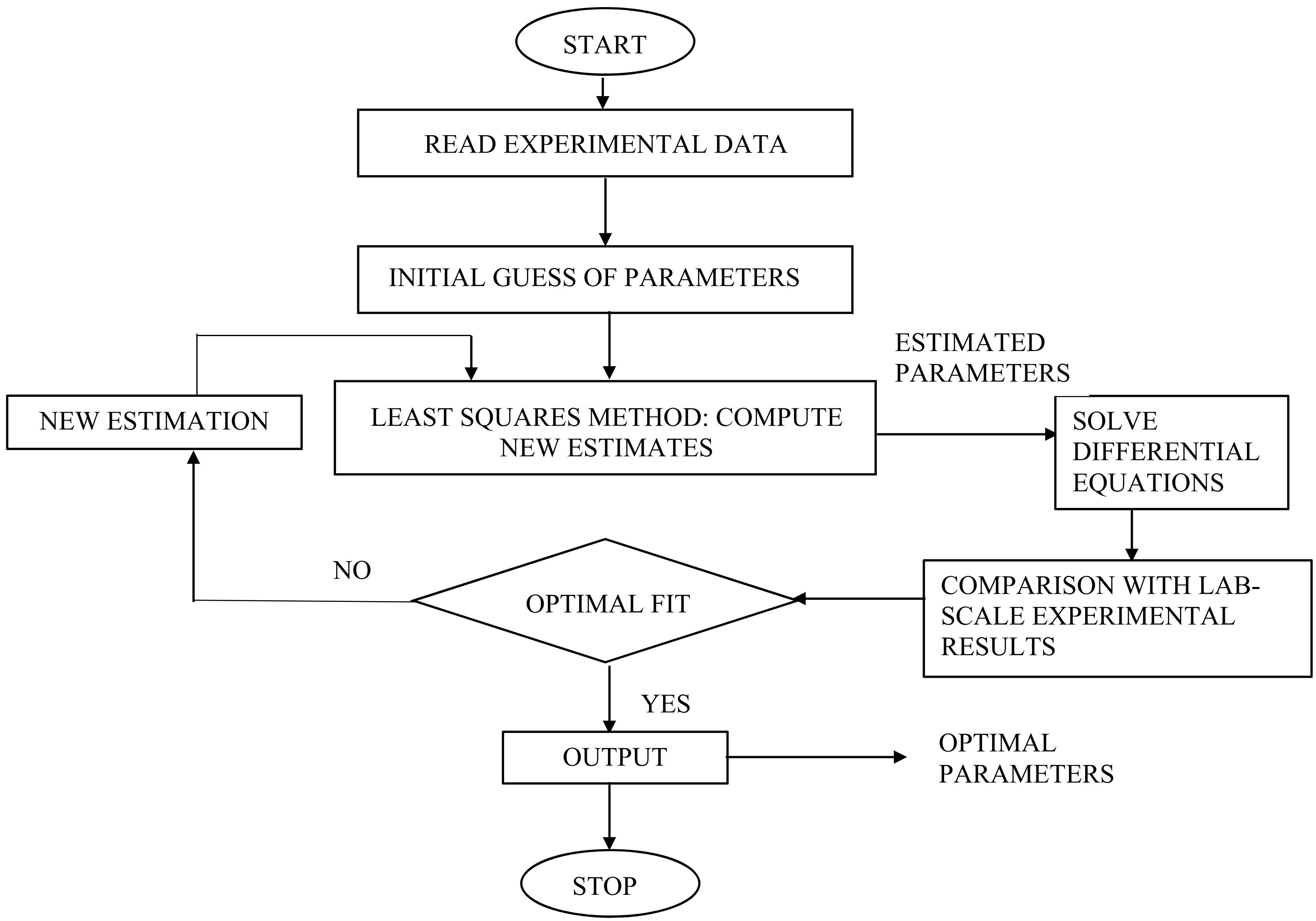


Figure 2. Flow chart for the estimation and optimization of kinetic parameters in the anaerobic co-digestion of swine manure and corn stover.

3. Results and discussion

3.1 Chemical characteristics of swine manure and corn stover

The chemical characteristics of swine manure and corn stover are shown in Table 1. The low C/N ratio of swine manure and high C/N ratio of corn stover indicate that these two substrates are suitable for co-digestion. The C/N ratio of swine manure was similar to the values which were 14.4 and 10.2 reported by Li et al. (2011) and Cuetos et al. (2011), respectively. The C/N ratio of corn stover was higher than the values which were 70.9 and 26.66 reported by Zhong et al. (2012) and Wu et al. (2010), respectively.

Table 1. Characteristics of swine manure and corn stover used in the experiment.

Parameter	Swine Manure	Corn Stover
C/N Ratio	11.51	90.07
COD (mg/g)	372	2031
TOC (mg/L)	3.59×10^4	-
TKN (mg/L)	1.805×10^4	-
pH	6.77	6.65
TS (%)	38.1	92.60
VS (% of TS)	84.5	94.68
TC (%)	38.2	41.43
TN (%)	3.32	0.46

3.2 Kinetic parameters

Initially, the values of the parameters used in the model were taken from Gelegenis et al. (2007) and modified for thermophilic conditions according to Siegrist et al. (2002). Siegrist et al. (2002) stated that the influence of the temperature on the kinetic expressions (growth rate, Monod and inhibition constants) was assumed to be exponential $F(T) = e^{\theta(T-T_0)}$ with θ being the temperature coefficient in the mesophilic (30-40 ºC) and thermophilic temperature (50-60 ºC) range. The temperature coefficients (θ) for different kinetic parameters are shown in Table 2. All kinetic coefficients for thermophilic conditions (T = 50 ºC) are calculated on the basis of the mesophilic values (T_0 =35 ºC) and the results are presented in Table 3.

Table 2. Values of temperature coefficients (θ) for different kinetic parameters used in the model development*.

Coefficient	Unit	θ (ºC^{-1})
k_{H_C}	d^{-1}	0.024
k_{H_P}	d^{-1}	0.024
μ_{max_f}	d^{-1}	0.069
K_{S_f}	gCOD/m^3	0.069
$\mu_{max_{PP}}$	d^{-1}	0.055
$K_{S_{PP}}$	gCOD/m^3	0.1

Table 2 Continued

Coefficient	Unit	θ (ºC^{-1})
μ_{max_A}	d^{-1}	0.069
K_{S_A}	gCOD/m^3	0.1
$\mu_{max_{H_2}}$	d^{-1}	0.069
$K_{S_{H_2}}$	gCOD/m^3	0.08

*Values of θ were obtained from Siegrist et al. (2002).

Table 3. Values of kinetic parameters at 35 ºC and their modified values at 50 ºC used in the model development in this study*.

Parameter (unit)	k_{H_C} (d^{-1})	k_{H_P} (d^{-1})	μ_{max_f} (d^{-1})	K_{S_f} ($gCOD/m^3$)	$K_{i,f}^{A}$ ($gCOD/m^3$)	Y_f (gVSS/gCOD)	$\mu_{max_{PP}}$ (d^{-1})	$K_{S_{PP}}$ ($gCOD/m^3$)
@ 35 ºC	0.146	0.104	5.559	28	604	0.043	0.111	247
@ 50 ºC	0.209	0.149	15.65	78.82	604	0.043	0.253	1107

Parameter (unit)	$K_{i,a}^{A}$ ($gCOD/m^3$)	Y_{PP} (gVSS/gCOD)	μ_{max_A} (d^{-1})	K_{S_A} ($gCOD/m^3$)	Y_A (gVSS/gCOD)	$\mu_{max_{H_2}}$ (d^{-1})	$K_{S_{H_2}}$ ($gCOD/m^3$)	Y_{H_2} (gVSS/gCOD)
@ 35 ºC	181	0.018	0.167	56	0.026	0.695	0.13	0.018
@ 50 ºC	181	0.018	0.470	251	0.026	1.956	0.432	0.018

*Values of kinetic parameters at 35 ºC were obtained from Gelegenis et al. (2007).

3.3 Model calibration

Experimental results of the thermophilic anaerobic co-digestion of swine manure and corn stover in the batch CSTR were used to for model calibration. Cumulative methane yield was first simulated using the kinetic parameters at mesophilic and thermophilic conditions listed in Table 3. The results are shown in Figure 3 (a) and (b), respectively. It was observed that the simulated methane production results were over-predicted in comparison with the experimental data (Figure 3 (a) and (b)). The deviations could be attributed to the values of the kinetic parameters obtained and modified from Gelegenis et al. (2007) and Siegrist et al. (2002) who used different substrates in their anaerobic digestion studies. Thus, it is necessary to estimate the kinetic parameters for the anaerobic co-digestion of swine manure and corn stover.

