USING ANAEROBIC CO-DIGESTION WITH ADDITION OF MUNICIPAL ORGANIC WASTES AND PRE-TREATMENT TO ENHANCE BIOGAS PRODUCTION FROM WASTEWATER TREATMENT PLANT SLUDGE

by

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Abstract

In this project, by adding selected co-substrates and by incorporating optimum pre-treatment strategies, four experimental phases were conducted to assess the enhancement of biogas production from anaerobic co-digestion using wastewater treatment plant sludge as the primary substrate.

In the first phase, the feasibility of using municipal organic wastes (synthetic kitchen waste (KW) and fat, oil and grease (FOG)) as co-substrates in anaerobic co-digestion was investigated. KW and FOG positively affected biogas production from anaerobic co-digestion, with ideal estimated substrate/inoculum (S/I) ratio ranges of 0.80-1.26 and 0.25-0.75, respectively. Combined linear and non-linear regression models were employed to represent the entire digestion process and demonstrated that FOG could be suggested as the preferred co-substrate.

The effects of ultrasonic and thermo-chemical pre-treatments on the biogas production of anaerobic co-digestion with KW or FOG were investigated in the second phase. Non-linear regressions fitted to the data indicated that thermo-chemical pre-treatment could increase methane production yields from both FOG and KW co-digestion. Thermo-chemical pre-treatments of pH=10, 55 ℃ provided the best conditions to increase methane production from FOG co-digestions.

In the third phase, using the results obtained previously, anaerobic co-digestions with FOG were tested in bench-scale semi-continuous flow digesters at Ravensview Water Pollution Control Plant, Kingston, ON. The effects of hydraulic retention time (HRT), organic loading rate (OLR) and digestion temperature (37 ℃ and 55 ℃) on biogas production were evaluated. The best biogas production rate of 17.4 ± 0.86 L/d and methane content 67.9±1.46% was obtained with thermophilic (55℃) co-digestion at HRT=24 days and OLR=2.43±0.15 g TVS/L·d.

In the fourth phase, with the suitable co-substrate, optimum pre-treatment method and operational parameters identified from the previous phases, anaerobic co-digestions with FOG were investigated in a two-stage thermophilic semi-continuous flow co-digestion system modified to incorporate thermo-chemical pre-treatment of pH=10 at 55℃. Overall, the modified two-stage co-digestion system yielded a 25.14±2.14 L/d (with 70.2±1.4% CH_4) biogas production, which was higher than that obtained in the two-stage system without pre-treatment.

The positive results could provide valuable information and original contribution to justify full-scale investigation in a continuing research program and to the field of research on anaerobic co-digestion of municipal organic wastes.

Co-Authorship

Chapter 2 through 7 of this thesis have been accepted or will be submitted for publication in peer-reviewed scientific journals. The presented work was carried out by Chenxi Li, with the assistance of the indicated co-authors who provided valuable comments, suggestions and revisions to the manuscripts.

CHAPTER 2

Using anaerobic co-digestion with addition of organic wastes to enhance biogas production from wastewater treatment plant sludge: A review

Chenxi Li, Pascale Champagne and Bruce C Anderson

Manuscript will be submitted to *Waste Management*, Elsevier.

CHAPTER 3

Enhancing Biogas Production from Anaerobic Co-digestion Using Pre-treatment Methods: A Review

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CHAPTER 4

Evaluating and modelling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions

Chenxi Li, Pascale Champagne and Bruce C Anderson

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CHAPTER 5

Effects of ultrasonic and thermo-chemical pre-treatment on methane production from fat, oil and grease (FOG) and synthetic kitchen waste (KW) in anaerobic co-digestion

Chenxi Li, Pascale Champagne and Bruce C Anderson

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CHAPTER 6

Biogas production performance of mesophilic and thermophilic anaerobic co-digestion with fat, oil, and grease (FOG) in semi-continuous flow digesters: Effects of temperature, hydraulic retention time and organic loading rate

Chenxi Li, Pascale Champagne and Bruce C Anderson

Manuscript will be submitted to *Environmental Technology*, Taylor & Francis.

CHAPTER 7

Enhanced biogas production from anaerobic co-digestion of municipal wastewater treatment sludge and fat, oil, and grease (FOG) by a modified two-stage thermophilic digester system with thermo-chemical pre-treatment

Chenxi Li, Pascale Champagne and Bruce C Anderson

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λ	Lag Phase Duration (h)
ADS	Anaerobic Digester Sludge
B	Cumulative methane yield (mL/gTVS)
B_0	Ultimate Methane Yield (mL/gTVS)
BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
Es	Specific Ultrasonic Supplied Energy (kJ/kgTS)
FOG	Fat, Oil and Grease
GC	Gas Chromatography
HPH	High Pressure Homogenizer
HRT	Hydraulic Retention Time (h)
k	First-order Biogas Production Rate Constant (h^{-1})
KW	Kitchen Waste
LCFA	Long Chain Fatty Acid
MSW	Municipal Solid Waste
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate (gTVS/L d)
P	Ultrasonic Power (kW)
PRS	Primary Sludge
R_m	Maximum Methane Production Rate
SCOD	Soluble Chemical Oxygen Demand

S/I Ratio	Substrate to Inoculum Ratio on TVS Basis
SPC	Soluble Protein Concentration
SRT	Substrate Retention Time
$t_{exponential}$	Exponential Phase (h)
TKN	Total Kjeldahl Nitrogen
t_{steady}	Steady State (h)
TS	Total Solid (g/L)
TVS	Total Volatile Solid (g/L)
VFA	Volatile Fatty Acid
WAS	Waste Activated Sludge
WWTP	Wastewater Treatment Plant

Chapter 1

Introduction

1.1 Background Overview

Producing biogas, especially with a focus on methane, from organic residues (e.g. primary sludge and waste activated sludge (WAS)) through anaerobic digestion processes has been widely and conventionally applied in wastewater treatment plants to support the on-site, co-generation of electrical power and heat (Zitomer et al, 2008). This technology can significantly stabilize the organic residues and particularly reduce the operating costs at wastewater treatment facilities, as the biogas has been reported as a renewable energy source which could recover 20-40% of the on-site energy requirement of the plant (Crawford and Sandino, 2010). However, the anaerobic digestion process has a number of limitations including slow reaction rates compared to aerobic processes, long retention times, sensitivity to waste loads and toxic materials, and complex operation. The methane content of the biogas may also vary and depend on the nature of the substrate and digestion process operation (Lin et al., 1997; Hwang et al., 1997; Navia et al., 2002).

To enhance biogas production and assist in wastewater treatment and municipal organic waste management, anaerobic digestion with the addition of co-substrates, i.e. co-digestion, has been recognized as an effective, low-cost and commercially flexible approach to reduce process limitations and improve biogas yields (Alatriste-Mondragón et al., 2006; Natural Resource Canada, 2002). A number of studies have shown that co-digestion with municipally available organic wastes including fats, oils and grease (FOG) and kitchen waste (KW) or food waste, which can be collected in close proximity to the treatment facility, could be employed as potential co-substrates. Cotrell (2008) reported a 50% increase in biogas production at a full-scale digester using FOG as a co-substrate. Kabouris et al. (2008) also investigated FOG as a co-substrate and achieved significantly higher biogas production. Carucci et al. (2005), Gunaseelan (2004), Gómez et al. (2006), Labatut et al. (2010), and Li et al. (2002) evaluated food wastes, which are also the main components of KW, and were successful in enhancing biogas yields.

Besides adding co-substrates, pre-treating substrates using various pre-treatment methods prior to anaerobic digestion has also been reported as a potential approach to improve biogas production efficiency (Dohányos et al., 2004). Among the different pre-treatment approaches available, thermo-chemical and ultrasonic pre-treatments have been reported as effective and economically flexible methods (Apul and Sanin, 2010; Kim et al., 2003; Pilli et al., 2011; Rafique et al., 2010; Valo et al., 2004). Kim et al. (2003) obtained a greater than 34% methane increase from WAS using thermo-chemical pre-treatment. Pilli et al. (2011) reviewed ultrasonic pre-treatment and concluded that this was effective for sludge, but that the efficiency varied with the sludge characteristics. Although ultrasonic and thermo-chemical pre-treatments have been recognized as efficient processes for biogas production enhancement, they have primarily been investigated in the anaerobic digestion of wastewater treatment plant sludges (e.g. WAS and primary sludge) and most studies have been focused on traditional digestion without co-substrate addition (Apul and Sanin, 2010; Dhar et al., 2012; Kim et al, 2003; Tanaka and Kamiyama, 2002; Valo et al., 2004; Vlyssides and Karlis, 2004). Although FOG, KW and other municipal organic wastes have been reported by a number of researchers as potential co-substrates in the anaerobic co-digestion of municipal wastewater treatment plant sludges, to our knowledge they have seldom been utilized and evaluated in co-digestion with ultrasonic or thermo-chemical pre-treatments.

To determine the suitability of a specific organic substrate for anaerobic digestion, the biochemical methane potential (BMP) test has been proven to be a relatively economical and reliable method for comparison of the extent and rates of waste conversion to biogas. A number of studies have used BMP tests to evaluate the improvement in ultimate biogas production through co-digestion and conventional linear regression models have been widely applied to indicate the first order production rate (Carucci et al., 2005; Hansen et al., 2004; Heo et al., 2004). However, the application of modified non-linear regression models has seldom been employed in combination with BMP tests to model the full digestion process (Li et al., 2011).

In addition, most studies that have examined FOG or KW co-digestion performance to date have focused on the use of BMP tests, and little research has been conducted using bench-scale or pilot-scale continuous flow digesters. In continuous-flow anaerobic digestion, many parameters including substrate characteristics, flow rate, organic loading rate (OLR), hydraulic retention time (HRT), process temperature (mesophilic 37 °C or thermophilic 55 °C), pH and organic constituents (e.g. organic acids, proteins and nutrients) can affect the digestion performance and biogas production (Gerardi, 2003; Fezzani and Cheikh, 2010; Gómez et al., 2006; Kabouris et al., 2009). Hence, in order to ensure successful and efficient biogas production from co-digestion in continuous flow digesters, more research is necessary to provide additional information on co-digestion processes and applications at the larger scales.

Within the limited continuous-flow FOG and KW co-digestion studies reported to date, most tests have concentrated on conventional one-stage digestion systems but not the more common two-stage digestion process, which has been reported and recommended as an advanced anaerobic digestion system configuration to enhance the substrate hydrolysis and biogas production (Demirer and Chen, 2005; Parawira et al., 2008). Furthermore, pre-treatment has seldom been applied in the two-stage anaerobic digestion system, particularly during co-digestion (Fezzani and Cheikh, 2010; Kabouris et al., 2008; Kabouris et al., 2009; Park et al., 2005). It can be postulated that anaerobic co-digestion in an advanced two-stage thermophilic semi-continuous digestion system combined with pre-treatment could be expected to yield a higher biogas production than conventional one-stage or two-stage digestion, but research to identify process efficiency and potential limitations on this process is still required and is the subject of this work.

The City of Kingston (Ontario, Canada), a typical medium-size Canadian municipality, recently upgraded one of its two wastewater treatment facilities (Ravensview Water Pollution Control Plant) to yield higher quantities of biogas to decrease on-site operational costs. The results from the research project described in this thesis should be considered as providing useful information and operational parameters to promote more successful and efficient anaerobic co-digestion implementation in Kingston’s and other Canadian municipalities’ wastewater treatment plants.

1.2 Research Objectives

The main objectives of this research were to:

1) Understand the fundamentals of anaerobic co-digestion processes, the wastes that could be utilized as potential substrates, and the general development of various pre-treatment methods that could enhance biogas production from anaerobic co-digestion.
2) Identify and test suitable co-substrates (e.g. FOG and KW) and substrate/inoculum mixing ratios for maximum biogas production through a series of BMP tests and non-linear regression models.
3) Investigate effective pre-treatment methods (e.g. thermo-chemical and ultrasonic) and optimum ranges of operational conditions for biogas production enhancement from the co-digestion using wastewater treatment plant sludges as primary substrates and selected co-substrates (e.g. FOG and KW) through a series of BMP tests and non-linear regression models.
4) Operate bench-scale semi-continuous flow digesters for co-digestion tests and explore the effects of operational parameters such as temperature (mesophilic 37 ℃ or thermophilic 55 ℃), HRT and OLR on digester effluent characteristics and biogas production enhancement.
5) Develop a novel bench-scale two-stage semi-continuous flow co-digestion system combined with thermo-chemical pre-treatment using suitable operational parameters selected from the previous work.

1.3 Thesis Organization

This thesis is composed of eight chapters. Chapter 1 is a general introduction that presents the background information, objectives and the organization of the thesis. In order to achieve the abovementioned objectives, which are complementary to each other, a series of integrated experiments were designed and conducted based on recent literature in the field (Chapter 2 and Chapter 3). The experimental results were analyzed, discussed and are presented in Chapters 4, 5, 6 and 7. The relationships of Chapters 2 to 7 are illustrated in Figure 1.1. The six separate manuscripts (Chapter 2 to Chapter 7) have been accepted or will be submitted for publication in peer-reviewed scientific journals. Chapter 8 concludes the entire thesis with recommendations for future work.

Chapter 2 presents a comprehensive review on fundamental knowledge regarding anaerobic co-digestion and its application. Available organic resources that have been or could be utilized as co-substrates to enhance biogas production from wastewater treatment plant sludge are reviewed and compared. Optimum operational parameters and research recommendations for co-digestion with the addition of potential co-substrates are discussed and summarized. Digesters and digestion strategies that could further improve anaerobic co-digestion performance are also mentioned.

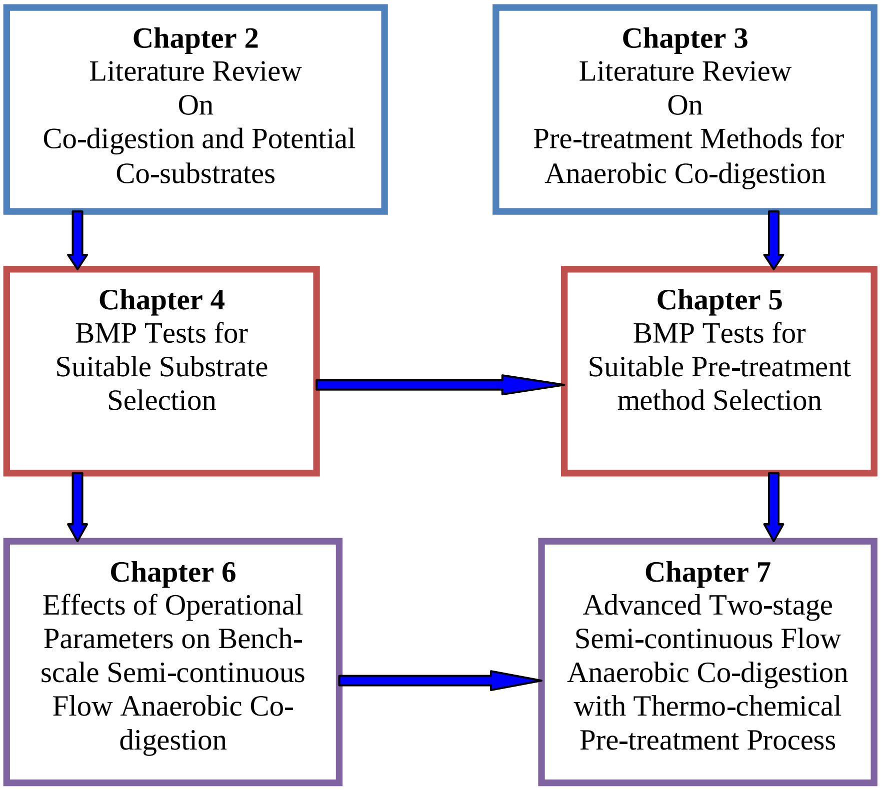

Figure 1.1 Relationships of the Chapters 2 to 7 and the project flowchart.

Chapter 3 is a literature review on various recent pre-treatment methods that have been applied for enhancing biogas production from anaerobic digestion. Mechanisms and fundamentals of mechanical, thermal, chemical, thermo-chemical, ultrasonic, ozonation, microwave and biological pre-treatments are introduced and compared. Furthermore, research on pre-treatment applications in anaerobic co-digestion for various organic wastes is also reviewed and discussed.

Chapter 4 is an original research paper aiming to identify and select suitable co-substrates through a series of BMP tests. Based on the information reviewed in Chapter 2, KW and FOG were tested as potential co-substrates. Biogas production potentials of KW and FOG were compared. Linear and non-linear regression models were developed to assist in the interpretation of the results. The suitable co-substrate and mixing ratios between substrate and inoculums selected from this work provided useful information for subsequent tests described in Chapters 5 and 6.

Chapter 5 presents an investigation on the optimum range of pre-treatment conditions for biogas production enhancement from co-digestion through a series of BMP tests. According to the various pre-treatment methods reviewed and compared in Chapter 3, the effects of thermo-chemical and ultrasonic pre-treatment methods were evaluated on co-digestions with suitable co-substrates selected from the tests described in Chapter 4. Biogas production enhancement and the influential variables were analyzed and discussed. Non-linear regression models were also developed to assist in the interpretation of the results.

Chapter 6 focuses on investigating and comparing biogas production from bench-scale semi-continuous flow co-digestions. The tests were originally conducted based on the information yielded by the research described in Chapters 4 and 5. The effects of operational parameters such as temperature (mesophilic 37 ℃ or thermophilic 55 ℃), HRT and OLR on biogas production were compared. Various physical and chemical effluent characteristics including pH, solid contents, organic constituent composition and biogas production were monitored. The investigations were aiming to derive operational conditions for subsequent two-stage bench-scale semi-continuous digestions and future large-scale applications.

Chapter 7 compares and characterizes the biogas and methane production from two-stage semi-continuous flow co-digestion systems with and without pre-treatment. The digestion system operational conditions were established based on the optimum operational parameters identified in the previous Chapters 4, 5 and 6.

Chapter 8 concludes the thesis with proposed recommendations for future work.

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Chapter 2

Using Anaerobic Co-digestion with Addition of Organic Wastes to Enhance Biogas Production from Wastewater Treatment Plant Sludge: A Review

Abstract: Biogas produced from the anaerobic digestion of sludge at wastewater treatment plants (WWTP) is categorized as renewable energy. A variety of materials such as municipal wastes, agricultural residues, and industrial effluents can be utilized as potential co-substrates in anaerobic co-digestion for enhancing methane production from wastewater treatment plant sludge. The application is also beneficial for waste treatment and environment protection. This review is intended to introduce and summarize fundamental information about potential organic wastes that can be applied in anaerobic co-digestion, particularly in the process with wastewater treatment plant sludge as primary substrate. The most current literature investigating eligible organic waste resources as co-substrates, analysis of approaches in biogas production from co-digestion, and strategies for the operation of anaerobic co-digesters are introduced and reviewed.

Keywords: Biogas; organic wastes; sludge; wastewater treatment plant; anaerobic co-digestion.

2.1 Introduction

Anaerobic digestion is a biological process that stabilizes organic biomass while producing a methane-rich biogas. It can reduce pathogens and odours and requires little land space for organic waste treatment (Salminen and Rintala, 2002a). Methane produced from anaerobic digestion processes is categorized as renewable energy. A number of waste feedstocks are considered eligible sources of biodegradable biomass, including agricultural manures, municipal and industrial sludges and biosolids, municipal solid waste, agricultural and forestry residues, and energy crops (Demirbaş, 2001). These natural organic materials are stored sources of chemical energy that is transformed from solar energy through photosynthesis. They can be biodegraded as substrates and co-substrates through anaerobic digestion processes, releasing methane as a renewable green energy (Demirbaş, 2001; Metcalf and Eddy, 2003).

Although the anaerobic digestion process has many advantages, several limitations exist, including: slow reaction rate, long retention times (20-30 days), sensitivity to waste loads and toxic materials, and its potentially complex operation (Lin et al., 1997; Hwang et al., 1997; Navia et al., 2002). Anaerobic digester gas production rates depend on a number of variables including volatile solids concentration, mixing performance, pH, temperature control, and other chemical and physical operational parameters. The energy content of the gas may also vary, depending on the nature of the sludge and performance of the digestion reactors.

Given these limitations, anaerobic co-digestion has been suggested as an effective method to enhance the digestion performance, biodegradation rate, as well as biogas production quantity and rate. For instance, Alatriste-Mondragón et al. (2006) conducted a survey of the literature on the application of anaerobic co-digestion for municipal, agricultural, and industrial organic wastes. Anaerobic co-digestion has been applied in the anaerobic digestion of sludges from wastewater treatment processes for methane production (Bolzonella et al., 2006; Davidsson et al., 2007; la Cour Jansen et al., 2004). However, it was noted that surprisingly few reports included full-scale applications of this concept. The successful implementation of this approach and the selection of suitable potential co-substrates for anaerobic co-digestion with wastewater treatment plant sludge as primary substrate in Canadian municipalities has been limited (De Baere, 2000; Mata-Alvarez et al., 2000; Natural Resources Canada, 2002).

To determine the susceptibility of an organic substrate for anaerobic degradation to methane and to compare methane yields during anaerobic decomposition processes, biochemical methane potential (BMP) tests, which are usually conducted in small-volume serum-bottles, are often undertaken prior to bench-scale or pilot-scale anaerobic digester tests. This method is the most rapid and economical way to select suitable single digestion and co-digestion substrates for subsequent larger scale studies and can be applied as a fundamental step in anaerobic digestion research. Cumulative biogas production, organic concentration changes, suitable pH levels, optimum operational temperatures, and inhibition or toxicity avoidance for different biomass substrates can be quickly and easily evaluated through the BMP tests. A number of studies have directly used or modified the serum-bottle method developed by Owen et al. (1979) and advanced specific BMP tests for application in their respective anaerobic digestion studies (Shelton and Tiedje, 1984; Jawed and Tare, 1999; Demirer et al., 2000; Gunaseelan, 2004; Hansen et al., 2004; Heo et al., 2004; Wilkie et al,, 2004). Bench-scale batch and continuous-flow reactor studies are typically conducted to supply fundamental information about wastes such as specific methane production capacity, co-digestion performance, effect of pre-treatment and temperature, simple kinetics, and model studies for future full-scale simulations (Alatriste-Mondragón et al., 2006). Two-phase continuous-flow anaerobic digesters have also been employed in energy recovery studies (Yu et al., 2002). Hence, engineered BMP tests and bench-scale reactor studies are beneficial approaches that provide valuable information for larger scale applications.

The objective of this review is to introduce fundamental knowledge regarding anaerobic co-digestion and its application. Available organic resources that have been or could be utilized as co-substrates to enhance biogas production from wastewater treatment plant sludge (e.g. primary sludge, activated sludge, anaerobic digester sludge) in wastewater treatment plant (WWTP) anaerobic digestion are reviewed and compared. Optimum operational parameters and research recommendations for co-digestion with the addition of potential co-substrates are discussed and summarized in detail. Digesters and digestion strategies that have been utilized by researchers and could further improve anaerobic co-digestion performance are also mentioned.

2.2 Methane Production and Anaerobic Co-digestion

The most widely used traditional fuel is fossil fuel, which is transformed in the earth from plant or animal remains, in a process that requires millions of years. Fossil fuels generally include coal, oil, natural gas, oil shales, and tar sands (U.S. Environmental Protection Agency, 2006). It is non-renewable resource and upon combustion produces large amounts of carbon dioxide, nitrogen oxides, sulfur dioxide, volatile organic compounds, radioactive materials and heavy metals that can pollute the environment.

In recent years, the acquisition of fossil fuels has become more costly for economic and political reasons. The current approach to overcome these issues is to continue developing renewable energy from natural resources, such as geothermal, hydro, solar, tidal power and biomass. It has been suggested that one of the natural, renewable carbon resources that is large enough to be used as a substitute for fossil fuel is biomass (Klass, 2004). Biomass is the term used to describe all biologically produced matter (Demirbaş, 2001). All non-fossil-based living or dead organisms and organic materials that have intrinsic chemical energy content are included. Solid, gaseous, or liquid fuels produced from biomass are biofuels. Biogas is one type of biofuel that can be produced by the biological breakdown of organic matter. One type of biogas is produced by anaerobic digestion or fermentation of biodegradable biomass such as wastewater treatment sludge, municipal waste, agricultural and forestry waste, human and animal wastes and energy crops (Demirbaş, 2001).

This type of biogas is composed primarily of methane and carbon dioxide, and is continuing to gain attention in Canada and throughout the world. For example, in Canada the predominant solid waste disposal practice tends to be landfilling because of the availability of land. However, insufficient pollution control for aging waste management systems and difficulties in securing new land for future landfilling of waste is attracting more public attention. Hence, consideration of energy recovery options including anaerobic digestion processes for these organic wastes are gaining in popularity.

Energy from wastes is suggested to be the most sensible way of dealing with this environmental concern (Natural Resources Canada, 2002). As an example, in the mid 1800s biomass (primarily woody biomass) supplied over 90% of U.S. energy and fuel needs (Klass, 2004), via a simple combustion process with a low power generation efficiency. After the 1970s and as a result of the global oil crisis, countries like the US, Japan and Germany focused their attention and investment on bioenergy and biofuel development, including processes for enhanced methane production.

The methane generated from these biological conversion processes can be combusted or oxidized to release energy. Methane produced from biomass can be regarded as a form of solar energy, since photosynthesis combines atmospheric carbon dioxide with water in the presence of sunlight to form the biomass. The process can be simply presented by Equation 2.1 (Schuck, 2006):

$$CO_2 + 2H_2O \rightarrow ([CH_2O] + H_2O) + O_2 \quad (2.1)$$

where $[CH_2O]$ represents the biomass (as carbohydrates) and oxygen is also produced as a side benefit of this reaction. The carbon dioxide bound in the biomass, which is released during the bioenergy recovery process, can be seen as the balance of that utilized in photosynthesis and can be available to produce new biomass (Peppley, 2006). Thus, methane as a biogas produced from biomass through anaerobic digestion processes can be considered as an economical and environmental friendly biofuel.

The anaerobic digestion process is an effective method for the treatment of solid organic wastes, which, in the absence of oxygen, converts organic materials into carbon dioxide and methane. Anaerobic digestion consists of a series of microbial conversions that can be simply considered to occur in two phases (Carucci et al., 2005; Geradi, 2003). In the first phase a group of microorganisms referred to as “acid formers” break down complex organic matter. This begins with the microbial hydrolysis of complex organic compounds such as protein, fat and cellulose in the absence of oxygen. The soluble products are biologically converted into short-chain volatile acids, alcohols, and ketones. In the second phase, another group of microorganisms referred to as “methane formers” break down the output from the first phase to form biogas. Anaerobic digestion is widely used to treat wastewater treatment plant sludge and municipal organic wastes. It has been reported that anaerobic digestion has many advantages, such as providing for volume and mass reduction of the input material. As part of an integrated waste management system, it can reduce pathogens and odors and requires little land space for treatment (Salminen and Rintala, 2002a). The nutrient-rich solids left after anaerobic digestion can be used as fertilizer. Kelleher (2007) pointed out that the market for the anaerobic digestion of municipal solid wastes in Europe had grown exponentially over the last decade. This might indicate that the treatment of solid organic wastes using anaerobic digestion will be the trend for future waste disposal and bio-energy production.

Theoretical values for maximum methane production in full degradation can be calculated from the Buswell formula, given as Equation 2.2 if the chemical composition of the substrate is known.

$$\begin{aligned} & C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \\ & \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \end{aligned} \qquad (2.2)$$

However, the chemical formula of solid organic wastes are not always known, since the wastes are composed of various components with different biodegradation pathways and rates.

Co-digestion is considered as an option for improving the yield of biogas from anaerobic digestion. In most co-digestion cases, the improvement of biogas yields has been seen to be due to positive synergisms established in the digestion medium, the supply of missing nutrients by the co-substrate, or the dilution of toxicants by the moisture available in the co-substrates (Mata-Alvarez et al., 2000; Salminen and Rintala, 2002). However, it is also noted that surprisingly few reports have focused on actual industrial applications of this concept, and the method has been less applied than expected in full-scale systems (Mata-Alvarez et al., 2000; De Baere, 2000). Hence, optimizing co-digestion operations and promoting its full-scale application should be the subject of future research, which can economically increase and enhance biogas production and its reliable use as a biofuel.

The co-digestion process can also be applied in domestic wastewater treatment plants for enhanced methane production from the sludge, to provide power generation and heating (la Cour Jansen et al., 2004; Bolzonella et al., 2006; Davidsson et al., 2007). Alatriste-Mondragón et al. (2006) conducted a survey of the current literature on the application of anaerobic co-digestion from municipal, farm, and industrial organic wastes. This survey indicated that municipal wastewater sludge, the organic fraction of municipal solid waste, and manures were the main wastes most often used in co-digestion processes. Agricultural wastes including manures were also mentioned as one of the main sources of co-substrates usually applied in co-digestion application. However, it was reported by Callaghan et al. (1999) that the high ammonia concentrations of chicken manure could contribute to inhibition of the digestion process and consequently lead to reductions in methane production. Hence, although co-digestion can provide missing nutrients, it is noted that the characteristics and components of the co-substrates must be carefully evaluated to ensure a viable process results.

2.3 Potential Co-substrates for Anaerobic Co-digestion

2.3.1 Organic Fraction of Municipal Solid Waste (OFMSW)

Municipal solid waste (MSW) is a heterogeneous material with varying composition. The composition usually depends on such factors as region, climate, extent of recycling, collection frequency, season, and cultural practices (Gunaseelan, 1997). The most appropriate MSW that has been applied as a potential co-substrate in anaerobic digestion is the easily biodegradable organic fraction of MSW (OFMSW) with a moisture content below 85-90% (Mata-Alvarez et al., 2000). The OFMSW has been categorized by Mata-Alvarez et al. (1990), Gunaseelan (1997) and Dereli et al. (2010), as mechanically-sorted OFMSW (MS-OFMSW), source-sorted OFMSW (SS-OFMSW) and hand-sorted OFMSW (HS-OFMSW). It has been generally recognized that MS-OFMSW usually contains more suspended, non-biodegradable solids including plastic, wood and paper than other kinds of OFMSW. Hence, the OFMSW reviewed here includes only SS-OFMSW and HS-OFMSW, which are all referred to as OFMSW in this review.

Anaerobic co-digestion of wastewater treatment plant sludge and OFMSW for enhancing methane production is not a new concept. Early references to this appeared in the late 1970s (Miller et al., 1978). Currently, co-digestion of wastewater treatment plant sludge with the addition of various OFMSW as co-substrates has been widely investigated and applied in bench-scale to full-scale applications for enhancement of biogas production, improvement in digester performance, and as a supply of on-site energy in WWTPs (Mata-Alvarez et al., 2011). According to the relevant literature published during the last decade, it is noted that most OFMSW tested in anaerobic co-digestion studies was sourced from mixed household waste (Angelidaki et al., 2006; Fernández et al., 2010; la Cour Jansen et al., 2004), kitchen waste separated from household waste (Wang et al., 2006), food waste from households or restaurants (Kim et al., 2003; Kim and Oh, 2011; Park et al., 2008), and fruit and vegetable waste from households or commercial markets (Gunaseelan, 2004). Hence, this section focuses on the literature involving co-digestion with these sources of OFMSW.

Bench-scale research on the anaerobic co-digestion of OFMSW and wastewater treatment plant sludge (65:35 mixture by volume of primary sludge and thickened WAS) was conducted by Hamzawi et al. (1998), where the pre-treatment of the substrate was also discussed. The results showed that the most anaerobically biodegradable components of the OFMSW tested in this work were paper and grass from household wastes, which increased the biodegradability of the waste mixtures. However, the authors did not present or discuss the specific cumulative methane production or the changes in biogas composition. Stroot et al. (2001) tested the feasibility of co-digestion of wastewater treatment plant sludge with the addition of simulated typical U.S. OFMSW using bench-scale semi-continuous flow digesters. The biogas (a combination of carbon dioxide and methane) production reached 0.56 L/g VS with a methane content of 59.6%. The optimum operation for this system were daily feeding in semi-continuous co-digestion under minimal mixing levels, with hydraulic retention time (HRT) = 20 days and organic loading rate (OLR) = 3.5 g VS/L ꞏd. This study confirmed that continuous mixing was not necessary for the semi-continuous digester and that higher loading rates could inhibit the digestion process.

Sosnowski et al. (2003) conducted an anaerobic co-digestion analysis for OFMSW from household waste (fruit and vegetables, bread, paper, rice and kitchen wastes) and wastewater treatment plant sludge in bench-scale upflow anaerobic sludge blanket (UASB) reactors. Methane yields from digestion with only sludge and from co-digestion with OFMSW addition were compared and discussed. The biogas volume produced from the mixture of sludge and OFMSW was twice that obtained from the sludge alone, which was due to higher loading of biodegradable organic matter in the substrate. It was also noted that, although the higher organic load did not result in a similar digestion inhibition as reported by Stroot et al. (2001), the increase in biogas production was achieved more slowly at higher rather than at lower organic loads. This study also recommended that a co-digestion comparison between thermophilic and mesophilic conditions should be further researched. Following on this, Li et al. (2002) conducted research on a high solids anaerobic co-digestion process for food waste composed of fruits, vegetables, meat, fish and staple food. This study did not digest the food waste with wastewater treatment plant sludge, but did indicate that a thermophilic condition (55 ℃) was more effective for reducing lipids and yielding more methane, and had a higher loading capacity than the mesophilic condition (35 ℃) for digestion of the food waste OFMSW.

Kim et al. (2003) also considered food waste (from a Korean dining hall) as a co-substrate in the anaerobic digestion of wastewater treatment plant sludge. It was observed that an adequate fraction of food waste could enhance methane production both under mesophilic and thermophilic conditions. Consistent with Li et al. (2002), thermophilic conditions (55 ℃) were shown to be better than mesophilic conditions (35 ℃) for methane yield in single-stage batch co-digestion. Kim et al. (2011) tested the co-digestion of wastewater treatment plant sludge and food waste using an anaerobic thermophilic sequencing batch reactor. The results demonstrated that the thermophilic sequencing digestion provided better performance for methane production and organic solids digestion than the conventional mesophilic process. However, it was noted by Nosrati et al. (2004) that although biogas production from food waste digestion under thermophilic conditions could be improved, inhibition resulting from the presence of long chain fatty acids could negatively affect the biogas production. Murto et al. (2004) conducted a study on anaerobic co-digestion using food wastes as the co-substrate for the digestion of sewage sludge and pig manure. When the starch-rich potato processing wastes were added into the sewage wastes, the digestion became sensitive and exhibited poor buffering capacity, and finally failed.

Hence, careful characterization and consideration of the substrate waste is essential for successful co-digestion. In order to discern the effects of the food waste fraction, organic loading and hydraulic retention time on the co-digestion of food waste and sewage sludge, Heo et al. (2004) conducted similar batch analysis to Kim et al. (2003) and further tested the co-digestion in semi-continuous digesters. The food wastes and sludge were mixed on a volatile solids (VS) basis. The ratios of food wastes to sludge were 10:90, 30:70, 50:50, 70:30, and 90:10. The biodegradability of the mixtures increased to as high as 82.6% when the food waste proportion of the mixture was 90% (VS). The best overall digestion performance (including buffer capacity, effluent volatile solids concentration, and methane production) was obtained in a mixture of 50:50 with a 13 day hydraulic retention time, and the results of this study indicated that various mixture ratios could affect the results significantly. Carucci et al. (2005) also used food industry wastes as the co-substrate for the anaerobic co-digestion of sludge from a wastewater treatment plant using bench-scale batch reactors, and concluded that the methane yield of the co-digestion was more efficient than that of digestion with a single substrate.

Gunaseelan (2004) tested the biochemical methane production potential of 54 fruit and vegetable waste samples and showed that the fruit and vegetable waste, which is also the main fraction of OFMSW, could be utilized as suitable substrates for biogas production enhancement. Fruit and vegetable waste from OFMSW was also tested as the co-substrate for wastewater treatment plant sludge digestion (Gómez et al., 2006). The results indicated that the co-digestion of mixtures of sludge and OFMSW achieved better biogas production than digestion of sludge alone. Similar to Stroot et al. (2001), the study discussed the effects of different mechanical mixing conditions on co-digestion performance. Low mixing conditions (80 rpm) were reported as more effective than high mixing speeds (200 rpm). From the results, it was shown that adjusting the mixing speed could significantly affect the digestion and biogas production rates. Rizk et al. (2007) tested anaerobic co-digestion of fruit and vegetable waste and wastewater treatment plant sludge in a 70 L reactor with no mixing system at room temperature (25±5℃）with a residence time of 105 days. This process produced the most methane and biogas during the first month of operation. According to the research conducted by Gómez et al., 2006, it was suggested that installation of a mixing system in the large (70 L) reactor was needed to improve the digestion performance. Park et al. (2012) co-digested fruit and vegetable waste from a supermarket with first-stage and second-stage anaerobic digester sludge from a WWTP two-stage digestion system. Co-digestion with fruit and vegetable waste addition significantly increased the methane production for both sludges. It was suggested that, compared to the co-digestion with second-stage anaerobic digester sludge, co-digestion with first-stage sludge achieved the best methane production capability of 0.514 L/g VS. This would suggest that the OFMSW could be added to the first stage of a multi-stage anaerobic digestion system to maximize methane generation and achieve ideal solid waste treatment.