Different methods have been used for parameter estimation, such as least squares, least-modulus, or maximum likelihood (Donoso-Bravo et al., 2011). In this study, least squares method was employed for the estimation of the parameters (Figure 2). It is assumed that the standard deviation of the measurement errors, which can be known or unknown is constant (Donoso-Bravo et al., 2011), as shown in Equation (16). In equation (16), S is the objective function, y_{exp} are the collected measurements, y_{sim} are the model-predicted outputs, α represents the parameter to be determined and N is the number of measurements. The least-squares estimate of α is simply the value of α that minimizes S(α).

$$S(\alpha) = \min \sum_{t=1}^{N} \left(y_{exp}(t) - y_{sim}(t, \alpha) \right)^2 \qquad (16)$$

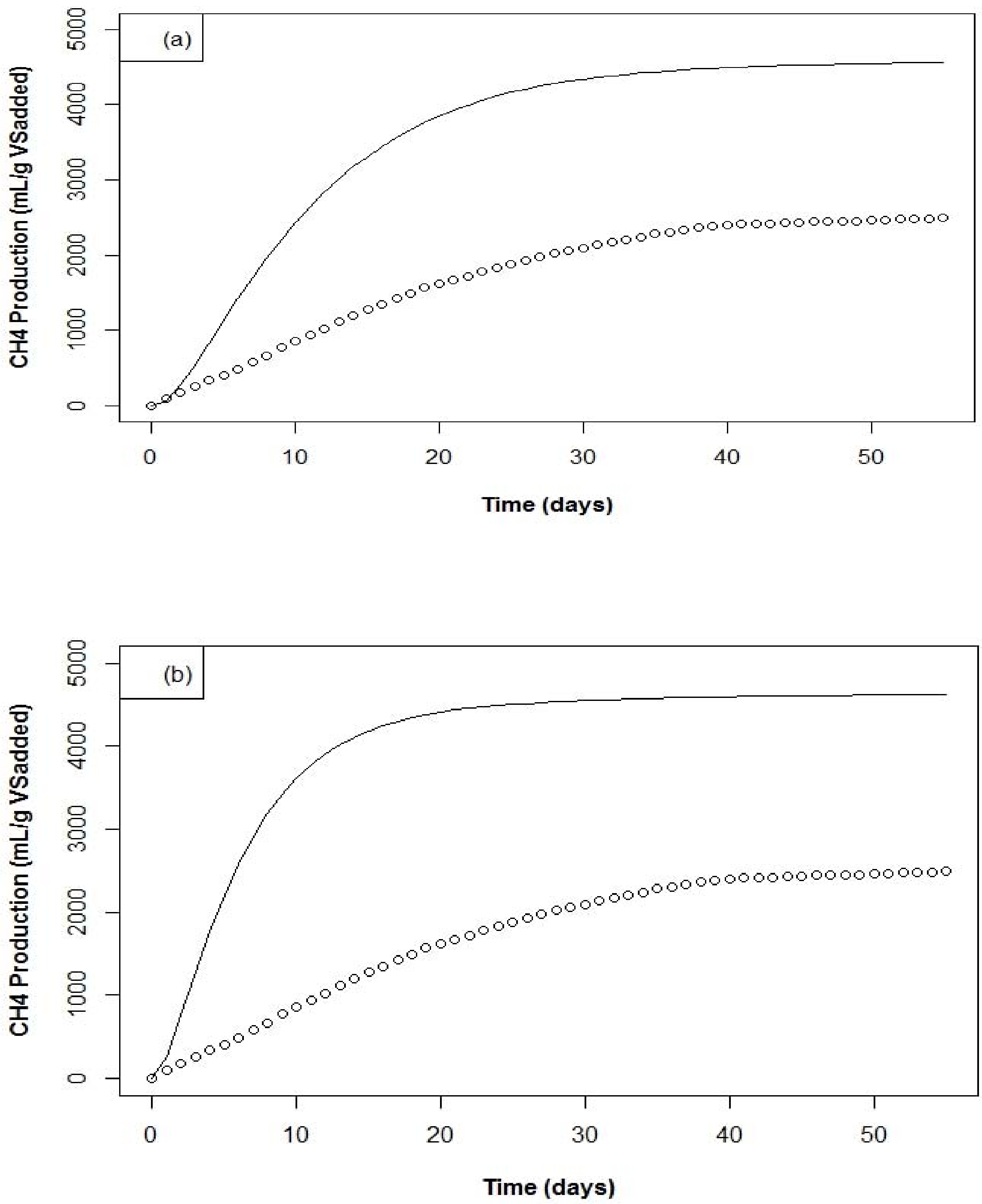


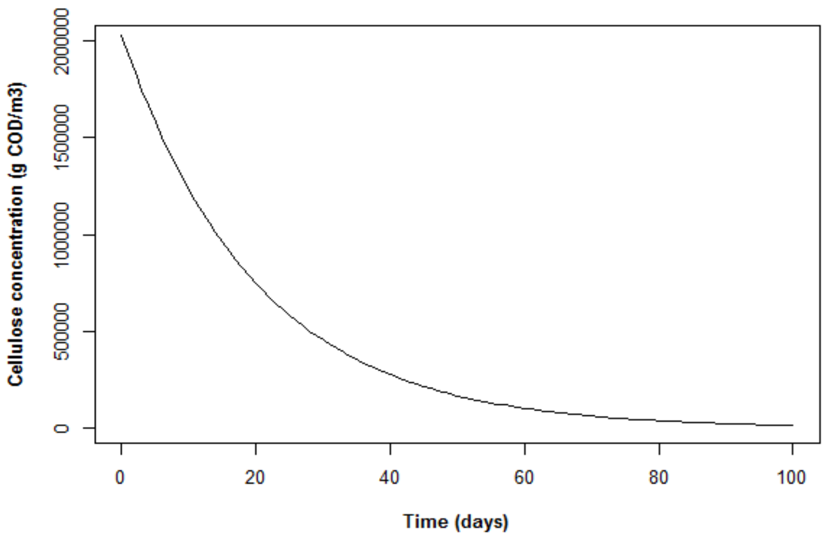
Figure 3. Simulation of methane generation in batch experiments using different values of parameters: (a) mesophilic kinetic values; (b) calculated thermophilic kinetic values (○ experimental data; —model simulation).

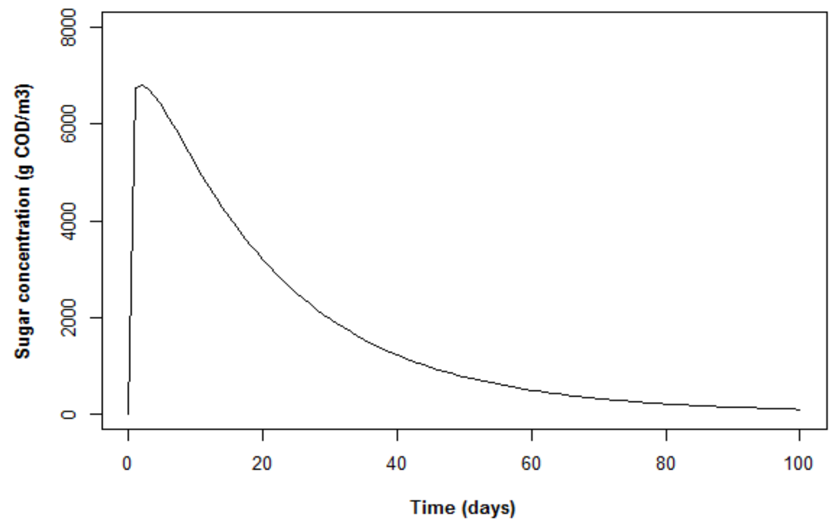
As shown in Figure 2, curve fitting was used to estimate and optimize the kinetic parameters in the modeling. The first step was to set the initial values to all kinetic parameters. The initial values of all parameters were those at 50 ºC listed in Table 3 except the hydrolysis constant of cellulose was manually set at 0.04 which was recommended by Gujer et al. (1983). Simulations were undertaken to fit the model outputs to the experimental data using ordinary differential equations (ODE) solver and least square algorithms in RStudio®. Optimal parameter values that best fit the experimental results are presented in Table 4. The simulated results of the variation of each variable in the model are shown in Figure 4. Simulation of cumulative methane yield using optimal parameters in our model and experimental results are shown in Figure 5. It can be seen from Figure 5that the simulation results agree with the experimental data very well.

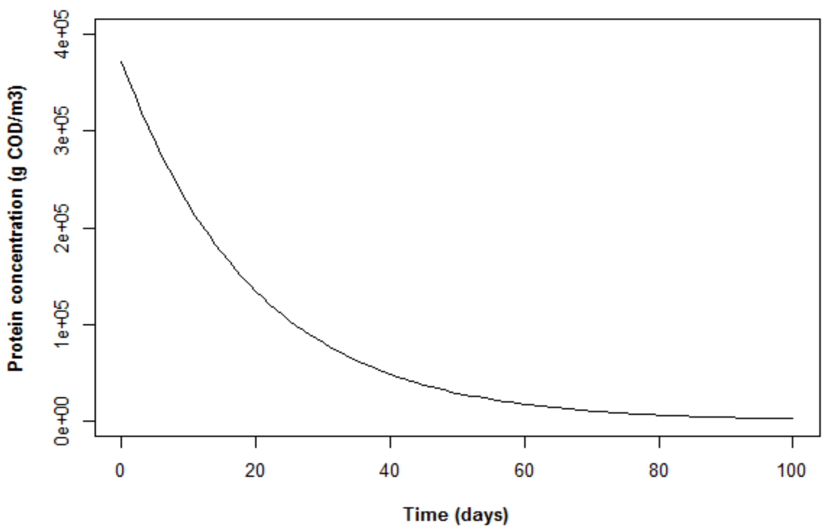
Table 4. Optimized kinetic parameter values for thermophilic anaerobic co-digestion of swine manure with corn stover using least squares method.

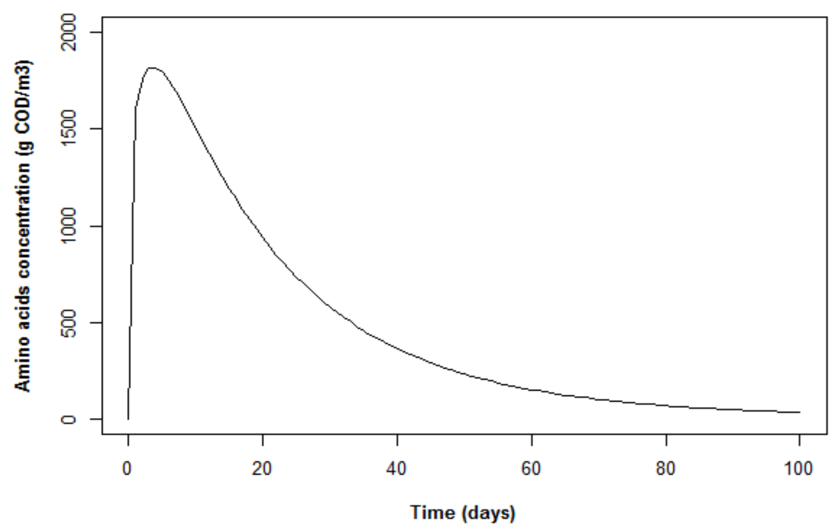
Parameter (unit)	k_{H_C} (d^{-1})	k_{H_P} (d^{-1})	μ_{max_f} (d^{-1})	K_{S_f} (gCOD/m^3)	$K_{i,f}^{A}$ (gCOD/m^3)	Y_f (gVSS/gCOD)	$\mu_{max_{PP}}$ (d^{-1})	$K_{S_{PP}}$ (gCOD/m^3)
Value	0.050	0.051	15.66	78.73	604.1	0.047	0.281	1107
	$K_{i,a}^{A}$ (gCOD/m^3)	Y_{PP} (gVSS/gCOD)	μ_{max_A} (d^{-1})	K_{S_A} (gCOD/m^3)	Y_A (gVSS/gCOD)	$\mu_{max_{H_2}}$ (d^{-1})	$K_{S_{H_2}}$ (gCOD/m^3)	Y_{H_2} (gVSS/gCOD)
Value	181.0	0.017	0.341	361.4	0.012	2.072	0.516	0.012

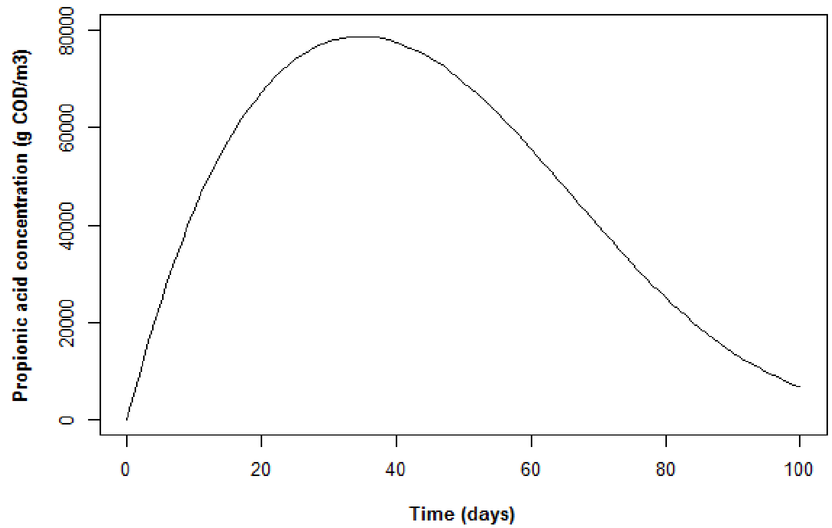
Figure 4. The outputs of simulated variation of each variable in the developed model using the optimal values of the parameters

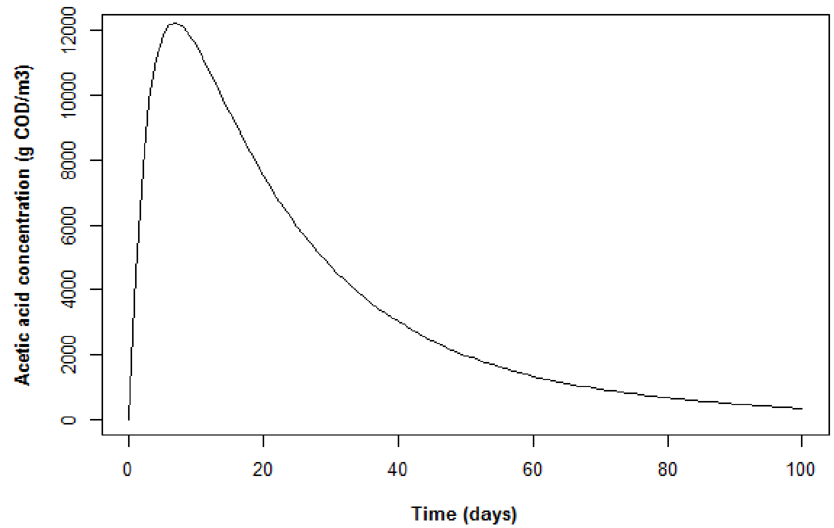

Cellulose concentration (g COD/m3)
0
500000
1000000
1500000
2000000
0
20
40
60
80
100
Time (days)

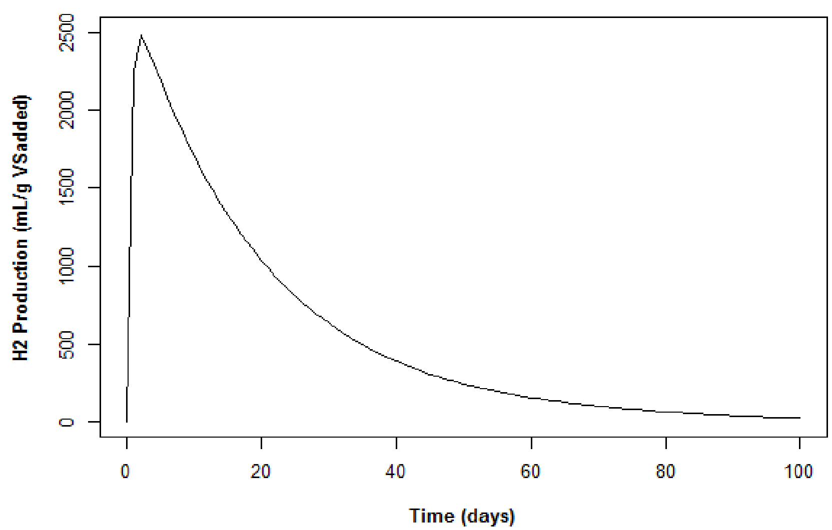

Sugar concentration (g COD/m3)
0
2000
4000
6000
8000
0
20
40
60
80
100
Time (days)

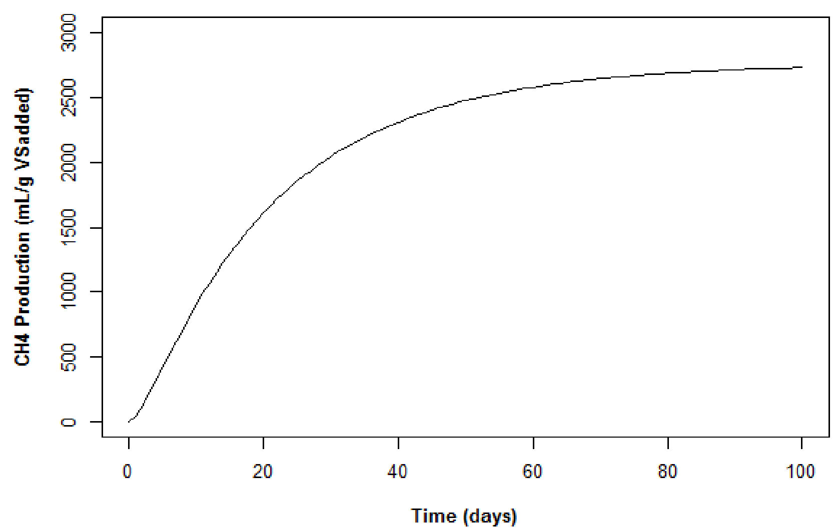

Protein concentration (g COD/m3)
0e+00
1e+05
2e+05
3e+05
4e+05
0
20
40
60
80
100
Time (days)


Amino acids concentration (g COD/m3)
0
500
1000
1500
2000
0
20
40
60
80
100
Time (days)


Propionic acid concentration (g COD/m3)
0
20000
40000
60000
80000
0
20
40
60
80
100
Time (days)


Acetic acid concentration (g COD/m3)
0
2000
4000
6000
8000
10000
12000
0
20
40
60
80
100
Time (days)


H2 Production (mL/g VSadded)
0
500
1000
1500
2000
2500
0
20
40
60
80
100
Time (days)


CH4 Production (mL/g VSadded)
0
500
1000
1500
2000
2500
3000
0
20
40
60
80
100
Time (days)