The anaerobic co-digestion of wastewater treatment plant sludge and OFMSW has also been investigated in full-scale applications in WWTPs. In order to develop an integrated solution to upgrade the Florence WWTP to enhance WAS disposal and biogas production, Caffaz et al. (2008) tested the co-digestion of WAS and OFMSW in an experimental plant with 200 L working volume batch-fed CSTR digesters. The co-digestion with OFMSW and WAS yielded 10 times the biogas production with significant organic removal efficiencies compared to the single-digestion with WAS alone. Bolzonella et al. (2006) co-digested WAS with OFMSW at the Viareggio (Italy) WWTP and obtained a 50% increase in biogas production. Full-scale co-digestion of wastewater treatment plant sludge (a mixture of primary sludge and WAS) and OFMSW has also been investigated by Zupančič et al. (2008), at the Velenje municipal WWTP in Slovenia. The results indicated that biogas production could be increased by 80% and the excess biogas generated could increase electrical energy production by 130% and heat energy production by 55%.

Studies that have provided typical operational recommendations for co-digestion of wastewater treatment plant sludge and OFMSW are summarized in Table 2.1. It is noted that various OFMSW including household, kitchen, food and fruit and vegetable wastes have been all reported as ideal potential co-substrates to increase biogas production from WWTP sewage sludge. Several specific operational parameters including reactor configuration, digestion temperature, reaction time (e.g. HRT), system mixing regime, substrate fraction and loading are stated as having significant effects on the methane production performance. It can be concluded from the reviewed literature and the information summarized in Table 2.1 that, for co-digestion of wastewater treatment plant sludge and OFMSW, a thermophilic multi-stage digestion system with lower level mixing regime (i.e. 80 rpm or minimal mixing) and suitable substrate loading could effectively improve methane production and organic degradation efficiency. It is also important to mention that an HRT below 12 days could inhibit the co-digestion of wastewater treatment plant sludge and OFMSW in continuous flow digesters due to the overloading of organics (Bouallagui et al., 2003; Heo et al., 2004; Li et al., 2002).

Table 2.1 Typical research on anaerobic co-digestion of wastewater treatment sludge and OFMSW

Sludge	**OFMSW**	**Digester**	**Temp ℃**	**Biogas Production**	**Recommendations**	**Reference**
Primary sludge and thickened WAS	Simulated mixture of household wastes	Batch digester 800 mL	Mesophilic 37	Significant biogas production enhancement	OFMSW could be used as the potential co-substrate	Hamzawi et al. (1998)
Primary sludge and WAS	Simulated typical U.S. OFMSW	Semi-continuous digester 1 L	Mesophilic 37	Biogas 0.56 L/g VS with 59.6% methane	Minimal mixing better than continuous mixing for semi-continuous digester	Stroot et al. (2000)
Primary sludge and WAS	Mixture of household wastes	Semi-continuous UASB 9 L	Mesophilic 37	Biogas 0.58 L/g VSS with 60% methane	Higher organic loading can result in inhibition and low biogas production rate	Sosnowski et al. (2003)
WWTP sewage Sludge	Food waste	Batch digester 100 mL	Mesophilic 35 and Thermophilic 55	Methane 0.344 L/g VS	Thermophilic should be better condition in batch operation	Kim et al. (2003)
WAS	Food waste	Batch digester 500 mL and Semi-continuous digester 4 L	Mesophilic 35	Methane 0.321 L/g VS from semi-continuous digestion	Suitable OFMSW fraction could provide optimum digestion performance	Heo et al. (2004)

Table 2.1 Typical research on anaerobic co-digestion of wastewater treatment sludge and OFMSW (Continued)

Sludge	OFMSW	Digester	Temp ℃	Biogas Production	Recommendations	Reference
Sludge from WWTP thickener	Food waste	Two-stage sequencing batch reactor 4 L	Thermophilic 55	Methane 0.2 L/g VS	Thermophilic should be better condition in continuous flow operation	Kim et al. (2011)
Primary sludge	Fruit and vegetable waste	Semi-continuous digester 3 L	Mesophilic 35	Biogas 0.6 L/g VS Methane was not measured	Low level mixing (80 rpm) could obtain better digestion performance	Gómez et al. (2006)
First stage and second stage anaerobic digester sludge	Fruit and vegetable waste	Batch digester 150 mL	Mesophilic 37	Methane 0.514 L/g VS from first stage sludge co-digestion	OFMSW would be better added to the first stage of multi-stage anaerobic digestion operation	Park et al. (2012)

2.3.2 Fat, Oil and Grease (FOG)

FOG is a term commonly used to define the lipid-rich waste material from restaurants, cooking, and food processing. It can be categorized as waste cooking oil (yellow grease) and grease trap and interceptor wastes (brown grease), which is in turn comprised of yellow grease, food solids, and water (Fonda et al., 2004). The direct release of FOG into municipal wastewater collection systems is illegal in many North American municipalities, since FOG can accumulate or deposit on pipe walls through chemical or physical aggregation processes. These hardened deposits and accumulation can lead to sanitary sewer overflows and cost municipalities millions of dollars each year in cleaning, repairing and maintenance fees (Kabouris et al., 2008). Hence, in many North American and European municipalities, FOG and related wastes are usually collected and disposed of separately.

As most FOG streams are from restaurants, cooking and food processing, the primary constituents of FOG are biodegradable lipids, which exist as neutral fats (i.e. triglycerides) and saturated or unsaturated long chain fatty acids (LCFAs) (Masse et al., 2002; Cavaleiro et al., 2008). In the anaerobic digestion process, the lipids in FOG are first hydrolyzed to glycerol and LCFAs. LCFAs can be degraded anaerobically via the β-oxidation pathway to acetate, and glycerol can also be degraded to acetate. The acetates degraded from LCFAs and glycerols are subsequently anaerobically degraded to methane (Kabouris et al., 2008; Madigan et al., 2006; Wakelin and Forster, 1997). Hence, in theory anaerobic digestion of FOG could yield higher biogas production since the degradable fraction of the lipids is higher than that of the typical carbohydrates and proteins found in sewage sludge (Pavlostathis and Giraldo-Gomez, 1991). Although these components have led to FOG being recognized as an energy value-added material and reported as beneficial for incineration and biodiesel production processes, it has been frequently noted that anaerobic co-digestion of FOG should be a better disposal process since it requires minimal pre-treatment and produces enhanced amounts of biogas (Canakci and Van Gerpen, 2001; Jeganathan et al., 2006). In addition, other methods such as landfilling and incineration have been reported as not being acceptable by some European municipalities (Davidsson et al., 2007). Co-digestion of FOG with wastewater treatment plant sludge has therefore gained particular attention in the last 5 years and most peer-reviewed publications on this process appeared after 2006, when the FOG stream disposal was recognized as an important environmental issue in urban areas, it was recognized that the lipid-rich materials were poorly biodegradable if they were not mixed with other more degradable wastes, and it was observed that significant enhancement of biogas production could generally be achieved through co-digestion (Alatriste-Mondragón et al., 2006; Bond et al., 2012).

Davidsson et al. (2007) have stated that co-digestion of wastewater treatment plant sludge from WWTPs with grease trap sludge is a better way of making use of the methane potential in the grease trap sludge than is single-substrate digestion, and also a more efficient approach to simultaneously improve organic waste management. Following on this, Davidsson et al. (2008) conducted co-digestion tests using both batch and semi-continuous flow digesters. The addition of grease trap sludge to wastewater treatment plant sludge from a WWTP increased the methane yield by 9-27% without additional waste sludge production. However, the methane production capacity in the semi-continuous digesters was decreased compared to that yielded in batch tests, which suggested that the optimum HRT and OLR should be tested and adjusted. Kabouris et al. (2008) also noted that HRT is one of the factors influencing the continuous flow co-digestion performance.

Zhu et al. (2011) and Li et al. (2011) used batch digesters to evaluate ultimate methane production potential from FOG co-digestion. Specifically, Li et al. (2011) used the factor S/I ratio (substrate to inoculum ratio, on a VS basis) to configure the co-substrate addition. They also utilized non-linear regression analysis to estimate the biogas production performance, which provided a mathematical evaluation approach for this co-digestion process. Although the substrates used in these two studies were from different sources, it was observed in both studies that biogas production increased with FOG addition, but process inhibition and reduction in biogas production occurred at higher FOG loadings. The results suggested the importance of optimized FOG loading rates for the implementation of anaerobic co-digestion. Luostarinen et al. (2009) reported ideal methane production enhancement from the co-digestion of grease trap sludge and also pointed out that biogas production would decrease at an OLR higher than the optimum range in semi-continuous digesters. Similar observations were provided by Liu and Buchanan (2011), Silverstre et al. (2011), and Wan et al. (2011). In addition to the optimum OLR, all 3 studies also suggested HRT<10 day may inhibit the co-digestion process and HRT>20 day could ensure sufficient co-digestion and steady biogas production. Hence, it can be noted (based on this literature) that HRT and FOG loadings are two of the most important parameters affecting co-digestion performance.

The co-digestion of FOG can also be affected by operating temperature. Kabouris et al. (2009) conducted thermophilic semi-continuous flow co-digestion of FOG and wastewater treatment plant sludge based on the fundamental information obtained by Kabouris et al. (2008) which indicated that the co-digestion with FOG under ideal organic loading could be successfully operated in semi-continuous digesters. The optimum methane production from the thermophilic semi-continuous condition in their study was 0.512 L/g VS, which was significantly higher than the 0.45 L/g VS obtained under mesophilic condition. Martín-González et al. (2010) successfully investigated FOG co-digestion in mesophilic semi-continuous digestion with a FOG feeding fraction of 15% (VS basis) and improved methane production compared to digestion with OFMSW alone. Based on the mesophilic results, thermophilic semi-continuous co-digestion experiments were subsequently conducted by Martín-González et al. (2011) who reported that the thermophilic condition improved anaerobic digestion and methane production conditions. According to the reviewed literature, although thermophilic conditions (~55 ℃) have been reported as more effective for biogas production, it is noted that relatively few studies have investigated co-digestion of FOG under thermophilic conditions or compared this to mesophilic conditions (35-37 ℃). This may be due to the energy consumption for heating and cost minimization considerations.

The reviewed literature on FOG co-digestion is summarized in Table 2.2. Despite the benefits of effective FOG co-digestion with wastewater treatment plant sludge, it can be observed that research on FOG co-digestion is not as widespread as those on OFMSW co-digestion, and more research interests has appeared in the past 5 years. This could be due to a wide assortment of process operational challenges including sludge flotation, digester foaming and blockages of pipes and pumps which have been reported by Jeganathan et al. (2006) and reviewed by Long et al. (2012). Hence, as an economically and environmentally sustainable method of FOG disposal and as an approach for improving biogas production, a better understanding of FOG and wastewater treatment plant sludge co-digestion is still needed, as is a larger pool of laboratory, pilot or full-scale experimental data to establish accurate models for industrial implementation, which would provide an alternative approach to effectively treat FOG wastes and enhance energy production at WWPTs.

Table 2.2 Reviewed research on anaerobic co-digestion of wastewater treatment sludge and FOG

Sludge	FOG	Digester	Temp ℃	Biogas Production	Recommendations	Reference
Primary sludge and WAS	Grease trap sludge	Batch digester 2 L and semi-continuous digester 35 L	Mesophilic 35	Batch methane 0.68 L/g VS and semi-continuous methane 0.34 L/g VS	Semi-continuous operation needs optimum HRT and OLR adjustment to achieve maximum methane production capability	Davidsson et al. (2008)
Primary and secondary sludge	FOG	Batch digester 2 L and semi-continuous digester 2 L	Mesophilic 35	Batch methane 1.01 L/g VS	HRT should be longer than 13 days for semi-continuous operation	Kabouris et al. (2008)
Primary sludge and WAS	FOG	Semi-continuous digester 30 L	Mesophilic 35 and thermophilic 55	Biogas production rate 1.8 L/h with 106% increase at 55 ℃	Thermophilic condition should be better than mesophilic condition for co-digestion operation	Suto et al. (2006)
Primary and secondary sludge	FOG	Semi-continuous digester 4 L	Mesophilic 35 and thermophilic 52	Methane 0.51 L/g VS at 52 ℃	Thermophilic condition should have better operation but the energy consumption should be considered	Kabouris et al. (2009)
Sewage sludge	FOG	Semi-continuous digester 5 L	Thermophilic55	Methane 0.49 L/g VS	Thermophilic condition should have better operation than mesophilic	Martín-González et al. (2011)
Sewage sludge from WWTP	Grease trap sludge	Semi-continuous digester 5 L	Mesophilic 35	Methane 0.463 L/g VS	Too high FOG fraction (71%, VS basis) feeding could result in biogas production decrease	Luostarinen et al. (2009)

Table 2.2 Reviewed research on anaerobic co-digestion of wastewater treatment sludge and FOG (Continued)

Sludge	FOG	Digester	Temp ℃	Biogas Production	Recommendations	Reference
Primary and secondary sludge	Grease trap sludge	Batch digester 100 mL	Mesophilic 35	Methane production increased by 65% with FOG addition	Proper FOG loading rate is important for the implementation of co-digestion	Zhu et al. (2011)
WAS	FOG	Batch digester 250 mL	Mesophilic 37	Methane 0.42 L/g TVS	Ideal FOG fraction could maximize the biogas production	Li et al. (2011)
Primary sludge and WAS	Grease	Semi-continuous digester 20 L	Mesophilic 37	Methane production increased by 25% with FOG addition	HRT around 20 days could ensure successful digestion	Liu and Buchanan (2011)
Primary sludge and WAS	Grease trap sludge	Semi-continuous digester 7 L	Mesophilic 35	Methane production increased by 138% with FOG addition	HRT around 20 days could ensure successful digestion	Silvestre et al. (2011)
WAS	FOG	Semi-continuous digester 4 L	Mesophilic 37	Methane production increased 137% with FOG addition	HRT<10 days could inhibit the digestion and decrease biogas production	Wan et al.(2011)

2.3.3 Lipid-rich Industrial Effluent

Slaughterhouse and olive mill wastes are the two main kinds of industrial effluents that have been tested and reported as potential co-substrates for biogas production. They are also lipid and protein-rich organic wastes that can be degraded to methane through the anaerobic digestion process.

Slaughterhouse waste usually contains various process by-products including blood, offal, bone, meat and other wastes from animals, which may depend on the process conditions and operations. This waste contains high amounts of different proteins and lipids, with degradation pathways that have been reviewed by Salminen and Rintala (2002a). The proteins can be hydrolysed to polypeptides and amino acids. Similar to FOG lipid hydrolysis, lipids in slaughterhouse waste can be hydrolysed to LCFA and glycerol. After hydrolysis, these polypeptides, amino acids, LCFA and glycerol are biologically converted to intermediate volatile fatty acids (VFAs). Consequently, the VFAs can eventually be degraded to acetic acid and then methane. As anaerobic digestion has been recognized as a process that competes well with other treatment processes for slaughterhouse waste, over the past two decades a large number of studies have shown that slaughterhouse waste can effectively increase biogas production (Salminen and Rintala, 2002a; Tritt and Schuchardt, 1992).

The digestion of slaughterhouse waste for biogas production has been widely tested in bench and pilot-scale continuous flow digesters, and it has even been applied in full-scale applications. Salminen and Rintala (2002b) operated 2 L semi-continuous digesters to test the effects of HRT and OLR on anaerobic digestion with solid poultry slaughterhouse waste. The digesters were operated at 31 °C with HRT ranging from 13-100 days and OLR ranging from 0.5-2.1 kg VS/m^3 d. The results indicated that higher OLR (1.0-2.1 kg VS/m^3 d) and shorter HRT (13-25 days) inhibited and overloaded the digestion process through the accumulation of VFAs and LCFAs, subsequently decreasing the methane yield. To examine the biogas improvement through co-digestion, Alvarez and Lidén (2008) compared the biogas production from digestions with slaughterhouse waste and co-digestions with slaughterhouse waste, manure, and fruit and vegetable wastes. The results indicated that the co-digestion exhibited higher capacity for improving biogas production. Furthermore, with co-digestion the OLR that inhibited the single-digestion could be avoided. Cuetos et al. (2008) investigated the effects of HRT and OLR on co-digestion of slaughterhouse waste and OFMSW and compared the results with single-digestion using only slaughterhouse waste. Consistent with the conclusions of Alvarez and Lidén (2008), compared to the single-digestion process, co-digestion could sustain higher OLR with effective biogas production enhancement. In addition, the process HRT could also be decreased from 50 days to 25 days.

The digestion temperature (mesophilic ~35 °C and thermophilic ~55 °C) has been also reported as one operational parameter affecting the digestion performance. However, contrary to the co-digestion work with OFMSW or FOG, co-digestion with slaughterhouse waste has been negatively influenced by thermophilic conditions (Battimelli et al., 2009; Bayr et al., 2012; Hejnfelt et al., 2009). It has been widely recognized that slaughterhouse waste contains various nitrogen-rich compounds and can release relatively high ammonia concentrations during the anaerobic digestion process, particularly under thermophilic operation (Salminen and Rintala, 2008a; Hejnfelt et al., 2009; Hensen et al.,1998). As such, it was noted that mesophilic conditions were a better choice for anaerobic digestion of nitrogen-rich substrates.

In order to enhance biogas production from the co-digestion of slaughterhouse waste, pre-treatment methods have been investigated in bench-scale experiments in some studies (Battimelli et al., 2010; Cuetos et al., 2010). It has been suggested that thermo-chemical pre-treatment with the addition of sodium hydroxide at 120 ℃ was a better pre-treatment to improve for biogas production (Battimelli et al., 2009; Battimelli et al., 2010). Anaerobic co-digestion of slaughterhouse waste has been also tested and applied in full-scale studies (Miranda et al., 2005; Nery et al., 2008). However, process limitations including slow lipid degradation and inhibition through high ammonia release have been frequently reported (Miranda et al., 2005; Palatsi et al., 2011). In addition, it is noted from the reviewed literature and from Table 2.3 that slaughterhouse waste has seldom been co-digested with wastewater treatment plant sludge from WWTPs, which has been attributed to the transportation costs or various policies for waste disposal, as well as the limited research on this concept.

Table 2.3 Typical research on anaerobic co-digestion of slaughterhouse waste

Sludge	Co-substrates	Digester	Temp °C	Biogas Production	Recommendations	Reference
NO	solid poultry slaughterhouse waste	Semi-continuous digester 2 L	31	Methane 0.55 L/g VS	Shorter HRT and higher OLR could inhibit the biogas production	Salminen and Rintala (2002b)
NO	Solid slaughterhouse waste, manure, fruit and vegetable wastes	Semi-continuous digester 2 L	35	Methane 0.35 L/g VS	Co-digestion could receive higher OLR and have better biogas production than single-digestion	Alvarez and Lidén (2008)
NO	Solid slaughterhouse waste and OFMSW	Semi-continuous digester 3 L	34	Biogas 8.6 L/day	Co-digestion could not receive higher OLR but can decrease HRT	Cuetos et al. (2008)
NO	Mixture of slaughterhouse by-products	Semi-continuous digester 5 L	37 and 55	Methane 0.357 L/g VS at 37 °C	Mesophilic should be better than thermophilc condition for anaerobic co-digestion of slaughterhouse waste	Hejnfelt and Angelidaki (2009)
NO	Rendering plant and slaughterhouse wastes	Semi-continuous digester 13 L	35 and 55	Methane 0.527 L/g VS at 35 °C	Mesophilic should be better than thermophilc condition for anaerobic co-digestion of slaughterhouse waste	Bayr et al. (2012)
NO	Slaughterhouse waste	Batch digester 5 L	35	Methane 0.617 L/g VS	Thermo-chemical pre-treatment should be better pre-treatment method to enhance biogas production from slaughterhouse waste	Battimelli et al. (2010)

Olive mill waste digestions have been widely reported by researchers from Mediterranean countries including Spain, Greece and Tunisia. This is due to the importance of olive tree cultivation and the olive oil industry in terms of wealth and tradition in these countries, with the production of olive mill waste being a consequence of this industry (Roig et al., 2006). Olive mill waste including solid waste and wastewaters usually contains high amounts of phenolic compounds, suspended solids and organic compounds, and low amounts of nitrogen and alkalinity (Fezzani and Cheikh, 2007a; Gannoun et al., 2007). Co-digestion of olive mill waste with other substrates has been reported as an effective way to dilute the higher phenolic concentration and to provide sufficient ammonia for the enhancement of biogas production from olive mill waste (Fezzani and Cheikh, 2007b; Zarkadas and Pilidis, 2011).

Similar to other co-digestion scenarios, HRT and OLR can affect the biogas production in continuous-flow digesters (Gelegenis et al., 2007; Fezzani and Cheikh, 2010; Rincón et al., 2010). It is noted from Fezzani and Cheikh (2010) that HRTs shorter than 20 days can result in inhibition, and a HRT around 24 days represents a more effective operational condition for co-digestion of olive wastes in semi-continuous digesters at mesophilic temperature conditions. Contrary to co-digestion with slaughterhouse waste, but similar to co-digestions with OFMSW or FOG, it has also been observed that thermophilic conditions can enhance biogas production from olive mill wastes (Gannoun et al., 2007; Goberna et al., 2010). According to recent peer-reviewed publications regarding the co-digestion of olive mill waste, it could be seen that this waste stream has seldom been co-digested with wastewater treatment plant sludge, but it has been co-digested with animal manure. In addition, as olive mill wastes are mainly produced in the Mediterranean countries, the process has only been researched in that area (i.e. not in North America). For this reason, co-digestion of olive mill waste is not reviewed in more detail here.

2.3.4 Agricultural Wastes

Agricultural wastes, also referred to as farm wastes, consist of agricultural plant residues and animal manures. The most common agricultural plant residues include rice husks, sugar cane fiber (bagasse), coconut husks and shells, groundnut shells, wheat straws and other crop residues, which depend on agricultural regions and harvesting seasons. Animal manures usually include cattle, chicken and pig manures (Demirbaş, 2001). In some developing countries, agricultural wastes are usually utilized as combustible fuels for cooking and heating. However, since agricultural wastes are mostly organic in composition, many researchers have reported that the anaerobic digestion or co-digestion of these wastes for methane production can be a more efficient way to provide renewable energy and to reduce greenhouse gas emissions. Various studies that have focused on anaerobic co-digestion of wastes including farm wastes have been reviewed by Alatriste-Mondragón et al. (2006).

According to the existing literature, animal wastes and manures have been widely tested. Mladenovska et al. (2003) co-digested cattle manure with a lipid co-substrate addition (glycerol trioleate). The results of the co-digestion were compared with the single-substrate (cattle manure) digestion, and it was noted that the co-digestion of the manure and lipid mixture exhibited a significantly higher methane yield. Gelegenis et al. (2007) co-digested olive-oil mill wastewater using diluted poultry manure as the co-substrate under mesophilic conditions in continuous-flow reactors, and concluded that adding diluted poultry manure could slightly increase biogas production with no negative effects on the pilot-plant digestion. Comino et al. (2009) developed an *in situ* pilot-scale anaerobic co-digestion process with cow manure and whey mix and found that the co-digestion yielded the best biogas production.

Similar to the co-digestions with other substrates, co-digestion with animal manure can be influenced by HRT, OLR and operational temperature (Callaghan et al., 1999; Demirer and Chen, 2005; Kaparaju et al., 2008; Wen et al., 2007). From these studies, it can be summarized that thermophilic (55 ℃) digestion with suitable HRT (~20 days) and ideal OLR ranges (depending on the characteristics of the substrates) are the optimum operational conditions. However, the main concern with the digestion of manure is the high ammonia concentrations. Many researchers have reported that high ammonia concentrations released from manures can contribute to inhibition of the digestion process and a reduction of methane production (Callaghan et al., 1999; Garcia and Angenent, 2009; Hansen et al., 1998; Nakakubo et al,, 2008; Procházka et al., 2012). Hence, manures need to be co-digested with the addition of substrates with lower alkalinity and higher C/N ratios (Mata-Alvarez et al., 2011). This would be the primary reason that manures have been widely reported as being co-digested with slaughterhouse wastes and agricultural residues.

Although anaerobic digestion or co-digestion of agricultural wastes has been studied and reviewed by a number of researchers, large quantities of agricultural wastes incur high transportation costs from farms to the WWPT, and as a result there have been limited studies and applications of the co-digestion of agricultural waste and sewage sludge in WWPTs (Alatriste-Mondragón et al., 2006; Nakakubo et al., 2008; Parawira et al., 2008; Ward et al., 2008).

2.3.5 Wood Wastes

Wood wastes are typically generated from wood processing industries. The advantage of the utilization of wood processing wastes is the avoidance of high transportation costs if the energy conversion can be operated at or near the industrial site. Generally, wood wastes are composed of tightly bound fibers, having a hard external surface. They are polysaccharides and long-chain natural polymers. It is noted that cellulose, hemicellulose, and lignin are contained in the woody plants and wastes, and these materials can determine the utilization efficiency (McKendry, 2002). It is also mentioned that with a low moisture content, wood wastes are usually more economically suited to gasification, pyrolysis or combustion. These thermal conversion processes are more efficient for wood wastes in the production of liquid fuels, such as methanol. The anaerobic digestion of wood waste has seldom been reported due to the low moisture and high lignin contents, as conventional anaerobic digestion processes are unable to degrade lignin effectively.

2.4 Experimental Procedures in Anaerobic Co-digestion Research

2.4.1 Biochemical Methane Potential (BMP) Tests

To determine and compare the ultimate methane production from various organic substrates during the anaerobic decomposition processes, laboratory-scale biochemical methane potential (BMP) tests are often conducted prior to bench or pilot-scale testing. Through a comparison of ultimate methane yields, suitable substrates or organic wastes can be selected for further examination. Commonly, methods based on and modified from the experiment conducted and replicated by Owen et al. (1979), Shelton and Tiedje (1984), and Chynoweth et al. (1993) continue to be widely utilized for laboratory-scale BMP tests. Over the last decade, with the development of better analytical techniques, many researchers have modified the original method and made the BMP tests more accurate through the use of advanced or economical analytical approaches (Demirer et al., 2000; Hansen et al., 2004; Heo et al., 2004; Jawed and Tare, 1999; Wilkie et al., 2004). From this work, the BMP testing procedure developed by Hansen et al. (2004) has been highly recommended by Davidsson et al. (2007) and Li et al. (2011) as a more reliable and accurate approach, with its utilization of pressure-lock syringes and a gas chromatograph (GC) for the analysis of gas composition. The BMP tests have thus been generally shown to be a relatively simple and reliable method for comparison of the extent and rates of waste conversion to methane.

Generally, in order to obtain ideal and comparable results, it is important to ensure that the biodegradation in the BMP test is not limited by nutrients, inoculum, substrate toxicity, pH, oxygen toxicity or substrate overload. Glass bottles with rubber septums are always used as the reactors, but the volumes of the bottles are determined by the volume of the substrates added and the biogas production expected. Normally, the bottles range from 125 mL to 2 L in volume. Gunaseelan (2004) used 135 mL serum bottles with 75 mL biomass addition. 250 mL serum bottles capped with natural rubber sleeve stoppers, with a total volume of 100 mL inoculum and organic wastes, were utilized by Isci and Demirer (2007) to examine the biogas production potential of cotton waste. 500 mL Erlenmeyer flasks have also been reported as reactors for BMP testing, with 250 mL inoculum and waste addition (Heo et al., 2004). 2 L glass bottles with a thick rubber septum were used as the reactors with 100 mL diluted (10%) organic waste and 400 mL inoculum in the work by Hansen et al. (2004).

The bioreactor set-up from the study by Hansen et al. (2004) is shown in Figure 2.1, as an example of a commonly used experimental set-up. The biogas samples are periodically taken from the headspace of the reactors using a pressure-lock syringe to test the biogas composition using GC. In order to ensure an anaerobic atmosphere, Owen et al. (1979) first mentioned using a 30% CO_2 and 70% N_2 gas mixture to flush the BMP serum bottles. According to other approaches reported in the reviewed literature, CO_2 and N_2 gas mixtures or pure N_2 gases are all considered suitable as flushing gases.

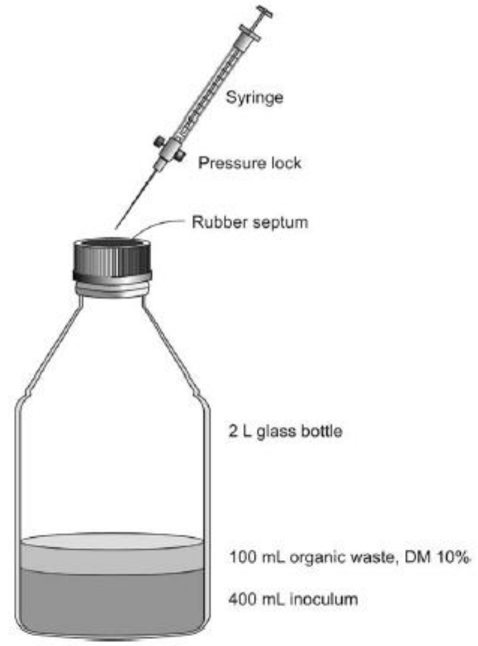

Figure 2.1 BMP reactor set-up with advanced method of sample extraction and analysis by Hansen et al. (2004). DM 10% = 10% dry matter

The mixing ratio between substrate and inoculum can affect the digestion reaction in the BMP test. Active inocula from industrial plants or from synthetic microbiological solutions are necessary to provide sufficient active bacteria to support anaerobic conversion of the substrates and biogas production. Hence, an important parameter is the S/I ratio, which is defined as substrate to inoculum ratio usually based on volatile solid contents, which has been used to evaluate the ideal organic loading (Heo et al., 2004; Li et al., 2011).

The incubation period for the BMP test is typically 30 days, which ensures virtually complete decomposition of biodegradable organics in most cases. Some organics may require a longer period for acclimation. As such, it is necessary to select the period depending on the specific waste and operational conditions. It is important to mention that many researchers have reported an ultimate methane production peak before or around the 10th day (Owen et al., 1979; Jawed and Tare, 1999).

The cumulative methane production in BMP tests is described using units referring to sample volume (m^3 CH_4/m^3 sample), sample mass (m^3 CH_4/kg sample), or sample organic content (m^3 CH_4/kg COD), depending on which unit is convenient in the specific test. The degradation of substrates and biogas production performance have been assumed to follow a first order rate of decay and is generally described using Equation 2.3 (Gunaseelan, 2004; Lo et al., 2010):

$$B = B_0(1 - e^{-kt}) \quad (2.3)$$

where B_0 is the ultimate methane yield, B is the cumulative methane yield at time t, and k is the methane production rate constant.

However, linear regression models cannot accurately describe and predict cumulative methane production through the entire process, particularly the lag and exponential phases. Hence, in order to assess and compare the methane production from different substrates through the digestion process, non-linear regressions were utilized to achieve representative simulations and predictions. The modified Gompertz equation as shown in Equation 2.4 has been applied and reported in BMP tests (Donoso-Bravo et al., 2010; Li et al., 2011; Lo et al., 2010):

$$B = B_0 \exp\left\{-\exp\left[\frac{R_m e}{B_0}(\lambda - t) + 1\right]\right\} \quad (2.4)$$

where R_m is the maximum methane production rate, and λ is the lag phase duration time.

2.4.2 Batch and Continuous Flow Digester Tests

Similar to other biochemical processes, batch- and continuous-flow reactors are typically utilized in anaerobic digestion research. Bench-scale batch digester testing including BMP tests are usually applied in laboratory studies, from which fundamental information regarding the wastes such as specific methane production capacity, co-digestion performance, effects of pre-treatment and temperature, and simple kinetic studies can be obtained (Alatriste-Mondragón et al., 2006). Batch reactors have been used by many researchers to study the basic characteristics of the anaerobic digestion of various wastes. Gavala et al. (1996) used 10 L digesters for the co-digestion of agricultural wastes to compare the digestion rates and to derive reaction constants. Ergüder et al. (2001) digested cheese whey using upflow anaerobic sludge blanket (UASB) reactors after the batch experiments provided fundamental information on substrate characteristics and loading rate ranges. Other researchers including Stroot et al. (2000), Sosnowski et al. (2003), Çinar et al. (2004) and Shin et al. (2010) have conducted experiments using batch reactors or using batch digester tests to support the continuous-flow digester experimental design. Continuous-flow reactors (bench- and pilot-scale) can be utilized after obtaining fundamental information from batch tests to simulate the actual industrial processes, in which the organic wastes are loaded continuously. However, considering the cost and operational flexibility, most laboratory-scale continuous-flow experiments have been conducted in semi-continuous flow digesters with intermittent loading.

2.4.3 One-stage, Two-stage, and Two-phase Digestions

Generally, anaerobic digestion can be conducted using three different configurations: one-stage digestion, two-stage digestion, and two-phase digestion. In one-stage digestion systems, hydrolysis, acetogenesis and methanogenesis occur in one digester (Gerardi, 2003). In two-stage digestion systems, hydrolysis and methanogenesis occur simultaneously and continuously in the first digester and sludge thickening and further organic reduction take place in the following digester. In two-phase digestion systems, hydrolysis and acetogenesis occurs in the first phase and methanogenesis takes place in the second phase. Among the three configurations, one-stage and two-stage digestions are conventional anaerobic digestion strategies that have been investigated in most laboratory-scale studies and operated in most full-scale WWTP for organic degradation and biogas production (Mata-Alvarez et al., 2000). The two-stage digestion can have a lower overall retention time than a single-stage system (Alatriste-Mondragón et al., 2006). Recently, a number of studies evaluating anaerobic digester performance considered the possibility of the two-phase configuration.

Pohland and Ghosh (1971) firstly proposed the concept of the two-phase anaerobic digestion configuration for wastewater treatment plant sludge digestions and enhanced the overall process stability and control. According to their research, total digestion time was considerably lower than the conventional one-stage digestion. Demirel and Yenigün (2002) reviewed the two-phase anaerobic digester and concluded that for better acid phase digestion, the HRT, pH, sludge retention time, temperature and sludge recycle are the influential process factors. It can be suggested that the longer HRT could make the acid phase more efficient. Most of the literature reviewed by Demirel and Yenigün (2002) showed that a pH ranging from 5.0 to 8.0 was the most suitable condition for the reactions. It was also concluded that two-phase anaerobic digestion needs shorter HRT than one-stage digestion, and can obtain higher organic removal efficiencies. Some researchers claim that two-phase digestion has other advantages as well, such as increased hydrolysis and methanogenesis rates. Demirer and Othman (2008) used a two-phase anaerobic digestion system (thermophilic hydrolysis and acetogenesis, mesophilic methanogenesis) to digest WAS. This system achieved higher chemical oxygen demand solubilization, volatile solids destruction, gas production and indictor pathogen reduction with shorter HRT and higher OLR then one-stage digestion. De La Rubia et al. (2009) tested the phase of hydrolysis-acetogenesis in a mesophilic anaerobic digestion process with various HRTs and OLRs. It is noted from their study that the hydrolysis and acetogenesis of the substrates were mainly influenced by OLRs but not HRTs. Park et al. (2005) upgraded a two-phase digestion system with the inclusion of a pre-treatment process to enhance the hydrolysis of the substrates. Thermo-chemical pre-treatment effectively increased the biogas production and organic reduction of the two-phase digestion system. Pérez-Elvira et al. (2011) compared four different anaerobic digestion strategies and concluded that two-phase digestion systems exhibited better performance for biogas production, pathogen reduction and sludge digestion than the conventional one-stage process. Although the two-phase anaerobic digestion configuration has been recognized as having many advantages, it has also been reviewed by Gunaseelan (1997) and De Baere (2000) that the economic feasibility and complexity of the operation limits its utilization for large-scale industrial applications.

2.4.4 Mesophilic and Thermophilic Operating Temperature

In anaerobic digestion processes, mesophilic digestion typically occurs in the temperature range 30-38 ℃, while thermophilic digesters have a temperature range of 49-57 ℃. In bench-scale and pilot-scale tests, thermophilic digestions are suggested to offer better operational conditions than mesophilic systems. Gunaseelan (1997) presented a detailed comparison between thermophilic and mesophilic digestion, which indicated that the methane production capacity and organic treatment efficiency were improved under thermophilic conditions. Gallert and Winter (1997) tested the methane production of the organic fraction of household wastes under mesophilic and thermophilic anaerobic digestion, and showed that the thermophilic condition degraded more protein than mesophilic digestion. Kim et al. (2003) obtained higher methane production from the anaerobic co-digestion of sewage sludge under thermophilic conditions. Gannoun et al. (2007) compared the mesophilic and thermophilic anaerobic co-digestion of olive mill wastewaters and abattoir wastewaters, and reported that thermophilic digestion achieved higher biogas yields under the same HRT and OLR as the mesophilic system.

It can therefore generally be noted that under the same operational conditions, thermophilic temparatures would result in better anaerobic digester performance than mesophilic temperatures. For most research and industrial applications, digestions are usually initially operated at mesophilic temperatures after which the thermophilic conditions are established. Although full-scale applications need to consider the economic impacts of construction and operation (for instance the addition and operation of a heating system for the thermophilic operation), researchers and technology suppliers continue to provide information on the operation of, and the facilities for, thermophilic anaerobic digestion (De Baere, 2000). This is due to the fact that thermophilic digestion can treat the waste at higher temperatures and thereby enhance pathogen die-off during the process. Higher biogas production and biodegradation rates can also be achieved. It can therefore be suggested that thermophilic operational conditions could be considered as a beneficial future research focus under suitable economic considerations (Kaparaju and Rintala, 2006; Feng et al., 2009; Palatsi et al., 2010; Siles et al., 2010).