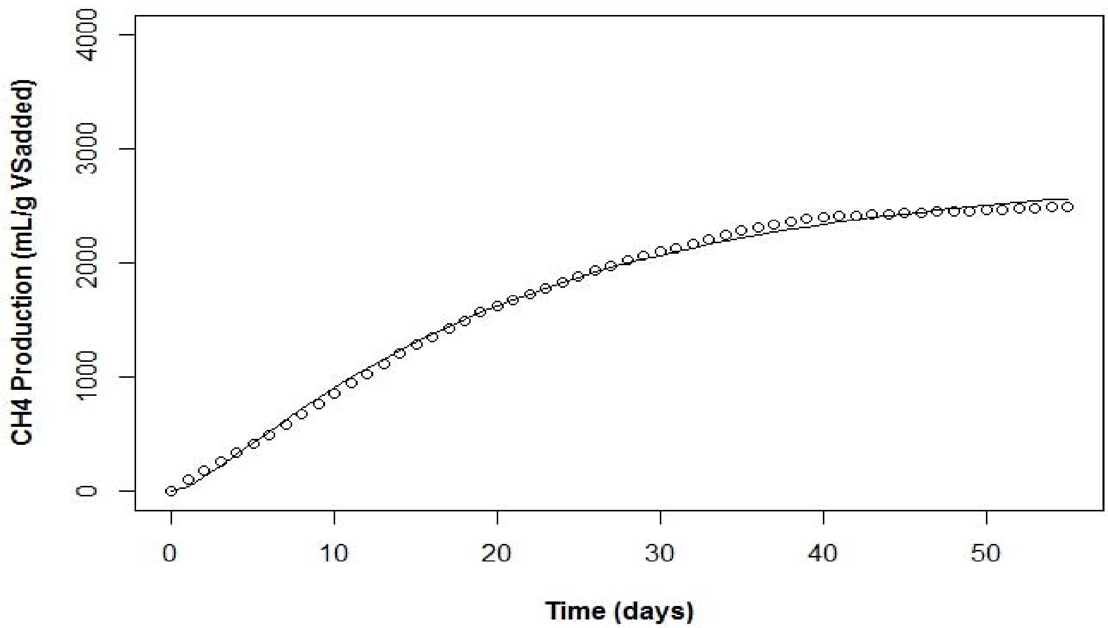


Figure 5. Simulation of methane generation in batch experiments using optimized values of kinetic parameters (○ experimental data; — model simulation).

It can be seen from Table 4 that there is a notable difference between the optimized hydrolysis constants (0.050, 0.051) and the calculated hydrolysis constants shown in Table 3 (0.209, 0.149). This may be due to the sensitivity of hydrolysis constants and different experimental conditions and substrates used in this study (Gavala et al., 2003; Vavilin et al., 2008). The hydrolysis constant (k) for the production of biogas from straw/pig manure mixture was estimated to be 0.02 d^{-1} in a batch experiment (Llabrés-Luengo et al., 1987). Gujer et al. (1983) reported the typical values of kinetic coefficient of the first-order rate of hydrolysis of cellulose was between 0.04 and 0.13 d^{-1}. Christ et al. (2000) reported the typical values of kinetic coefficient of the first-order rate of hydrolysis of proteins was between 0.015 and 0.075 d^{-1} at 55 ºC. The values of estimated hydrolysis constants (0.050, 0.051) in this study are within the typical ranges.

3.4 Estimation of uncertainty in model parameters

The uncertainty of kinetic parameters is important in their estimation and optimization. The purpose of uncertainty analysis is to evaluate the quality of the fitting process and the accuracy of the estimation of the parameters (Dochain et al., 2001). Bootstrap technique is used for estimating parameter uncertainty in this study. The steps for the bootstrap are followed:

firstly, the least squares parameter estimates β, the fitted values $\hat{y}(t_1),\ldots, \hat{y}(t_n)$, and the residuals $e_1,\ldots, e_n$ are determined using the non-linear least squares. Then a new bootstrapped data set $y^{(b)}(t_1), \ldots, y^{(b)}(t_n)$ is formed by adding fitted values to a randomly chosen residual from the collection of residuals of the original fit. That is $y^{(b)}(t_i) = \hat{y}(t_i) + e^*$, where e^* is drawn with replacement from the collection $\{e_1, \ldots, e_n\}$. A bootstrapped set of parameter estimates $\beta^{(b)}$ can be determined from the bootstrapped data set using non-linear least squares. If the formation of bootstrapped data set is replicated N times, a collection of $\{\beta^{(1)}, \ldots, \beta^{(N)}\}$ can be determined. The collection $\{\beta^{(1)}, \ldots, \beta^{(N)}\}$ can be used as a surrogate for the sampling distribution of β. A standard error for a parameter β can be estimated by the standard deviation of $\{\beta^{(1)}, \ldots, \beta^{(N)}\}$, or empirical quantiles of $\{\beta^{(1)}, \ldots, \beta^{(N)}\}$ can be used to estimate a confidence interval (CI) for a parameter β.

The R codes for bootstrapping the parameter estimates are shown in Appendix. The bootstrap replicates is set at 1000. Table 5 presents the results of bootstrap-based standard error and 95% CI of the model parameters. The 95% CI of k_{H_C} and k_{H_P} are in the ranges of 0.046-0.053 and 0.041-0.063, respectively while the calibrated values of k_{H_C} and k_{H_P} are 0.050 and 0.051 as shown in Table 4. The small standard error and narrow ranges of 95% CI of k_{H_C}, k_{H_P} indicated the accuracy of these parameters in the calibration step. Large standard error and broad ranges of 95% CI of parameters such as $\mu_{max_{PP}}$, $\mu_{max_{H_2}}$ indicated that additional data need to be collected which is suggested by Dochain et al., 2001.

Table 5. Estimation of the bootstrap-based standard error and 95% confidence interval for the model parameters in this study

Parameter (unit)	Bootstrap-based standard error	95% Confidence Interval
k_{H_C} (d^{-1})	0.002	(0.046, 0.053)
k_{H_P} (d^{-1})	0.005	(0.041, 0.063)
μ_{max_f} (d^{-1})	14.8	(15, 69.1)
K_{S_f} (gCOD/m^3)	25.1	(78.6, 134.2)
$K_{i,f}^{A}$ (gCOD/m^3)	5.91	(603.7, 605.2)
Y_f (gVSS/gCOD)	0.0004	(0.046, 0.048)
$\mu_{max_{PP}}$ (d^{-1})	20.9	(0.5, 90.3)
$K_{S_{PP}}$ (gCOD/m^3)	2.3	(1107, 1108)
$K_{i,a}^{A}$ (gCOD/m^3)	27.1	(181, 285)
Y_{PP} (gVSS/gCOD)	0.008	(0.002, 0.03)
μ_{max_A} (d^{-1})	0.062	(0.25, 0.50)
K_{S_A} (gCOD/m^3)	0.008	(361, 415)
Y_A (gVSS/gCOD)	0.0002	(0.012, 0.013)
$\mu_{max_{H_2}}$ (d^{-1})	12.5	(1.1, 57.5)
$K_{S_{H_2}}$(gCOD/m^3)	18.2	(0.24, 56)
Y_{H_2} (gVSS/gCOD)	0.0002	(0.012, 0.013)

3.5 Model Validation

After calibrating the model using the cumulative methane yield from thermophilic anaerobic co-digestion of swine manure and corn stover in a batch CSTR, a validation study was conducted in order to evaluate the quality of the calibrated parameters and the applicability of the model. Owing to limitation of thermophilic co-digestion data, experimental results from Cuetos et al. (2011) who investigated the co-digestion of swine manure with corn residues under mesophilic condition in batch tests were used to validate the mathematical model, assessing the agreement between predicted and measured data for cumulative methane yield and acetic acid (an important intermediate in the process) concentration. Considerng the experimental results from Cuetos et al. (2011) were achieved at mesophilic condition, the parameter values for validation were calculated on the basis of optimal parameter values at thermophilic condition by applying the equation $\mathrm{F(T)} = e^{\theta(T-T_0)}$ and the results are shown in Table 6. The comparsion between model simulated results and experimental data from Cuetos et al. (2011) of methane yield and acetic acid concentration are shown in Figure 6(a) & (b), respectively. As shown in Figure 6(a), the model could predict the trends of cumulative methane yield fairly well. The simulation results closely followed the experimental trends at the first 25 days but underestimated the cumulative methane yield after 30 days. This might be due to the character hypothesis of substrates in the model since it only considered cellulose and proteins but ignored other components which might potentially contribute to the methane production.

It can be found from Figure 6(b) that the simulation results of acetic acid concentration followed the experimental trends well for the first 8 days of the experiments and overestimated in the later days. These deviations may be caused by two reasons: one is that the model included the acetic acids inhition in the fermentatin and acetogenesis, but no acetic acid inhibtion was observed in Cuetos et al. (2011) study; the other is that only experimental results of cumulative methane production were used for model calibration and optimization of the kinetic parameters. The lack of experimental results of acetic acid concentration in the model calibration resulted in that the simulation of acetic acid concentration was not as good as cumulative methane production.

Table 6. Values of kinetic parameters at mesophilic condition (35 ºC) used for model validation in this study.