2.5 Conclusions

Anaerobic co-digestion is an efficient process to enhance waste disposal and energy production. Organic wastes including OFMSWs, FOG, industrial waste effluents and agricultural wastes can be utilized as important organic waste sources for anaerobic co-digestion in WWPTs to enhance biogas production from wastewater treatment plant sludge. Among these potential co-substrate sources, organic wastes including industrial effluents and agricultural wastes have seldom been utilized in bench or full-scale co-digestion to assist in the improvement of biogas production in WWPT anaerobic digesters, mainly because of transportation costs. Most agricultural wastes such as manures have been reported to be co-digested with industrial effluents to provide sufficient ammonia and other nutrients. Hence, municipal organic wastes including

OFMWS and FOG, which could be collected from local proximity with lower transportation costs, should be considered the reasonable and economical co-substrates for enhancing biogas production in WWTPs.

OFMSWs have been the co-substrates most widely tested in municipal wastewater treatment plant sludge co-digestions. Although FOG could theoretically yield higher biogas production than OFMSWs, since the degradable fraction of the lipids is higher than that of the typical carbohydrates and proteins found OFMSWs, it has been comparelatively less studied in co-digestion applications with sludge due to operational limitations including sludge flotation, digester foaming and blockages of pipes and pumps. Hence, co-digestion of FOG and wastewater treatment plant sludge still requires a better understanding to provide an alternative approach to effectively treat FOG wastes and enhance energy production at WWPTs. Consequently, co-digestion of FOG with wastewater treatment plant sludge has therefore gained more attention in the last 5 years and needs more specific research in the future.

In order to identify suitable co-substrates and their addition, biochemical methane potential (BMP) tests are typically conducted prior to bench-scale or pilot-scale anaerobic investigations. The BMP assay has proven to be a relatively simple and reliable method for comparison of the extent and rate of conversion to methane, which can provide fundamental information for larger scale digestion tests and for selection of suitable HRT and OLR ranges. Constituent characterization and co-substrate mixing ratios (S/I ratio) must be considered and compared during the study to avoid inhibition and failure of the digestion.

Many operational configurations, including batch, continuous-flow, one-stage, two-stage and two-phase digestions, have generally been tested and compared for anaerobic digestion with various substrates. However, although the two-phase strategy has been shown to be a better configuration for performance consideration, it is not as widely used as one-stage or two-stage configurations in full-scale applications due to its operational complexity. Various operational parameters including OLR, HRT, pH, and temperature can affect the process performance. The thermophilic operating temperature regime has been proven to be a better condition for anaerobic co-digestion than mesophilic operating conditions, except for co-digestion with slaughterhouse wastes. However, extra energy consumptions of heating systems need to be carefully evaluated.

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Chapter 3

Enhancing Biogas Production from Anaerobic Co-digestion Using Pre-treatment Methods: A Review

Abstract: To enhance biogas production and assist in municipal organic waste management, anaerobic digestion with the addition of more than one kind of substrates, i.e. anaerobic co-digestion has been considered a more effective, low-cost, and commercially flexible approach than conventional anaerobic digestion to reduce process limitations and improve methane yields. Although a number of pre-treatment methods including: mechanical, chemical, thermal, thermo-chemical, ultrasonic, ozonation, microwave, and biological (enzymatic) have been tested as effective approaches to promote biogas production from anaerobic digestion of municipal wastewater treatment plant sludges or organic biosolids, their application in anaerobic co-digestion has been limited and not been widely reported. With advancements in co-digestion research using a variety of organic wastes as co-substrates, more specific examination of effective pre-treatment methods and operational parameters for co-digestion are necessary. This review first introduces the pre-treatment methods that have been tested to enhance anaerobic digestion, and then compares and summarizes pre-treatment applications in the co-digestion of various organic wastes. The review aims to advance the understanding of pre-treatment methods and to provide reference for future pre-treatment selection to improve biogas production from anaerobic co-digestion.

Keywords: Anaerobic co-digestion; organic wastes; biogas; methane; pre-treatment.

3.1 Introduction

Anaerobic digestion is the breakdown of organic materials by micro-organisms in the absence of oxygen. It is an effective method to treat solid organic wastes by converting the organic materials into carbon dioxide and methane. Anaerobic digestion consists of a series of microbial events that can be considered to occur in two main phases (Carucci et al., 2005; Geradi, 2003). In the first phase, which is typically referred to as fermentation, a group of microorganisms referred to as "acid formers" break down complex organic materials. The phase begins with bacterial hydrolysis of complex organic materials such as protein, fat, and cellulose. The soluble products are then biologically converted into short-chain volatile fatty acids (VFA), alcohols, and ketones. In the second phase, a collection of microorganisms referred to as "methane formers" convert the output of the first phase to form biogas. The biogas generated by anaerobic digestion is primarily composed of methane and carbon dioxide (with trace amounts of other gases such as hydrogen sulphide). Theoretical values of maximum methane production from full degradation can be calculated from the Buswell formula, given in Equation 3.1, when the chemical composition of the substrate is known (Hansen et al., 2004).

$$\begin{aligned}&C_nH_aO_bN_c+(n-\frac{a}{4}-\frac{b}{2}+\frac{3c}{4})H_2O\rightarrow\\&(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}-\frac{3c}{8})CH_4+(\frac{n}{2}-\frac{a}{8}+\frac{b}{4}+\frac{3c}{8})CO_2+cNH_3\end{aligned} \qquad (3.1)$$

Anaerobic digestion is widely used to treat wastewater sludges and other organic wastes (e.g. animal manure) with high water contents. It has been reported that anaerobic digestion has many advantages in addition to the production of biogas, such as providing mass and volume reductions of the input material. As part of an integrated waste management system, anaerobic digestion reduces the emission of greenhouse gases into the atmosphere if the generated biogas is captured for beneficial reuse. It can reduce pathogens and odors and require little land space for treatment. The nutrient-rich solids remaining after digestion can generally be used as fertilizer in agricultural land spreading (Salminen and Rintala, 2002).

Although this process has many advantages, several limitations also exist. These include the relatively slow reaction rates, the sensitivity of the system to shock loads and toxic materials, and the somewhat complicated operation (Lin et al., 1997). Other disadvantages such as long retention time due to slow processing of the wastes (20-30 days) and the instability of the anaerobic process have also been mentioned (Hwang et al., 1997; Navia et al., 2002). These limitations and disadvantages can certainly affect waste treatment and biogas production performances. In order to overcome these limitations, it has been reported that enhanced biogas and methane productions could be achieved with the pre-treatment of the feed substrates (Mata-Alvarez et al., 2000).

In the anaerobic digestion process, valuable carbon in the feed substrates is contained within the organic wastes. In order to obtain higher methane yields, it is necessary to release the carbon components for conversion in the digestion process. In addition, according to the phases of the anaerobic digestion process and from Equation 3.1, it has also been suggested that enhancing the hydrolysis process could increase the disintegration of organics and microorganism, and lead to improvements in the rate and efficiency of methane production, as well as in the solid waste treatment efficiency (Feitkenhauer, 2003; Kopp et al., 1997). Various processes including mechanical, thermal, chemical, thermo-chemical, ultrasonic, ozonation, microwave, and biological methods have been reported as effective pre-treatment methods to enhance biogas production from anaerobic digestion (Apul and Sanin, 2010; Kim et al., 2003; Pilli et al., 2011; Rafique et al., 2010; Valo et al., 2004). These pre-treatments can shorten the stabilization time, increase the biodegradation process efficiency, and improve the biogas production by increasing the hydrolysis of organic wastes.

While the conventional digestion process has been well studied, during the last decade, in efforts to enhance biogas production and assist in wastewater treatment and municipal organic waste management, anaerobic co-digestion with the addition of various co-substrates to the digestion process has been increasingly recognized as a potentially effective, low-cost, and commercially flexible approach to improve methane yields (Alatriste-Mondragón et al., 2006; Natural Resources Canada, 2002). It has been demonstrated by some researchers that co-digestion with municipally available organic wastes including food waste, fats, oils and grease (FOG), or animal manure, which can be collected in close proximity to the treatment facility, could be employed as the potential co-substrates with favourable results (Carucci et al., 2005; Davidsson et al., 2008; Gunaseelan, 2004; Kabouris et al., 2008; Li et al., 2011).

Although pre-treatments have been recognized as efficient processes to enhance methane production, they have mainly been tested in the anaerobic digestion of wastewater treatment plant sludge (e.g. waste activated sludge, primary sludge), particularly waste activated sludge (WAS), and a large number of studies were concerned with traditional digestion without co-substrate addition (Apul and Sanin, 2010; Dhar et al., 2012; Tanaka and Kamiyama, 2002; Vlyssides and Karlis, 2004). Nonetheless, some studies have reported on pre-treatments for digestion or co-digestion with various substrates (Blank and Hoffmann, 2011; Elbeshbishy et al., 2011; Fdez.-Güelfo et al., 2011; Luste et al., 2009; Navia et al., 2002; Saha et al., 2011; Saifuddin and Fazlili, 2009). However, with the development of co-digestion techniques, it is also important to conduct more specific research to provide information on reliable pre-treatment methods and operational parameters for effective pre-treatment applications in co-digestion, with the aim of enhancing biogas production and methane yield.

The objective of this article is to review the recent literature on various pre-treatment applications for enhancing biogas production from anaerobic digestion. Mechanisms and fundamentals of mechanical, thermal, chemical, thermo-chemical, ultrasonic, ozonation, microwave, and biological pre-treatments are introduced and compared. Furthermore, research on pre-treatment applications in anaerobic co-digestion for various organic wastes is also reviewed and discussed.

3.2 Mechanical Pre-treatment

Mechanical methods can reduce the size of substrate particles and increase the substrate specific surface area, which could consequently lead to a more rapid anaerobic digestion process and improvement in biogas production. Several mechanical methods can be used for pre-treatment with the most widely reported being: high pressure homogenizer (HPH), stirred ball mills, and the jetting and colliding method (Choi et al., 1997; Engelhart et al., 2000; Müller, 2000; Toreci et al., 2011). Although mechanical pre-treatment methods differ in energy consumption and the suitability of the equipment for practical applications, their main principles are similar in that they are designed to change the substrate characteristics by stressing the substrate cell walls and disrupting the microbial cells by physical processes without chemical addition (Hartmann et al., 2000; Müller, 2000).

Through the HPH system, the substrate is usually pumped under high pressure into the homogenizer. The resulting intense energy release can lead to interior pressure differences in the substrate and the formation of cavitation bubbles to disrupt the cell membranes. Engelhart et al. (2000) studied the effects of mechanical disintegration on anaerobic biodegradability of sewage excess sludge. The HPH was set at three pressure levels (50, 300, 600 bar) for testing. Higher pressure levels led to higher degrees of disintegration of the substrates. Consequently, a 25% increase in volatile solid (VS) reduction and a higher specific biogas production was achieved with the mechanical pre-treatment energy input. Most researchers reported that significant biogas production improvements were achieved from HPH pre-treated substrates at pressure levels higher than 300 bar (Kopplow et al., 2004; Stephenson et al., 2007). However, it has been also reported by Onyeche (2007) that 150 bar HPH operation could yield a 30% increase in biogas production and achieve a 23% sludge volume reduction. Dhar et al. (2011) reported that even 75 psi (5.2 bar) of pressurization of waste activated sludge (WAS) could result in a 10% improvement in biogas production and a decrease in methyl mercaptan generation. However, it should be noted that the HPH pre-treatment approach utilized by these authors was combined with the addition of chemicals including hydrogen peroxide (H_2O_2) and ferrous chloride ($FeCl_2$).

The stirred ball mill technique can disrupt the cellular wall and membrane by the agitation or shear forces produced by two counter rotating plates or grinding beads. The substrate can be mechanically crushed by the forces and pressure, which increases the membrane rupture efficiency. Baier and Schmidheiny (1997), Müller (2000), and Perez-Elvira et al. (2006) reported that, with reliable operation and no odor generation, this technique could effectively increase the disintegration of substrate. However, economic factors including corrosion and mechanical abrasion have been mentioned as drawbacks. In addition, it is noted that most research has concentrated on the pre-treatment effects on changes in substrate characteristic rather than changes in biogas production.

With the application of the jetting and colliding pre-treatment method, the substrate is usually pressurized up to 50 bar and jetted to the collision plate or a smash-plate after going through a nozzle. Consequently, the treated substrate cell wall can be disrupted (Carrère et al., 2010). Hwang et al. (1997) and Choi et al. (1997) pre-treated WAS to improve the methane production with jetting and colliding pre-treatment method under pressurized conditions (5-50 bar) with different thickening times. The soluble protein concentration (SPC) in the sludge was attributed to the cell rupture. The results indicated that cell rupture increased with the pressure and thickening time. However, biogas production and its improvement as a result of the pre-treatments was not directly assessed during their study. Nah et al. (2000) applied mechanical pre-treatment to WAS prior to pilot-scale anaerobic digestion. The pre-treatment equipment was composed of a high-pressure pump, needle valve, and 30 L tank containing a nozzle and collision-plate, which could solubilize the sludge through jetting-and-colliding at high pressure. The pressure was adjusted to 30 bar. In this study, microphotographs at 400 times magnification were used to show that most of the aerobic microorganisms were hydraulically converted to low-molecular-weight components, and parts of the intracellular substance was released from the interior of the cells. Both chemical oxygen demand (COD) and soluble protein concentration (SPC) were tested as reference parameters to illustrate the degradation process efficiency, and both increased with the mechanical energy input. Digestion results showed that the pre-treatment decreased the substrate retention time (SRT) from 13 to 6 days, and enhanced volatile solids (VS) reduction and unit biogas production. The advantages of the mechanical jet and smash process were also summarized in this study. The process requires simple facility modifications for applications at existing plants, needs no additional equipment in the sludge transport pipeline between the sludge stock tank and the digester, provides improvements in anaerobic digestion performance and reduces particle size.

According to the literature involving mechanical pre-treatments, it can be summarized that these processes are simple in operation and economical in terms of installation or retrofitting to existing reactors. Overall, disintegration and biogas production increase with the energy input. However, mechanical pre-treatment has mainly been applied to the treatment of wastewater treatment sludge, particularly WAS, but not to other organic wastes which could be used as co-substrates. Researchers have also not reported on the biogas production improvement from anaerobic co-digestion with mechanical pre-treatment.

3.3 Thermal Pre-treatment

Thermal pre-treatment is usually operated in the temperature range of 40-150 ℃ (Müller, 2000). Substrate hydrolysis can be accelerated by thermal pre-treatment with the long-chain biomolecules being solubilized at high temperature (Appels et al., 2008). However, it has been reported that when the pre-treatment temperature is higher than 175 ℃, biogas production in anaerobic digestion decreases (Bonmatí et al., 2001).

Most studies have concentrated on testing thermal pre-treatment of wastewater treatment plant sludge (e.g. primary sludge or WAS sludge). Wang et al. (1997) applied thermal pre-treatment with temperatures of 60 ℃, 80 ℃ and 100 ℃ to pre-treat WAS and found that a 60 ℃ thermal pre-treatment increased the methane generation by 30%. Schieder et al. (2000) applied thermal pre-treatment (160-200 ℃) accompanied with high pressure (up to 40 bar) in a laboratory-scale digestion test. By increasing pressure and temperature, the organic waste was split up in the first step into short-chain fragments that were well suited for biodegradation. The resultant anaerobic digestion was faster, more stable, and yielded more biogas. Kim et al. (2003) observed a significant increase of 30% in VS reduction, 67.8% in soluble chemistry oxygen demand (SCOD) removal, and 34.3% methane production for the sludge pre-treated at 121 ℃ for 30 minutes. Gavala et al. (2003) estimated the effect of thermal pre-treatment at 70 ℃ on mesophilic (35 ℃)

and thermophilic (55 ℃) anaerobic digestions of primary and secondary (WAS) sludge. The results showed that the pre-treatment provided positive effects on the methane production upon subsequent thermophilic digestion of the primary sludge. For the WAS, the production rate of methane was also positively affected by the pre-treatment, however, the methane production of the WAS was only increased by the pre-treatment when the subsequent step was mesophilic digestion. Graja et al. (2005) utilized a thermal pre-treatment at 175 ℃ and 40 min to promote the hydrolysis of wastewater treatment plant sludge from a wastewater treatment plant. Higher dewaterability of biosolids and a decrease of the hydraulic retention time were achieved. The methane production was tested in a semi-continuous digester, with significant enhancement. The hydraulic retention time of the digester was shortened and a nearly stoichiometrically complete conversion of organics to methane was observed. Takashima (2008) conducted thermal pre-treatment on wastewater treatment sludge (primary and WAS ratio of 2.8:1 on a TS basis) at 120 ℃ for 1h. The mesophilic continuous-flow anaerobic digestion of non-pretreated sludge yielded an average of 168 mL of methane/g VS fed daily, compared to a methane production of up to 204 mL/g VS fed in the digestion of pre-treated sludge, which indicated the thermal pre-treatment effectively enhanced the biogas production in this study. Donoso-Bravo et al. (2010) used thermal pre-treatment (175 ℃ for 30 minutes) and reached approximately 90% and 80% increases in maximum biogas production rate and total biogas production, respectively, from WAS sludge from a municipal wastewater treatment plant. Dhar et al. (2012) also reported a biogas production increase from thermally pre-treated municipal WAS. According to the results obtained from these research projects, it can be suggested that thermal pre-treatment has been widely applied to enhance the anaerobic digestion of municipal sludge. Temperature and treatment time have been found to be the key parameters controlling the process.

Compared to the numerous applications in municipal wastewater treatment plant sludge digestion, thermal pre-treatment has seldom been utilized to pre-treat other organic wastes. During the past decade, there have only been a few published reports that discuss enhanced biogas production from other organic wastes after being processed by thermal pre-treatment. In the study of Wang et al. (2006a), processing at 150 ℃ for 1 h achieved better pre-treatment than 70 ℃ for 0.5- 2 h on food waste. Thermal pre-treatment (< 90 ℃) was tested in the mesophilic anaerobic digestion of pig slurries by Bonmatí et al. (2001). In that study, thermal effects at 80 ℃ were reported to be beneficial for organic degradation and enhanced methane production, as opposed to enzymatic effects at 60 ℃. In addition, for a pig slurry with low ammonia concentration, the methane production rates increased significantly. However, the thermal pre-treatment did not show the same positive effects on the digestion of older pig slurry with high ammonia content. Mladenovska et al. (2006) thermally pre-treated a mixture of cattle and swine manure at 100-140 ℃ for 20-40 minutes prior to anaerobic digestion. The methane yield was faster and greater than from the digestion with non-pretreated manure. The optimum pre-treatment condition was suggested as being 140 ℃ for 40 minutes. Carrère et al. (2009) also maximized the production of methane from pig manure. The methane production increased with increasing temperature but temperatures higher than 135 ℃ were noted to be necessary to significantly improve the methane production. The best results were obtained with the highest temperature of 190 ℃. González-Fernández et al. (2012) and Menardo et al. (2012) also investigated the thermal pre-treatment of algae and agricultural by-products, respectively. González-Fernández et al. (2012) used 70 ℃ and 90 ℃ thermal pre-treatments to treat algal biomass (mainly composed of *Scenedesmus* sp.) for 3 hours and achieved the best anaerobic biodegradability of 48% and significantly higher methane production from digestion of the pre-treated biomass at 90 ℃. In the study reported by Menardo et al. (2012), the effect of thermal pre-treatments at 90 ℃ and 120 ℃ for 30 minutes were investigated for four kinds of agricultural by-products (wheat, barley, rice straw, and maize stalks). The best methane production performance was obtained from the digestion of 120 ℃ thermally pre-treated wheat straw, which had an increase of 64% in methane yield.

As the research on the effects of pre-treatment on biogas production from other organic wastes or biomass is limited, it is even more difficult to find thermal pre-treatment applications in anaerobic co-digestion experiments. The tests reported by Cuetos et al. (2010) might be the most current report of thermal pre-treatment utilization in the anaerobic co-digestion process, even though the thermal pre-treatment at 133 ℃ in this study was combined with increased pressure (>3bar). As expected, the biogas production was greatly enhanced by co-digestion. The co-digestion of slaughterhouse waste and municipal solid waste achieved significantly higher biogas production than the digestion with only a single substrate. However, the biogas production from digestion with pre-treated substrates was slightly lower than that from digestion with untreated substrates. According to the characteristics of the pre-treated substrates, it was noted that the pre-treated substrate had higher organic concentrations (COD and VFA), which resulted in the inhibition of the digester reactions. This would suggest that, as the pre-treatment could effectively increase the organic solubility of substrates, it is still necessary and important to conduct specific research to identify effective pre-treatment methods and operational parameters for anaerobic co-digestion.

In general, thermal disintegration processes have higher energy consumption than mechanical methods, but low temperature condition thermal pre-treatment processes can use low cost thermal sources of energy instead of electrical energy (Müller, 2000). Climent et al. (2007) compared the effects of thermal and mechanical pre-treatments of secondary sludge (WAS) on biogas production, and obtained the best biogas production from low temperature (70 ℃) thermally pre-treated sludge. Hence, thermal pre-treatment at a lower temperature (below 100 ℃) becomes more attractive (Gavala et al., 2003).

According to the research on thermal pre-treatment reviewed in this section, it can be summarized that temperature influences the thermal pre-treatment more than operation time. Doh ányos et al. (2004) pointed out that in a longer thermal pre-treatment period, biologically active compounds could be inactivated and some toxic products formed. It is important to note that when the pre-treatment temperature is higher than 175 ℃, biogas production in anaerobic digestion may decrease. This is probably the reason that most investigations examined temperatures between 70 ℃ and 150 ℃ in these thermal pre-treatment studies. Similar to other pre-treatment methods, the effects of thermal pre-treatment on anaerobic co-digestion with various co-substrates needs to be further researched.

3.4 Chemical Pre-treatment

Chemical pre-treatment includes acidic and alkaline pre-treatment methods that can break down the complex organic compounds by adding strong mineral acids or alkalis (Doh ányos et al., 2004).

Acids including HCl and H_2SO_4 have been employed as pre-treatment chemical agents. However, it has been suggested by Heo et al. (2003) and Carballa et al. (2009) and proven by Lin and Lee (2002) and Jan et al. (2008) that alkaline addition is more efficient than acid addition for improving anaerobic digestion. Chen et al. (2007a) and Chen et al. (2007b) observed higher COD solubilization of WAS under alkaline pre-treatment (pH=11) compared to that of acid pre-treatment (pH=4). This can explain why acidic pre-treatment is not as widely utilized as the alkaline pre-treatment for improvement of anaerobic digestion. For alkaline pre-treatment, chemicals including NaOH, KOH, $Mg(OH)_2$, and $Ca(OH)_2$ have been investigated (Beccari et al., 1999). Of these potential alkaline pre-treatment agents, NaOH has been reported as the most efficient for enhancing organic hydrolysis and anaerobic digestion by Navia et al. (2002) and Kim et al. (2003). Lin et al. (1997) and Lin et al. (1999) enhanced the anaerobic digestion of waste activated sludge using sodium hydroxide (NaOH) solutions of 20 meq/L and 40 meq/L in the pre-treatment process. When a 1% total solid (TS) waste activated sludge was pre-treated by 20 meq/L and 40 meq/L NaOH solutions, 33% and 30% biogas production increases were observed, respectively. When a 2% (TS) sludge was pre-treated by the 20 meq/L NaOH solution, a 163% increase in biogas production was obtained. The results indicated that alkaline pre-treatment can solubilize the wastes and increase the biogas production, but the biogas production might not increase linearly with the concentration of the sodium hydroxide solution. Chiu et al. (1997) combined alkaline pre-treatment of 40 meq/L NaOH with ultrasonic pre-treatment and obtained effective recovery of volatile fatty acids from activated sludge, which can increase methane production in anaerobic digestion. However, it has been also reported that too high a concentration of Na^+ may cause subsequent inhibition of anaerobic digestion (Mouneimne et al., 2003). Therefore, NaOH dosage control by adjusting pre-treatment condition pH has been utilized as an effective way to avoid this inhibition. Moreover, it has been reported by many researchers that a pH=12 should be the optimum alkaline pre-treatment operational level with the addition of NaOH to enhance biogas production (Carballa et al., 2009; Neyens et al., 2003; Valo et al., 2004).

During the past 10 years, chemical pre-treatment applications on anaerobic co-digestion processes have been tested by some researchers. Heo et al. (2003) used alkaline addition to pre-treat a mixture of WAS and food waste. An 88% methane production increase was obtained when compared to the control tests. Alkaline pre-treatment applied in kraft mill sludge treatment for further enhancement of anaerobic digestion was investigated by Navia et al. (2002). The results showed that the alkaline pre-treatment increased soluble COD, which would likely lead to an increase in methane yield and efficiency in the subsequent anaerobic digestion. Pang et al. (2008) and Li et al. (2009) pre-treated the co-digested substrates cattle manure and corn stover. Compared to the single substrate digestion, 7.4% more biogas production was achieved, which indicated that co-digestion with NaOH pre-treatment could represent a good option for efficient biogas production and waste treatment. However, Hidalgo et al. (2012) reported that chemical pre-treatment did not appear to have significant effects on biogas production from the co-digestion of food wastes. Hence, for the anaerobic co-digestion of various organic wastes, further research is needed to investigate the optimum chemical pre-treatment operational conditions.

Based on this review, it can be concluded that alkaline pre-treatment with the addition of NaOH represents a good chemical pre-treatment method. Biogas production might not increase significantly with an increasing concentration of the sodium hydroxide solution, but the pH adjustment would be an effective way to control the process. This pre-treatment has been well studied in anaerobic digestion with wastewater treatment plant sludge, but the optimum chemical pre-treatment for the co-digestion of various substrates still needs more investigation.

3.5 Thermo-chemical Pre-treatment

Thermo-chemical pre-treatment is the combination of thermal and chemical methods. As discussed, the chemicals added in thermo-chemical pre-treatments are often alkaline such as NaOH.

The combination of thermal and chemical pre-treatments has been investigated in a number of studies in which the enhancement of the anaerobic digestibility of sludge was reported. Patel et al. (1993) obtained the best biomass degradation and biogas production when substrates were pre-treated at pH=11 and 121 ℃. They also reported that NaOH was a better chemical agent than HCl in the thermo-chemical pre-treatment process. Tanaka et al. (1997) compared the individual effects of chemical (NaOH), thermal, and thermo-chemical (with NaOH) pre-treatments on WAS. Thermo-chemical pre-treatment yielded the best results with methane production increases of more than 200%. Tanaka and Kamiyama (2002) used the same optimum thermo-chemical pre-treatment (130 ℃, 5 minutes, 0.3 gNaOH/gVSS) to treat WAS. The subsequent mesophilic anaerobic digestion (HRT=8 d) of the pre-treated WAS exhibited a significant improvement in biogas production and removal of organics. Park et al. (2005) compared the efficiency of thermo-chemical and biological pre-treatments, and obtained 80% higher biogas production with thermo-chemical pre-treatment. Rafique et al. (2010) also compared the effects of thermal, chemical and thermo-chemical pre-treatments to enhance methane production from pig manure. The best biogas production enhancement was noted from the thermo-chemical pre-treated substrate. It was also indicated that the best temperature for thermo-chemical pre-treatment was in the range of 25-100 ℃ depending on the chemical dosages.

Penaud et al. (2000) and Delgenès et al. (2000) examined the characteristics of the sludge digestion after thermo-chemical pre-treatment. The optimum pre-treatment condition was reported as 140 ℃, pH=12, and 30 min treatment time, from which the biogas production was significantly increased when compared to the control experiment. Vlyssides and Karlis (2004) investigated thermo-chemical pre-treatment at temperatures in the range of 50-90 ℃. The optimal condition was found to be 90 ℃ and pH=11. In addition, a satisfactory correlation between the hydrolysis rate coefficient, pH and temperature was reported. Kim et al. (2003) observed that the thermo-chemical pre-treatment of WAS increased the methane production by 34.3% and organic removal by 67.8% with optimum thermo-chemical pre-treatment of pH=12, and 121 ℃ for 30 minutes. Valo et al. (2004) compared the thermal and thermo-chemical pre-treatment for biogas production from WAS. The results indicated that thermo-chemical pre-treatment of pH=12 and 170 ℃ yielded a biogas production increase of 54%. Rani et al. (2012) also tested low temperature thermo-chemical pre-treatment (50, 60, 70 and 80℃; pH values of 10, 11 and 12) on WAS. The thermo-chemical pre-treatment of 60 ℃ and pH=12 achieved the best biogas production. The results reported from the various studies summarized above would indicate that since thermo-chemical pre-treatment combines chemical and thermal technologies the pre-treatment time, chemical dosage, temperature, and quantity of treated substrate would be the parameters expected to affect the effectiveness of the pre-treatment process, which must be considered and optimized for specific digestion applications, including co-digestion.

According to the thermo-chemical pre-treatment application in the digestion outlined above, it can be noted that thermo-chemical pre-treatment is better than thermal or chemical processes for biogas production enhancement from conventional digestion. However, comparisons between alkaline, thermal and thermo-chemical pre-treatments in the study conducted by Hamzawi et al. (1998) showed that alkaline pre-treatment resulted in a higher substrate biodegradability than thermo-chemical treatment to enhance biogas production from co-digestion. According to the method used by Hamzawi et al. (1998), it was observed that inhibition happened during the thermo-chemical pre-treatment of mixture of 25% organic fraction of municipal solid waste and 75% sludge (65% primary sludge and 35% thickened WAS) due to the long pre-treatment time (1 hour) and high chemical dosage. It was also noted in Section 3.3 (Thermal Pre-treatment) that long thermal pre-treatment times can lead to inhibition in the anaerobic digestion process. With the addition of chemicals, thermo-chemical pre-treatment should enhance organic solubilization and thus introduce higher soluble organic substrates to the subsequent digestion process, as long as inhibition does occur. As was discussed in Section 3.4 (Chemical Pre-treatment), a pH control strategy during pre-treatment would represent a better alternative than chemical dosage control (g chemical/g substrate) to optimize the pre-treatment process. Wu et al. (2009) pre-treated meat and bone meal using thermo-chemical pre-treatments at two temperatures (55 ℃ and 131 ℃) with NaOH (0, 1.25, 2.5, 5, 10 and 20 g/L) addition. Higher methane production was observed from the co-digestion with thermo-chemical pre-treatment of 55 ℃ and 10 g/L NaOH. It was also noted that methane production was inhibited by the addition of NaOH at 131 ℃. However, similar to other pre-treatment methods, thermo-chemical pre-treatment has not been widely tested for anaerobic co-digestion of organic wastes other than wastewater treatment sludge (particular WAS), at least during last 10 years.

Although the thermo-chemical pre-treatment of sludge results in an increased biodegradability, the process consumes a substantial amount of energy, as well as chemicals. High operation and maintenance costs and corrosion problems due to the use of predominantly alkaline chemicals and high temperature need to be considered when this pre-treatment is being applied on a large scale. In addition, similar to other pre-treatment methods, thermo-chemical pre-treatment has not been widely tested for anaerobic co-digestion of organic wastes other than wastewater treatment sludge.

3.6 Ultrasonic Pre-treatment

Ultrasonic disintegration is essentially a physical process. Ultrasound is a sound wave at a frequency above the normal hearing range of humans (>20 kHz). When the ultrasound wave propagates in a medium, it generates a repeating pattern of compressions and rarefactions in the medium. Due to the low pressure of the rarefactions, a liquid or slurry is torn apart and microbubbles are formed. The sudden and violent collapse of a large number of microbubbles generates powerful hydromechanical shear forces that can disrupt adjacent bacterial cells and break the cell wall and membrane (Khanal et al., 2007). In addition, the high temperature and pressure resulting from ultrasound can also assist in the disintegration of the materials.

In the ultrasound application, some terms need to be defined, i.e. *ultrasonic intensity* relates to the power supplied per transducer area (W/cm^2), *ultrasonic density* relates to the power supplied per sample volume (W/L), *ultrasonic dose* relates to the energy supplied per sample volume and time (W/s·L), and *specific supplied energy* (E_s, kJ/kg TS) is defined using Equation 3.2 (Bougrier et al., 2006):

$$E_s = \frac{Pt}{vTS_0} \quad (3.2)$$

where P is the ultrasonic power (kW), t is the sonication time (h), v is the sample volume (L), and TS_0 is the initial total solids concentration (kg/L). Researchers have used different terms to describe the level of ultrasonic treatment, which makes the comparison of the performance of different treatment conditions difficult. However, Tiehm et al. (2000) investigated the effects of ultrasonic pre-treatment at various frequencies ranging from 41 to 3217 kHz and reported that the sludge disintegration was most significant at low frequency. It can also be noted from the reports published since 1997 that ultrasound within the frequency range of 20-40 kHz has been widely applied for pre-treatment (Apul and Sanin, 2010; Neis et al. (2000); Tiehm et al., 1997; Tiehm et al., 2000; Wang et al., 1999). Furthermore, over the past 10 years, most ultrasonic pre-treatments applied in studies aimed at enhancing biogas production from municipal wastewater treatment sludge have been conducted at a frequency of 20 kHz (Benabdallah El-Hadj et al., 2007; Mao and Show, 2006; Saha et al., 2011).

Literature reviews on ultrasonic pre-treatment have been published recently and provided comprehensive fundamental knowledge on this technique and indicate that this pre-treatment approach has been widely investigated for the anaerobic digestion of wastewater treatment plant sludges (Khanal et al, 2007; Pilli et al., 2011). Wang et al. (2005) and Wang et al. (2006b) analyzed the components released from ultrasonicated waste activated sludge (WAS), discussed the mechanisms of sludge disintegration, and fitted kinetic models to the disintegration process. They reported that ultrasound can destroy the extracellular polymeric substances in WAS, which is important to the sludge flocs structure. Hence, more substances inside the substrate cells can be effectively released into the aqueous phase.

According to the mechanisms of the ultrasound treatment and its potential for medium disintegration, many researchers consider it as the preferred pre-treatment method for improving biogas production from anaerobic digestion of wastewater treatment sludges. For example, Tiehm et al. (1997) noted significant increases in biogas production from ultrasonicated primary sludge in a semi-continuous digester and they also observed a 44.3% reduction of volatile solids (VS). Wang et al. (1999) used ultrasonic pre-treatment in the anaerobic digestion of WAS. In the pre-treatment, a 9 kHz frequency was operated for 0, 10, 20, 30 and 40 min. The results indicated that the methane generation increased with ultrasonication time up to 30 min. However, when the pre-treatment time was extended to 40 min, the increase in methane generation (69%) was not significantly different than the increase noted at 30 min (64%). It is interesting that Wang et al. (1999) used a 9 kHz frequency ultrasound treatment, which outside the frequency range most applied by other researchers, especially during the last decade.

Mao and Show (2006) utilized various ultrasound densities of 0.18 W/mL, 0.33 W/mL, and 0.52 W/mL to treat WAS from a wastewater treatment plant. The improved solids degradation increased biogas production by 1.4-2.5, 1.9-3.0, and 1.6-3.1 times, respectively. Increases in methane composition of 2-17% were also noted in all digesters fed with ultrasonicated sludge. Bougrier et al. (2005) supplied 20 kHz ultrasound as the pre-treatment for WAS. Biogas production increased with the increase in organic solubility resulting from the pre-treatment. Bougrier et al. (2006) also conducted ultrasonic pre-treatment studies using 225W power and 20 kHz ultrasound for the WAS pre-treatment. However, two different specific supplied energies (6250 kJ/kg TS and 9350 kJ/kg TS) were tested separately. Biogas production showed significant increases under the different specific supplied energies, but there was not a significant difference between the two conditions for methane production. This implies that ultrasonication with energy inputs higher than 7000 kJ/kg TS does not have much effect on the methane production. Yin et al. (2006) used ultrasound at 600 W/m^2 intensity and 20 kHz frequency to treat WAS for 1, 1.5 and 30 min. It was found that 1 min was the best treatment time for ultrasound pre-treatment. This result was consistent with the findings of Wang et al. (1999) and Bougrier et al. (2006), who reported that improvement in biogas production from wastewater treatment plant sludge cannot be ensured if the ultrasonic pre-treatment input power is too high.

Currently, with the development of advanced techniques for anaerobic digestion or co-digestion with the addition of various organic materials, ultrasonic pre-treatment has been applied and reported for pre-treating organic substrates other than wastewater treatment sludge to promote the substrate potential for biogas production. Luste et al. (2009), Elbeshbishy et al. (2011) and Saha et al. (2011) reported that ultrasonic pre-treatment with frequencies around 20 kHz could effectively enhance the biogas production from meat-industry by-products, hog manure, and pulp mill wastewater treatment sludge, respectively. During the last 2 years, with increasing research interest in anaerobic digestion and the wide application of mature ultrasonic technologies, ultrasonic pre-treatment methods have been investigated by some researchers in anaerobic co-digestion studies. Kameswari et al. (2011) compared the effects of ultrasonic pre-treatment and ozonation pre-treatment on the co-digestion of tannery solid wastes. The ultrasonic pre-treatment obtained better biogas improvement (53%). In addition, when considering the optimum contact time, energy cost, and potential for implementation, ultrasonic pre-treatment was considered the most appropriate and viable pre-treatment process. Luste et al. (2012) co-digested dairy cattle slurry and meat-industry by-products with ultrasonic pre-treatment, which was considered as one of the most suitable pre-treatment methods for the co-digestion based on its effectiveness in promoting the organic degradation and methane production.

Ultrasonic pre-treatment has a number of advantages over other pre-treatment techniques, e.g. it neither generates secondary toxic compounds nor contributes additional chemical compounds. Meanwhile, many toxic and recalcitrant organic pollutants such as aromatics, chlorinated aliphatics and organic dyes can be broken down into simpler forms (Khanal et al., 2007). One of the major issues with ultrasonic pre-treatment is the high capital and operational costs. Long-term performance data from full-scale ultrasound systems are still limited. This may discourage the application of the process in full-scale application. According to the reviewed literature, the mechanisms and effectiveness of the ultrasonic process are influenced by three factors: supplied energy, ultrasonic frequency and the nature of the influent. Based on this review, 20 kHz is suggested as the optimal ultrasonic frequency. Higher ultrasonic density and influent concentration can negatively affect biogas production in the subsequent anaerobic digestion. Although some researchers have applied ultrasonic pre-treatment in some anaerobic co-digestion processes, as summarized here, the body of literature about the effects of ultrasonic pre-treatment on biogas production from co-digestion with various organic wastes is still considerably less than that on the effects on digestion with sewage sludge.

3.7 Ozonation Pre-treatment

Ozone (O_3) is a powerful oxidant and disinfectant. It has strong cell lytic activity and reacts with the polysaccharides, proteins, lipids and various complex organic compounds to transform them into simpler compounds. The reactions and transformations occurring during the ozonation process can result in efficient cellular membrane rupture and the release of more soluble and easily biodegradable organics from treated substrates. As such, improvements in biogas production from the subsequent anaerobic digestion can be expected (Ahn et al., 2002; Chu et al., 2009; Elliott and Mahmood, 2007; Weemaes et al., 2000).

It has been observed that biogas production increases with increasing ozone dosage. However, the optimum ozone dosage depends on the type of substrate and the operational conditions. Weemaes et al. (2000) ozonized a sludge mixture (primary sludge and WAS) and then digested the mixture in a batch digester. The ozone flow rate was 200 L/h with a concentration of 35 mg O_3/L gas. The ozonation time ranged from 3 h 12 min to 3 h 38 min to achieve ozone dosages of 0.05 and 0.1 g O_3/g COD. Microscopic pictures showed that the sludge after ozonation had obvious destruction. The anaerobic digestion with 0.1 g O_3/g COD pre-treatment achieved the highest methane production. In a study conducted by Yeom et al. (2002), various ozone dosages of 0, 0.02, 0.05, 0.1, 0.2 and 0.5 g O_3/g SS were applied to pre-treat the sludge collected from a municipal wastewater treatment plant in Seoul prior to anaerobic digestion. The pre-treatment using a 0.2 g O_3/g SS ozone dosage showed the highest increase in biogas and methane production. Although the 0.5 g O_3/g SS pre-treated sludge achieved the best cumulative biogas production and organic solubilization performances, they were not substantially higher than those obtained under the 0.2 g O_3/g SS condition. Considering oxygen consumption and system cost, it was suggested that a 0.2 g O_3/g SS ozone dosage was the ideal choice for municipal sludge pre-treatment. Bougrier et al. (2006) also investigated the effect of ozonation pre-treatment for improving biogas production from waste activated sludge (WAS) using ozone dosages of 0.10 and 0.16 g O_3/g TS, and obtained better methane production and biodegradability with the 0.16 g O_3/g TS pre-treatment. According to the ozonation pre-treatment methods reviewed by Carrère et al. (2010), it was suggested that the optimum ozone dosage should be between 0.05 and 0.5 g O_3/g TS.

One interesting phenomenon is that most researchers have concentrated on investigating the effects of ozonation pre-treatment on substrate organic solubilization and solids reduction but have seldom mentioned these effects on biogas production (Bougrier et al, 2007; Goel et al., 2003a; Goel et al., 2003b). In addition, most studies were conducted to treat the municipal wastewater treatment plant sludge, but not other potential organic wastes for anaerobic co-digestion. Therefore, from the limited reports on enhancing biogas production from ozonation pre-treated substrates, some literature is reviewed here.

Some researchers have examined ozonation pre-treatment on potential organic wastes other than wastewater treatment sludge. Benitez et al. (1997) compared the methane yields from the anaerobic digestion of olive mill wastewaters with and without ozonation. Temperature, pH and ozone partial pressures were adjusted to the desired values of 20℃, 4.85 and 1.30 kPa, respectively. The ozone was fed to the organic waste with a flow rate of 40 L/h for up to 8 hours. Compared to the methane production from anaerobic digestion without pre-treatment, the methane obtained in the digestion combined with ozonation was 266 mL CH_4/g COD, which was higher than the 194 mL CH_4/g COD obtained from digestion without ozonation. However, it should be noted that in this research, the ozone dosages were not normalized to the widely used unit of g O_3/g TS, which makes the results difficult compare to other studies. Martín et al. (2002) compared the digestion of vinasse pre-treated with ozone and ozone plus ultraviolet light. Although the ozonation pre-treated sludge exhibited no apparent methane production enhancement, it did provide the best methane production kinetic and biogas yield coefficient.

For ozonation pre-treatment in anaerobic co-digestion with more than one kind of substrate, few studies have been published during the past 15 years. In their work Kameswari et al. (2011) identified the effects of ozonation pre-treatment processes on co-digestion of tannery solid wastes in 650 mL batch digesters. The ozone was purged through substrates at dosages of 0.15, 0.18 and 0.20 g O_3/g TS. The best organic solubilization as indicated by the highest SCOD concentration was achieved by the 0.18 g O_3/g TS pre-treatment, which was identified in the study as the optimum ozonation condition. The ozonation pre-treatment effectively increased the cumulative biogas production by 45%. An increase in organic solubilization and solids reduction was also reported.

Besides the effects on organics solubilization and increased biogas production, other benefits of the ozonation pre-treatment process include its effect as a disinfectant to kill a wide range of microorganisms, and excellent removal of taste and odors. A major disadvantage is that, due to its instability, ozone must be generated on-site before use. The equipment and operating costs can be quite high, which would not be attractive for use of this technique in large-scale industrial applications (Goel et al., 2003a; Kameswari et al., 2011). In addition, ozonation as a pre-treatment method for improving biogas production from anaerobic digestion or co-digestion of substrates other than municipal sludge has not been widely researched.

3.8 Microwave Pre-treatment

The microwave technique is not a new technique, and has been widely applied in food processing industry. However, most microwave technique applications have seen little commercialization in the field of environmental engineering for the lack of knowledge concerning the design of large scale microwave equipment (Jones et al., 2002). It is an electromagnetic radiation in the frequency range of 0.3 to 300 GHz, which results in wavelengths from 1 mm to 1 m (Park et al., 2004). The alternating electric field caused by microwave irradiation causes a rapid alignment and realignment of dipoles in a polar solvent, thus resulting in heat generation (Collins et al., 1991). The microwave process can generate heat both internally and at the surface of the materials that absorb microwave energy and can also result in a change of the structure of microorganisms. According to the mechanisms of the microwave technique, it can be interpreted that microwaves can disintegrate the organic waste and release potential constituents locked inside the cell walls.

Although the microwave oven is widely used in homes and is familiar to most people, it is considered a novel pre-treatment method for anaerobic digestion (Park et al., 2004; Eskicioglu et al., 2008). In the study of Park et al. (2004), a microwave system (2450 MHz, 700 W) was used to treat WAS from a wastewater treatment plant. Irradiation times were set at 0, 3, 5, 7, 9, 11 and 15 minutes. The treated secondary sludge was anaerobic digested in 5 L digesters with hydraulic retention times (HRT) of 8, 10, 12 and 15 days. Microwave pre-treatment was found to enhance the disintegration of the sludge, with up to 22% increase in SCOD concentration after pre-treatment. The maximum rates of COD removal and methane production were obtained from the anaerobic digestion of the pre-treated sludge, which were 64% and 79% higher than those yielded by the digestion of non-pre-treated sludge, respectively. Eskicioglu et al. (2008) also used a microwave system to treat thickened WAS (3% TS) prior to anaerobic digestion, and they tested the methane production from the treated sludge using biochemical methane potential (BMP) tests. Consistent with the results obtained by Park et al. (2004), sludges pre-treated by microwave (175^{o}C) yielded 31% higher biogas production and enhanced sludge dewaterability by 75%. Park and Ahn (2011) optimized the microwave pre-treatment conditions to maximize methane production from sewage sludge digestion. They obtained the maximum methane yield by using a microwave pre-treatment temperature of 92^{o}C. Toreci et al. (2009) also investigated microwave-pre-treated activated sludge in semi-continuous digestions and obtained significant increases in biogas production rates. Conversely, Climent et al. (2007) pre-treated secondary sludge with a domestic microwave oven (Sharp R-234, 800 W, 2450 MHz) at various power inputs from 520 to 13000 kJ/kg SS, but no significant biogas increase was obtained.

Based on this review, it can be noted that most recent research on microwave pre-treatment has focused on wastewater treatment plant sludge. Although microwave pre-treatment has seldom been applied in co-digestion tests, some applications on substrates other than wastewater treatment plant sludge have been published. Jin et al. (2009) pre-treated dairy manure through a microwave-based thermo-chemical pre-treatment configuration and improved methane production with an optimized microwave treatment of 147° C for 25.3 minutes. Saifuddin and Fazlili (2009) microwaved palm oil mill effluent for 3 minutes at 2450 MHz and 700 W and reported a large increase in methane production. Beszédes et al. (2011) analyzed the effects of microwave pre-treatment on biogas production from dairy and meat industry sludges, respectively. Microwave conditions of 5W/g substrate for 30 minutes was reported as the optimum operational conditions to enhance biogas production from both sludges. In another study, source-separated kitchen waste was pre-treated by microwave (1200 W, 2450 MHz) and a slightly improved cumulative methane production was obtained (Marin et al., 2010). However, contrary to the results obtained from their batch analysis, they observed that the methane production rate decreased using microwave pre-treated substrate in semi-continuous digestion tests (Marin et al., 2010).

The microwave technique generally has the potential to be an effective pre-treatment to enhance biogas production from anaerobic digestion. However, it has also been reported that microwave pre-treatment can cause a reduction in biogas production (Climent et al., 2007; Marin et al., 2010). In addition, most experiments were undertaken using household sized microwave ovens with limited space and volume that can only treat small quantities of waste. For industrial pilot or full-scale applications, reports are limited and cannot effectively support a mature and full-scale microwave pre-treatment facility application, especially for the co-digestion of substrates other than wastewater treatment sludge.

3.9 Biological Pre-treatment

Biological pre-treatment can accelerate hydrolysis through the catalysis of enzymes including lipases, proteinases, cellulases and hydrolases (Doháyos et al., 2004; Mshandete et al., 2005; Schieder et al., 2000).

Hasegawa et al. (2000) added extracellular hydrolytic enzymes including proteases and amylases, which they isolated from thermophilic aerobic bacteria, into the pre-treatment of organic sludge. 40% of the organic wastes were solubilized after biological pre-treatment for 1-2 days. The sludge after the pre-treatment resulted in higher biogas production during anaerobic digestion, and was 1.5 times greater than that from untreated sludge. Mendes et al. (2006) pre-treated a lipid-rich dairy industry wastewater using commercially available low-cost lipase as the enzyme. The best results were obtained when the enzymatic hydrolysis was performed for 12 hours, with higher COD removal and biogas formation.

Although many researchers have reported that biological pre-treatment with enzyme addition can effectively enhance the hydrolysis of the organic compounds, it has also been noted that the biological process is more effective at hydrolyzing smaller cellulose particles but not lipid or grease-rich cells (Fdez-Güelfo et al., 2011; Luste et al., 2011; Mshandete et al., 2005; Zhong et al., 2011). Masse et al. (2001) used the commercially available enzyme Pancreatic Lipase 250 (PL-250; Genencor International, Rochester NY) in the pre-treatment of a lipid-rich slaughterhouse wastewater, to test the hydrolysis and size reduction of fat particles. The enzyme concentrations ranged from 125 mg/L to 1000 mg/L. The higher enzyme concentration was seen to have better particle size reduction and long chain fatty acid (LCFA) degradation. However, in this study the effects of the enzymatic pre-treatment on anaerobic digestion and biogas production were not tested. In order to evaluate the effects of enzymatic PL-250 pre-treatment on anaerobic digestion, Masse et al. (2003) used 250 mg/L PL-250 enzyme to treat 2000 mg/L slaughterhouse wastewater containing pork fat particles for 5.5 h at 25 ℃. The methane production was slightly increased by the pre-treatment. Conversely, Luste et al. (2009) evaluated the methane production from the anaerobic digestion of enzymatically pre-treated meat industry by-products, and they observed that the methane production decreased with the pre-treatment.

From the research summarized here, it is noted that the enzymes applied in the biological pre-treatment for anaerobic digestion can be obtained commercially (Mendes et al., 2006; Masse et al., 2003) or by isolating them from activated organic wastes (Hasegawa et al., 2000). Enzyme isolation would be considered more cost effective, but pure constituents in the commercial enzymes can ensure efficient catalysis and avoid possible inhibition. The complicated structure of the enzymes and the mechanisms of enzymatic reaction dictate that specific enzymes can only function on the adapted substrate hydrolysis. Therefore, the specific components of the substrates need to be evaluated and a suitable enzyme application rate needs to be determined. These would be the main reasons limiting the biological pre-treatment application in anaerobic digestion, particularly in co-digestion.

3.10 Comparison of Pre-treatment Methods

As can be seen from Table 3.1, all pre-treatment methods have been shown to be effective in enhancing biogas production to varying degrees.

Table 3.1 Summary of the comparison of various pre-treatment methods

Methods	Recommended Operational Conditions	Disadvantages	Application to Co-digestion
Thermal	1. Depending on the treatment time, optimum operation temperature should be from 40-150 ℃ but not higher than 175 ℃ (Bonmatí et al., 2001; Müller, 2000). 2. Process below 100 ℃ is more attractive (Gavala et al., 2003).	The process has high energy consumption.	1. Co-digestion of cattle and swine manure by Mladenovska et al. (2006) 2. Co-digestion of slaughterhouse waste and municipal solid waste by Cuetos et al. (2010)
Chemical	1. NaOH is a more effective chemical than acid and other alkalies including KOH, $Mg(OH)_2$, and $Ca(OH)_2$. 2. Pretreatment condition with pH=12 would be the optimum operation	The process has high costs for chemical addition.	1. Co-digestion of WAS and food waste by Heo et al. (2003) and Hidalgo et al. (2012) 2. Co-digestion of cattle manure and corn stover by Pang et al. (2008) and Li et al. (2009)
Thermo-chemical	1. NaOH should be the best agent for thermo-chemical pretreatment (Patel et al., 1993) 2. Pretreatment condition with pH>10 would be the optimum operation (Penaud et al., 2000; Vlyssides and Karlis, 2004) 3. Temperature should be between 50 ℃ and 150 ℃, depending on the treatment time (Kim et al., 2003; Rani et al., 2012)	Thermo-chemical process presents better capability than thermal or chemical pretreatments alone. However, the process consumes amounts of energy and chemicals. High operation and maintenance costs and corrosion problems due to the use of chemicals and high temperature.	Thermo-chemical pretreatment has not been widely tested within anaerobic co-digestion of organic wastes other than sewage sludge or WAS, at least during last decade (Wu et al., 2009, Zarkadas and Pilidis, 2011).

Table 3.1 Summary of the comparison of various pre-treatment methods (Continued)

Methods	**Recommended Operational Conditions**	**Disadvantages**	**Application to Co-digestion**
Mechanical	1. HPH system with 300-600 bar high pressure Engelhart et al., 2000; Kopplow et al., 2004) 2. Ball mills with two counter rotating plates or grinding beads depending on treatment time (Perez-Elvira et al., 2006) 3. Jetting and colliding pre-treatment usually with 5-50 bar pressurized conditions (Carrère et al., 2010).	Operation should be simple and economical but dangerous for high pressure application. Negative factors including corrosion and mechanical abrasion have been mentioned.	Seldom applied in co-digestion
Ultrasonic	1. Ultrasound within the frequency range of 20-40 kHz has been widely applied for pre-treatment (Apul and Sanin, 2010; Neis et al. (2000); Tiehm et al., 1997; Tiehm et al., 2000; Wang et al., 1999). 2. During current decade, most ultrasonic pre-treatment methods applied for enhancing the biogas production from wastewater treatment sludge were set with the frequency of 20 kHz (Benabdallah El-Hadj et al., 2007; Mao and Show, 2006; Saha et al., 2011). 3. Ultrasonic pre-treatment could be defined using power supplied per transducer area (W/cm^2), ultrasonic density relates to the power supplied per sample volume (W/L), ultrasonic dose relates to the energy supplied per sample volume and time (W/s·L), and specific supplied energy (E_s , kJ/kgTS) (Bougrier et al., 2006).	The process does not generate secondary toxic compounds or contribute additional chemical compounds. However, high capital, installation and operation costs are found.	1. Co-digestion of sewage sludge, waste activated sludge (WAS), and municipal kitchen waste (Blank and Hoffmann, 2011). 2. Co-digestion of tannery solid wastes (Kameswari et al, 2011) 3. Co-digestion of dairy cattle slurry and meat-industry by-products (Luste et al., 2012)

Table 3.1 Summary of the comparison of various pre-treatment methods (Continued)

Methods	**Recommended Operational Conditions**	**Disadvantages**	**Application to Co-digestion**
Ozonation	1. The optimum ozone dosages should be between 0.05 and 0.5 g O_3/g TS (Carrère et al., 2010; Yemo et al., 2002). 2. The specific ozone dosage depends on the type of substrate and the operation.	Ozone must be generated on-site before use due to its instability. The equipment and operating costs can be quite high, which may restrict the industrial application.	1. Co-digestion of primary and secondary sludge Weemaes et al. (1999). 2. Co-digestion of tannery solid wastes (Kameswari et al., 2011).
Microwave	1. The 2450 MHz household microwave oven is widely used in the microwave pre-treatment tests (Climent et a., 2007; Park et al., 2004). 2. The specific treatment temperature and time depend on the type of substrate and the operation.	The mature and large volume microwave facilities are rare and must be developed for industrial application.	Seldom applied in co-digestion
Biological	1. The pre-treatment depends on the substrate and dosage of enzymes including lipases, proteinases, cellulases and hydrolases (Dohányos et al., 2004; Mshandete et al., 2005; Schieder et al., 2000). 2. The enzymes can be obtained by purchasing commercially or by isolating in the research laboratory (Hasegawa et al., 2000; Masse et al., 2003; Mendes et al., 2006).	The process is environmental friendly. However, the commercial enzymes are expensive and the specific isolated enzymes are complicated to achieve.	Seldom applied in co-digestion

However, to date, due to their relative process complexities, feasibility of industrial scale implementation, and the high costs and/or energy consumption associated with their installation and maintenance, pre-treatments like ozonation and microwaving have seldom been investigated in pilot or full-scale studies. Although biological pre-treatment can be viewed as the most environmental friendly process, the economical aspects and the need to find the most effective enzyme(s) for the specific substrate(s) would be the main issues limiting its wider application. In addition, it can be seen from Table 3.1 that mechanical, ozonation, microwave, and biological pre-treatments have seldom been tested or applied in anaerobic co-digestion research. Thermo-chemical pre-treatment has not been studied thoroughly within co-digestion processes; nevertheless, it is postulated that this could have a better potential than thermal or chemical pre-treatments alone for the enhancement of anaerobic co-digestion, since it is the combination of these methods and has already been reported as a suitable method for the digestion of sewage sludge (Rafique et al., 2010; Valo et al., 2004).

3.11 Conclusions

In examining the selected articles from 1995 to the present, mechanical, chemical, thermal, thermo-chemical, ultrasonic, ozone, microwave, and biological (enzymatic) methods are the most current and common pre-treatment methods that have been reported for the promotion of biogas production in anaerobic digestion. Each method has shown various advantages for the reduction of organic waste size, homogenization of samples, interruption of the cell structure, and increase in the biodegradation efficiency. However, these different methods have seldom been combined together and compared directly with other pre-treatments.

Most pre-treatment processes are still limited in application to the laboratory-scale. Long-term performance data of pilot or full-scale pre-treatment systems remain limited. For example, microwave experiments have mainly been conducted using household-sized microwave ovens with limited space and volume that can only treat small quantities of waste. Therefore, how to treat large quantities of organic wastes efficiently in industrial, pilot or full-scale applications, and how to achieve mature and large volume treatment facilities remain important questions.

Although various pre-treatments have been well researched for the improvement of anaerobic digestion with wastewater treatment plant sludge, only thermal, chemical, ultrasonic, and ozonation have been tested by a few researchers for anaerobic co-digestion with other organic wastes as co-substrates. This would indicate that, since the pre-treatment could effectively increase the organic solubility of substrates and result in enhanced biogas production, it is still necessary to conduct further research to provide information on reliable pre-treatment methods and operational parameters for pre-treatment application in anaerobic co-digestion.

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Chapter 4

Evaluating and Modeling Biogas Production from Municipal Fat, Oil, and Grease and Synthetic Kitchen Waste in Anaerobic Co-digestions

Abstract: The feasibility of using synthetic kitchen waste (KW) and fat, oil, and grease (FOG) as co-substrates in the anaerobic digestion of waste activated sludge (WAS) was investigated using two series of biochemical methane potential (BMP) tests. Ranges of ideal substrate to inoculum (S/I) ratio were determined for the FOG (0.25-0.75) and KW (0.80-1.26) as single substrates in the first experiment. The second experiment, which estimated the methane production performances of FOG and KW as co-substrates for WAS co-digestion, was conducted based on the optimal parameters selected from the results of the first experiment. Results indicated that co-digestions with FOG and KW enhanced methane production from 117±2.02 mL/gTVS (with only WAS) to 418±13.7 mL/gTVS and 324±4.11 mL/gTVS, respectively. FOG exhibited more biogas production than KW as co-substrate. Non-linear regression results showed that co-substrate addition shortened the lag phases of organic biodegradation from 81.8 h (with only WAS) to 28.3 h with FOG and 3.90 h with KW.

Keywords: Anaerobic co-digestion; fat, oil, and grease; synthetic kitchen waste; methane production; linear and non-linear modelling.

4.1 Introduction

Producing methane from organic residues (e.g. primary and activated sludge) through anaerobic digestion processes has been applied to the on-site, co-generation of electrical power and heat in wastewater treatment plants (Zitomer et al, 2008). This technology can significantly reduce the operating costs at wastewater treatment facilities and stabilize the organic residues. A significant reduction in greenhouse gas emissions can also be realized. However, the anaerobic digestion process has a number of limitations including slow reaction rates compared to aerobic processes, long retention time requirements (20-30 days), sensitivity to waste loads and toxic materials, and its complex operation (Lin et al., 1997; Hwang et al., 1997; Navia et al., 2002). The energy content of the gas may also vary and is dependent on the nature of the substrate.

To enhance biogas production and assist in municipal organic waste management, anaerobic digestion with the addition of co-substrates, i.e. co-digestion, has been considered an effective, low-cost, and commercially flexible approach to reduce process limitations and improve methane yields (Alatriste-Mondragón et al., 2006). However, surprisingly few reports have focused on full-scale applications of this concept, and consequently the successful implementation of this approach in Canadian municipalities has been limited (De Baere, 2000; Mata-Alvarez et al., 2000; Natural Resource Canada, 2002). The City of Kingston (Ontario, Canada), a typical medium-size Canadian municipality, recently upgraded one of its two wastewater treatment facilities (Ravensview Water Pollution Control Plant) to yield higher quantities of methane to decrease on-site operational costs. In this case, an anaerobic co-digestion process with the addition of low-cost municipal organic wastes could also be considered for implementation as an efficient and economical solution. As such, municipally available organic wastes including fats, oils and grease (FOG) and kitchen waste (KW), which can be collected in close proximity to the treatment facility, could be employed as the potential co-substrates. Cotrell (2008) reported a 50% increase in biogas production at a full-scale digester using FOG as a co-substrate. Kabouris et al. (2008) also investigated FOG as a co-substrate and achieved significantly higher methane production. Carucci et al. (2005), Gunaseelan (2004), Gómez et al. (2006), Labatut et al. (2010), and Li et al. (2002) evaluated food wastes, which are also the main components of KW, and were successful in enhancing methane yield production.

To determine the suitability of a specific organic substrate for anaerobic digestion, the biochemical methane potential (BMP) test has been proven to be a relatively simple and reliable method for the comparison of the extent and rates of waste conversion to methane (Hansen et al., 2004; Owen et al., 1979). To evaluate co-digestion using BMP tests, it is important to consider the substrate to inoculum ratio (S/I) on a total volatile solids basis (TVS). Determining the ideal S/I ratio is necessary to achieve the maximum ultimate methane production. Chynoweth et al. (1993) indicated that a S/I ratio = 0.5 may be required to achieve the maximum rate of methane production. A S/I ratio = 0.2 was employed by Heo et al. (2004) for co-digestion using mixtures of food waste and activated sludge. However, it should be noted that the characteristics of organic wastes and inocula can vary considerably, and the organic waste loading rate to the anaerobic digesters is critical in pilot or full-scale applications (Gelegenis et al., 2007). Hence, the selection of a suitable S/I ratio for a specific co-substrate is not only important from a BMP evaluation perspective, but also valuable to provide information for the design of future research and industrial applications. A number of studies have used BMP tests to evaluate the improvement in ultimate methane production through co-digestion and conventional linear regression models have been widely applied to indicate the first order production rate (Carucci et al., 2005; Hansen et al., 2004; Heo et al., 2004). However, the application of both linear regression and modified non-linear regression models has seldom been employed in combination with BMP tests to estimate the time required to reach full digestion and to characterize reaction phases (e.g. lag phase, exponential phase, and steady phase), particularly in the BMP tests using fat and oil-rich wastes (Davisson et al., 2008; Luostarinen et al., 2009; Zhu et al., 2011). Hence, if each reaction phase with specific substrate or co-substrate addition could be estimated and compared using BMP tests, the results could also provide valuable information for future work (e.g. continuous-flow reactor configuration) aimed at determining optimal substrate addition ratios and loading rates.

The main objective of this research was to identify and select suitable co-substrates through a series of biochemical methane potential (BMP) tests. A series of co-substrate mixtures were evaluated under applicable operating conditions and the best co-substrates and mixing ratios were identified. Methane production capacities of the co-substrates were compared. Linear and non-linear regression models were developed to assist in the interpretation of the results. The results obtained from this study will provide valuable fundamental information for future research and renewable energy development in Canadian municipalities.

4.2 Material and Methods

4.2.1 Inocula and Substrates

The inoculum used for initiating the digestions was anaerobic digester sludge (ADS) collected from the mesophilic anaerobic digesters at the Cataraqui Bay Wastewater Treatment Plant (Kingston, Ontario, Canada). The sludge was collected and stored at 4 ℃ for no more than 2 days before utilization. Municipally available organic wastes including concentrated waste activated sludge (WAS), fats, oils and grease (FOG) and synthetic kitchen waste (KW) collected in close proximity to the treatment facility were investigated as potential co-substrates and tested in single-substrate digestion and the subsequent co-digestion BMP tests.

The WAS used throughout the experiments was obtained from the Cataraqui Bay Wastewater Treatment Plant. The sludge was stored at 4 ℃ for 2 days, and then the supernatant was decanted to produce the concentrated waste activated sludge (WAS). The FOG was collected from the garbage oil receptacle of the Graduate Club, a restaurant located on the Queen's University campus (Kingston, Ontario, Canada), which consisted of a mixture of waste frying oil, bacon grease, and animal fat. Since the FOG was produced from similar materials that were used on a daily basis in the restaurant and presented similar characteristics as shown in Table 4.1, it was collected before each test and stored in a glass bottle at 4 ℃. The synthetic KW was generated using a mixture of potato (40 g), strawberry (16 g), orange (32 g), tomato (72 g), chicken breast (52 g), apple (24 g), green peas (40 g), cabbage (50 g), pork (20 g), and distilled water (24 mL) as per the synthetic kitchen waste recipe advanced by Neves et al. (2002) and Li et al. (2002). It should be noted that, as the components of the actual KW can generally vary considerably, the synthetic KW was employed to provide results that were reproducible for the two experiments, and to ensure that the results from these experiments were reliable and comparable. The fruits, vegetables, and meats applied in this synthetic KW recipe were obtained from the municipal disposal facilities (e.g. green bins for organic wastes around the City of Kingston, Ontario, Canada).

Characteristics of the inocula and substrates utilized in the study are listed in Table 4.1.

Table 4.1 Characteristic of the inocula and substrates in single-substrate digestion and co-digestion experiments

	Single-substrate Digestion Experiment			***Co-digestion Experiments***			
Substrates	*ADS	*KW	*FOG	*ADS	*WAS	*KW	*FOG
Density (g/mL)	0.99 ±0.01	1.11 ±0.05	1.01 ±0.01	1.03 ±0.01	1.01 ±0.001	1.08 ±0.01	0.96 ±0.001
pH	7.5	4.7	4.0	7.7	7.1	4.4	4.1
TS(mg/g substrate)	23.6 ±0.02	124 ±4.45	947 ±33.8	31.5 ±0.12	5.9 ±0.18	118 ±1.51	999 ±0.67
TVS(mg/g substrate)	16.7 ±0.10	114 ±6.30	942 ±33.9	21.4 ±0.28	4.6 ±0.68	111 ±1.37	961 ±10.1
TVS/TS %	70.9	92.1	99.6	67.9	77.4	93.6	96.2
Total COD (g/L)	--	--	--	11.6	3.50	52.4	375

ADS=Anaerobic digester sludge; WAS=Waste activated sludge; KW=Synthetic kitchen waste; FOG=Fat, oil, and grease; *=With standard deviation.

4.2.2 Experimental Procedure and Sample Analysis

In this study, two series of BMP tests were conducted to assess methane production with different organic wastes. In the single-substrate digestion experiment, the substrates including FOG and KW were investigated as single substrates mixed with the ADS inoculum at different S/I ratios (Table 4.2) on a total volatile solid (TVS) basis. Ideal single substrates and S/I ratios were selected based on the results obtained from the single-substrate digestion experiment for the subsequent co-digestion experiment. In the co-digestion experiment, the KW and FOG were alternatively tested as co-substrates to enhance the biogas production from the WAS substrate. The co-substrates were mixed with prepared WAS and inocula within optimized S/I ratio ranges based on the results obtained from the single-substrate digestion experiment. The experimental conditions are shown in Table 4.2. In co-digestion experiments, various operating parameters can influence biogas production. Initial and/or final pH, digestion time, chemical oxygen demand (COD), total solids (TS), and total volatile solids (TVS) were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1995). Volatile fatty acids (VFA) in the final solutions were analyzed using ion chromatography (Dionex ICS-3000, column IONPAC AS11).

Table 4.2 Inoculum and substrate compositions in single-substrate digestion and co-digestion experiments

Experiment 1				***Experiment 2***				
Duplicated Sample	*Inoculum ADS (mL)*	*Single Substrates (KW or FOG) (g)*	*S/I Ratio*	*Duplicated Sample*	*Inoculum ADS (mL)*	*Substrate AS (mL)*	*Co-Substrates (KW or FOG) (g)*	*S/I Ratio*
B01 B02 BLANK	40	0	0.00	B01 B02 BLANK	40	0	0	0.00
B03 B04 KW	40	12	2.08	B03 B04 Semi-Blank	40	15	0	0.08
B05 B06 KW	40	6	1.03	B05 B06 KW	40	15	3	0.46
B07 B08 KW	40	3	0.52	B07 B08 KW	40	15	6	0.83
B09 B10 FOG	40	1.4	2.00	B09 B10 KW	40	15	9	1.20
B11 B12 FOG	40	2.8	4.00	B11 B12 KW	40	15	12	1.59
B13 B14 FOG	40	0.7	1.00	B13 B14 FOG	40	15	0.2	0.30
B15 B16 FOG	40	0.35	0.50	B15 B16 FOG	40	15	0.35	0.46
				B17 B18 FOG	40	15	0.7	0.85
				B19 B20 FOG	40	15	1.4	1.61

ADS=Anaerobic digester sludge; WAS=Waste activated sludge; KW=Synthetic kitchen waste; FOG=Fat, oil, and grease; S/I Ratio=Substrate to inoculums ratio based on total volatile solid; B=BMP test, e.g. B01=BMP Test 01.

In the two BMP experiments, the mixtures of 40 mL inocula and substrates were transferred to 250 mL septum top glass bottles based on S/I ratios with a headspace volume around 190 mL (Table 4.2) and flushed with high pressure N_2 gas for 30 seconds prior to sealing to ensure all the bottles were under anaerobic conditions. The flushing method was modified from the approaches of Owen et al. (1979) and Hansen et al. (2004). The bottles were then incubated in a New Brunswick 4500 incubation shaker at 37 ℃ and 100 rpm. Biogas samples were collected from the headspaces of the BMP bottles using a pressure lock syringe (VICI® A-2). The contents of the gas samples including carbon dioxide and methane were analyzed by gas chromatography (Varian 3400 with HayeSep® Q Micropacked column, TCD detector). The results of the gas analysis were reported on the basis of cumulative methane at standard temperature and pressure (STP, 101.325kPa, 273.15 °K) and normalized in terms of total original organic matter present in each sample with units of CH_4 mL/g TVS (Mladenovska et al., 2006; Moller et al., 2004; Nielsen et al., 2004; Owen et al., 1979). All experiments were conducted in duplicate.

4.2.3 Data Analysis

Empirical linear and non-linear regression models were developed and fitted to evaluate co-digestion performance under each of the BMP testing conditions. The aim of the linear regression model was to estimate the first-order biogas production rate (k, h^{-1}) and the time to reach steady state (t_{steady}, h) for each experimental condition. As the linear regression models are generally assumed to follow first order regression, they cannot be employed to accurately predict the entire degradation process which includes lag, and steady state phases in the biogas production process. Hence, the aim of the non-linear regression model was to estimate the lag phase duration time (λ,h), predict the ultimate cumulative biogas production (B_0, mL/gTVS), and indicate maximum biogas production rate (R_m, mL/gTVS h). The coefficient of determination (R^2) and confidence intervals which were derived from statistical analysis using mean, degrees of freedom, the standard error, and critical t value were quantified to indicate the accuracy of the models.

Generally, the lag and exponential phases of biogas production can be assumed to follow a first order rate and be described by the linear regression model shown in Equation 4.1 (Gunaseelan, 2004):

$$B = B_0(1 - e^{-kt}) \qquad (4.1)$$

where B_0 is the ultimate methane yield or methane production potential (mL/gTVS), B is the cumulative methane yield (mL/gTVS) at incubation time t (h), k is the first order methane production rate constant with the unit of h^{-1}, and e is equal to 2.7183.

However, linear regression models cannot accurately describe and predict cumulative methane production through the entire process, especially after the exponential phase. Hence, in order to assess and compare the methane production from different substrates through the digestion process, non-linear regressions were utilized to achieve representative simulations and predictions. In this study, the modified Gompertz equation was applied and is shown in Equation 4.2 (Donoso-Bravo et al., 2010; Lo et al., 2010):

$$B = B_0 \exp\left\{ - \exp\left[\frac{R_m e}{B_0}(\lambda - t) + 1 \right] \right\} \qquad (4.2)$$

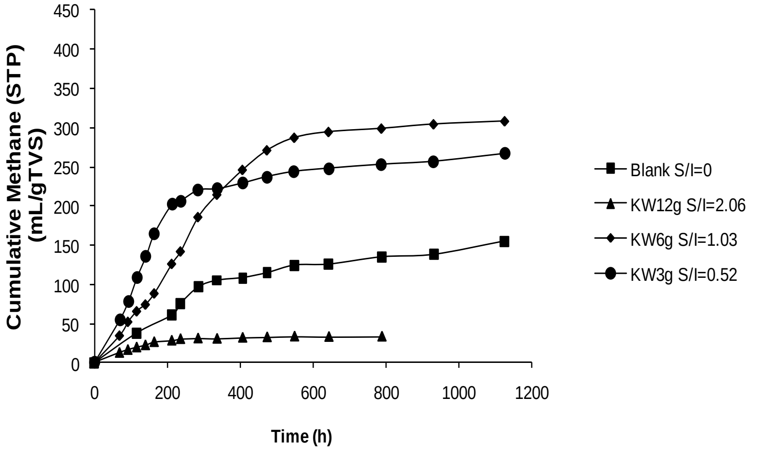
where R_m is the maximum methane production rate (mL/gTVS·h), and λ is the lag phase duration time (h).

According to the assumptions of the linear regression model, the time to steady state (t_{steady}, h) could be estimated using Equation 1. Combined with the lag phase duration time (λ, h) estimated from Equation (2), the exponential phase ($t_{\mathrm{exponential}}$, h) of the methane production process could be assessed.