Parameter (unit)	k_{H_C} (d^{-1})	k_{H_P} (d^{-1})	μ_{max_f} (d^{-1})	K_{S_f} (gCOD/m^3)	$K_{i,f}^{A}$ (gCOD/m^3)	Y_f (gVSS/gCOD)	$\mu_{max_{PP}}$ (d^{-1})	$K_{S_{PP}}$ (gCOD/m^3)
Value	0.035	0.036	5.564	27.97	604.1	0.047	0.123	247
	$K_{i,a}^{A}$ (gCOD/m^3)	Y_{PP} (gVSS/gCOD)	μ_{max_A} (d^{-1})	K_{S_A} (gCOD/m^3)	Y_A (gVSS/gCOD)	$\mu_{max_{H_2}}$ (d^{-1})	$K_{S_{H_2}}$ (gCOD/m^3)	Y_{H_2} (gVSS/gCOD)
Value	181	0.017	0.121	80.64	0.012	0.736	0.155	0.012

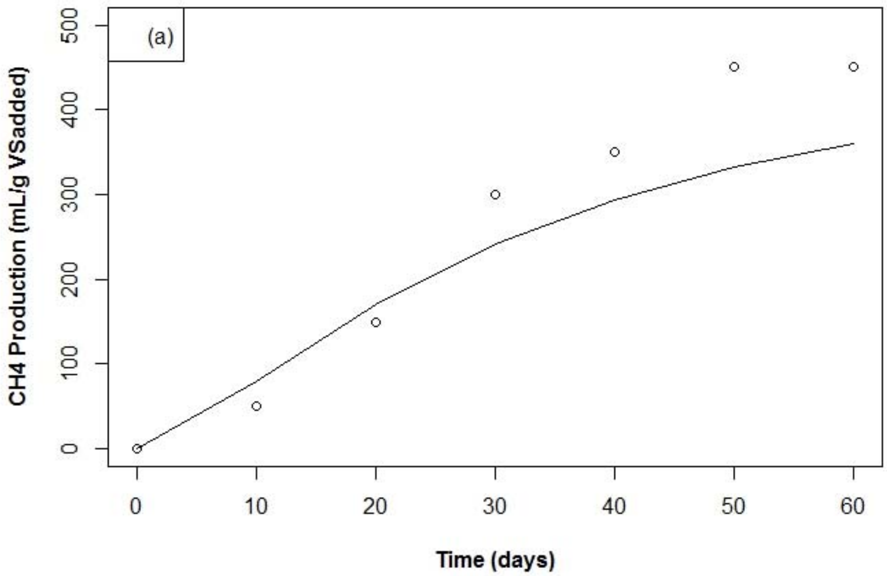


Figure 6. Simulation of methane yield and acetic acid concentration by proposed model in comparison with experimental data from Cuetos et al. (2011) (○ experimental data; —model simulation; the values of model parameter under mesophilic condition were converted from thermophilic calibrated parameter values based on the equation $\mathrm{F(T)} = e^{\theta(T-T_0)}$).

4. Conclusions

A mathematical model from Gelegenis et al. (2007) and Hublin et al. (2013) has been modified to describe swine manure and corn stover co-digestion process under thermophilic anaerobic conditions. The model was calibrated using the experimental results from the thermophilic anaerobic co-digestion of swine manure and corn stover in a batch CSTR and optimal kinetic parameters in the model were obtained by applying the least square method. The validation of the model was conducted by comparing experimental results of methane yield and acetic acid concentration from Cuetos et al. (2011) with the model simulation results. The comparison between experimental and model results suggests that the model is basically valid in predicting cumulative methane yield. However, further improvement of the model with experimental data of the intermediates is needed for the model to predict the intermediates well.

REFERENCES

APHA. (1998). *Standard methods for the examination of water and wastewater* (20th ed). New York, USA: American Public Health Association.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V, Pavlostathis, S. G., & Rozzi, A., et al. (2002). The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science & Technology*,*45*(10), 65–73.

Boubaker, F., & Ridha, B. C. (2008). Modelling of the mesophilic anaerobic co-digestion of olive mill wastewater with olive mill solid waste using anaerobic digestion model No. 1 (ADM1). *Bioresource Technology*, *99*(14), 6565–6577.

Christ, O., Wilderer, P.A., Angerhofer, R., & Faulstich, M., (2000). Mathematical modelling of the hydrolysis of anaerobic processes. *Water Science & Technology*, *41*(3), 61-65.

Costello, D.J., Greenfield, P.F., & Lee, P.L. (1991). Dynamic modelling of a single-stage high-rate anaerobic reactor - I. Model derivation. *Water Research*, *25*(7), 847–858.

Cuetos, M. J., Fernández, C., Gómez, X., & Morán, A. (2011). Anaerobic co-digestion of swine manure with energy crop residues. *Biotechnology and Bioprocess Engineering*, *16*(5), 1044–1052.

De Gioannis, G., Muntoni, A., Cappai, G., & Milia, S. (2009). Landfill gas generation after mechanical biological treatment of municipal solid waste. Estimation of gas generation rate constants. *Waste Management*, *29*(3), 1026–1034.

Derbal, K., Bencheikh-Lehocine, M., Cecchi, F., Meniai, A-H., & Pavan, P. (2009). Application of the IWA ADM1 model to simulate anaerobic co-digestion of organic waste with waste activated sludge in mesophilic condition. *Bioresource Technology*, *100*(4), 1539–1543.

Dochain, D., Vanrolleghem, P.A. (2001). Dynamic modelling and estimation in wastewater treatment processes. *IWA publishing*, London, UK.

Donoso-Bravo, A., Mailier, J., Martin, C., Rodríguez, J., Aceves-Lara, C. A., & Wouwer, A. Vande. (2011). Model selection, identification and validation in anaerobic digestion: A review. *Water Research*, *45*(17), 5347–5364.

Esposito, G., Frunzo, L., Panico, A, & Pirozzi, F. (2011). Model calibration and validation for OFMSW and sewage sludge co-digestion reactors. *Waste Management*, *31*(12), 2527–2535.

Flotats, X., Palatsi, J., Ahring, B. K., & Angelidaki, I. (2006). Identifiability study of the proteins degradation model , based on ADM1 , using simultaneous batch experiments. *Water Science & Technology*, *54*(4),31-39.

Gavala, H. N., Angelidaki, I., & Ahring, B. K. (2003). Kinetics and modelling of anaerobic digestion process. *Advances in Biochemical Engineering*, *81*, 57-93.

Gelegenis, J., Georgakakis, D., Angelidaki, I., & Mavris, V. (2007). Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy*, *32*(13), 2147–2160.

Gujer, W., Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. *Water Science & Technology*, *15*(8-9), 127-167.

Hublin, A., & Zelic, B. (2013). Modelling of the whey and cow manure co-digestion process. *Waste Management & Research, 31*(4), 353–360.

Keshtkar, A., Ghaforian, H., Abolhamd, G., & Meyssami, B. (2001). Dynamic simulation of cyclic batch anaerobic digestion of cattle manure. *Bioresource Technology*, *80*(1), 9-17.

Koch, K., Lübken, M., Gehring, T., Wichern, M., & Horn, H. (2010). Biogas from grass silage - Measurements and modeling with ADM1. *Bioresource Technology*, *101*(21), 8158–8165.

Lauwers, J., Appels, L., Thompson, I. P., Degrève, J., Van Impe, J. F., & Dewil, R. (2013). Mathematical modelling of anaerobic digestion of biomass and waste: Power and limitations. *Progress in Energy and Combustion Science*, *39*(4), 383–402.

Li, Y., Yan, X.-L., Fan, J.-P., Zhu, J.-H., & Zhou, W.-B. (2011). Feasibility of biogas production from anaerobic co-digestion of herbal-extraction residues with swine manure. *Bioresource Technology*, *102*(11), 6458–6463.

Llabrés-Luengo, P., & Mata-Alvarez, J. (1987). Kinetic study of the anaerobic digestion of straw-pig manure mixtures. *Biomass*, *14*(2), 129–142.

Lo, H. M., Kurniawan, T. a, Sillanpää, M. E. T., Pai, T. Y., Chiang, C. F., Chao, K. P., Liu, M. H., et al. (2010). Modeling biogas production from organic fraction of MSW co-digested with MSWI ashes in anaerobic bioreactors. *Bioresource Technology*, *101*(16), 6329–6335.

Palatsi, J., Illa, J., Prenafeta-boldú, F. X., Laureni, M., Fernandez, B., Angelidaki, I., & Flotats, X. (2010). Long-chain fatty acids inhibition and adaptation process in anaerobic thermophilic digestion : Batch tests , microbial community structure and mathematical modelling. *Bioresource Technology*, *101*(7), 2243–2251.

Rao, M. S., & Singh, S. P. (2004). Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yield--organic loading relationships for process optimisation. *Bioresource Technology*, *95*(2), 173–185.

Redzwan, G., & Banks, C. (2004). The use of a specific function to estimate maximum methane production in a batch-fed anaerobic reactor. *Journal of Chemical Technology and Biotechnology*, *79*,1174–1178.

Siegrist, H., Vogt, D., Garcia-Heras, J. L., & Gujer, W. (2002). Mathematical model for meso- and thermophilic anaerobic sewage sludge digestion. *Environmental Science & Technology*, *36*(5), 1113–1123.

Sosnowski, P., Klepacz-Smolka, A., Kaczorek, K., & Ledakowicz, S. (2008). Kinetic investigations of methane co-fermentation of sewage sludge and organic fraction of municipal solid wastes. *Bioresource Technology*, *99*(13), 5731–5737.

Vavilin, V. A., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. *Waste Management*, *28*(6), 939–951.

Weiland, P. (2000). Anaerobic waste digestion in Germany-status and recent developments. *Biodegradation*, *11*(6), 415–421.

Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology*, *101*(11), 4042–4047.

Xie, S., Lawlor, P. G., Frost, J. P., Hu, Z., & Zhan, X. (2011). Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technology*, *102*(10), 5728–5733.

Zhang, L., Lee, Y. W., & Jahng, D. (2011). Anaerobic co-digestion of food waste and piggery wastewater: focusing on the role of trace elements. *Bioresource Technology*, *102*(8), 5048–5059.

Zhong, W., Zhang, Z., Luo, Y., Qiao, W., Xiao, M., & Zhang, M. (2012). Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresource Technology*, *114*, 281–286.

CHAPTER 6: CONCLUSIONS AND FUTURE RESEARCH

1. Overall conclusions

This dissertation contributes to the application and expansion of the thermophilic anaerobic co-digestion technology. Four studies had been conducted to investigate the performance of anaerobic co-digestion of swine manure and corn stover under thermophilic condition.

Temperature change from mesophilic (35 ºC) to thermophilic (50 ºC) condition using one-step temperature jump was successfully carried out in anaerobic continuously stirred tank reactor (CSTR) treating swine wastewater and corn stover. During the transition period, low methane content and production were observed due to the accumulation of acetate. When the acetate was consumed, the methane production increased back to normal. It had been demonstrated that the thermophilic anaerobic digestion showed better performance of daily biogas production, specific gas yield and chemical oxygen demand (COD) removal compared with that under mesophilic condition.

Alkaline pretreatment method proved to be an effective method in improving biodegradability and biogas production of corn stover in anaerobic digestion. In general, higher specific methane and biogas yield were observed when the corn stover was pretreated with NaOH, lime and combination of NaOH and lime compard with that of untreated corn stover. The improved biodegradability of alkaline pretreated corn stover was indicated by the increased total solids (TS) and volatile solids (VS) reduction.

The effect of TS concentration on thermophilic anaerobic co-digestion of swine manure and corn stover was investigated. It was observed that the methane yield decreased with the increasing TS concentration for the substrates investigated. The lower gas yield achieved at higher TS concentration could be partially attributed to the overloading of the substrates.

A mathematical model had been developed to describe the anaerobic co-digestion of swine manure and corn stover process. The model assumes that methane is generated as a result of the simultaneous performance of all phases in anaerobic digestion, i.e. hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The kinetic parameters used in the model were adjusted and optimized for thermophilic conditions in this study. Least square method was used for parameter estimation and bootstrap is used for estimating parameter uncertainty. The validation of the model indicated that the model was applicable in predicting methane yield for anaerobic co-digestion of swine manure and corn stover.

2. Suggestions for future work

In addition to alkaline pretreatment, the effect of other promising pretreatment technologies on biodegradability of corn stover can be investigated. It is also recommended to measure the cellulose crystallinity, lignin content and pore size of pretreated corn stover to study the changes in physical and chemical properties of corn stover after pretreatment.

Apart from hydraulic retention time (HRT) and TS concentration, it is of interest to investigate the effect of other crucial factors such as pH, substrate/inoculum (S/I) ratio on biogas production of thermophilic anaerobic co-digestion of swine manure and corn stover.

Future research on model development can consider incorporating the conversion of the waste organic particles to carbohydrates and protein, i.e., the disintegration process or pretreatment technologies such as alkaline pretreatment. Measurements of concentration of intermediate products such as propionate, acetate and hydrogen are also highly recommended to simulate their variation in the process.

APPENDICES

APPENDIX A. SAS CODES FOR CHPATER 3

This SAS code evaluates the difference in averages of daily biogas production for NaOH-treated corn stover and untreated corn stover.

```
data NaOH;
input reactor production;
datalines;
1 3848
1 3848
1 4056
1 4472
1 3952
1 4472
1 4680
1 4472
1 4368
1 4680
1 4680
1 4368
1 5096
1 4992
1 4680
2 6608
2 6384
2 6384
2 6272
2 5936
2 6608
2 6608
2 6384
2 6384
2 6384
2 6608
2 6384
2 6496
2 6608
2 6608
run;
proc glm data=NaOH;
class reactor;
model production= reactor;
run;
```

This SAS code evaluates the difference in averages of daily biogas production for lime-treated corn stover and untreated corn stover.

```
data Lime;
input reactor production;
datalines;
1 3744
1 3848
1 3848
1 3744
1 3952
1 3744
1 4056
1 3848
1 3952
1 3640
1 3640
1 3744
1 3744
1 3640
1 3640
2 5824
2 5712
2 5712
2 5712
2 5824
2 5600
2 5712
2 5600
2 5712
2 5264
2 5600
2 5488
2 5712
2 5712
2 5600
run;
proc glm data=Lime;
class reactor;
model production= reactor;
run;
```

This SAS code evaluates the difference in averages of daily biogas production for combined NaOH and lime-treated corn stover and untreated corn stover.

```
data Combo;
input reactor production;
datalines;
1 3744
1 3848
1 4368
1 4472
1 3848
1 4368
1 4056
1 4472
1 4368
1 3952
1 3640
1 3744
1 3848
1 3952
1 3744
2 5936
2 6832
2 6832
2 6608
2 6496
2 6384
2 6048
2 6832
2 6384
2 6160
2 6048
2 6272
2 6832
2 6608
2 6720
run;
proc glm data=Combo;
class reactor;
model production= reactor;
run;
```

APPENDIX B. SAS CODES FOR CHPATER 4

This SAS code evaluates the difference in averages of daily biogas production at different total solids concentration.

```
data DBP;
input TS production;
datalines;
2.5 16
2.5 16
2.5 15
2.5 16
2.5 17
2.5 16
2.5 16
2.5 15
2.5 16
2.5 17
2.5 15
2.5 16
2.5 16
2.5 17
2.5 16

4 24
4 26
4 24
4 26
4 24
4 26
4 26
4 26
4 24
4 24
4 24
4 24
4 24
4 26
4 24

5.5 22
5.5 22
5.5 21
5.5 20
5.5 22
5.5 22
5.5 23
5.5 21
5.5 20
5.5 21
5.5 19
5.5 20
5.5 19
5.5 19
5.5 21
run;
proc glm data=DBP;
```

```
class TS;
model production= TS;
lsmeans TS/cl pdiff adj=tukey;
run;
```

This SAS code evaluates the difference in averages of daily methane production at different total solids concentration.

```
data DMP;
input TS production;
datalines;
2.5 8
2.5 7
2.5 7
2.5 8
2.5 8
2.5 8
2.5 8
2.5 7
2.5 8
2.5 8
2.5 7
2.5 7
2.5 8
2.5 8
2.5 8


4 11
4 12
4 11
4 12
4 11
4 12
4 12
4 12
4 11
4 11
4 11
4 11
4 11
4 12
4 11


5.5 9
5.5 9
5.5 8
5.5 8
5.5 9
5.5 9
5.5 9
5.5 8
5.5 8
5.5 8
```

```
5.5 8
5.5 8
5.5 8
5.5 8
5.5 8
run;
proc glm data=DMP;
class TS;
model production= TS;
lsmeans TS/cl pdiff adj=tukey;
run;
```

This SAS code evaluates the difference in averages of specific biogas yield at different total solids concentration.

```
data SBY;
input TS production;
datalines;
2.5 0.304
2.5 0.291
2.5 0.287
2.5 0.308
2.5 0.314
2.5 0.308
2.5 0.306
2.5 0.285
2.5 0.304
2.5 0.317
2.5 0.283
2.5 0.291
2.5 0.300
2.5 0.319
2.5 0.298

4 0.286
4 0.305
4 0.282
4 0.307
4 0.275
4 0.304
4 0.308
4 0.308
4 0.277
4 0.279
4 0.279
4 0.286
4 0.305
4 0.279

5.5 0.185
5.5 0.190
5.5 0.178
5.5 0.171
5.5 0.183
5.5 0.187
```

```
5.5 0.192
5.5 0.177
5.5 0.171
5.5 0.176
5.5 0.159
5.5 0.172
5.5 0.163
5.5 0.165
5.5 0.176
run;
proc glm data=SBY;
class TS;
model production= TS;
lsmeans TS/cl pdiff adj=tukey;
run;
```

This SAS code evaluates the difference in averages of specific methane yield at different total solids concentration.

```
data SMY;
input TS production;
datalines;
2.5 0.143
2.5 0.137
2.5 0.132
2.5 0.145
2.5 0.148
2.5 0.142
2.5 0.144
2.5 0.140
2.5 0.143
2.5 0.149
2.5 0.133
2.5 0.137
2.5 0.141
2.5 0.150
2.5 0.143

4 0.134
4 0.144
4 0.132
4 0.144
4 0.129
4 0.143
4 0.145
4 0.145
4 0.131
4 0.130
4 0.131
4 0.131
4 0.134
4 0.144
4 0.131

5.5 0.074
5.5 0.078
```

```
5.5 0.071
5.5 0.070
5.5 0.075
5.5 0.077
5.5 0.081
5.5 0.071
5.5 0.070
5.5 0.072
5.5 0.065
5.5 0.071
5.5 0.067
5.5 0.068
5.5 0.072
run;
proc glm data=SMY;
class TS;
model production= TS;
lsmeans TS/cl pdiff adj=tukey;
run;
```