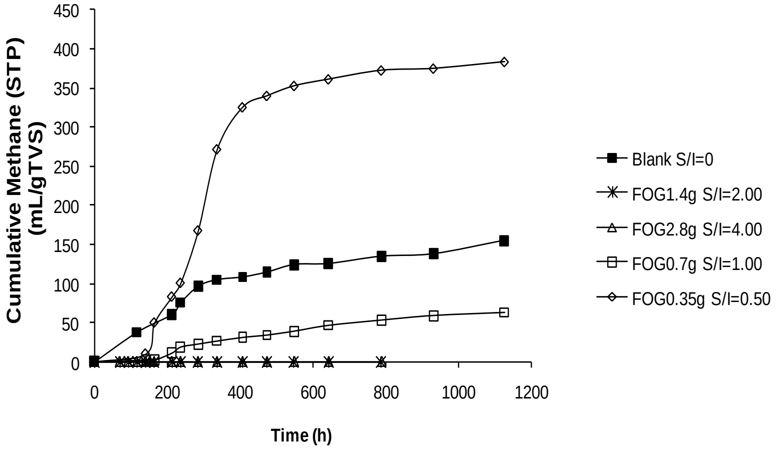
4.3 Results and Discussion

4.3.1 Single-Substrate Digestion: Selection of Suitable Substrates and S/I ratio ranges

Locally collected FOG and synthetic KW were first tested as single substrates through BMP tests to estimate the ideal S/I ratios for the subsequent co-digestions (Table 4.2). The cumulative methane production obtained from the different mixtures is presented in Figure 4.1 (a) and (b). Experimental results showed that the digestion using 6 g KW with S/I ratio=1.03 had a higher cumulative methane production (308±6.89 mL/gTVS, STP) than the other digestions using KW with different S/I ratios. Similarly, the digestion using 0.35 g FOG with S/I ratio=0.50 yielded higher cumulative methane production (383±7.19 mL/gTVS, STP) than other FOG digestions. According to Figure 4.1(a), most digestions using KW with the exception of 12.0 g KW yielded higher methane productions than the blank controls. However, in FOG digestions, only the 0.35 g FOG digestion yielded higher cumulative methane production than the blanks. It can also be seen from Figure 4.1(b) that digestions using more than 1.40 g FOG and S/I ratio higher than 2.00 resulted in negligible methane accumulation. This would indicate that the anaerobic consortium was much more sensitive to FOG than KW as the initial TVS and COD concentrations were much higher in FOG than in KW. Hence, the substrate loading in FOG digestions is likely one of the most important parameters in the process and the suitable S/I ratio ranges obtained from the BMP tests could contribute fundamental information regarding the organic and substrate design loading rates for pilot or full-scale reactor operations.



(a)



(b)

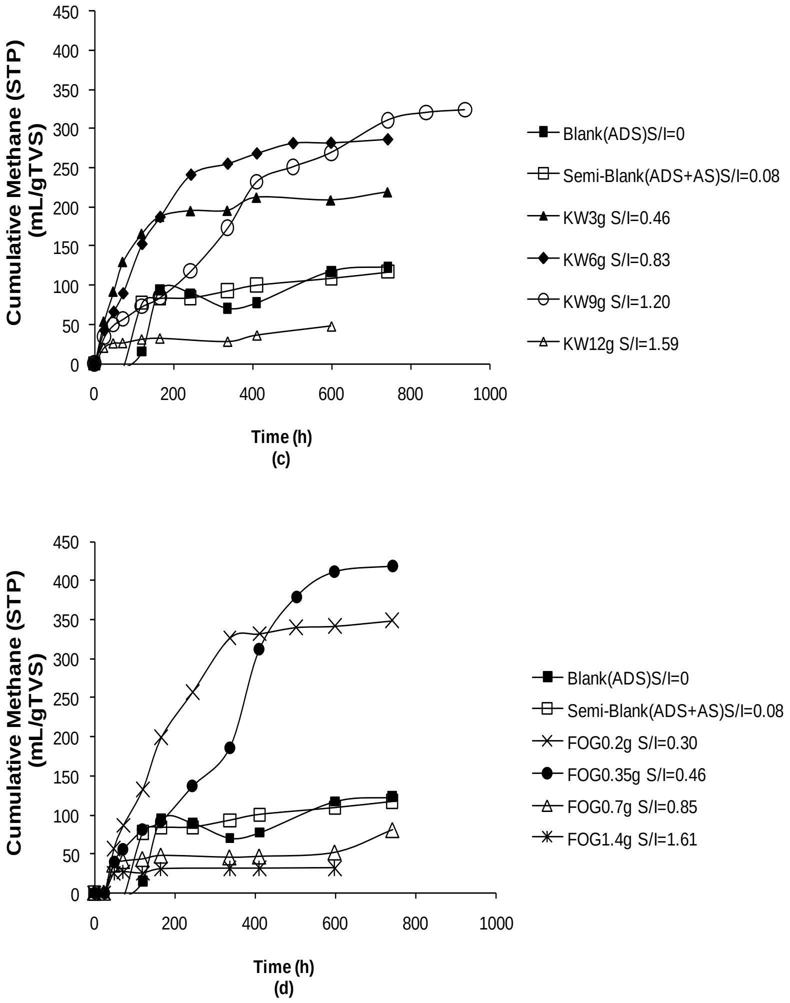
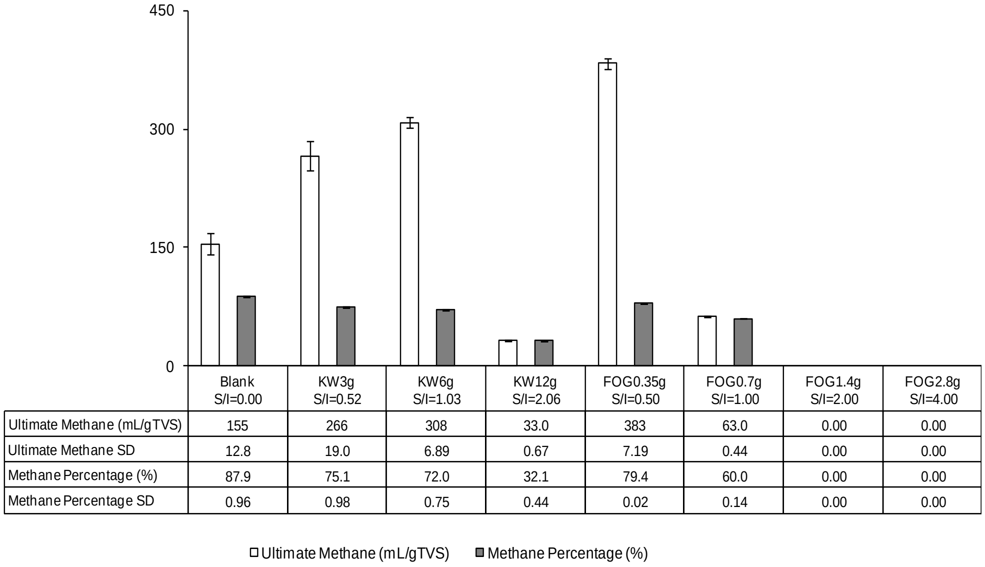


Figure 4.1 Cumulative methane production (STP, mL/gTVS) from single-substrate digestion experiment using (a) KW (synthetic kitchen waste) and (b) FOG (fat, oil, and grease) as the single substrates and from co-digestion experiment using (c) KW and (d) FOG as the co-substrates.

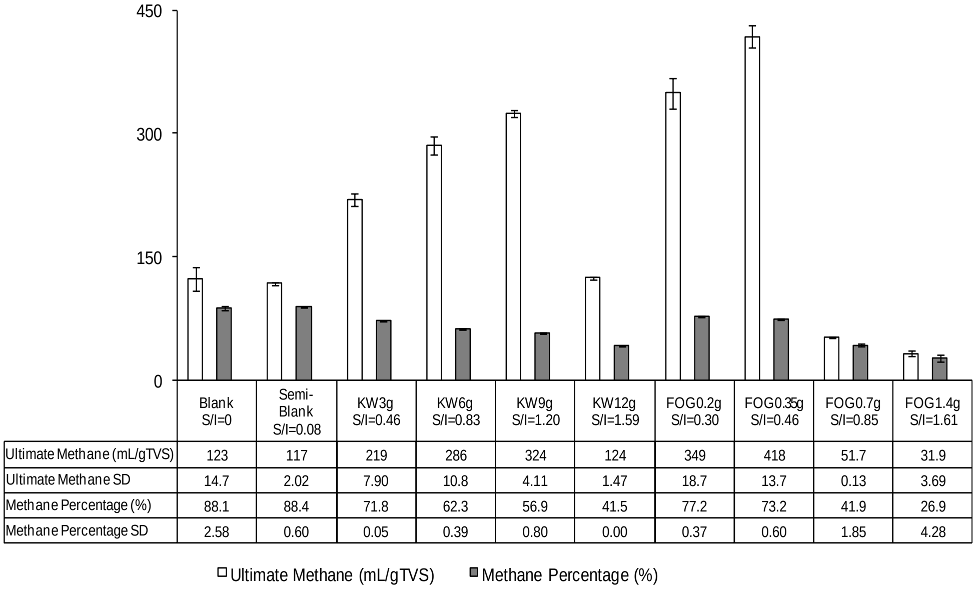
From Figure 4.2(a), it can be noted that 0.35 g FOG achieved the highest methane production, the ultimate methane percentage of the digestion was 79.4±0.02%, which was 9.4% (P=0.045) higher than the percentage obtained from the digestion with 6.0 g KW with a S/I ratio=1.03. Although the ultimate methane percentage decreased with increasing S/I ratios, the ultimate methane production volume increased with increasing S/I ratios until the S/I ratio was higher than 1.03 and 1.00 in KW and FOG, respectively.

The relations between S/I ratios and ultimate methane production volumes, and the relations between S/I ratios and the ultimate methane percentages were examined and are presented in Figure 4.3(a) and (b). Based on Figure 4.3(a), it can be estimated that the optimal ultimate methane volume and percentage from KW could be achieved from digestions with S/I ratios between 0.80 and 1.26. As the 1.4 g and 2.8 g FOG digestions generated negligible methane production, the ideal S/I ratio for FOG digestion was estimated to be between 0.25 and 0.75. Comparing Figures 4.2 (a) and (b) and Figure 4.3(a) and (b), it could be concluded that, based on the S/I ratios investigated in this study, ideal single-substrate digestions were obtained from 6 g KW with a S/I ratio=1.03 and 0.35g FOG with a S/I ratio= 0.50. In addition, as 0.35g FOG achieved the highest ultimate methane percentage, it could be considered the best for methane production within these single-digestions.



	Blank S/I=0.00	KW3g S/I=0.52	KW6g S/I=1.03	KW12g S/I=2.06	FOG0.35g S/I=0.50	FOG0.7g S/I=1.00	FOG1.4g S/I=2.00	FOG2.8g S/I=4.00
Ultimate Methane (mL/gTVS)	155	266	308	33.0	383	63.0	0.00	0.00
Ultimate Methane SD	12.8	19.0	6.89	0.67	7.19	0.44	0.00	0.00
Methane Percentage (%)	87.9	75.1	72.0	32.1	79.4	60.0	0.00	0.00
Methane Percentage SD	0.96	0.98	0.75	0.44	0.02	0.14	0.00	0.00

(a)



	Blank S/I=0	Semi-Blank S/I=0.08	KW3g S/I=0.46	KW6g S/I=0.83	KW9g S/I=1.20	KW12g S/I=1.59	FOG0.2g S/I=0.30	FOG0.35g S/I=0.46	FOG0.7g S/I=0.85	FOG1.4g S/I=1.61
Ultimate Methane (mL/gTVS)	123	117	219	286	324	124	349	418	51.7	31.9
Ultimate Methane SD	14.7	2.02	7.90	10.8	4.11	1.47	18.7	13.7	0.13	3.69
Methane Percentage (%)	88.1	88.4	71.8	62.3	56.9	41.5	77.2	73.2	41.9	26.9
Methane Percentage SD	2.58	0.60	0.05	0.39	0.80	0.00	0.37	0.60	1.85	4.28

(b)

Figure 4.2 Comparison of methane production capacities in (a) different single-substrate digestion experiments using KW (synthetic kitchen waste) and FOG (fat, oil, and grease) as single substrates and in (b) different co-digestion experiments using KW and FOG as co-substrates with standard deviation (SD).

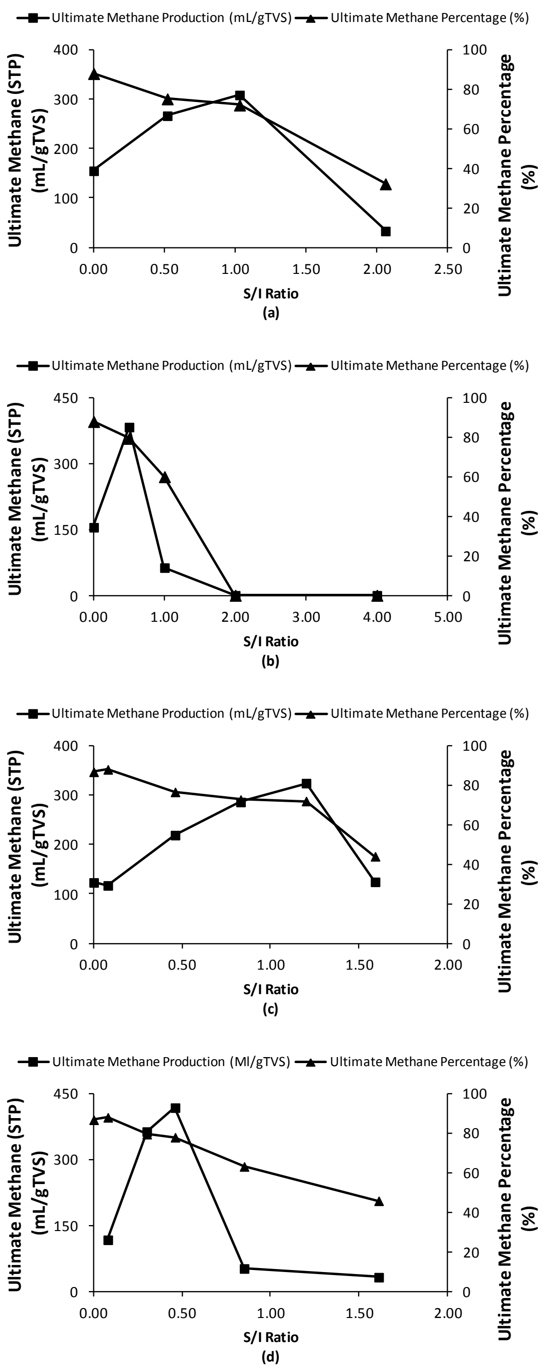


Figure 4.3 Correlations of the (a) KW(synthetic kitchen waste) and (b) FOG(fat, oil, and grease) S/I ratios, ultimate methane production (STP, mL/gTVS) and ultimate methane percentage (%) in single-substrate digestion experiment and correlations of the (c) KW and (d) FOG I/S ratios, ultimate methane production (STP, mL/gTVS) and ultimate methane percentage (%) in co-digestion experiment.

4.3.2 Co-Digestion Experiment: Biogas Production in Co-digestions

4.3.2.1 *Effects of Co-substrate Addition on Methane Production Performance*

Based on the results obtained from the single-substrate digestion experiment, the co-digestion experiment was elaborated using the ideal S/I ratios for KW and FOG in the co-digestion with WAS (Table 4.2). With the use of ideal S/I ratios, all co-digestions accumulated substantial volumes of methane. However, it can be noted from Figure 4.1(c) and (d) that the co-digestions using 12 g KW with S/I=1.59, 0.7 g FOG with S/I=0.85, and 1.4 g FOG with S/I=1.61 did not improve methane production. The methane production limitation could be attributed to the high S/I ratios which were not within the ideal ranges suggested by single-substrate digestion experiment and described earlier. In addition, from Table 4.3 it can be seen that the final VFA and COD concentrations of these three co-digestions were much higher than others, which led to the lower pH conditions observed in these co-digestions and could have impacted the anaerobic digestion process. Anaerobic digestions need a critical mass of microorganisms and adequate nutrient concentrations in the inocula to promote methanogenic activity for methane production (Caruci et al., 2005). The VFA and COD analyses (Table 4.3) suggested that higher organic mass loading could have resulted in the inhibition of these methanogens to support effective methanogenic activity. Consequently, although the organic loading in 12.0 g KW, 0.7 g FOG, and 1.4 g FOG co-digestions could produce sufficient microorganisms from the inocula to degrade the co-substrates into simple organic compounds (e.g. VFAs) in the hydrolysis and acetogenesis phases, further methanogenesis to form methane was limited (Kabouris et al., 2008). The other co-digestions generally presented significant improvements in methane production. 9.0 g KW with S/I=1.20 and 0.35 g FOG with S/I=0.46 achieved higher ultimate methane volumes and methane percentages than the other treatments, and it is noted that these two co-digestions had S/I ratios within the ranges recommended from the results of the single-substrate digestion experiment. However, it should also be noted that although 3.0 g KW and 0.35 g FOG co-digestions had the same S/I ratios (0.46), their digestion performances were different, which would imply that the optimal S/I ratios for KW and FOG are likely very different and dependent on the composition of organic constituents. As shown in Figure 4.2(b), similar to the single-digestion results, the 0.35 g FOG co-digestion achieved the highest methane production (418 ± 13.7 mL/gTVS) and the low final VFA concentrations. The increase in ultimate methane production and methane percentage did not follow the S/I ratio linearly. However, it could be noted from Figure 4.2(b) and Figure 4.3 that the methane percentage did decrease with the increase of the S/I ratios and the addition of the co-substrates. The relationships between the S/I ratios and methane production capabilities were estimated in Figure 4.3(c) and (d) to ascertain the optimal S/I ratio that would maximize the ultimate methane production based on volume and percentage. Based on Figure 4.3 (c) and (d), the optimal S/I ratios for KW and FOG in these co-digestions were proposed to be around 0.80 and 0.46, and within the ideal S/I ratio ranges from single-substrate digestion experiment, respectively.

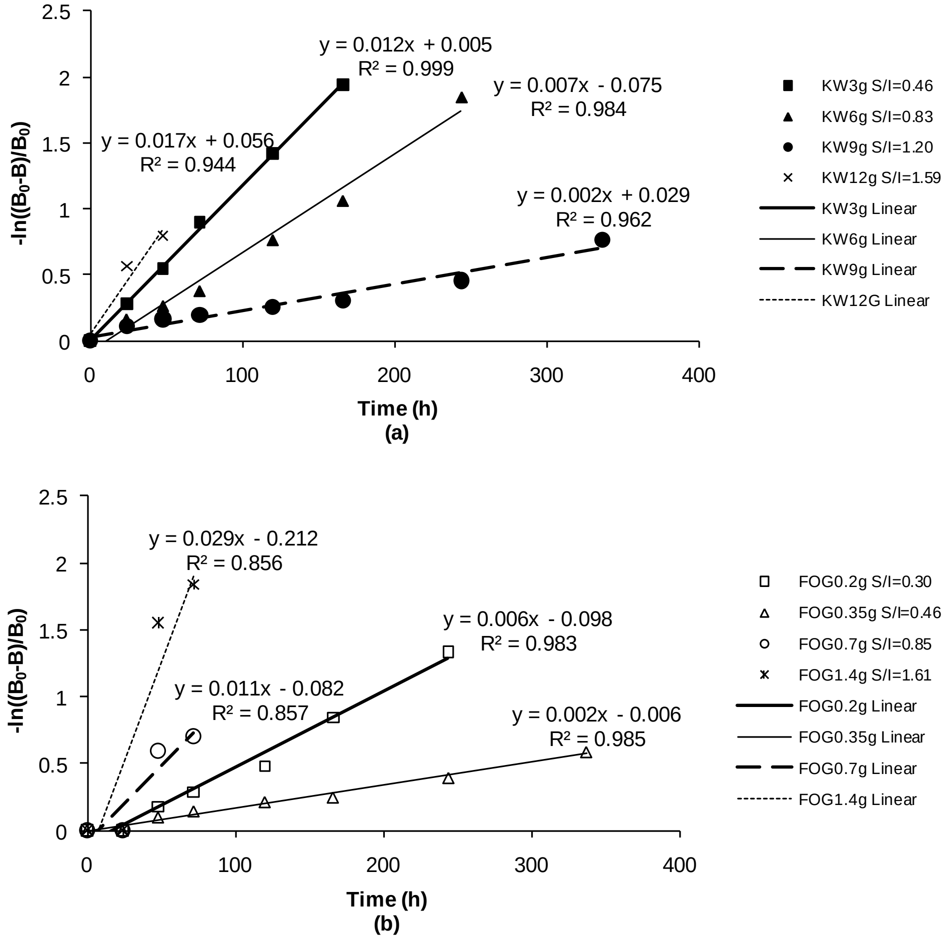
Table 4.3 Composition of final BMP test solutions after co-digestion experiment

SAMPLE	*Acetic acid (mg/L)*	*Propionic acid (mg/L)*	*Isobutyric acid (mg/L)*	*Butyric acid (mg/L)*	*pH*	**COD (g/L)*	**Ultimate CH4 (mL/gTVS)*
Blank S/I=0	<2.5	<2.5	174	<2.5	8.2	8.06±0.24	123±14.7
Semi-Blank S/I=0.08	<2.5	<2.5	129	<2.5	7.9	5.81±0.16	117±2.02
KW3g S/I=0.46	<2.5	<2.5	120	<2.5	7.8	4.68±0.14	219±7.90
KW6g S/I=0.83	<5	<5	113	<5	7.9	6.93±0.21	286±10.8
KW9g S/I=1.20	<5	<5	222	<5	7.9	7.50±0.22	324±4.11
KW12g S/I=1.59	6980	2950	995	<250	7.0	26.6±0.78	124±1.47
FOG0.2g S/I=0.30	<2.5	85.9	<2.5	<2.5	7.7	4.12±0.16	364±18.7
FOG0.35g S/I=0.46	28.4	90.3	<2.5	<2.5	7.6	8.62±0.30	418±13.7
FOG0.7g S/I=0.85	1310	1480	2470	<100	6.8	21.0±0.65	51.7±0.13
FOG1.4g S/I=1.61	1810	1940	1130	141	6.8	18.8±0.57	31.9±3.69

*=With Standard Deviation

4.3.2.2 *Modelling of the Co-digestions*

Generally, linear regression models as shown in Equation 4.1 assume that the lag phase and exponential phase of the biogas production follow a first order rate of production. Linear regressions can be used to estimate the first order methane production rate constant k (h^{-1}) and the onset of steady state biogas production. However, as previously stated, linear regression cannot be used to describe the full digestion process accurately and therefore cannot estimate total biogas production effectively. Hence, to evaluate the co-digestion performance parameters, linear and non-linear regressions were jointly utilized, with these results shown in Figures 4.4 to 4.6. The more critical estimated digestion parameters are summarized in Table 4.4.



(a)

(b)

Figure 4.4 Linear regression for the cumulative methane production during co-digestion at different I/S ratios with (a) KW(synthetic kitchen waste) and (b) FOG(fat, grease, and oil) as co-substrates.

Table 4.4 Parameters estimated from linear and non linear regressions for co-digestion experiment

	Non-linear Regression				*Linear Regression*			*Experimental CH4 Production**	*Estimated Exponential Time*
	Estimated B_0 (mL/gTVS)	λ (h)	R_m (mL/gTVS h)	R^2	*k* (h^{-1})	*t steady* (h)	R^2	Experimental B_0 (mL/gTVS)	*t exponential* (h)
Blank	95.2	116	3.93	0.913	-	-	-	123 ±14.7	-
Semi-Blank	98.4	81.0	2.21	0.964	-	-	-	117 ±2.02	-
KW3 g S/I=0.46	203	3.90	2.06	0.980	0.012	166	0.949	219 ±7.90	162
KW6 g S/I=0.83	274	13.6	1.45	0.990	0.007	244	0.984	286 ±10.8	230
KW9 g S/I=1.20	335	16.1	0.59	0.990	0.002	337	0.962	324 ±4.11	321
KW12g S/I=1.59	38.2	10.2	1.95	0.917	0.017	48	0.944	124 ±1.47	37.8
FOG0.2 g S/I=0.30	343	28.3	1.51	0.992	0.006	244	0.983	363 ±18.7	216
FOG0.35g S/I=0.46	468	72.3	0.93	0.991	0.002	337	0.985	418 ±13.7	265
FOG 0.7g S/I=0.85	46.1	33.6	2.83	0.980	0.011	72	0.857	51.7 ±0.13	38.4
FOG 1.4g S/I=1.61	29.6	38.6	3.32	0.971	0.029	72	0.856	31.9 ±3.69	33.4

KW=Synthetic kitchen waste; FOG=Fat, oil, and grease; B_0= Ultimate cumulative biogas production; λ = Lag phase duration time; R_m = Maximum biogas production rate; *k* =First-order biogas production rate; t_{steady}= Time to reach steady state; $t_{exponential}$= Length of the exponential time; *=With standard deviation.

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Figure 4.4 presents the linear regression of the methane production during the lag and exponential phases of the co-digestions. Figure 4.4(a) shows that, as the S/I ratios increased with KW addition as the co-substrate, the methane production rate decreased through the lag and exponential phases until the S/I ratio was higher than 1.59. It should be noted that although the KW co-digestion with S/I ratio=1.59 presented a 0.017(h^{-1}) first order constant which was higher than other KW co-digestions, this condition did not correspondingly improve ultimate methane production significantly as was noted from Figures 4.1 (c) and (d) and Figure 4.2(b). In addition, as the R^2 of the linear regression for the 12 g KW co-digestion was relatively low (0.944), the linear regression cannot be used to fit the lag and exponential phases of the co-digestion. This would indicate that KW co-digestions with S/I ratio ≥ 1.59 resulted in limited substrate biodegradation and methane production, while the co-digestion performance did not change with S/I ratio for other KW co-digestions. This result is consistent with the results shown in Figure 4.2(b). Similarly, and consistent with Figure 4.1(d) and Figure 4.2(b), as shown in Figure 4.4(b) co-digestions with FOG additions ≥ 0.7 g or I/S ratios ≥ 0.85 did not result in any significant increases in cumulative methane production. The FOG co-digestion that achieved highest methane production (418 mL/gTVS) was with the addition of 0.35g FOG. The results from the 0.35 g FOG co-digestion process fitted the linear regression model with R^2=0.985, which was higher than the R^2=0.857 (14.94%, P=017) and R^2=0.856 (15.07%, P=0.010) values obtained for the 0.7 g FOG and 1.4 g FOG co-digestion processes, respectively. In addition, the best KW co-digestion using 9 g KW with a 324±4.11 mL/g TVS methane production presented a better fit to the linear regression model with a R^2=0.999 which was higher than the R^2=0.944 obtained in the 12 g KW co-digestion. Based on the best coefficient of determination (R^2) obtained for each co-digestion using linear regression, the onset of steady state for each co-digestion could be estimated and are summarized in Table 4.4. It could also be noted that the time to reach steady state in the co-digestions aimed at methane production enhancement with the mass of co-substrate addition.

Figure 4.5 (e) and (f) show the non-linear regressions of the blank treatment with ADS and the single digestion with WAS (semi-blank) with confidence intervals. The non-linear regressions were shown to fit the entire co-digestion process phases including the lag, exponential, and steady state phases, which could enable discussions using mathematical and statistical parameters estimated from the regression models. It can be noted from Table 4.4 that the lag phases of the blank and the single digestion were 116 h and 81.8 h, respectively, which implied that a longer incubation time was necessary for microbial growth and accumulation in the original ADS inoculums to ensure the full digestion of the substrates. With the addition of the single substrate WAS, the estimated lag phase decreased to 81.8 h. Hence, co-substrate addition would be expected to shorten the lag phase as various nutrients present in the co-substrates should positively affect digestion performance. The non-linear regressions for KW and FOG as co-substrates shown in Figures 4.5 and 4.6, and the estimated parameters summarized in Table 4.4 further support these assumptions.

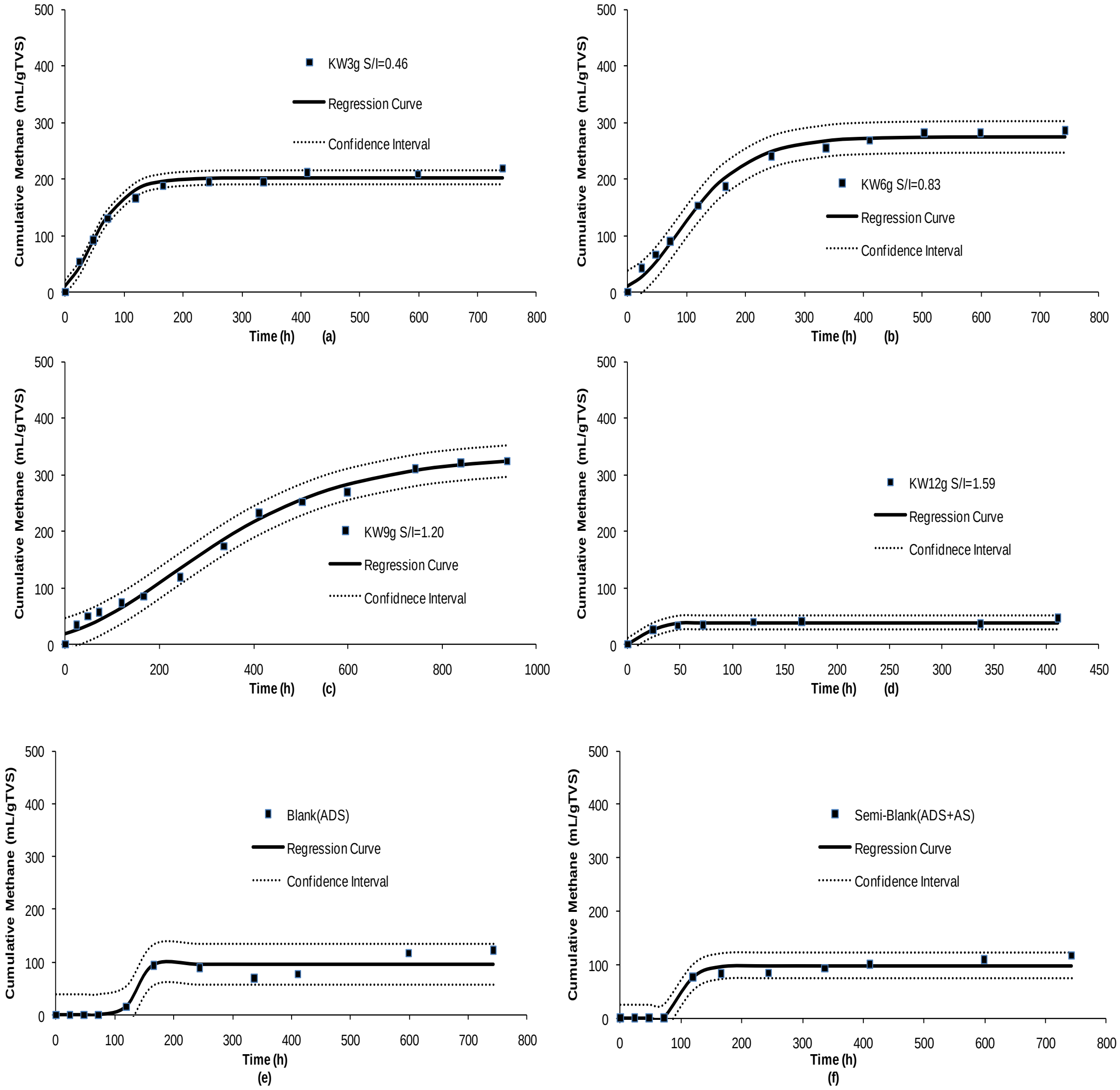

Figure 4.5 Non-linear regression for the cumulative methane production (STP, mL/gTVS) during co-digestion of KW (synthetic ketchen waste) (a) 3g I/S ratio=2.19, (b) 6g I/S ratio=1.20, (c) 9g I/S ratio=0.83, and (d)12g I/S=0.63; and non-linear regression for the cumulative methane production during co-digestion of (e) Blank and (f) Semi-Blank treatments.

Figure 4.5 illustrates the non-linear regressions of the KW co-digestions. Similar to the predictions based on Figure 4.5 (e) and (f), the lag phase decreased as shown in Table 4.4. All lag phases of the KW co-digestions were much shorter than those observed for the ADS single-digestion. All non-linear regressions fit the KW co-digestions well except for 12 g KW and S/I ratio=1.59, which is consistent with the linear regression results. This indicates that 12 g KW co-digestions did not follow the modified Gomertz growth curve and that the KW mass loading was likely too high to be biodegraded efficiently. Other KW co-digestions fit the modified Gomertz regression well and the maximum methane production rates decreased with increasing S/I ratio, which is consistent with the discussion presented based on the linear regressions. Therefore, to ensure sufficient biodegradation of the organics and efficient methane production enhancement in KW co-digestions, an S/I ratio ≤ 1.59 should be employed.

Non-linear regressions of the FOG co-digestions are presented in Figure 4.6. All lag phases of the FOG co-digestions were shorter than those of the single-digestions as predicted. However, all FOG co-digestion lag phases were longer than those of the KW co-digestions. This could be attributed to the FOG composition, which would consist of a higher concentration of more complex long chain fatty acids that require longer times for degradation and result in higher final concentrations of VFA and COD as shown in Table 4.3 (Wakelin and Forster, 1997). The co-digestion using 0.35 g FOG with S/I ratio = 0.46 required 72.3 h to reach the exponential phase and obtained the highest estimated ultimate methane production (468 mL/gTVS) in the FOG co-digestions. Although the co-digestions using 1.4 g and 0.7 g FOG exhibited shorter lag phases than the 0.35 g FOG co-digestion, it was noted that their ultimate methane productions were lower than the ultimate methane obtained from the blank and WAS single-substrate digestions. Therefore, only 0.2 g and 0.35 g FOG co-digestions exhibited sufficient methanogenic activities. Similar to the discussion for the KW co-digestion regressions, the maximum methane production rates in the 0.2 g and 0.35 g FOG co-digestions increased with decreasing S/I ratio, which was consistent with the results from the linear regressions. Hence, in order to achieve efficient methane production performance, the maximum S/I ratio for FOG co-digestions should be lower than 0.85.

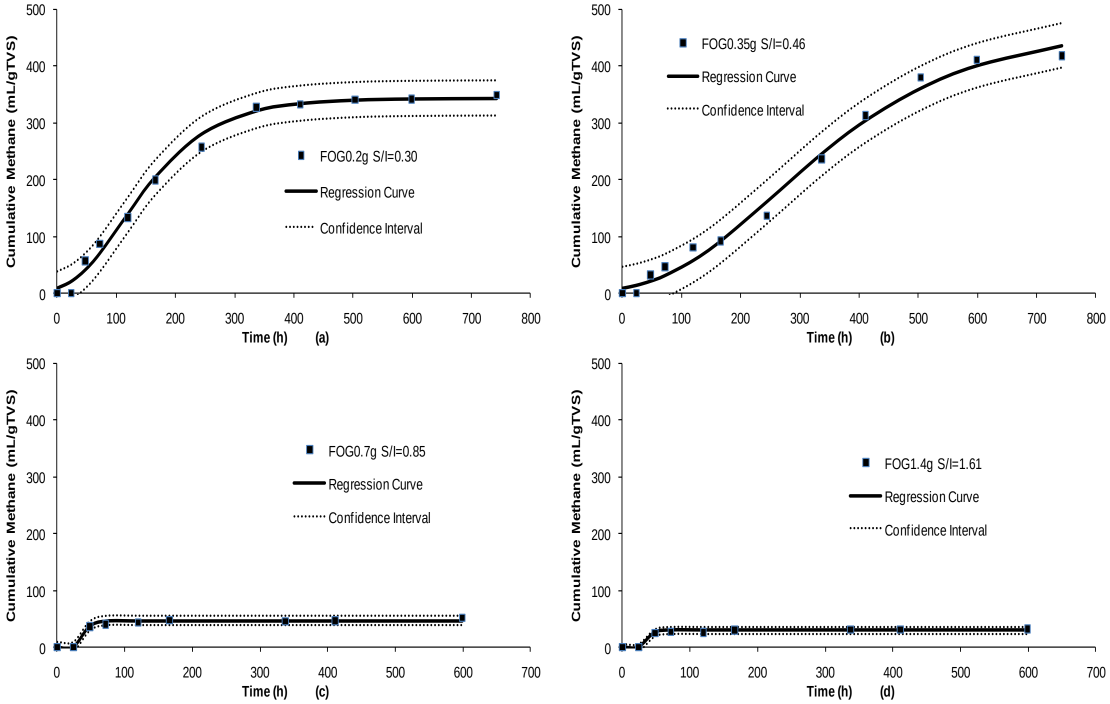

Figure 4.6 Non-linear regression for the cumulative methane production (STP, mL/gTVS) during co-digestion of FOG (fat, oil, and grease) (a) 0.2 g I/S ratio=3.37, (b) 0.35 g I/S ratio=2.17, (c) 0.7 g I/S ratio=1.18, and (d)1.4 g I/S=0.62

It could be noted from Table 4.4 that the estimated lag phase duration time (λ, h) for each co-digestion with methane production enhancement (e.g. 3g KW, 6g KW, 9g KW, 0.2g FOG, and 0.35g FOG) increased with the co-substrate mass addition and S/I ratio. Using the onset of steady state (t_{steady}, h) estimated using the linear regression model, as well as the lag phase duration time (λ, h), the exponential phase time ($t_{\text{exponential}}$, h) estimated from non-linear regression models, were evaluated and these are shown in

Table 4.4. According to the experimental results and estimated parameters in Figure 4.2, Table 4.3, and Table 4.4, FOG was considered to be a better co-substrate than KW for it required less mass loading per unit methane production, presented better methane production capability, achieved higher ultimate methane percentage, and exhibited a shorter estimated exponential time within the ideal S/I ratio ranges.

4.4 Conclusions

BMP tests with optimized substrate to inoculum (S/I) ratios demonstrated that KW and FOG were potential substrates that could be applied in WAS co-digestions. KW and FOG positively affected methane production with ideal estimated S/I ratios of 1.20 and 0.46, respectively. Co-digestion with FOG within the ideal S/I ratio range would be suggested as the preferred co-substrate. Combined linear and non-linear regression models were employed to represent the entire digestion process. Co-substrate addition effectively decreased the lag phase of co-digestion. Estimated parameters from regression models could provide valuable information for future experiments under various operating conditions and for larger-scale testing.

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Chapter 5

Effects of Ultrasonic and Thermo-chemical Pre-treatment on Methane Production from Fat, Oil and Grease (FOG) and Synthetic Kitchen Waste (KW) in Anaerobic Co-digestion

Abstract: The effects of ultrasonic and thermo-chemical pre-treatments on the methane production potential of anaerobic co-digestions with waste activated sludge (WAS) as primary substrate and municipal organic wastes including synthetic kitchen waste (KW) or fat, oil and grease (FOG) as co-substrates were investigated using biochemical methane potential (BMP) tests. Non-linear regressions were fitted to accurately assess and compare the methane production from co-digestion under the various pre-treatment conditions and to achieve representative simulations and predictions. Ultrasonic pre-treatment was not found to improve methane production effectively from FOG co-digestion or from KW co-digestion. Thermo-chemical pre-treatment could increase methane production yields from both FOG and KW co-digestions. A comprehensive evaluation indicated that the thermo-chemical pre-treatments of pH=10, 55 ℃ and pH=8, 55 ℃ provided the best conditions to increase methane production from FOG and KW co-digestions, respectively. The most effective enhancement of biogas production (288 ± 0.85 mL CH_4/g TVS) was achieved from thermo-chemically pre-treated FOG co-digestion, which was 9.9 ± 1.5% higher than FOG co-digestion without thermo-chemical pre-treatment.

Keywords: Anaerobic co-digestion; biochemical methane potential (BMP); pre-treatment; fat, oil, and grease; synthetic kitchen waste; non-linear regression.

5.1 Introduction

To enhance biogas production and assist in wastewater treatment and municipal organic waste management, anaerobic digestion with the addition of co-substrates, i.e. co-digestion, has been recognized as an effective, low-cost, and commercially viable approach to improve methane yields (Alatriste-Mondragón et al., 2006; Natural Resource Canada, 2002). A number of studies have shown that co-digestion with municipally available organic wastes including fats, oils and grease (FOG) and kitchen waste (KW) or food waste, which can be collected in close proximity to the treatment facility, could be employed as potential co-substrates (Carucci et al., 2005; Davidsson et al., 2008; Gunaseelan, 2004; Kabouris et al., 2008; Li et al., 2011; Martín-González et al., 2010).

Pre-treating substrates using various pre-treatment methods has also been reported as a potential approach to improve methane production efficiency (Dohányos et al., 2004). Among the different pre-treatment approaches available, thermo-chemical and ultrasonic pre-treatments have been reported as effective and economically viable methods (Apul and Sanin, 2010; Kim et al., 2003; Pilli et al., 2011; Rafique et al., 2010; Valo et al., 2004). Kim et al. (2003) obtained a greater than 34.3% methane increase from waste activated sludge (WAS) using thermo-chemical pre-treatment. Pilli et al. (2011) reviewed ultrasonic pre-treatment and concluded that this was effective for sludge, but that the efficiency varied with the sludge characteristics.

Although ultrasonic and thermo-chemical pre-treatments have been recognized as efficient processes to enhance methane production, they have primarily been investigated in the anaerobic digestion of wastewater treatment plant sludges (e.g. WAS, primary raw sludge) and most studies have focused on traditional digestion without co-substrates (Apul and Sanin, 2010; Dhar et al., 2012; Kim et al, 2003; Tanaka and Kamiyama, 2002; Valo et al., 2004; Vlyssides and Karlis, 2004). However, a few studies have reported the use of ultrasonic or thermo-chemical pre-treatments on the digestion or co-digestion of a range of substrates (Blank and Hoffmann, 2011; Elbeshbishy et al., 2011; Fdez.-Güelfo et al., 2011; Luste et al., 2009; Navia et al., 2002; Saha et al., 2011; Saifuddin and Fazlili, 2009). Although FOG, KW and other municipal organic wastes have been reported by a number of researchers as potential co-substrates in the anaerobic co-digestion of municipal wastewater treatment plant sludges, to our knowledge they have seldom been utilized and evaluated in co-digestion with ultrasonic or thermo-chemical pre-treatments.

In addition, a number of studies have reported on the effects of various pre-treatments on biogas production based on comprehensive experimental analyses and observations including COD solubilization, solids content and volatile fatty acid (VFA) concentrations, which have provided valuable information (Bougrier et al, 2005; Bourgrier et al, 2006; Lin et al., 1999). However, the use of empirical regression models, particularly non-linear regressions, has seldom been applied to provide a mathematical relationship to support and explain experimentally-derived anaerobic digestion observations, particularly for co-digestions with pre-treatment (Donoso-Bravo et al., 2010). It is believed that non-linear regression models could be applied to integrate the results and allow for the prediction of optimum digestion configurations and operating conditions.

As such, the main objective of this study was to identify and investigate an optimum range of pre-treatment conditions to enhance methane production from the co-digestion of wastewater treatment plant sludges with FOG and municipal KW as co-substrates through a series of BMP tests. Methane production results from the pre-treated co-digestion experiments were compared and the variables controlling methane production were analyzed and discussed. Non-linear regression models were also developed to assist in the interpretation of the results.

5.2 Material and Methods

5.2.1 Inocula and Substrates

Anaerobic digester sludge (ADS) was used as the inoculum for initiating the digestion reactions in each test. Four L of ADS was collected from the mesophilic anaerobic digesters at the Cataraqui Bay Wastewater Treatment Plant (Kingston, Ontario, Canada) and stored at 4 ℃ in the refrigerator for no more than 2 days prior to utilization.

WAS, obtained from the aeration basin of the same facility, was employed as the primary substrate throughout all co-digestion BMP tests. 4 L of WAS was collected and stored at 4 ℃ for 2 days, and then the supernatant was decanted to produce the concentrated WAS, which was then employed in the experiments.

Municipal organic wastes including FOG and synthetic KW collected in close proximity to the treatment facility were selected as potential co-substrates. The FOG was collected from the waste oil receptacle of the Graduate Club, a restaurant located on the main campus of Queen’s University (Kingston, Ontario, Canada), and consisted of a mixture of waste frying oil, bacon grease and animal fat. 1 L of FOG was collected before each test and stored in a glass bottle at 4 ℃. As the constituents of KW can generally vary considerably, a synthetic KW was employed to ensure that the results from these experiments were reliable and comparable. The synthetic KW was generated using a mixture of potato (40 g), strawberry (16 g), orange (32 g), tomato (72 g), chicken breast (52 g), apple (24 g), green peas (40 g), cabbage (50 g), pork (20 g) and distilled water (24 mL) as per the synthetic kitchen waste recipe presented by Neves et al. (2002) and Li et al. (2011). The fruits, vegetables and meats applied in this synthetic KW recipe were obtained from local municipal disposal facilities (e.g. green bins for organic wastes around the City of Kingston, Ontario, Canada). Characteristics of the inocula and substrates utilized in the study are presented in Table 5.1.

Table 5.1 Characteristics of inocula and substrates utilized in co-digestion experiments with ultrasonic and thermo-chemical pre-treatment.

	Ultrasonic Pre-treatment		**Thermo-Chemical Pre-treatment**	
	TVS (g/L)	***pH***	***TVS (g/L)***	***pH***
ADS	18.0 ±0.30	7.7	15.4 ±0.07	7.8
WAS	9.90 ±0.33	7.3	13.5 ±0.08	7.3
FOG	938 ±35.5	4.1	941 ±18.8	4.1
KW	121 ±2.46	4.6	126 ±3.49	4.5

ADS=Anaerobic digester sludge; WAS=Waste activated sludge; KW=Synthetic kitchen waste; FOG=Fat, oil, and grease.

5.2.2 Experimental Procedure and Sample Analysis

In this study, two series of BMP tests were conducted to assess methane production from the anaerobic co-digestion using ultrasonically and thermo-chemically pre-treated municipal organic wastes as the co-substrates. To evaluate co-digestion using BMP tests, it is important to consider the substrate to inoculum ratio (S/I) on a total volatile solids (TVS) basis, since identifying and using an S/I ratio in the ideal range is necessary to obtain adequate methane generation and achieve the maximum ultimate methane production per unit mass of substrate. Ideal S/I ratios of 0.46 and 1.20 have been previously reported for FOG and KW co-digestion with WAS, respectively (Li et al., 2011). FOG and KW, which were individually investigated as co-substrates, were mixed with the primary substrate WAS and the inocula ADS within a previously determined range of ideal S/I ratios. Prior to mixing with ADS, mixtures of WAS and the co-substrates were pre-treated under various conditions presented in Table 5.2. The pre-treated mixtures were then mixed with inocula in 250 mL septum top glass bottles as shown in Table 5.2. The bottles were then flushed with N_2 gas for 30 seconds prior to sealing to ensure all the bottles were under anaerobic conditions. All BMP bottles were incubated in a New Brunswick 4500 incubation shaker at 37 ℃ with a shaking velocity of 100 rpm. In the ultrasonic pre-treatment experiments, mixtures of WAS and co-substrates were pre-treated in 100 mL beakers using a Fisher Scientific Model 500 ultrasonic probe (20kHz) for specified treatment times and power inputs. In the thermo-chemical pre-treatment tests, sodium hydroxide (NaOH) (Fisher Scientific) solutions (6 g/L) was added as an alkaline agent to adjust the pH value of the substrate mixtures to 8,10 and 12 and the substrate mixtures were stirred at 55 ℃ for 1.5 hours. The experimental conditions are shown in Table 5.2. All experiments were conducted in duplicate.

Table 5.2 Sample composition and operational conditions employed in the ultrasonic and thermo-chemical pre-treatment experiments

Ultrasonic Pre-treatment

Duplicate Samples	*Inoculum ADS (mL)*	*Substrate WAS (mL)*	*Co-substrate (g)*	*S/I Ratio*	*Power Input (J)*	*Treatment Time (min)*
UB01B02	50	0	0	0	0	0
UB030B4	50	15	0	0.21	0	0
UB05B06	50	15	0.3 FOG	0.48	0	0
UB07B08	50	15	0.3 FOG	0.48	2400 (5300kJ/kg TS)	5
UB09B10	50	15	0.3 FOG	0.48	4600 (10000kJ/kg TS)	10
UB11B12	50	15	0.3 FOG	0.48	8800 (19600kJ/kg TS)	20
UB13B14	50	15	0.3 FOG	0.48	16000(36000kJ/kg TS)	40
UB15B16	50	15	8 KW	1.15	0	0
UB17B18	50	15	8 KW	1.15	2100 (1700kJ/kg TS)	5
UB19B20	50	15	8 KW	1.15	4600 (3800kJ/kg TS)	10
UB21B22	50	15	8 KW	1.15	8000 (6700kJ/kg TS)	20
UB23B24	50	15	8 KW	1.15	16000(14000kJ/kg TS)	40

Thermo-Chemical Pre-treatment

Duplicate Samples	*Inoculum ADS (mL)*	*Substrate WAS (mL)*	*Co-substrate (g)*	*S/I Ratio*	*Substrate Mixture pH (with NaOH Addition)*	*Temp °C*
TB01B02	65	0	0	0	--	Room
TB030B4	65	15	0	0.2	7.5	Room
TB05B06	65	15	0.3 FOG	0.48	6.5	Room
TB07B08	65	15	0.3 FOG	0.48	6.4	55
TB09B10	65	15	0.3 FOG	0.48	8 (0.5mL NaOH)	55
TB11B12	65	15	0.3 FOG	0.48	10 (2mL NaOH)	55
TB13B14	65	15	0.3 FOG	0.48	12 (5mL NaOH)	55
TB15B16	65	15	7.5 KW	1.16	6.1	Room
TB17B18	65	15	7.5 KW	1.16	5.6	55
TB19B20	65	15	7.5 KW	1.16	8 (2.5mL NaOH)	55
TB21B22	65	15	7.5 KW	1.16	10 (6mL NaOH)	55
TB23B24	65	15	7.5 KW	1.16	12 (13.5mL NaOH)	55

ADS=Anaerobic digester sludge; WAS=Waste activated sludge; FOG=Fat, oil and grease; S/I Ratio=Substrate to inoculum ratio based on total volatile solids; UB=BMP test with ultrasonic pre-treatment; TB=BMP test with thermo-chemical pre-treatment, e.g. UB01=BMP Test 01 with ultrasonic pre-treatment; NaOH=6 g/L NaOH solution.

Parameters that can affect biogas production including initial and final pH, chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD), total solids (TS) and total volatile solids (TVS) were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Volatile fatty acids (VFA) in the solutions were analyzed using ion chromatography (Dionex ICS-3000, column IONPAC AS11). Biogas samples were collected from the headspaces of the BMP bottles using a pressure-lock syringe (VICI® A-2) (Hansen et al., 2004; Li et al., 2011). The contents of the gas samples (CH_4 and CO_2) were analyzed by gas chromatography (Varian 3400 with HayeSep® Q Micropacked column, TCD detector). The results of the gas analysis were reported on the basis of cumulative methane at standard temperature and pressure (STP, 101.325 kPa, 273.15 K) and normalized in terms of the total initial organic matter present in each sample, with units of mL CH_4/g TVS.

5.2.3 Data Analysis

Empirical non-linear regression models were developed that estimate three variables including the duration of the lag phase, the ultimate cumulative methane production, and the maximum methane production rate. In this study, the modified Gompertz equation was applied and is shown in Equation 5.1 (Donoso-Bravo et al., 2010; Li et al., 2011):

$$B = B_0 \exp\left\{ -\exp\left[\frac{R_m e}{B_0}(\lambda - t) + 1 \right] \right\} \quad (5.1)$$

Where B_0 is the estimated ultimate cumulative methane yield or methane production potential (mL/g TVS), B is the cumulative methane yield (mL/g TVS) at incubation time

t (h), e is equal to 2.7183, R_m is the maximum methane production rate (mL/g TVS·h), and λ is the lag phase duration time (h).

The coefficient of determination (R^2) and confidence intervals, which were derived from statistical analyses using the mean, degrees of freedom, standard error and critical t value, were calculated to indicate the accuracy of the models. The purpose for applying the non-linear regression model was to accurately assess and compare the methane production from co-digestion under the various pre-treatment conditions and to achieve representative simulations and predictions.

5.3 Results and Discussion

5.3.1 Co-digestions with Ultrasonic Pre-treatment

5.3.1.1 *Methane Production Results of Ultrasonic Pre-treated Co-digestions*

According to the experimental results shown Figure 5.1 and Table 5.3 in detail, compared to the digestions with only ADS and WAS, co-digestions with and without the utilization of ultrasonic pre-treatment consistently produced higher volumes of methane. However, ultrasonic pre-treatment did not improve the ultimate methane production from the FOG co-digestions (Figure 5.1(a)) and the FOG co-digestion without ultrasonic pre-treatment obtained a higher experimental ultimate methane production (285 ± 2.00 mL/g TVS) than other treatments. KW co-digestions with ultrasonic pre-treatment achieved higher ultimate methane production than the KW co-digestion without ultrasonic pre-treatment (Figure 5.1(b) and Table 5.3). The KW co-digestion exposed to 10 minutes of 4600 J (3800 kJ/kg TS) ultrasonic pre-treatment obtained the highest ultimate methane production (237±1.58 mL/g TVS) amongst all KW co-digestions with ultrasonic pre-treatment, which was 7.7±2.6% higher than that yielded from KW co-digestion without pre-treatment (Figure 5.1 (b)). However, the improvement was not statistically significant (P=0.2). Hence, the appeared to results indicated that ultrasonic pre-treatment cannot significantly enhance methane production from KW co-digestion.

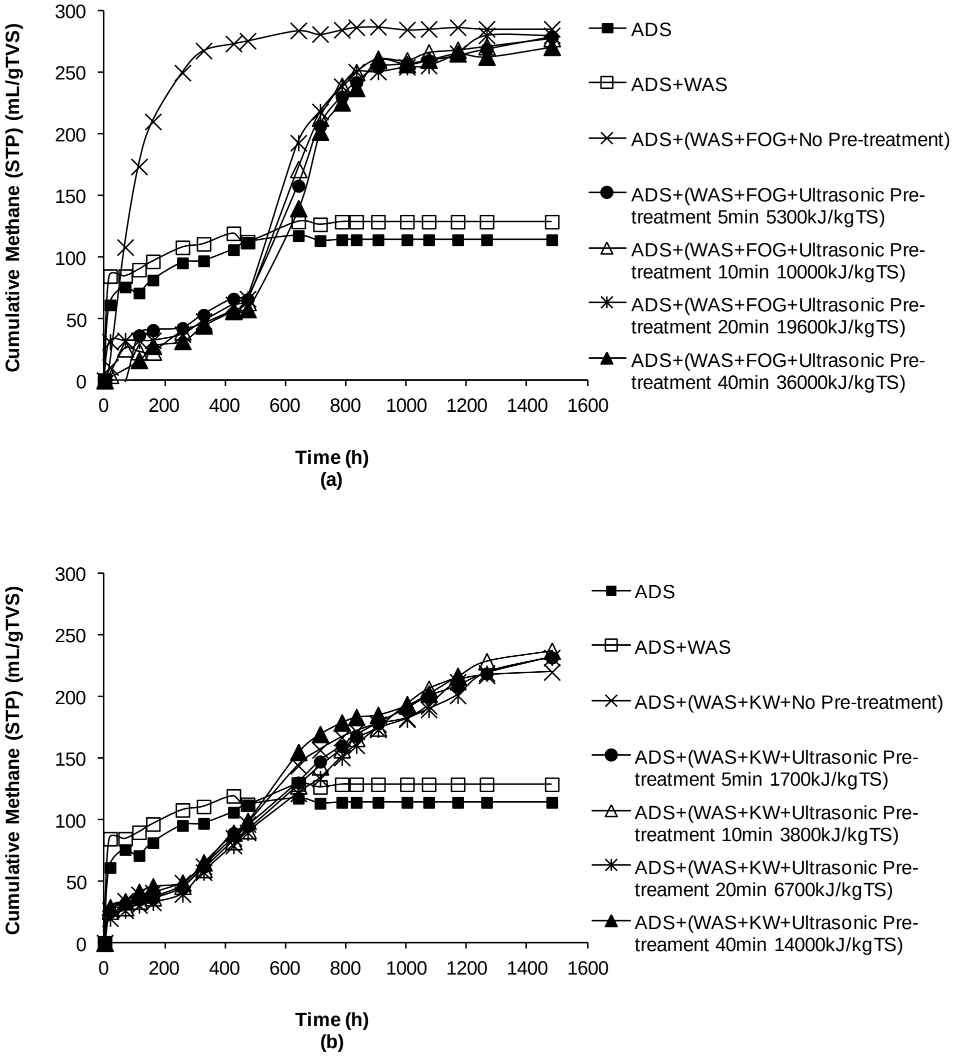


Figure 5.1 Average cumulative methane production (STP, mL/gTVS) from (a) FOG co-digestions with ultrasonic pre-treatment and (b) KW co-digestions with ultrasonic pre-treatment.

Table 5.3 Experimental results and parameters estimated from non-linear regressions for FOG and KW co-digestions with ultrasonic and thermo-chemical pre-treatments.

Ultrasonic Pre-treatment

Duplicate Co-digestion Samples	*Pre-treatment Condition (min, kJ/kgTS)*	*Experimental B0 (mL/g TVS)*	*Estimated B0 (mL/g TVS)*	*λ (h)*	*Rm (mL/g TVS h)*	*R^2*
ADS	--	114±0.01	110	0.00	0.85	0.780
ADS+WAS	--	129±1.05	118	4.40	6.69	0.790
FOG	--	285±2.00	281	10.4	1.64	0.992
FOG	5, 5300	279±1.82	290	237	0.43	0.976
FOG	10,10000	278±2.67	282	312	0.55	0.980
FOG	20, 19600	280±6.18	281	290	0.54	0.968
FOG	40, 36000	271±4.53	280	319	0.52	0.983
KW	--	220±5.23	241	6.40	0.22	0.989
KW	5, 1700	232±4.80	258	19.4	0.21	0.995
KW	10, 3800	237±1.58	278	33.6	0.21	0.995
KW	20, 6700	232±4.73	264	45.1	0.20	0.996
KW	40, 14000	226±2.87	242	12.3	0.24	0.987

Thermo-Chemical Pre-treatment

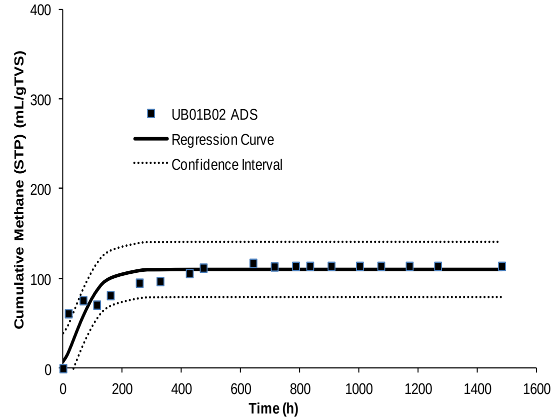
Duplicate Co-digestion Samples	*Pre-treatment Condition (pH, ℃)*	*Experimental B0 (mL/g TVS)*	*Estimated B0 (mL/g TVS)*	*λ (h)*	*Rm (mL/g TVS h)*	*R^2*
ADS	--	96.4±3.39	89.2	20.4	0.17	0.949
ADS+WAS	--	120±6.14	98.4	9.95	1.46	0.821
FOG	--	262±3.23	249	3.46	2.53	0.956
FOG	--,55	269±1.80	257	-37.2	0.88	0.957
FOG	8, 55	269±1.00	255	-37.4	0.93	0.948
FOG	10, 55	288±0.85	282	-4.46	0.80	0.991
FOG	12, 55	287±1.98	285	-10.8	0.65	0.994
KW	--	221±2.45	219	184	0.42	0.995
KW	--,55	226±2.87	224	200	0.47	0.993
KW	8, 55	242±2.11	236	116	0.57	0.993
KW	10, 55	214±9.58	211	69.0	0.49	0.995
KW	12, 55	221±9.88	219	35.7	0.44	0.998

ADS=Digestion with ADS; ADS+WAS= Digestion with ADS and WAS; FOG= Co-digestion with ADS as inoculum, WAS as primary substrate and FOG as co-substrate; KW= Co-digestion with ADS as inoculum, WAS as primary substrate and KW as co-substrate; -- means no pre-treatment applied; B_0= Ultimate cumulative biogas production; λ = Length of the lag phase; R_m = Maximum biogas production rate.

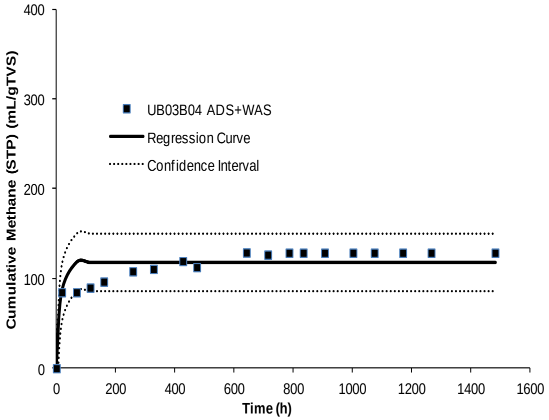
As can also be seen in Figure 5.1(a), compared to the FOG co-digestion without ultrasonic pre-treatment, the lag phases of the FOG co-digestions with ultrasonic pre-treatment were much longer and their exponential phases appeared to be delayed. Although all FOG co-digestions eventually yielded similar ultimate methane production, those with ultrasonic pre-treatment reached these yields much later. Conversely, it can be noted from Figure 5.1(b) that, compared to the KW co-digestion without ultrasonic pre-treatment, the exponential phases of the KW co-digestions with ultrasonic pre-treatment were not delayed as much as the FOG co-digestions. In addition, it can be shown by Figure 5.1 that the reaction rates during the exponential phases of the KW co-digestions were lower than those of the FOG co-digestions.

5.3.1.2 *Modelling and Influential Parameters on Methane Production from Ultrasonic Pre-treated Co-digestions*

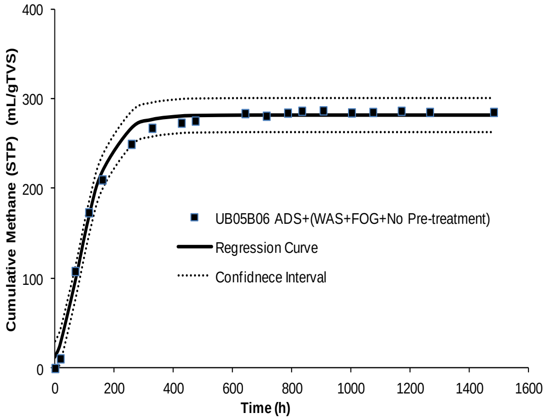
In order to further assess the effectiveness of the ultrasonic pre-treatment on methane production from co-digestions, empirical non-linear regression models (Equation 5.1) were fitted to the data. The regression results are shown in Figure 5.2.


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB01B02 ADS
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

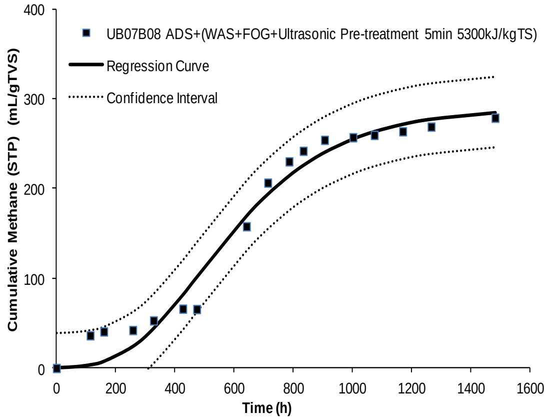
(a)


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB03B04 ADS+WAS
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

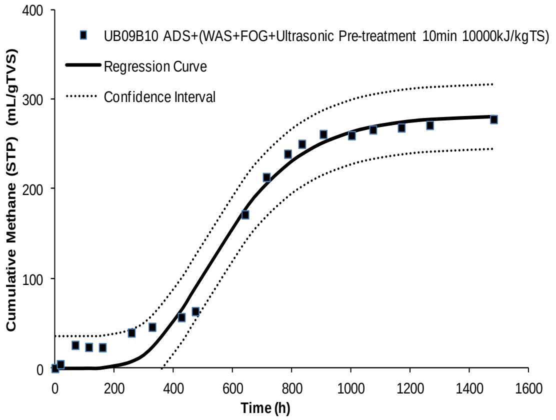
(b)


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB05B06 ADS+(WAS+FOG+No Pre-treatment)
Regression Curve
Confidnece Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

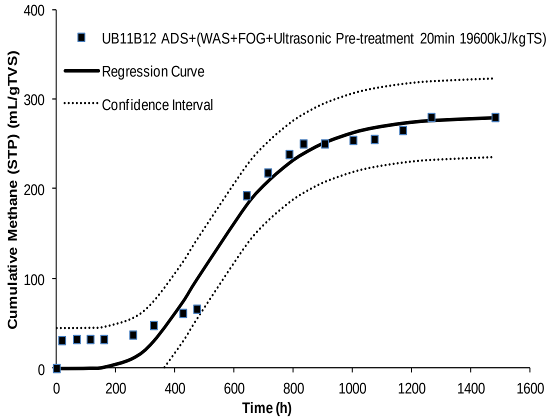
(c)


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB07B08 ADS+(WAS+FOG+Ultrasonic Pre-treatment 5min 5300kJ/kgTS)
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

(d)


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB09B10 ADS+(WAS+FOG+Ultrasonic Pre-treatment 10min 10000kJ/kgTS)
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

(e)


Cumulative Methane (STP) (mL/gTVS)
400
300
200
100
0
UB11B12 ADS+(WAS+FOG+Ultrasonic Pre-treatment 20min 19600kJ/kgTS)
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
1600
Time (h)

(f)

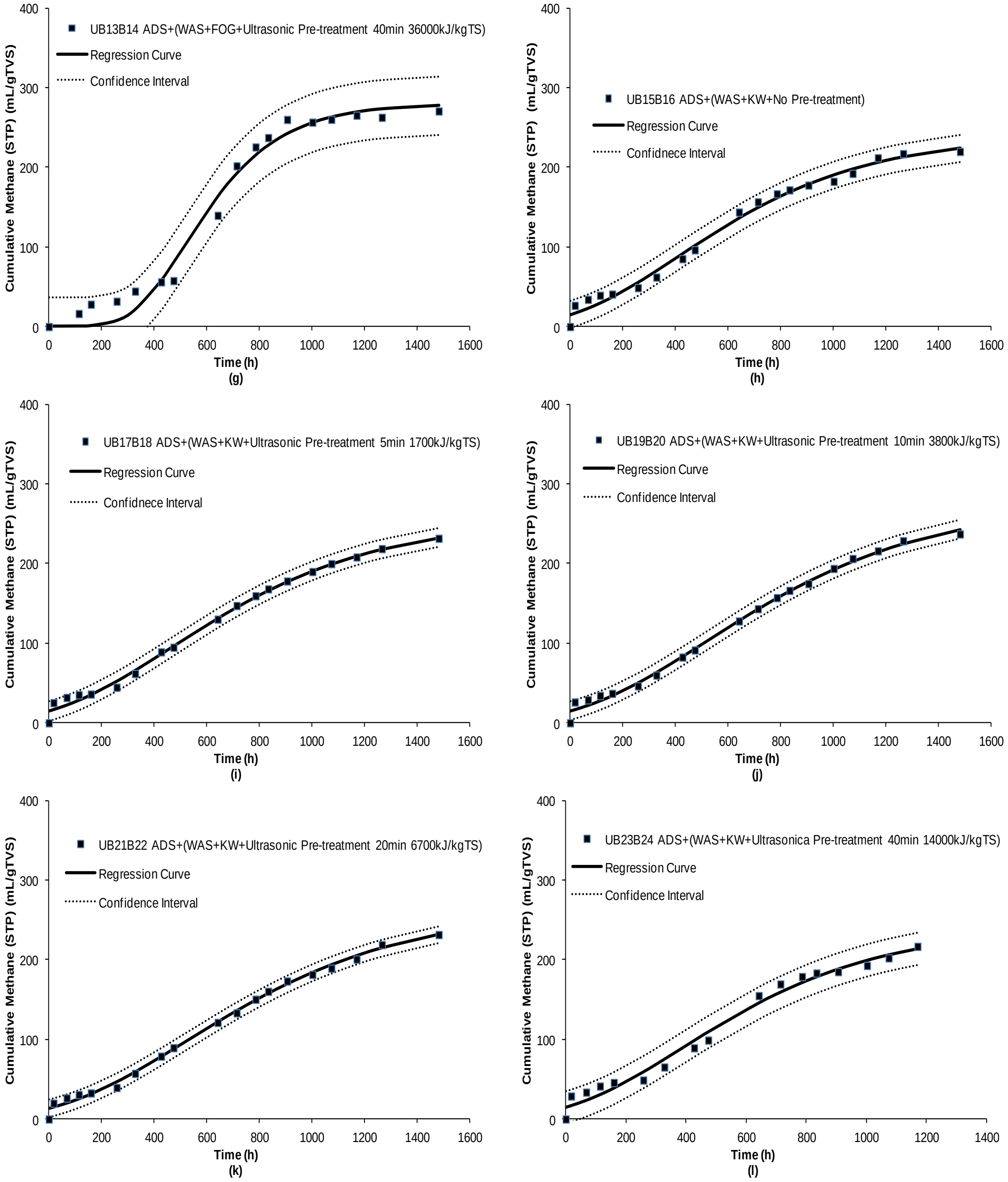


Figure 5.2 Non-linear regressions for the cumulative methane production during digestions of (a) ADS and (b) ADS+WAS; and non-linear regression for the cumulative methane production (STP, mL/g TVS) during co-digestions of FOG (from (c) to (g)) and KW (from (h) to (l)) with various ultrasonic pre-treatment conditions.

Figure 5.2(a) and (b) illustrate the non-linear regression results of digestion without substrate and digestion with WAS alone. Figure 5.2(c) to (g) and Figure 5.2(h) to (l) present the non-linear regression results of FOG co-digestions and KW co-digestions, respectively, with and without ultrasonic pre-treatment. The experimental ultimate methane production and the important digestion parameters estimated from the regression analysis are summarized in Table 5.3. In addition, other experimental parameters that may influence methane production, including pre-treatment conditions, solid contents, COD, SCOD and VFA are presented in Table 5.4.

From Figure 5.2(c) to (g), it can be noted that the regression curves fit the FOG co-digestions with and without ultrasonic pre-treatment well. However, compared to the KW co-digestion regressions, the confidence intervals of the FOG co-digestion regressions were much larger, with the exception of the FOG co-digestion without ultrasonic pre-treatment (Figure 5.2(c)). This would imply that ultrasonic pre-treatment impeded FOG co-digestion. It can also be noted from the estimated lag phase duration times (λ) in Table 5.3, that the lag phase increased with the ultrasonic power input, which suggested that a longer incubation time was necessary to reach the methanogenesis phase in FOG co-digestions with ultrasonic pre-treatment. This can be also supported by the estimated maximum methane production rates R_m shown in Table 5.3. In the FOG co-digestions, the R_m decreased from 1.64 mL/g TVS·h to 0.52 mL/g TVS·h, which corresponded with the delay in the establishment of the exponential methanogenesis phase. Similar results were also noted by Luste et al. (2009) where 24 kHz (5600 kJ/kg TS) ultrasonic pre-treatment did not significantly change the ultimate methane production potential in the digestion of grease trap sludge, but did prolong the lag phase by 8 days (192 hours) as compared to digestion without ultrasonic pre-treatment.

Table 5.4 Sample (mixture of inoculum and pre-treated FOG and KW co-substrates) characteristics after ultrasonic and thermo-chemical pre-treatment

Ultrasonic Pre-treatment

Duplicate Samples	***Acetic Acid (mg/L)***	***TVS (g/L)***	***SCOD (%)***
WAS+FOG 0min	70.0	23.2 ±4.45	47.3 ±0.05
WAS+FOG 5min	21.9	18.5 ±0.38	21.9 ±2.14
WAS+FOG 10min	20.5	19.2 ±0.07	26.7 ±0.32
WAS+FOG 20min	33.6	19.3 ±0.35	27.3 ±1.73
WAS+FOG 40min	38.6	19.7 ±0.10	30.7 ±0.26
WAS+KW 0min	518	29.6 ±2.21	30.5 ±0.94
WAS+KW 5min	340	27.5 ±0.42	30.6 ±1.22
WAS+KW 10min	641	28.2 ±1.08	35.1 ±1.54
WAS+KW 20min	508	28.4 ±1.50	41.5 ±7.24
WAS+KW 40min	629	29.2 ±0.35	40.8 ±11.6

Thermo-chemical Pre-treatment

Duplicate Samples	***Acetic Acid (mg/L)***	***TVS (g/L)***	***SCOD (%)***
WAS+FOG	< 5	22.2 ±3.24	47.7 ±2.65
WAS+FOG 55 °C	18.8	22.1 ±1.05	47.5 ±1.17
WAS+FOG 55 °C pH=8	26.2	22.2 ±0.45	48.7 ±0.76
WAS+FOG 55 °C pH=10	25.7	24.0 ±1.50	51.0 ±1.79
WAS+FOG 55 °C pH=12	9.31	23.1 ±0.46	68.7 ±1.19
WAS+KW	51.5	32.2 ±0.88	34.0 ±1.32
WAS+KW 55 °C	< 5	32.6 ±0.53	37.9 ±2.32
WAS+KW 55 °C pH=8	< 5	26.8 ±0.56	39.0 ±1.18
WAS+KW 55 °C pH=10	209	26.0 ±1.11	30.0 ±2.99
WAS+KW 55 °C pH=12	286	26.2 ±0.90	22.1 ±1.54

These observations regarding the inhibitory effects of ultrasonic pre-treatment on FOG co-digestions were further supported by the VFA analysis, particularly for acetic acid, as presented in Table 5.4. Specifically, the acetic acid concentration in FOG co-digestion without ultrasonic pre-treatment was around 70.0 mg/L prior to the co-digestion, while the acetic acid in other FOG co-digestions with ultrasonic pre-treatment decreased from 70.0 mg/L to around 20.5 mg/L. Acetic acid (CH_3COOH) is the primary intermediate product from the hydrolysis of complex organics that supports the methanogenesis phase in anaerobic digestion (Gerardi, 2003). Hence, based on the results presented in Table 5.4, ultrasonic pre-treatment appeared to lead to a decrease in the acetic acid concentration in the FOG co-digestions and inhibited methanogenesis, which in turn resulted in the longer lag phases observed in these FOG co-digestions. Luste et al. (2009) and Luste et al. (2011) also reported that ultrasonic pre-treatment should have degraded the complex lipid-rich compounds, but instead the VFA might also have degraded during the pre-treatment phase and other hydrolysis by-products (e.g. long chain fatty acid or re-crystallization compounds which are the primary constituents in lipid-rich organics) might have subsequently inhibited methanogenesis and methane formation.