APPENDIX C. R CODES FOR CHPATER 5

This R code simulates methane yield using parameter values at 35 ºC.

```
rm(list=ls())

require(deSolve)

experiment <-
data.frame(day=c(0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,
29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55),
production=c(0,102,189,266,341,415,494,583,672,774,862,945,1028,1120,1204,1284,1356,1427
,1499,1567,1629,1676,1724,1779,1828,1879,1929,1977,2023,2060,2097,2134,2171,2207,2242,2
282,2309,2335,2361,2384,2399,2411,2418,2424,2430,2436,2442,2448,2452,2456,2462,2468,24
75,2481,2487,2491))

source('C:/Users/lzhimin2010/Desktop/Model/Final final code and results/Methane_rv.r')
require(deSolve)# load the deSolve package into memory if it isn't loaded already
parms <-
list(a=0.146,b=0.104,c=5.559,d=28,e=604,f=0.043,g=0.111,h=247,i=181,j=0.018,k=0.167,l=56,
m=0.026,n=0.695,o=0.13,p=0.018)
time <- seq(0,55,by=1)
y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density

output <- ode(y=y.initial,time=time,func=methane.ode,parms=parms)
par(mfrow=c(2,1))

with(experiment,plot(production~day,xlim=c(0,55),ylim=c(0,5000),xlab="Time(days)",ylab="m
L CH4/g VSadded",font=1,font.lab=2))
lines(output[,1],output[,9])
legend("topleft",c("(a)"))
```

This R code simulates methane yield using parameter values at 50 ºC.

```
rm(list=ls())

require(deSolve)

experiment <-
data.frame(day=c(0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,
29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55),
production=c(0,102,189,266,341,415,494,583,672,774,862,945,1028,1120,1204,1284,1356,1427
,1499,1567,1629,1676,1724,1779,1828,1879,1929,1977,2023,2060,2097,2134,2171,2207,2242,2
282,2309,2335,2361,2384,2399,2411,2418,2424,2430,2436,2442,2448,2452,2456,2462,2468,24
75,2481,2487,2491))
```

```
source('C:/Users/lzhimin2010/Desktop/Model/Final final code and results/Methane_rv.r')

require(deSolve)# load the deSolve package into memory if it isn't loaded already
parms <-
list(a=0.209,b=0.149,c=15.649,d=78.823,e=604,f=0.043,g=0.253,h=1106.977,i=181,j=0.018,k=0
.470,l=250.975,m=0.026,n=1.956,o=0.432,p=0.018)
time <- seq(0,55,by=1)
y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density

output <- ode(y=y.initial,time=time,func=methane.ode,parms=parms)

with(experiment,plot(production~day,xlim=c(0,55),ylim=c(0,5000),xlab="Time(days)",ylab="m
L CH4/g VSadded",font=1,font.lab=2))
lines(output[,1],output[,9])
legend("topleft",c("(b)"))
```

This R code shows parameter estimation and optimization using least squares method and simulation of methane yield using optimized parameter.

```
rm(list=ls())

require(deSolve)

experiment <-
data.frame(day=c(0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,
29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55),
production=c(0,102,189,266,341,415,494,583,672,774,862,945,1028,1120,1204,1284,1356,1427
,1499,1567,1629,1676,1724,1779,1828,1879,1929,1977,2023,2060,2097,2134,2171,2207,2242,2
282,2309,2335,2361,2384,2399,2411,2418,2424,2430,2436,2442,2448,2452,2456,2462,2468,24
75,2481,2487,2491))

methane.ode <- function(t,y,parms){
#this function calculates and returns derivatives for methane generation in thermophilic batch
experiment
#y has eight components:
#'A'is concentration of cellulose
#'B'is concentration of sugar
#'C'is concentration of protein
#'D' is concentration of amino acids
#'E' is concentration of propionic acid
#'F' is concentration of acetic acid
#'G' is concentration of hydrogen
#'H' is concentration of methane
#params is a named list of parameters.  its contents are:
#a:hydrolysis constant for cellulose
#b:hydrolysis constant for protein
```

```
#c:maximum reaction velocity for fermentation reaction
#d:saturation concentration for fermentation reaction
#e:acetate inhibition constant for fermentation reaction
#f:biomass yield coefficient for fermentation reaction
#g:maximum reaction velocity for acetogenesis
#h:saturation concentration for acetogenesis
#i:acetate inhibition constant for acetogenesis
#j:biomass yield coefficient for acetogenesis
#k:maximum reaction velocity for aceticlastic methanogenesis
#l:saturation concentration for aceticlastic methanogenesis
#m:biomass yield coefficient for aceticlastic methanogenesis
#n:maximum reaction velocity for hydrogenotrophic methanogenesis
#o:saturation concentration for hydrogenotrophic methanogenesis
#p:biomass yield coefficient for hydrogenotrophic methanogenesis
A <- y["A"]
B <- y["B"]
C <- y["C"]
D <- y["D"]
E <- y["E"]
F <- y["F"]
G <- y["G"]
H <- y["H"]
a <- parms$a
b <- parms$b
c <- parms$c
d <- parms$d
e <- parms$e
f <- parms$f
g <- parms$g
h <- parms$h
i <- parms$i
j <- parms$j
k <- parms$k
l <- parms$l
m <- parms$m
n <- parms$n
o <- parms$o
p <- parms$p
dA.dt <- -a*A
dB.dt <- a*A-((c*B)/(d*(1+F/e)+B))*B
dC.dt <- -b*C
dD.dt <- b*C-((c*D)/(d*(1+F/e)+D))*D
dE.dt <- ((c*B)/(d*(1+F/e)+B))*B*f +((c*D)/(d*(1+F/e)+D))*D*f-((g*E)/((h+E)*(1+F/i)))*E
dF.dt <- ((c*B)/(d*(1+F/e)+B))*B*f+((c*D)/(d*(1+F/e)+D))*D*f+((g*E)/((h+E)*(1+F/i)))*E*j-
(k*(F/(l+F)))*F
```

```
dG.dt <- ((c*B)/(d*(1+F/e)+B))*B*f+((c*D)/(d*(1+F/e)+D))*D*f+((g*E)/((h+E)*(1+F/i)))*E*j-
(n*(G/(o+G)))*G
dH.dt <- (k*(F/(l+F)))*F*m+(n*(G/(o+G)))*G*p
return(list(c(dA.dt,dB.dt,dC.dt,dD.dt,dE.dt,dF.dt,dG.dt,dH.dt)))
}

ss.experiment <- function(th){

y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density
parms <-
list(a=th[1],b=th[2],c=th[3],d=th[4],e=th[5],f=th[6],g=th[7],h=th[8],i=th[9],j=th[10],k=th[11],l=t
h[12],m=th[13],n=th[14],o=th[15],p=th[16]) # named list of parameters

parRange <- data.frame(min=c(0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0))

times <- experiment$day # sequence of times for which we want a solution

# generate the numeric solution
output <- ode(y=y.initial,times=times,func=methane.ode,parms=parms)

ss <- sum((experiment$production - output[,9])^2)

return(ss)
}

methane.fit <-
optim(c(0.04,0.149,15.649,78.823,604,0.043,0.253,1106.977,181,0.018,0.470,250.975,0.026,1.9
56,0.432,0.018),ss.experiment)

y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density
parms <-
list(a=methane.fit$par[1],b=methane.fit$par[2],c=methane.fit$par[3],d=methane.fit$par[4],e=me
thane.fit$par[5],f=methane.fit$par[6],g=methane.fit$par[7],
h=methane.fit$par[8],i=methane.fit$par[9],j=methane.fit$par[10],k=methane.fit$par[11],l=metha
ne.fit$par[12],m=methane.fit$par[13],n=methane.fit$par[14],
o=methane.fit$par[15],p=methane.fit$par[16]) # named list of parameters
times <- experiment$day # sequence of times for which we want a solution
output <- ode(y=y.initial,times=times,func=methane.ode,parms=parms)

with(experiment,plot(production~day,xlim=c(0,55),ylim=c(0,4000),xlab="Time(days)",ylab="m
L CH4/g VSadded",font=1,font.lab=2))
lines(output[,1],output[,9])# generate the numeric solution
```