The results of the TVS, COD, and SCOD analyses on the pre-treated samples, which are shown in Table 5.4, also support this hypothesis. A number of studies have reported that ultrasonic pre-treatment could promote biogas production as a result of an increase in COD solubilization (Apul and Sanin, 2010; Bougrier et al., 2005; Bougrier et al., 2006; Dhar et al., 2012; Elbeshbishy et al., 2011; Saifuddin and Fazlili, 2009). COD solubilization can be represented using the SCOD/COD (%) ratio (Dhar et al., 2012). According to Table 5.4, comparing to the TVS (23.2 ±4.45 g/L) in the FOG co-substrates without ultrasonic pre-treatment, the TVS (g/L) in the FOG co-substrates after ultrasonic pre-treatment decreased. However, the SCOD/COD ratios of FOG co-digestions after ultrasonic pre-treatment did not increase correspondingly, which might also result in lower ultimate methane production. The substantial decrease in SCOD/COD ratio after ultrasonic pre-treatment could be due to temperature effects. The substrates temperature increased with ultrasonication period during the pre-treatment process, which might result in degradation of VFA and volatilization of other soluble organics. Although other studies have reported that ultrasonic pre-treatment could effectively increase COD solubilization, it should be noted that most of these ultrasonic pre-treatments were conducted on wastewater treatment plant sludge alone (Apul and Saninet, 2010; Pilli et al., 2011; Saha et al., 2011) or wastewater from treatment plants (Saifuddin and Fazlili, 2009), but rarely on lipid or grease-rich wastes (Luste et al., 2009). Based on the results from these previous studies, it was suggested that ultrasonic pre-treatment might effectively increase the solubilization of organics and enhance the ultimate methane production from the anaerobic co-digestions with protein-rich substrates including various wastewater treatment plant sludges and wastewaters. However, the results obtained in the current study and similar studies conducted by Luste et al. (2009), would indicate that the ultrasonic pre-treatment cannot effectively improve the methane production from co-digestions with lipid-rich substrate such as FOG.

Contrary to the results obtained with FOG co-digestions, it can be seen from Figure 5.1 (b), Figure 5.2, and the ultimate methane productions estimated using non-linear regressions (Table 5.3) that all KW co-digestions with ultrasonic pre-treatment yielded slightly improved ultimate methane production. Although the lag phases were noted to increase with ultrasonic pre-treatment, these were not as significant as those observed in the FOG co-digestions with ultrasonic pre-treatment. Interestingly, the maximum methane production rates did not decrease as a result of the pre-treatment. It can also be noted from Table 5.4 that the acetic acid concentrations in the ultrasonically pre-treated KW co-substrates were much higher than those found with the FOG co-substrates, which may have lead to the comparatively shorter lag phases noted for the KW co-digestions (Table 5.3). The VFA analysis would suggest that the ultrasonic pre-treatment did not inhibit the methanogenesis phase in the KW co-digestions. COD solubilization was found to increase from 30.5±0.94% (KW co-digestion without ultrasonic pre-treatment) to 41.5±7.24% (KW co-digestion with ultrasonic pre-treatment 30min 6700 kJ/kgTS), which corresponded to an improvement in ultimate methane production from KW co-digestions. Compared to the KW co-digestion without ultrasonic pre-treatment, the highest ultimate methane production was obtained from the 4590 J (3857 kJ/kg TS), 10 min ultrasonic pre-treatment with KW co-digestions, which represented an increase in methane production of 7.7±2.6 %. Hence, ultrasonic pre-treatment was found to be suitable to increase methane production in protein-rich KW co-digestions. These results are also consistent with those reported by Elbeshbishy et al. (2011) and Blank and Hoffmann (2011), where ultrasonic pre-treatment was found to enhance methane production from hog manure and municipal bio-waste (kitchen waste), respectively. However, the improvement was not statistically significant (P=0.2). Hence, the results appeared to indicate that for the ultrasonic pre-treatment conditions investigated in this study, this pre-treatment approach was not considered and effective approach to enhance ultimate methane production from KW co-digestions.

5.3.2 Co-digestions with Thermo-chemical Pre-treatment

5.3.2.1 Methane Production Results of Thermo-chemical Pre-treated Co-digestions

Figure 5.3 presents experimental results from the FOG and KW co-digestions with various thermo-chemical pre-treatments.

From Figure 5.3(a), it can be seen that the ultimate methane production from FOG co-digestions increased with alkaline dosage and corresponding pH level attained. The pre-treated FOG co-digestions at pH=10 (55 ℃) and pH=12 (55 ℃) achieved the highest ultimate methane productions of 288±0.5 mL/g TVS and 287±1.98 mL/g TVS, respectively. Although the FOG co-digestions with pH=10 and pH=12 pre-treatment had similar ultimate methane production, the thermo-chemical pre-treatment with pH=10 would represent a better operational alternative if the chemical dosages required to achieve target pre-treatment pH levels are a concern. The FOG co-digestion with pH=10 and 55 ℃ thermo-chemical pre-treatment improved the ultimate methane production significantly by 9.9±1.5 % (P=0.01) and 140±3.3% ($P=1.2\text{x}10^{-6}$) compared to the FOG co-digestion without pre-treatment and digestion with only WAS, respectively.

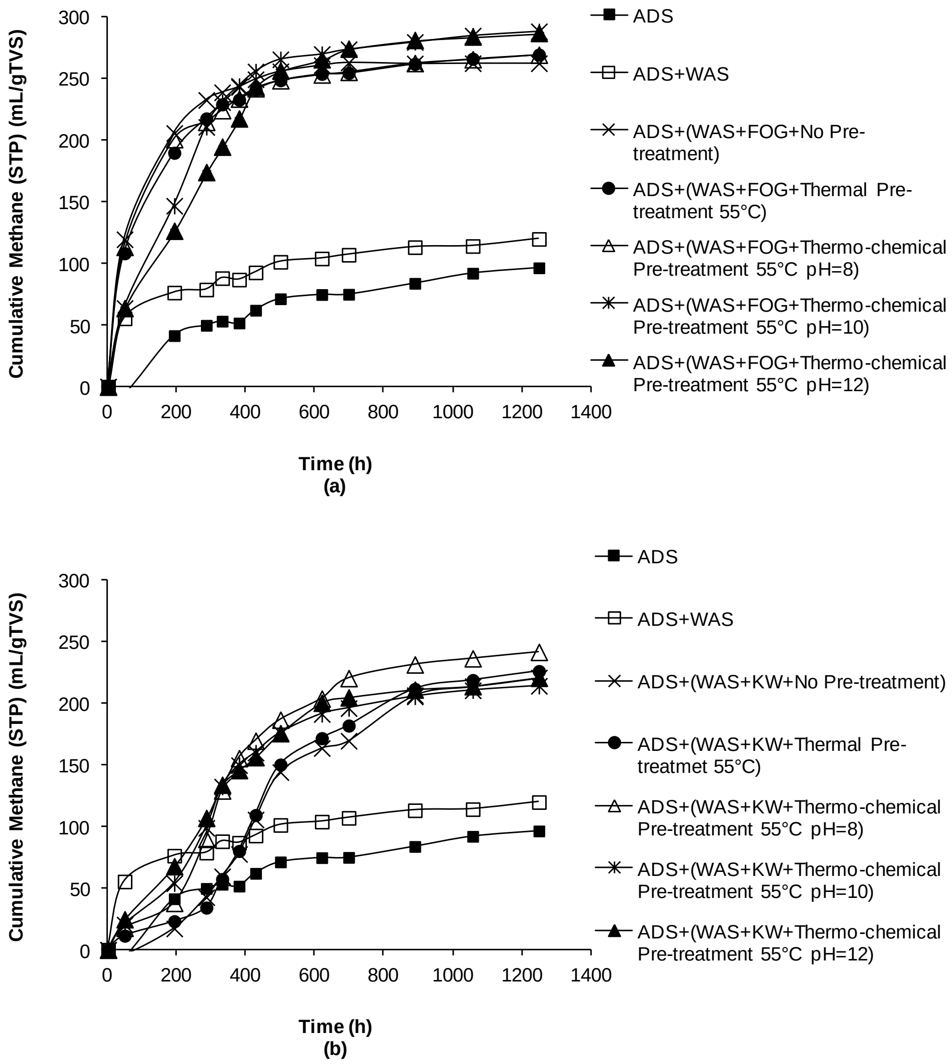


Figure 5.3 Cumulative methane production (STP, mL/gTVS) from (a) FOG co-digestions with thermo-chemical pre-treatment and (b) KW co-digestions with thermo-chemical pre-treatment.

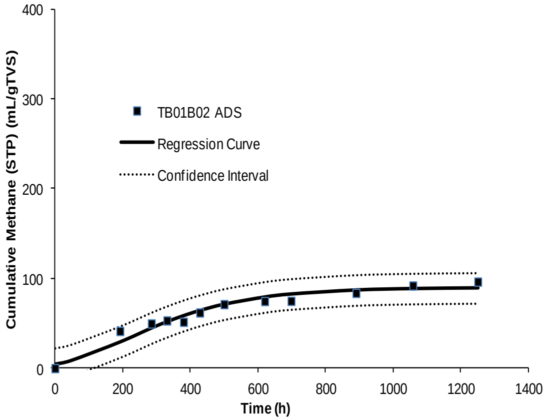
Figure 5.3(b) illustrates the methane production from KW co-digestions with thermo-chemical pre-treatment. Consistent with results previously reported by Li et al. (2011) and with the results from co-digestions with ultrasonic pre-treatment discussed above, KW co-digestions always achieved a lower ultimate methane production than FOG. In this research, KW co-digestion without thermo-chemical pre-treatment produced only 221 ±2.45 mL/g TVS ultimate methane, which was only 84.3 ±0.8% of the yield obtained for FOG co-digestion without thermo-chemical pre-treatment. The highest ultimate methane production (242 ±2.11 mL/g TVS) from KW co-digestion was obtained for the KW co-digestion with pH=8 at 55 ℃ pre-treatment, which enhanced the ultimate methane production significantly by 9.5 ±0.4% (P=0.01) when compared to KW co-digestion without thermo-chemical pre-treatment.

5.3.2.2 *Modelling and Influential Parameters on Methane Production from Thermo-chemical Pre-treated Co-digestions*

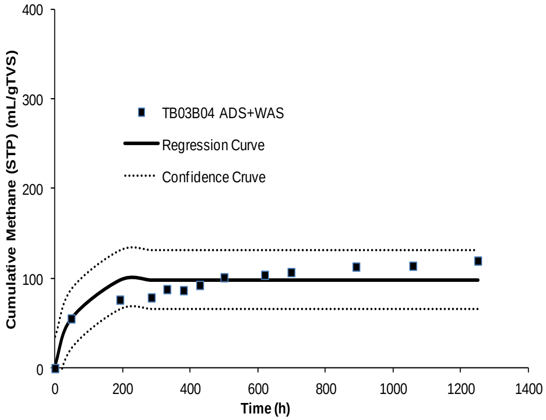
In order to demonstrate the effects of thermo-chemical pre-treatment on the co-digestion processes, a non-linear regression model was fitted to the results as presented in Figure 5.4. Experimental and estimated non-linear parameters that might affect the ultimate methane production are sumarized in Tables 5.3 and 5.4.

Contrary to the non-linear regression results obtained using ultrasonic pre-treatment, it can be seen from Figure 5.4(a) to (g) that the lag phases were decreased in FOG co-digestions with the addition of thermo-chemical pre-treatment. The non-linear parameters estimated in Table 5.3 also support this observation. The thermo-chemically pre-treated FOG co-digestions had no lag phases and the exponential phase was noted to start at the beginning of the digestion period. This was attributed to the VFA (acetic acids) concentrations, which were found to increase after thermo-chemical pre-treatment (Table 5.4) compared to the FOG co-digestion without thermo-chemical pre-treatment. This would have accelerated methanogenesis and methane production potential (Gerardi, 2003; Luste et al., 2009). This would suggest that thermo-chemical pre-treatment could effectively accelerate the FOG co-digestions and increase the ultimate methane production.

All FOG co-digestions achieved higher ultimate methane production after thermo-chemical pre-treatment, with the most improved being the FOG co-digestion with pH=10 at 55 ℃. This could also be attributed to the observed increase in the COD solubilization (SCOD/COD %) (Fdez.-Güelfo et al., 2011; Lin and Lee, 2002; Vlyssides and Karlis, 2004). As can be noted from Table 5.4, after thermo-chemical pre-treatment the FOG co-substrates exhibited increases in COD solubilization. The highest increases in FOG co-digestions were obtained with the pH=10 at 55 ℃ pre-treatment, consistent with the higher ultimate methane production obtained in these samples. Compared to the FOG co-digestion with only thermal pre-treatment at 55 ℃, all thermo-chemically pre-treated FOG co-digestions yielded higher ultimate methane productions, which is consistent with results reported in other studies that found that thermo-chemical pre-treatment has a better effect on methane production and COD solubilization than thermal pre-treatment alone (Rafique et al., 2010; Valo et al., 2004).


400
300
200
100
0
Cumulative Methane (STP) (mL/gTVS)
TB01B02 ADS
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
Time (h)

(a)


400
300
200
100
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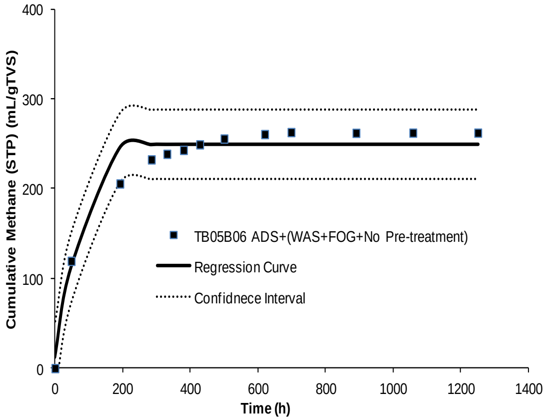
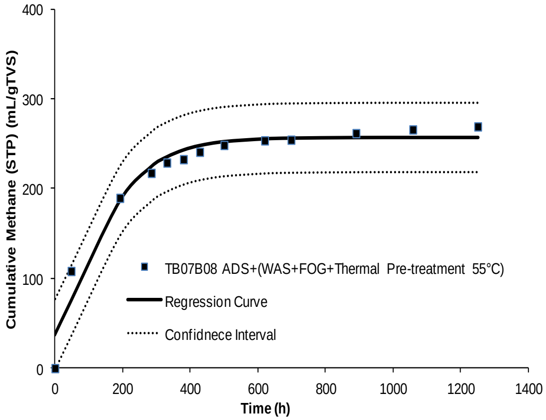
Cumulative Methane (STP) (mL/gTVS)
TB03B04 ADS+WAS
Regression Curve
Confidence Cruve
0
200
400
600
800
1000
1200
1400
Time (h)

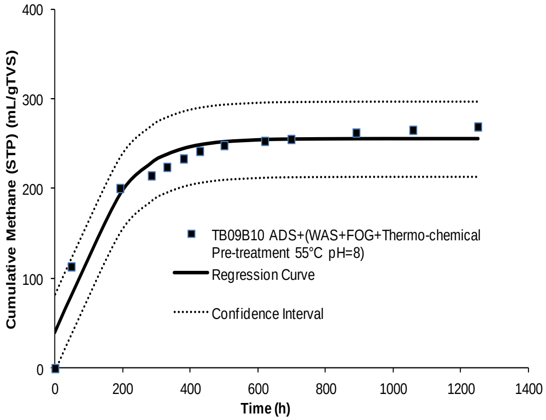
(b)


400
300
200
100
0
Cumulative Methane (STP) (mL/gTVS)
TB05B06 ADS+(WAS+FOG+No Pre-treatment)
Regression Curve
Confidnece Interval
0
200
400
600
800
1000
1200
1400
Time (h)

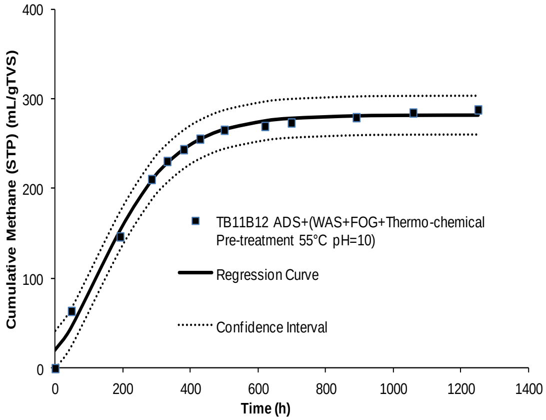
(c)


400
300
200
100
0
Cumulative Methane (STP) (mL/gTVS)
TB07B08 ADS+(WAS+FOG+Thermal Pre-treatment 55°C)
Regression Curve
Confidnece Interval
0
200
400
600
800
1000
1200
1400
Time (h)

(d)


400
300
200
100
0
Cumulative Methane (STP) (mL/gTVS)
TB09B10 ADS+(WAS+FOG+Thermo-chemical Pre-treatment 55°C pH=8)
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
Time (h)

(e)


400
300
200
100
0
Cumulative Methane (STP) (mL/gTVS)
TB11B12 ADS+(WAS+FOG+Thermo-chemical Pre-treatment 55°C pH=10)
Regression Curve
Confidence Interval
0
200
400
600
800
1000
1200
1400
Time (h)

(f)

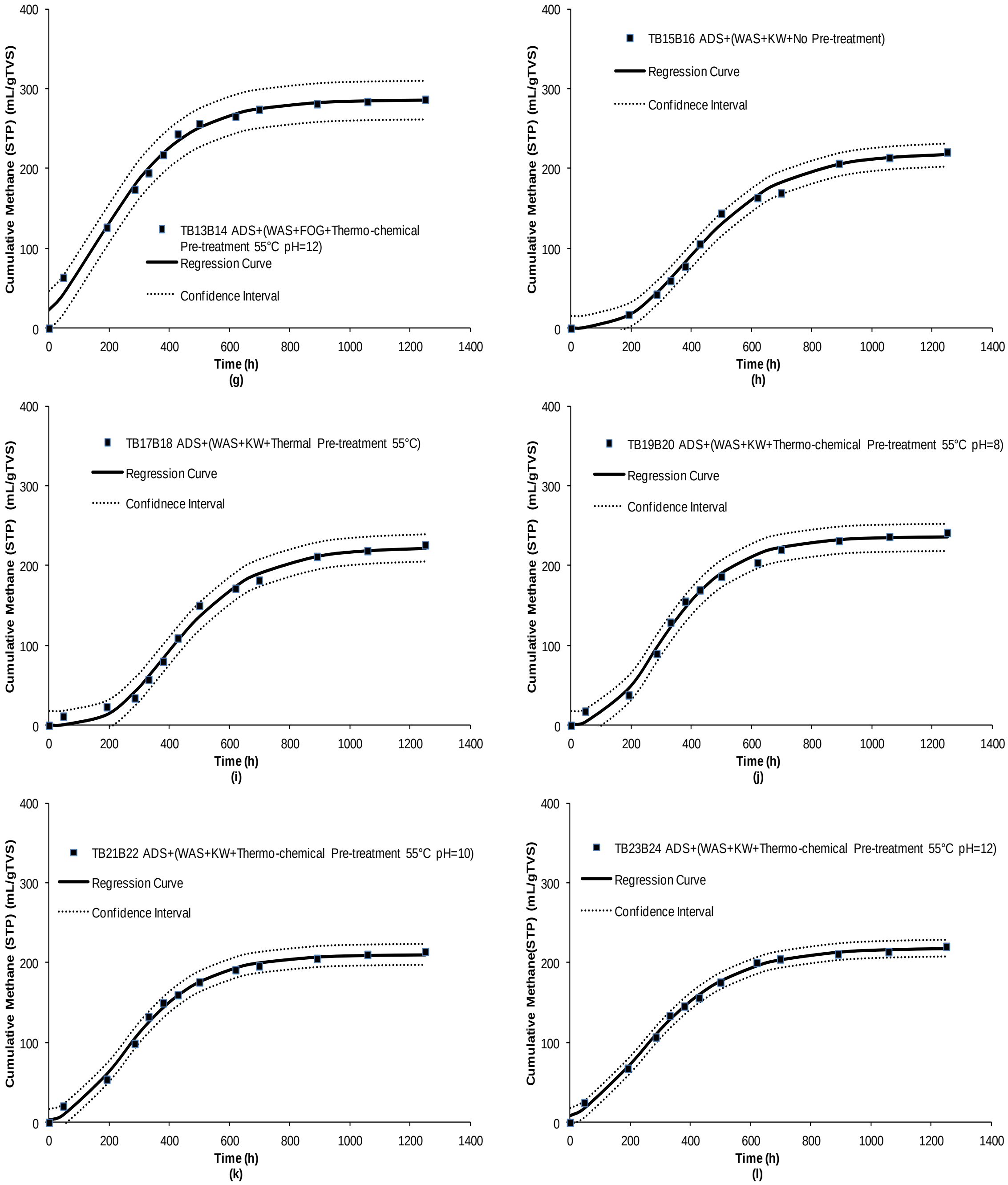


Figure 5.4 Non-linear regression for the cumulative methane production during digestions of (a) ADS and (b) ADS+WAS; and non-linear regressions for the cumulative methane production (STP, mL/gTVS) during co-digestions of FOG (from (c) to (g)) and KW (from (h) to (l)) with various thermo-chemical pre-treatment conditions.

According to Figure 5.4(h) to (l), the ultimate methane production from KW co-digestions with thermo-chemical pre-treatment was found to improve. However, as can be noted from Table 5.3, the methane production was not improved by the pre-treatment if the pH adjustment was higher than pH 8. This is supported by the results summarized in Table 5.4 where it is noted that, the thermo-chemical pre-treament appeared to effectively increase COD solubilization from 34.0±1.32 % (KW co-digestion without pre-treatment) to 39.0±1.18 % (KW co-digestion with thermo-chemical pretreatment of pH=8, 55 ℃) only up to an adjusted pH of 8. As such, the best thermo-chemical pretreatment to enhance the methane production from KW co-digestion was found to be a pH=8 adjustment at 55 ℃.

Based on these results and discussion, it may be suggested that the COD solubilization (SCOD/COD %) would be one of the most important parameters to be used in the assessment of the biodegradation and methane enhancement potential of a pre-treatment strategy. In addition, although the thermal pre-treatment achieved 226±2.78 mL/g TVS ultimate methane production (representing a 2.3±0.23% improvement over KW co-digestion without pre-treatment), the thermo-chemically pretreated KW co-digestion with pH=8 at 55 ℃ enhanced methane production by 9.5±0.4% (P=0.01) compared to KW co-digestion without thermo-chemical pre-treatment. This is consistent with FOG co-digestion with thermo-chemical pre-treatment and with other studies using similar substrates (Heo et al., 2003; Kim et al., 2003; Tanaka and Kamiyama, 2002). Hence, it can be suggested that the thermo-chemical pre-treatment represents a better alternative to enhance methane production from KW co-digestion than thermal pre-treatment alone.

5.4 Conclusions

Based on the results of this study, it can be concluded that ultrasonic pre-treatment was not found to improve methane production from FOG co-digestions. Although the KW co-digestion with a 10-minute, 20 kHz 4600J (3800 kJ/kg TS) ultrasonic pre-treatment achieved ultimate methane production enhancement by 7.7 ±2.6% as compared to KW co-digestion without ultrasonic pre-treatment, the improvement was not statistically significant or effective. The best thermo-chemical pre-treatment for FOG co-digestion and KW co-digestion was at pH=10 at 55 °C and pH=8 at 55 °C, which enhanced methane production by 9.9 ±1.5% (P=0.01) and 9.5 ±0.4% (P=0.01) compared to FOG co-digestion and KW co-digestion without thermo-chemical pre-treatment, respectively. It is important to note that the FOG co-digestion with a thermo-chemical pre-treatment of pH=10, 55 °C provided the highest (74.7 ±1.63%) ultimate methane percentage. Comparing the increases in ultimate methane production, non-linearly estimated lag phase periods and estimated maximum methane production rates of all co-digestions, thermo-chemical pre-treatment would be suggested as the best pre-treatment approach to enhance the methane production from co-digestions with municipal organic wastes.

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Chapter 6

Biogas Production Performance of Mesophilic and Thermophilic Anaerobic Co-digestion with Fat, Oil, and Grease (FOG) in Semi-continuous Flow Digesters: Effects of Temperature, Hydraulic Retention Time and Organic Loading Rate

Abstract: Anaerobic co-digestions with fat, oil, and grease (FOG) were investigated in semi-continuous flow digesters under various operating conditions. The effects of hydraulic retention time (HRT) of 12 and 24 days, organic loading rate (OLR) between 1.19 and 8.97 gTVS/L d, and digestion temperature of 37 ℃ and 55 ℃ on biogas production were evaluated. It was proposed that, compared to anaerobic digestion with wastewater treatment plant sludge (primary raw sludge), semi-continuous flow anaerobic co-digestion with FOG could effectively enhance biogas and methane production under ideal conditions. Thermophilic (55 ℃) co-digestions exhibited higher biogas production and organic conversion than mesophilic co-digestions. The best biogas production rate of 17.4 ±0.86 L/d and methane content 67.9 ±1.46% was obtained with a thermophilic co-digestion at HRT=24 days and OLR=2.43 ±0.15 gTVS/L d; these were 32.8% and 7.10% higher than the respective values from the mesophilic co-digestion under similar operating conditions.

Keywords: Anaerobic co-digestion; fat, oil, and grease; biogas production; mesophilic; thermophilic; semi-continuous digester.

6.1 Introduction

Biogas, specifically the methane, from wastewater treatment plant anaerobic digesters has been reported as a renewable energy source, which could allow one to recover 20-40% of the on-site energy requirement of a treatment plant (Crawford and Sandino, 2010). To enhance biogas production and assist in wastewater treatment and municipal organic waste management, anaerobic co-digestion has been recognized as an effective, low-cost and commercially viable approach to improve methane yields (Alatriste-Mondragón et al., 2006). It has been shown in a number of studies that co-digestion with municipal or food industry waste fat, oil and grease (FOG) employed as a co-substrate can increase methane production since FOG requires less mass loading per unit methane production and has demonstrated better biogas production potential than other organic wastes (Davidsson et al., 2008; Kabouris et al., 2008; Kabouris et al., 2009; Li et al., 2011; Martín-González et al., 2011).

Although FOG has been reported as a beneficial and effective co-substrate, most studies to date have been conducted through laboratory-scale (150-250 mL) batch investigations (e.g., using biochemical methane potential (BMP) tests). Surprisingly few reports have focused on pilot-scale or bench-scale FOG co-digestion in continuous flow digesters, and consequently the successful implementation of this approach in Canadian municipalities has been limited (Li et al., 2011). This can probably be attributed to the wide range of FOG co-digestion operational challenges including process inhibition, substrate transportation limitations, digester flotation and foaming, and blocking or clogging of the digestion pipeline systems (Long et al., 2011).

In continuous-flow anaerobic digestion, many parameters including substrate characteristics, flow rate, organic loading rate (OLR), hydraulic retention time (HRT), process temperature, pH and organic constituents (e.g. organic acids, proteins, and nutrients) can affect digestion performance and biogas production (Gerardi, 2003). Operational parameters including HRT between 10-36 days (Callaghan et al., 2002; Fezzani and Cheikh, 2010; Gerardi, 2003; Goberna et al., 2010), various OLR on the basis of total volatile solids (TVS) (Callaghan et al., 2002; Gómez et al., 2006; Kabouris et al., 2009), and digestion temperatures either as mesophilic (37 ℃) or thermophilic (55 ℃) (Kabouris et al., 2009; Gallert and Winter, 1997; Goberna et al., 2010; Zhang et al., 2009) have been tested to investigate their effects on the digestion process and performance. However, most research has not specifically focused on co-digestion with FOG. It has been reported in our previous BMP study that the FOG are more sensitive to the digestion configuration than other municipal organic wastes (Li et al., 2011). Hence, in order to ensure a successful and efficient biogas production from FOG co-digestion in continuous-flow digesters, more research is necessary. This research could provide further information for future FOG co-digestion tests and applications at the larger scales.

The main objective of this research was to identify ideal operational ranges to enhance biogas and methane production from semi-continuous flow co-digestion with FOG under mesophilic and thermophilic conditions. Various physical and chemical parameters including pH, solids content, organic constituent composition and methane production potential were monitored during each co-digestion, and the results were analyzed to derive operational conditions for pilot- and full-scale applications.

6.2 Material and Methods

6.2.1 Inocula and Substrates

In order to simulate an actual anaerobic digestion process and to obtain fresh substrate for daily use, the thermophilic semi-continuous anaerobic digester experiments were conducted onsite at the Ravensview Water Pollution Control Plant (Kingston, Ontario, Canada). Anaerobic digester sludge (ADS), used as the inoculum for initiating the digestions throughout these experiments, was collected from the full-size anaerobic digesters at Ravensview. Primary raw sludge (PRS), which was fed as the substrate to the digesters at the plant, was employed as the primary substrate in all experiments. This sludge was collected and stored at 4℃ for no more than 5 days prior to each use. Municipal FOG was collected from the garbage waste oil receptacle of the Graduate Club, a restaurant located on the Queen’s University main campus (Kingston, Ontario, Canada), and consisted of a mixture of waste frying oil, bacon grease and animal fat. It was collected in 1 L batches and stored in a 1 L glass bottle at 4℃. Characteristics of the substrates are listed in Table 6.1.

Table 6.1 Characteristics of the substrates fed into digesters in each of the three semi-continuous flow anaerobic co-digestion investigations S1, S2, and S3 of the tests.

	S1 HRT=12days		**S2 37 ℃; HRT=24days**		**S3 37 ℃; HRT=24days**	
Substrate	PRS	FOG	PRS	FOG	PRS	FOG
pH	6.1	4.5	5.9	4.4	5.9	4.5
TS (g/L)	41.9±1.07	1119±28.3	44.9±5.78	1119±28.3	39.8±1.86	1137±2.12
TVS (g/L)	31.6±3.33	951±15.6	31.5±3.29	951±15.6	28.5±1.45	995.2±22.2

6.2.2 Bench-scale Semi-continuous Anaerobic Digester Design

All tests were conducted using four 15 L pilot-scale semi-continuous flow anaerobic digesters with 12 L working volumes. The heating of these digesters to 37 ℃ or 55 ℃ was performed with electric heating cables and silicon heating blankets, respectively. The temperature was controlled using a Love Controls® option model 986 (with 2500 series temperature controller) portable thermo-control box combined with thermocouples. It is necessary to note that, as the digesters were made of plexiglass, heating of the digesters to 55 ℃ was staged and the temperature was increased and controlled gradually (increased 5-10 ℃ during 7 days) throughout the heating process. Each digester was equipped with an electric motor mixer (RW 205DS1, IKA Works, Inc., USA). All motor mixers were held by stainless steel stands. It has been reported by Stroot et al. (2001), Kaparaju et al. (2008) and Sulaiman et al. (2009) that minimal mixing or intermittent mixing improved biogas production compared to continuous mixing. Similar observations were also reported in other studies where gas circulation was employed to mix the substrates (Karim et al., 2005; Rico et al., 2011). This phenomenon was attributed to a better consumption of digestion by-products by slower hydrolysis and fermentation without affecting the syntrophic association during minimal or intermittent mixing. Hence, with comprehensive consideration of digester volume, mixing power input and practical operation, the mixers used in this study were adjusted to run at approximately 200 rpm and mixed all digesters continuously for 2 hours daily. Waste sludge extraction and substrate feed were conducted during the mixing period. For gas collection and monitoring, wet tip gas meters (invented by Dr. R.E. Speece and manufactured by Rebel Point Wet Tip Gas Meter Co., Nashwille, TN, USA) were connected to the headspaces of the digesters. The meters needed to be refilled and calibrated every two weeks given the water evaporation from the tip box. A feeding ball valve was fixed to the top of each digester and an effluent relief ball valve was located around the bottom-middle layer of the digester cylinder. Digesters were wasted and then fed manually at a 24 h interval. The feeding rate was an equal amount to the effluent removed. All digesters were installed and operated at the Ravensview Water Pollution Control Plant. The digester setup schematic is shown in Figure 6.1.

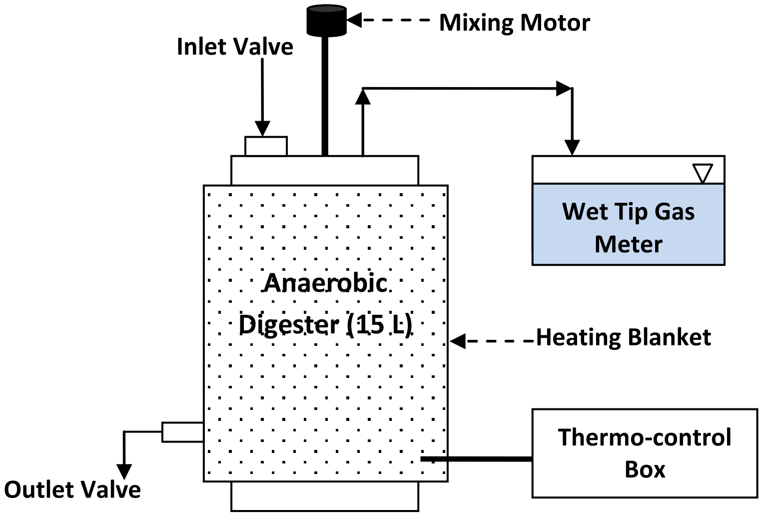

Figure 6.1 Schematic of bench scale (15L) semi-continuous flow anaerobic digester setup

6.2.3 Experimental Procedure

In this study, three investigations: 1) Investigation S1; 2) Investigation S2; and 3) Investigation S3 were conducted at the Ravensview Water Pollution Control Plant according to the procedures shown in Table 6.2. Four digesters were operated in parallel in each investigation, with different operational conditions including hydraulic loading rate (flow rate, L/d), HRT (d), OLR (gTVS/L d), where L represents the digester working volume), and temperature. At the beginning of each investigation, 12 L of ADS were fed into clean digesters as the inoculum to incubate for no less than 4-6 days. No substrates were fed into the digesters during the incubation period to allow for acclimation, until the digesters were warmed to the desired operational conditions and had reached biogas production rates similar to each other. PRS and FOG were then fed as the co-substrates into the digesters according to the scheduled OLR and HRT as shown in Table 6.2.

Table 6.2 Digester operation parameters for investigations S1, S2 and S3

	Investigation S1 OLR Selection					
	Inlet Flow Rate (PRS+FOG) (L/d)	***FOG Rate (mL/d)***	***HRT (d)***	***Temp*** (°C)	***OLR (gTVS/L d)***	***Initial S/I Ratio***
S1R1	1.0	20	12	37±2	4.22±0.28	0.29
S1R2	1.0	60	12	37±2	7.39±0.30	0.51
S1R3	1.0	80	12	37±2	8.97±0.29	0.62
S1R4	1.0	0	12	37±2	2.63±0.28	0.18
	Investigation S2 Mesophilic Co-digestion					
	Inlet Flow Rate (PRS+FOG) (L/day)	***FOG Rate (mL/d)***	***HRT (d)***	***Temp*** (°C)	***OLR (gTVS/L d)***	***Initial S/I Ratio***
S2R1	0.5	5	24	37±2	1.71±0.25	0.13
S2R2	0.5	15	24	37±2	2.50±0.23	0.20
S2R3	0.5	30	24	37±2	3.69±0.24	0.29
S2R4	0.5	0	24	37±2	1.31±0.17	0.10
	Investigation S3 Thermophilic Co-digestion					
	Inlet Flow Rate (PRS+FOG) (L/day)	***FOG Rate (mL/d)***	***HRT (d)***	***Temp*** (°C)	***OLR (gTVS/L d)***	***Initial S/I Ratio***
S3R1	0.5	5	24	55±2	1.60±0.13	0.13
S3R2	0.5	15	24	55±2	2.43±0.15	0.20
S3R3	0.5	30	24	55±2	3.68±0.17	0.30
S3R4	0.5	0	24	55±2	1.19±0.12	0.10

6.2.3.1 *Investigation S1: Mesophilic Co-digestion for Ideal HRT and OLR Selection*

In order to obtain efficient and stable biogas production in semi-continuous flow digesters, it is important to determine the ideal HRT and OLR, particularly for anaerobic co-digestion microbial consortia, which have been reported to be more sensitive to FOG than other substrate loads (Fezzani and Cheikh, 2010; Li et al., 2011). In our previous BMP study (Li et al., 2011), it was concluded that an ideal substrate to inoculum ratio (S/I) on a total volatile solids (TVS) basis could effectively enhance biogas production and would be necessary to achieve a maximum ultimate methane production per unit mass of substrate. The ideal S/I ratio for FOG co-digestion in BMP testing was found to range between 0.3 and 0.7, and 0.46 was suggested as the optimum S/I ratio to yield the best methane production efficiency. In addition, an OLR and S/I ratio based on TVS could be employed and would simplify process operation since TVS analysis is more practical than the analysis of other organic parameters (e.g. chemical oxygen demand, COD). Hence, the initial S/I ratio on a TVS basis was utilized to allow the determination of a suitable OLR (gTVS/L d) and the FOG/PRS feeding configurations. According to the estimated digestion time (265 h≈11days) of ultimate biogas production obtained from the previous BMP study (Li et al., 2011), and other empirical reports on HRT (Gerardi, 2003), an HRT of 12 days was selected for testing in Investigation S1. All experimental conditions are presented in Table 6.2.