This R code shows parameter uncertainty estimation using bootstrap technique.

```
rm(list=ls())

require(deSolve)

experiment <-
data.frame(day=c(0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,
29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55),
production=c(0,102,189,266,341,415,494,583,672,774,862,945,1028,1120,1204,1284,1356,1427
,1499,1567,1629,1676,1724,1779,1828,1879,1929,1977,2023,2060,2097,2134,2171,2207,2242,2
282,2309,2335,2361,2384,2399,2411,2418,2424,2430,2436,2442,2448,2452,2456,2462,2468,24
75,2481,2487,2491))

methane.ode <- function(t,y,parms){
#this function calculates and returns derivatives for methane generation in thermophilic batch
experiment
#y has eight components:
#'A'is concentration of cellulose
#'B'is concentration of sugar
#'C'is concentration of protein
#'D' is concentration of amino acids
#'E' is concentration of propionic acid
#'F' is concentration of acetic acid
#'G' is concentration of hydrogen
#'H' is concentration of methane
#params is a named list of parameters.  its contents are:
#a:hydrolysis constant for cellulose
#b:hydrolysis constant for protein
#c:maximum reaction velocity for fermentation reaction
#d:saturation concentration for fermentation reaction
#e:acetate inhibition constant for fermentation reaction
#f:biomass yield coefficient for fermentation reaction
#g:maximum reaction velocity for acetogenesis
#h:saturation concentration for acetogenesis
#i:acetate inhibition constant for acetogenesis
#j:biomass yield coefficient for acetogenesis
#k:maximum reaction velocity for aceticlastic methanogenesis
#l:saturation concentration for aceticlastic methanogenesis
#m:biomass yield coefficient for aceticlastic methanogenesis
#n:maximum reaction velocity for hydrogenotrophic methanogenesis
#o:saturation concentration for hydrogenotrophic methanogenesis
#p:biomass yield coefficient for hydrogenotrophic methanogenesis
A <- y["A"]
B <- y["B"]
C <- y["C"]
D <- y["D"]
```

```
E <- y["E"]
F <- y["F"]
G <- y["G"]
H <- y["H"]
a <- parms$a
b <- parms$b
c <- parms$c
d <- parms$d
e <- parms$e
f <- parms$f
g <- parms$g
h <- parms$h
i <- parms$i
j <- parms$j
k <- parms$k
l <- parms$l
m <- parms$m
n <- parms$n
o <- parms$o
p <- parms$p
dA.dt <- -a*A
dB.dt <- a*A-((c*B)/(d*(1+F/e)+B))*B
dC.dt <- -b*C
dD.dt <- b*C-((c*D)/(d*(1+F/e)+D))*D
dE.dt <- ((c*B)/(d*(1+F/e)+B))*B*f +((c*D)/(d*(1+F/e)+D))*D*f-((g*E)/((h+E)*(1+F/i)))*E
dF.dt <- ((c*B)/(d*(1+F/e)+B))*B*f+((c*D)/(d*(1+F/e)+D))*D*f+((g*E)/((h+E)*(1+F/i)))*E*j-
(k*(F/(l+F)))*F
dG.dt <- ((c*B)/(d*(1+F/e)+B))*B*f+((c*D)/(d*(1+F/e)+D))*D*f+((g*E)/((h+E)*(1+F/i)))*E*j-
(n*(G/(o+G)))*G
dH.dt <- (k*(F/(l+F)))*F*m+(n*(G/(o+G)))*G*p
return(list(c(dA.dt,dB.dt,dC.dt,dD.dt,dE.dt,dF.dt,dG.dt,dH.dt)))
}

ss.experiment <- function(th){

y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density
parms <-
list(a=th[1],b=th[2],c=th[3],d=th[4],e=th[5],f=th[6],g=th[7],h=th[8],i=th[9],j=th[10],k=th[11],l=t
h[12],m=th[13],n=th[14],o=th[15],p=th[16]) # named list of parameters
parRange <- data.frame(min=c(0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0))

times <- experiment$day # sequence of times for which we want a solution

# generate the numeric solution
output <- ode(y=y.initial,times=times,func=methane.ode,parms=parms)
```

```
ss <- sum((experiment$production - output[,9])^2)

return(ss)
}

methane.fit <-
optim(c(0.04,0.149,15.649,78.823,604,0.043,0.253,1106.977,181,0.018,0.470,250.975,0.026,1.9
56,0.432,0.018),ss.experiment)

y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density
parms <-
list(a=methane.fit$par[1],b=methane.fit$par[2],c=methane.fit$par[3],d=methane.fit$par[4],e=me
thane.fit$par[5],f=methane.fit$par[6],g=methane.fit$par[7],
h=methane.fit$par[8],i=methane.fit$par[9],j=methane.fit$par[10],k=methane.fit$par[11],l=metha
ne.fit$par[12],m=methane.fit$par[13],n=methane.fit$par[14],
o=methane.fit$par[15],p=methane.fit$par[16]) # named list of parameters
times <- experiment$day # sequence of times for which we want a solution
output <- ode(y=y.initial,times=times,func=methane.ode,parms=parms)

with(experiment,plot(production~day,xlim=c(0,55),ylim=c(0,4000),xlab="Time(days)",ylab="m
L CH4/g VSadded",font=1,font.lab=2))
lines(output[,1],output[,9])# generate the numeric solution
methane.fitted <- output[,9]
methane.residual <- experiment$production - methane.fitted
plot(experiment$day,methane.residual,xlab="day",ylab="residuals")


# bootstrap time

do.bootstrap <- function(X.boot=1000){

# pre-allocate storage
a.boot <- b.boot <- c.boot <- d.boot <-e.boot <-f.boot <-g.boot <-h.boot <-i.boot <-j.boot <-
k.boot <-l.boot <-m.boot <-n.boot <-o.boot <-p.boot <-rep(NA,X.boot)

for(Y.boot in 1:X.boot){

set.seed(Y.boot)
boot.data <- data.frame(day = experiment$day,
production = methane.fitted + sample(methane.residual, replace=TRUE))

ss.boot <- function(th){

y.initial <- c(A=2030600,B=0,C=372000,D=0,E=0,F=0,G=0,H=0) # initial density
```

```
parms <-
list(a=th[1],b=th[2],c=th[3],d=th[4],e=th[5],f=th[6],g=th[7],h=th[8],i=th[9],j=th[10],k=th[11],l=t
h[12],m=th[13],n=th[14],o=th[15],p=th[16]) # named list of parameters
times <- boot.data$day # sequence of times for which we want a solution

# generate the numeric solution
output <- ode(y=y.initial,times=times,func=methane.ode,parms=parms)

ss <- sum((boot.data$production - output[,9])^2)

return(ss)
}

fit.boot <-
optim(c(0.050,0.051,15.66,78.73,604.1,0.047,0.281,1107,181,0.017,0.341,361.4,0.012,2.072,0.5
16,0.012),ss.boot)

a.boot[Y.boot] <- fit.boot$par[1]
b.boot[Y.boot] <- fit.boot$par[2]
c.boot[Y.boot] <- fit.boot$par[3]
d.boot[Y.boot] <- fit.boot$par[4]
e.boot[Y.boot] <- fit.boot$par[5]
f.boot[Y.boot] <- fit.boot$par[6]
g.boot[Y.boot] <- fit.boot$par[7]
h.boot[Y.boot] <- fit.boot$par[8]
i.boot[Y.boot] <- fit.boot$par[9]
j.boot[Y.boot] <- fit.boot$par[10]
k.boot[Y.boot] <- fit.boot$par[11]
l.boot[Y.boot] <- fit.boot$par[12]
m.boot[Y.boot] <- fit.boot$par[13]
n.boot[Y.boot] <- fit.boot$par[14]
o.boot[Y.boot] <- fit.boot$par[15]
p.boot[Y.boot] <- fit.boot$par[16]
if(Y.boot %% 50 == 0) writeLines(paste(Y.boot,"bootstrap replicates completed!"))
}
return(list(a=a.boot,b=b.boot,c=c.boot,d=d.boot,e=e.boot,f=f.boot,g=g.boot,h=h.boot,i=i.boot,j=j
.boot,k=k.boot,l=l.boot,m=m.boot,n=n.boot,o=o.boot,p=p.boot))
}
theta<-do.bootstrap(1000)
```

This R code shows the simulation of methane yield and acetic acid concentration by proposed model in comparison with experimental data from Cuetos et al. (2011).

```
rm(list=ls())

require(deSolve)
experiment <- data.frame(day=c(0,10,20,30,40,50,60),
production=c(0,50,150,300,350,450,450)
)
source('C:/Users/lzhimin2010/Desktop/Model/Final final code and results/Methane_rv.r')
require(deSolve)# load the deSolve package into memory if it isn't loaded already
parms <-
list(a=0.035,b=0.036,c=5.564,d=27.968,e=604.100,f=0.047,g=0.123,h=247.023,i=181,j=0.017,k
=0.121,l=80.640,m=0.012,n=0.736,o=0.155,p=0.012)
time <- seq(0,60,by=10)
y.initial <- c(A=356523,B=0,C=18064,D=0,E=0,F=0,G=0,H=0) # initial density

output <- ode(y=y.initial,time=time,func=methane.ode,parms=parms)

with(experiment,plot(production~day,xlim=c(0,60),ylim=c(0,500),xlab="Time
(days)",ylab="CH4 Production (mL/g VSadded)",font=1,font.lab=2))
lines(output[,1],output[,9])
legend("topleft",c("(a)"))

rm(list=ls())

require(deSolve)
experiment <- data.frame(day=c(2,4,8,10,15,23,30,43,44,45,53,56),
concentration=c(2000,1500,3000,1500,600,500,0,0,0,0,0,0)
)
source('C:/Users/lzhimin2010/Desktop/Model/Final final code and results/Methane_rv.r')
require(deSolve)# load the deSolve package into memory if it isn't loaded already
parms <-
list(a=0.035,b=0.036,c=5.564,d=27.968,e=604.100,f=0.047,g=0.123,h=247.023,i=181,j=0.017,k
=0.121,l=80.640,m=0.012,n=0.736,o=0.155,p=0.012)
time <- seq(0,60,by=10)
y.initial <- c(A=356523,B=0,C=18064,D=0,E=0,F=0,G=0,H=0) # initial density

output <- ode(y=y.initial,time=time,func=methane.ode,parms=parms)

with(experiment,plot(concentration~day,xlim=c(0,60),ylim=c(0,3000),xlab="Time
(days)",ylab="Acetic acid concentration (mg/L)",font=1,font.lab=2))
lines(output[,1],output[,7])
legend("topleft",c("(b)"))
```