6.2.3.2 *Investigations S2 and S3: Mesophilic and Thermophilic Co-digestion Tests*

Investigations S2 and S3 were subsequently conducted based on the suitable operational parameters selected from Investigation S1. The digestion temperatures for these two investigations were adjusted to 37 ℃ and 55 ℃, respectively. The initial S/I ratios, OLRs and HRTs of these two investigations were similar to allow for a direct examination of the effect of digestion temperature on biogas production. These experimental conditions are presented in Table 6.2.

6.2.4 Sample Analysis

Parameters that were monitored throughout the tests, including pH, total solids (TS) and TVS, COD and soluble chemical oxygen demand (SCOD) were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Samples for soluble chemical oxygen demand (SCOD), volatile fatty acids (VFA), ammonia nitrogen (free ammonia NH_3-N) and total Kjeldahl nitrogen (TKN) analysis were extracted from the original sample supernatant after centrifuging for 15 minutes. VFA were analyzed using ion chromatography (Dionex ICS-3000, column IONPAC AS11). Free ammonia was analyzed using an Autoanalyzer System (SEAL AA3) and read colorimetrically at 630 nm. The TKN samples were prepared by acid digestion at 380 ℃ in the presence of a catalyst based on the standard method 4500-N (APHA, 2005) and analyzed with SEAL Analytical AACE 6.05 Autoanalyzer System reading colorimetrically at 660 nm. Biogas samples were collected from the headspace of the digesters using Cole-Parmer® Kynar dual-valve gas sampling bags. The contents of the gas samples (CH_4 and CO_2) were analyzed by gas chromatography (Agilent 3000 Micro GC with Plot U backflush module, TCD detector) calibrated on a volume basis (V/V). The results of the gas analysis were reported at standard temperature and pressure (STP, 101.325 kPa, 273.15 K). The hydrogen sulfide (H_2S) was measured using Dräger® tubes (0-200 ppm, Dräger, Germany). In this test, the digestion was considered to have achieved steady state when the biogas production rate varied by less than 5% for five consecutive days. Gas and liquid samples were collected 2-3 times per week with duplicate or triplicate analyses once the digesters had reached steady state.

6.3 Results and Discussion

6.3.1 Mesophilic Semi-continuous Co-digestions (Investigation S1) for Selection of Ideal OLR and HRT

From Figure 6.2(a), when compared to the mesophilic digestion with PRS feeding alone (S1R4), mesophilic co-digestions with FOG and PRS co-substrates at OLR 4.22±0.28g TVS/L·d (S1R1), OLR 7.39±0.30 gTVS/L·d (S1R2) and OLR 8.97±0.29 gTVS/L·d (S1R3) exhibited substantially higher biogas production rates during the first 6 days. The maximum biogas productions in these three co-digesters were 28.6 L/day, 28.0 L/day, and 30.7 L/day, respectively, compared to the 15.3 L/day obtained during the S1R4 digestion. However, it can also be observed from Figure 6.2(a) that the biogas productions from co-digestions at OLR 7.39±0.30 gTVS/L·d (S1R2) and OLR 8.97±0.29 gTVS/L·d (S1R3) decreased sharply after 6 days and eventually stopped. Although there was no similar suspension in biogas production observed in co-digestion at OLR

4.22±0.28 gTVS/L·d (S1R1), the biogas production rate also decreased consistently after 6 days and reached a similar rate to that of digester S1R4.

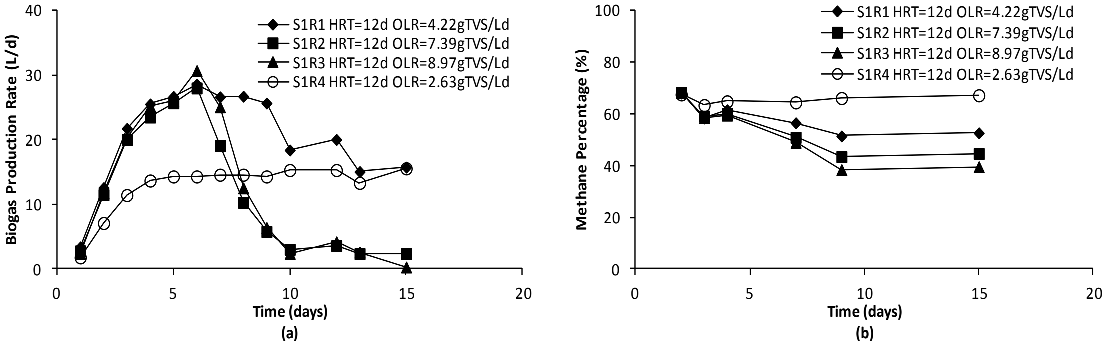

Figure 6.2 Investigation S1 semi-continuous flow digesters of mesophilic (37 ℃) co-digestions with 12 day HRT and various OLR: (a) biogas production rates of digesters and (b) methane percentage in the biogas.

It can be seen from Figure 6.2(b) and Table 6.3 that, although co-digesters S1R1, S2R2, and S3R3 produced more biogas (based on the maximum amount obtained) than S1R4 during their operation, the methane contents of the gas from these three digesters were much lower than that produced in S1R4 with PRS loading alone.

Table 6.3 Typical effluent and biogas characteristics of digesters in Investigation S1

	Initial S/I Ratio	**Final Effluent pH (after 6 days)**	**Final Effluent VFA (mg/L) (after 6 days)**	**Final Effluent TVS (g/L) (after 6 days)**	**Average Biogas Rate (L/d) (4-6 days)**	**Average CH4 Content (%) (4-6 days)**
S1R1	0.29	7.0±0.2	>3500	23.9±2.18	26.9±1.55	60.0±2.25
S1R2	0.51	6.7±0.4	>4000	28.9±2.45	25.7±2.25	59.6±0.71
S1R3	0.62	6.5±0.2	>4000	31.8±3.10	27.3±2.97	59.0±0.69
S1R4	0.18	7.2±0.1	<20	12.6±1.91	14.7±0.68	64.1±1.02

The typical effluent characteristics (after 6 days) of the four digesters in Investigation S1 are presented in Table 6.3. It can be noted from these characteristics that, at the end of the digestions, the total VFA (including acetic, propionic, butyric, and isobutyric acids) concentrations in the final effluents from the digesters with FOG and PRS co-substrates were much higher than those observed in digester S1R4 with PRS substrate alone. The total effluent VFA concentrations increased with increasing initial S/I ratios in the influent and OLR. In co-digesters S1R1, S1R2 and S1R3, the final total VFA concentrations were above 3500 mg/L, which would be one of the primary reasons for biogas production inhibition in these digesters. The pH values in the final effluents would also support VFA accumulation in these digesters, as the pH values were found to decrease with increasing S/I ratio and OLR. The final pH values of the co-digesters were under 7.0, which is not within the generally accepted optimal pH range (7.0 to 8.0) for co-digestion with FOG (Kabouris et al., 2008; Kabouris et al., 2009; Gelegenis et al., 2007; Martín-González et al., 2011; Li et al., 2011). In addition, consistent with the VFA accumulation, it can be also noted from Table 6.3 that the effluent TVS concentrations increased with initial S/I ratio and OLR. This could imply that higher initial S/I ratios and correspondingly higher OLR would lead to VFA and TVS accumulation, and subsequent inhibition and suspension of the anaerobic co-digestion processes. Moreover, although the ideal S/I ratio ranges for FOG co-digestions have generally been reported to be between 0.3 and 0.7 based on BMP tests as noted by Li et al. (2011), the initial S/I ratio for semi-continuous digestion should be lower since organic matter accumulation is likely with semi-continuous substrate feeding.

Based on observations from Figure 6.2 and Table 6.3, it can generally be summarized that semi-continuous flow anaerobic co-digestion with FOG could effectively enhance biogas and methane production with suitable organic loading. However, with the initial S/I ratio>0.51 and OLR>7.39±0.30 gTVS/L·d, a HRT=12 days as investigated in this study was too short to sustain efficient digestion and the degradation of the organic constituents, as well as the conversion of the intermediate products (e.g. VFA). Fezzani and Cheikh (2010) also reported similar observations, where they noted inhibition during their semi-continuous co-digestion with HRT=18 days, and that the inhibition was more significant in the co-digestion at higher OLR. They also pointed out that HRT=24 days could provide a more stable biogas production within a HRT range of 18 to 36 days. Other studies have also suggested that continuous flow anaerobic co-digestion with HRT>20 days could achieve steady state biogas production (Callaghan et al., 2002; Goberna et al., 2010; Gómez et al., 2006). Hence, it would be suggested that longer HRT and lower OLR (on a TVS basis) be utilized in semi-continuous flow anaerobic co-digestions to ensure optimum biogas and methane productions. Although the previous BMP batch tests (Li et al., 2011) provided essential information on potentially suitable co-substrate selection (e.g. FOG), ideal S/I ratio ranges and necessary retention time estimates, to assess effective performance accurately, the operational parameters for semi-continuous flow digestion also need to be evaluated from a series of tests conducted based on this fundamental information. As such, two investigations (Investigations S2 and S3) of semi-continuous co-digestions with FOG were undertaken with operational conditions of HRT=24days, initial S/I ratio<0.51, and OLR<7.39±0.30 gTVS/L·d as shown in Table 6.2. In order to compare the effect of digestion temperature, Investigations S2 and S3 were conducted under mesophilic (37 ℃) and thermophilic (55 ℃) conditions, respectively.

6.3.2 Comparison of Mesophilic (Investigation S2) and Thermophilc (Investigation S3) Semi-continuous Co-digestions

6.3.2.1 *Biogas and Methane Production in Mesophilic Co-digestions (Investigation S2)*

Figure 6.3 (a) and (b) illustrate the experimental results of the biogas production performances in four mesophilic semi-continuous flow digesters with OLR between 1.31 and 3.69 gTVS/L d. The typical characteristics of the effluents from these digesters at steady state (after 10 days) are summarized in Table 6.4.

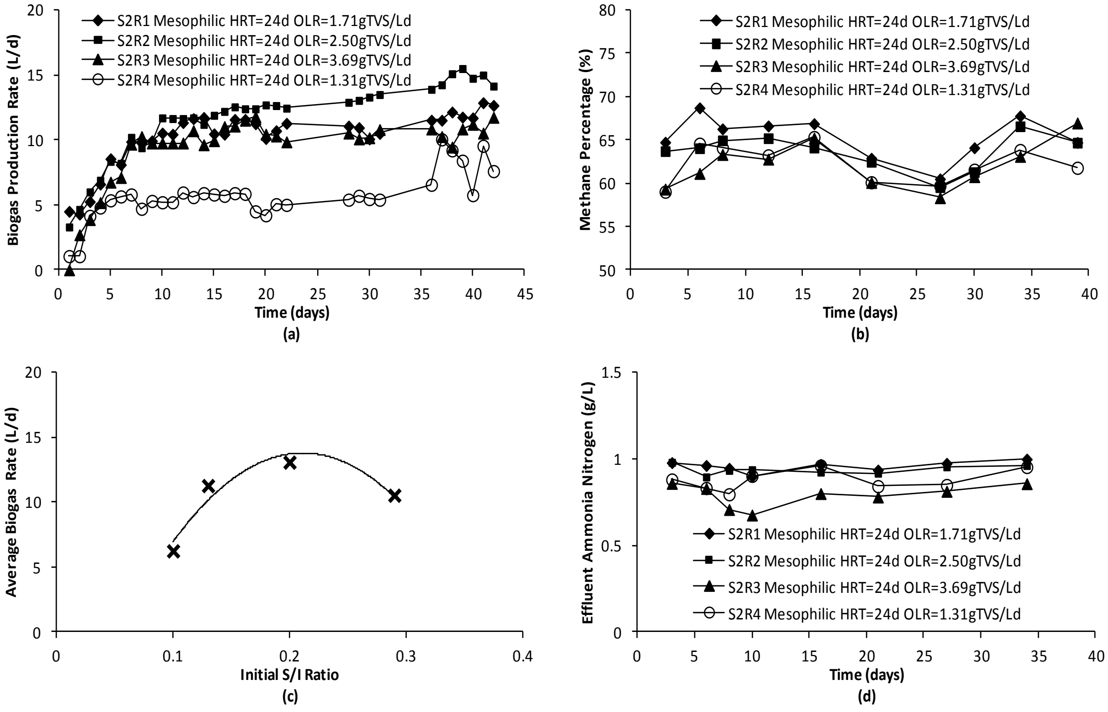

Figure 6.3 Investigation S2 semi-continuous flow digesters for mesophilic (37 ℃) co-digestions with 24 day HRT and various OLR: (a) biogas production rates of digesters, (b) methane percentage in the biogas, (c) average biogas production rate from digesters with various initial S/I ratio, and (d) effluent ammonia nitrogen concentrations.

Table 6.4 Typical effluent characteristics of digesters within steady state in Investigations S2 and S3

	STEP 2				**STEP3**			
Digester	***S2R1***	***S2R2***	***S2R3***	***S2R4***	***S3R1***	***S3R2***	***S3R3***	***S3R4***
Initial S/I Ratio	0.13	0.2	0.29	0.1	0.13	0.2	0.3	0.1
Average Gas Rate (L/d)	11.3 ±0.74	13.1 ±1.23	10.5 ±0.67	6.24 ±1.60	11.4 ±1.09	17.4 ±0.86	13.0 ±1.65	6.23 ±1.25
Average CH_4 (%)	64.8 ±2.56	63.4 ±2.44	62.4 ±3.00	62.2 ±2.03	67.2 ±1.01	67.9 ±1.46	64.5 ±2.18	66.8 ±1.09
H_2S(ppm)	5-10	25-30	80-100	5-10	5-10	15-30	70-100	5-10
pH	7.6 ±0.1	7.7 ±0.2	7.4 ±0.1	7.6 ±0.1	7.7 ±0.1	7.8 ±0.1	7.5 ±0.1	7.8 ±0.1
Acetic acid (mg/L)	147 ±31.1	190 ±34.4	146 ±55.3	55.2 ±5.68	113 ±15.6	265 ±10.5	154 ±15.1	67.2 ±3.06
TVS (g/L)	14.5 ±0.20	15.4 ±1.08	18.5 ±3.10	13.3 ±0.83	11.1 ±0.60	11.5 ±0.93	11.6 ±1.17	11.9 ±0.48
COD (g/L)	19.9 ±0.96	19.3 ±2.50	24.4 ±5.13	18.7 ±1.57	17.7 ±2.04	20.7 ±1.60	24.2 ±1.78	18.9 ±1.32
SCOD (g/L)	1.85 ±0.29	1.94 ±0.19	2.83 ±0.39	1.09 ±0.40	4.26 ±0.50	5.91 ±0.92	7.80+1.02	2.71 ±0.41
SCOD/COD (%)	9.30	10.1	11.6	5.83	24.1	28.6	32.2	14.3
Ammonia (g/L)	0.97 ±0.03	0.94 ±0.02	0.81 ±0.03	0.90 ±0.06	1.02 ±0.04	1.02 ±0.05	0.91 ±0.05	1.07 ±0.04
TKN (g/L)	1.01 ±0.05	1.02 ±0.07	0.92 ±0.03	0.92 ±0.05	1.34 ±0.07	1.37 ±0.17	1.27 ±0.41	1.17 ±0.27

It can be noted from Figure 6.3(a) that the digestions in Investigation S2 all reached a stable biogas production after 10 days, which implies that the initial S/I ratio and OLR within the ranges determined from Investigation S1 were appropriate and could allow sufficient reaction between the co-substrates and inocula. Consistent with the results obtained during Investigation S1, all co-digesters (S2R1, S2R2 and S3R3) with FOG co-substrate at OLRs between 1.71 to 3.69 gTVS/L·d achieved much higher biogas production rates and quantities than the digester with PRS alone as the substrate at OLR of 1.31±.017 gTVS/L·d (S2R4). From Figure 6.3(c), it can be seen that the biogas production rate increased with increasing initial S/I ratio (OLR) until the initial S/I ratio and OLR reached 0.20 and 2.50 gTVS/L·d in digester S2R2, respectively. Although co-digester S2R3 was fed with a higher OLR (3.29±0.24 gTVS/L·d), it yielded a lower average biogas production rate and average methane content than co-digesters S2R1 (OLR=1.17±0.25 gTVS/L·d) and S2R2 (OLR=2.50±0.23 gTVS/L·d) (Figure 6.3(b)). Within these three co-digesters receiving FOG, S2R2 with an initial S/I=0.20 and OLR=2.50±0.23 gTVS/L·d yielded the highest average biogas production rate (13.1±1.23 L/d) with an average methane content (63.4±2.44%) and lower H_2S content (25-30ppm) at steady state. Compared to digester S2R4 with PRS feeding alone, co-digester S2R2 (OLR=2.50±0.23 gTVS/L·d) improved the biogas production rate (13.1±1.23 L/d) to more than 2 times the rate from digester S2R4 (6.24±1.60 L/d). These results were consistent with our previous BMP tests (Li et al., 2011) and the co-digestion experiments with olive-oil rich wastewater reported by Gelegenis et al. (2007), where the biogas production increased with the initial S/I ratio and OLR on a TVS basis, but the biogas production were inhibited once the S/I ratio or OLR were higher than the ideal range of values.

It is generally recognized that most methane production from anaerobic digestion is from the biodegradation or conversion of acetic acid (Gerardi, 2003). According to the digester effluent characteristics summarized in Table 6.4, acetic acid (CH_3COOH) might be one of the factors influencing the biogas production rates and corresponding methane contents. Under mesophilic conditions, co-digestion in S2R2 yielded the highest biogas production rate (13.1±1.23 L/d) with a methane content of 63.4±2.44%, and released the highest average acetic acid concentration (190 mg/L). The co-digestion in S2R4 with the lowest OLR decreased the average acetic acid concentration to 55.3 mg/L during the steady state, which might limit methanogenesis and result in a lower biogas production rate (6.24±1.60 L/d) than in the other digesters.

Although ammonium ion (NH_4^+-N) has been widely investigated to evaluate its effect on biogas production (Chen et al., 2008; Gallert and Winter, 1997), it is also been well recognized that free ammonia (NH_3-N) has a much higher toxicity and has a greater effect on anaerobic digestion than the ammonium concentration since free ammonia can directly penetrate cells and disturb the metabolism of microorganisms (Angelidaki and Ahring, 1993). Hence, free ammonia could be recommended as a suitable indicator of anaerobic digester performance. In this study, the free ammonia was analyzed using a colorimetric method and the results are shown in Table 6.4. It can be noted from Table 6.4 and Figure 6.3 (d) that the free ammonia (NH_3-N) and TKN in co-digester at OLR=3.69±0.24 gTVS/L d (S2R3) were lower than these concentrations in co-digesters S1R1 (OLR=1.17±0.25 gTVS/L d) and S2R2 (OLR=2.50±0.23 gTVS/L d), which would be one of the other main reasons for the lower methane production in the digester S2R3. It should be noted that with a relatively lower average biogas production at steady state (10.5±0.67 L/d), the average free ammonia concentration in co-digester S2R3 (0.81±0.03 g/L) was lower than the concentrations in the other mesophilic co-digesters (0.97 g±0.03 g/L for S2R1 and 0.94±0.02 g/L for S2R2). In addition, the TKN in co-digester S2R3 was also lower than in the other co-digesters. Although it has been suggested in some studies that a free ammonia concentration higher than 200-400 mg/L could result in methane production inhibition, the actual inhibiting threshold ammonia concentration can be a function of differences in substrates and inocula, environmental conditions and acclimation periods (Chen et al., 2008; Gallert and Winter, 1997). Hansen et al. (1998) and Nakakubo et al. (2008) reported that methane production decreased if the free ammonia was higher than 1.1 g/L and 1.45 g/L in the anaerobic digestion of animal manure, respectively. Procházka et al., 2012 noted that high ammonia-nitrogen concentration (4.0 g/L) inhibited methane production, while low ammonia-nitrogen concentration (0.5 g/L) also resulted in low methane yields and loss of aceticlastic methanogenic activity. Hence, an ammonia increase within the ideal range could help to increase the stability of the anaerobic digestion process. The results presented here are consistent with this inference and could indicate that the biogas and methane production from the semi-continuous co-digestion of FOG under the suitable operational conditions (S/I range 0.1 to 0.3) were not inhibited by free ammonia concentrations under 1.0 g/L. Higher free ammonia concentrations in co-digesters S2R1 (0.97 g/L) and S2R2 (0.94 g/L) even appeared to promote the biogas and methane production rates. This may be due to the fact that, within the suitable ammonia concentration ranges, the digestion process was stable and the methanogens were adapted to a relatively high ammonia concentration. The increase in TKN and ammonia concentrations could allow the digestion process to be more resistant to instability and minimize the risk of ammonium-nitrogen limitation for the methanogens (Fezzani and Cheikh, 2010; Nakakubo et al., 2008).

According to the results presented in Figure 6.3 and Table 6.4, it can be summarized that in the mesophilic continuous-flow digestion investigation with FOG as the co-substrate, the effective OLR and S/I ratio with HRT=24 days would be 2.50±0.23 gTVS/L·d and 0.2, respectively, as evidenced by the performance of digester S2R2. The observation was similar to the results reported by Wan et al. (2011) where an OLR=2.34 gTVS/L·d produced an efficient mesophilic semi-continuous anaerobic co-digestion of FOG with the highest biogas production rate and methane content.

6.3.2.2 *Biogas and Methane Production in Thermophilic Co-digestions (Investigation S3)*

The biogas productions and methane contents obtained during the thermophilic co-digestion investigation are presented in Figure 6.4. Typical average digester effluent characteristics that could influence co-digestion performance are sumarized in Table 6.4.

The OLR and HRT applied in these thermophilic investigation (Investigation S3) were similar to those used during the mesophilic investigation (Table 6.2). The biogas production rate from co-digesters S3R1 at OLR=1.63±0.13 gTVS/L·d (11.4±1.09 L/d), S3R2 at OLR=2.43±0.15 gTVS/L·d (17.4±0.86 L/g), and S3R3 at OLR=3.68±0.17 gTVS/L·d (13.0±1.65 L/g) with FOG co-substrate all yielded higher biogas production than digester S3R4 (6.23±1.25 L/g) without FOG. Although the OLR in the thermophilic digester S3R4 with PRS alone was slightly lower than for the mesphilic digester S2R4 because of the seasonal variability in the TVS content of the PRS from the wastewater treatment plant (Table 6.1), the biogas production rate (6.23±1.25 L/d) was similar to the mesophilic digester (6.24±1.60 L/d), and the average methane content (66.8±1.09%) was higher during the thermophilic operation than that of the mesophilc digester S2R4 (62.2±2.03%). This would suggest that the thermophilc condition positively affected the methane production.

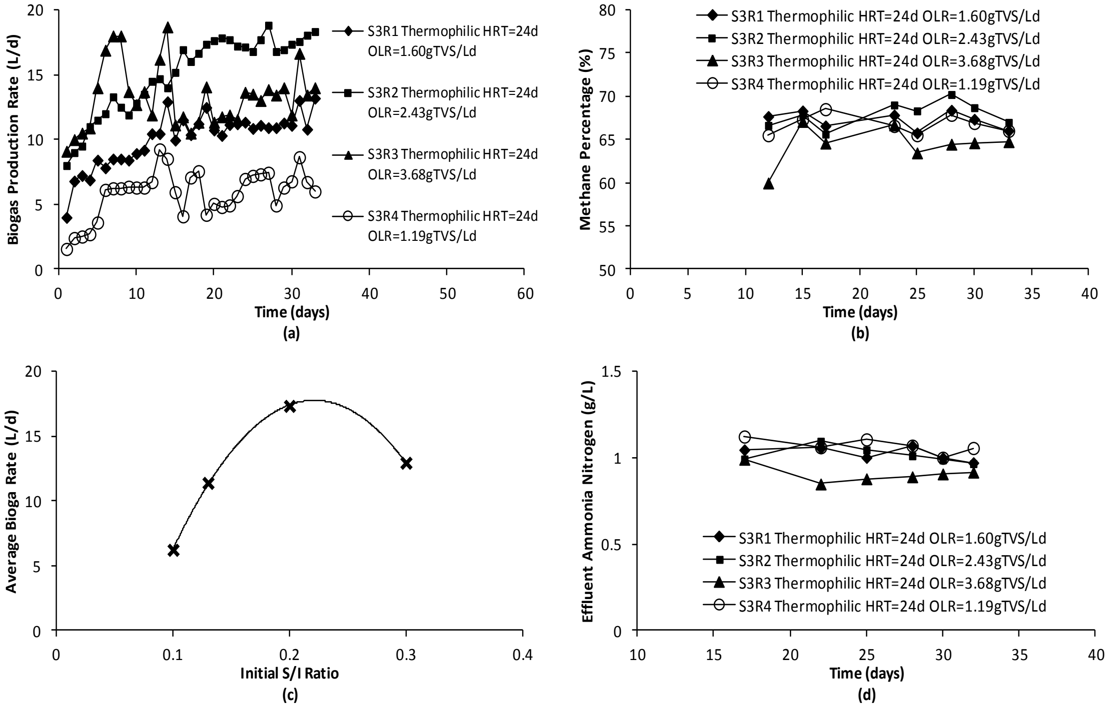

Figure 6.4 Investigation S3 semi-continuous flow digesters for thermophilic (55 ℃) co-digestions with 24 day HRT and various OLR: (a) biogas production rates of digesters, (b) methane percentage in the biogas, (c) average biogas production rate from digesters with various initial S/I ratio, and (d) effluent ammonia nitrogen concentrations.

It is noted from Table 6.4 that all co-digesters exhibited higher average biogas production performance under thermophilic operation than those under mesophilic conditions. In addition to the higher VFA (acetic acid) and nitrogen (free ammonia and TKN) concentrations, the overall biogas enhancement in the thermophilic co-digesters might also be attributed to an increase in COD solubilization (expressed in Table 6.4 as SCOD/COD %). Some studies have reported that higher COD solubilization could be achieved with an increase in digestion temperature, and this in turn could improve biogas production (Zhang et al., 2009; Valo et al., 2004). As can be seen from Table 6.4, the COD solubilization was significantly higher in the thermophilic co-digesters, compared to the results obtained during mesophilic operation. Conversely, it can be noted from Figure 6.4(a) that the thermophilic digester S3R3 with initial S/I=0.30 and OLR=3.68 gTVS/L d did not exhibit a stable biogas production, unlike during the comparable mesophilc conditions, and as observed in the other thermophilic co-digesters, even though the average biogas production rate 13.0±1.65 L/d (Table 6.4) was higher than that observed in the in mesophilic co-digester S2R3 10.5±0.67 L/d.

Although the average methane production rate and content from thermophilic co-digester S3R3 with the highest OLR were higher than those obtained under the comparable mesophilic condition (Table 6.4), these were lower than in the thermophilic co-digester S3R2. This observation was consistent with the results obtained under mesophilic condition and would suggest again that higher OLR could yield lower methane contents and digestion efficiency (Gelegenis et al., 2007). In addition, as discussed for the mesophilic co-digestions, the lower free ammonia (0.91±0.05 g/L) and TKN (1.27 g/L) might also have lead to the unstable performance in the S3R3 digester, and the lower methane production potential than in the other thermophilic co-digesters. Within the three thermophilic co-digesters with FOG co-substrate operating under steady state, S3R2 achieved the highest average biogas production rate (17.4±0.86 L/d) and methane content (67.9±1.46%) with relatively lower H_2S content (15-30 ppm). This is likely related to the higher VFA concentration (average acetic acids=265±10.5 mg/L) with progression of methanogenesis and methane production potential (Gerardi, 2003; Luste et al., 2009). The higher average TKN (1.37±0.17 g/L) and free ammonia (1.02±0.05 g/L) under steady state operation (Table 6.4 and Figure 6.4(d)) for S3R2 should also provide sufficient nutrients for the co-digestion and support an efficient and stable biogas production. The H_2S concentrations from the thermophilic co-digesters decreased slightly compared to the mesophilic systems. However, the S3R3 co-digester with the highest OLR yielded the highest H_2S production (70-100 ppm), which has been reported as a detrimental by-product of the ananerobic digestion process (Osorio and Torres, 2009). In addition, as the operational temperature was higher, substrate floatation was not found to be significant at the end of the thermophilic operation. From the results of Investigation S3 it can be summarized that an initial S/I ratio=0.2 with OLR=2.34±0.15 gTVS/L d provided the best conditions for enhanced methane production under thermophilic (55 °C) condition with HRT=24 days. Hence, under optimized operational conditions, thermophilic co-digestion can enhance biogas production compared to mesophilic co-digestion and could be recommended for future semi-continuous FOG co-digestion systems.

6.4 Conclusions

Based on these results, semi-continuous flow FOG co-digestion with initial S/I ratio=0.20 and OLR of approximately 2.50 gTVS/L d was found to be the best operational conditions to enhance biogas production under both mesophilic and thermophilic conditions with HRT=24 days. The appropriate thermophilic co-digestion in digester S3R2 at OLR=2.43±0.15 gTVS/L d exhibited higher biogas production rate (17.4±0.86 L/d) and methane content (67.9±1.46%), which were 32.8% and 7.10% higher than the highest biogas production from mesophilic co-digester S2R2 at OLR=2.50±0.23 gTVS/L d, respectively. It can be concluded that semi-continuous flow anaerobic co-digestion with FOG can effectively enhance the biogas and methane production under optimized HRT and OLR operating conditions. In addition, thermophilic co-digestion was found to yield higher biogas and methane production, lower hydrogen sulfide release, and more efficient organic matter conversion than mesophilic co-digestion.

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Chapter 7

Enhanced Biogas Production from Anaerobic Co-digestion of Municipal Wastewater Treatment Sludge and Fat, Oil and Grease (FOG) by A Modified Two-stage Thermophilic Digester System with Thermo-chemical Pre-treatment

Abstract: Anaerobic co-digestions with fat, oil and grease (FOG) were investigated in two-stage thermophilic (55 ℃) semi-continuous flow co-digestion systems. One two-stage co-digestion system (System I) was modified to incorporate a thermo-chemical pre-treatment of pH=10 at 55 ℃, which was the best pre-treatment condition for FOG co-digestion identified during laboratory-scale biochemical methane potential (BMP) testing. The other two-stage co-digestion system (System II) was operated without a pre-treatment process. The anaerobic digester of each digestion system had a hydraulic retention time (HRT) of 24 days. An organic loading rate (OLR) of 1.83 ± 0.09 g TVS/L ·d was applied to each digestion system. It was found that System I effectively enhanced biogas production as the thermo-chemical pre-treatment improved the substrate hydrolysis including increase COD solubilization and VFA concentrations. Overall, the modified System I yielded a 25.14 ± 2.14 L/d biogas production rate, which was substantially higher than the 18.73 ± 1.11 L/d obtained in the System II.

Keywords: Two-stage anaerobic co-digestion; fat, oil and grease; biogas production; pre-treatment; thermophilic temperature; semi-continuous digester.

7.1 Introduction

To enhance biogas production from wastewater treatment plant anaerobic digesters and to assist in municipal organic waste management, anaerobic co-digestion has been recognized as an effective, low-cost and commercially viable approach (Alatriste-Mondragón et al., 2006; Natural Resource Canada, 2002). It has been shown in a number of studies that the municipally available organic waste fat, oil and grease (FOG) could be employed as a potential co-substrate in the co-digestion of wastewater treatment process sludges in order to increase methane production, since FOG requires less mass loading and presents a better biogas production potential than other organic wastes (Davidsson et al., 2008; Kabouris et al., 2008; Kabouris et al., 2009; Li et al., 2011; Martín-González et al., 2010).

In addition to anaerobic co-digestion, pre-treating the substrates using thermo-chemical conditioning has also been widely reported as an efficient approach to improve methane production (Fdez-Güelfo et al., 2011; Luste et al., 2009; Kim et al., 2003; Rafique et al., 2010; Valo et al., 2004). However, in these studies, thermo-chemical pre-treatment has mainly been investigated in the anaerobic digestion of municipal wastewater treatment plant sludge alone (e.g. waste activated sludge (WAS) or primary sludge) and most studies employed traditional single-stage digestion without co-substrate addition (Kim et al, 2003; Tanaka and Kamiyama, 2002; Vlyssides and Karlis, 2004). To our knowledge, FOG has seldom been utilized and evaluated in co-digestions with thermo-chemical pre-treatment.

Most studies that have examined FOG co-digestion performance to date have focused on the use of biochemical methane potential (BMP) tests, and little research has been conducted using bench-scale or pilot-scale continuous flow digesters; hence the successful implementation of this approach in Canadian municipalities has been limited (Li et al, 2011; Natural Resource Canada, 2002). This can be attributed to a range of FOG co-digestion operational challenges including process inhibition, substrate transport limitation, digester flotation and foaming, and blocking or clogging of the digestion pipeline systems (Long et al., 2011). Moreover, because of the complexity of continuous-flow digester operations, a number of parameters including substrate characteristics, flow rates, organic loading rates (OLR), hydraulic retention times (HRT), process temperature, pH and organic constituents (e.g. organic acids, proteins, and nutrients) could affect digestion performance and biogas production (Gerardi, 2003).

It has been reported that thermophilic conditions can effectively improve biogas production and minimize digester content flotation when compared to mesophilic conditions (Goberna et al., 2010; Kabouris et al, 2009). In addition, within the limited continuous-flow FOG co-digestion studies reported to date, most tests have concentrated on conventional one-stage digestion systems, but not the more common two-stage digestion which has been reported and recommended as an advanced anaerobic digestion system configuration to enhance substrate hydrolysis and biogas production (Demirer and Chen, 2005; Parawira et al., 2008). Furthermore, thermo-chemical pre-treatment has seldom been applied in the two-stage anaerobic digestion system, particularly during the FOG co-digestion (Fezzani and Cheikh, 2010; Kabouris et al., 2008; Kabouris et al., 2009; Park et al., 2005). Hence, based on a review of the current literature, anaerobic co-digestion with FOG in an advanced two-stage thermophilic semi-continuous digestion system combined with thermo-chemical pre-treatment could potentially yield a higher biogas production than conventional one-stage or two-stage digestions.

The main objective of this research was to compare and characterize the biogas and methane productions from FOG co-digestions in two-stage semi-continuous flow co-digestion systems with thermo-chemical pre-treatment (System I) and without pre-treatment (System II). The operational conditions of the digester were established based on the optimum operational parameters identified in our previous BMP and semi-continuous flow digestion studies (Chapters 4 and 5). Various physical and chemical parameters including pH, solid contents, organic constituents and methane production potential during each co-digestion were analyzed and are discussed.

7.2 Material and Methods

7.2.1 Biochemical Methane Potential (BMP) Tests for Optimum Pre-treatment Selection

7.2.1.1 Inocula and Substrates

In the BMP experiments, 4 L of anaerobic digester sludge (ADS) collected from the anaerobic digesters at the Cataraqui Bay Wastewater Treatment Plant (Kingston, Ontario, Canada) were stored at 4 ℃ for no more than 3 days and used as the inoculum for initiating the digestions. 4 L waste activated sludge (WAS), also obtained from the Cataraqui Bay Wastewater Treatment Plant, were employed as the primary substrate throughout all BMP experiments. The WAS was stored at 4 ℃ for 2 days, and then the supernatant was decanted to produce a concentrated substrate prior to utilization. The FOG was collected from the waste oil receptacle of the Graduate Club, a restaurant located on the main campus at Queen’s University (Kingston, Ontario, Canada), that consisted of a mixture of waste frying oil, bacon grease and animal fat. 1 L FOG was collected prior to testing and stored in a glass bottle at 4 ℃. Characteristics of the inocula and substrates utilized in the BMP tests are listed in Table 7.1.

Table 7.1 Characteristics of the inocula and substrates in BMP tests and characteristics of the substrates fed into two-stage semi-continuous digestion systems

	BMP Tests for Optimum Pre-treatment Selection			**Two-stage Thermophilic Semi-continuous Digestion Systems with and without Pre-treatment**	
Substrate	ADS	WAS	FOG	PRS	FOG
pH	7.8	7.3	4.1	6.1	4.5
TS (g/L)	22.7 ±0.03	16.8 ±0.32	996 ±0.66	36.8 ±1.40	806 ±10.5
TVS (g/L)	15.4 ±0.07	13.5 ±0.08	941 ±18.8	25.0 ±1.87	765 ±26.5

WAS=Concentrated waste activated sludge; PRS=Primary raw sludge; FOG=Fat, oil and grease; OLR= Organic Loading Rate (g TVS/L·d).

7.2.1.2 BMP Experimental Procedure

Prior to the installation of the semi-continuous flow reactors, BMP tests were conducted to determine the range of ideal thermo-chemical pre-treatment conditions to be used (Li et al., 2011). To assess the methane production from the FOG co-digestion, it is important to consider the substrate to inoculum ratio (S/I) on a total volatile solids (TVS) basis, since determining the ideal S/I ratio is necessary to achieve the maximum ultimate methane production per unit mass of substrate. In our previous work (Li et al., 2011), an ideal S/I ratio of 0.46 was identified for FOG co-digestions during BMP experiments. Hence, in this experiment, FOG was mixed with the primary substrate WAS and the inocula ADS within the range of this ideal S/I ratio.

Table 7.2 Substrate composition and operational conditions in BMP and two-stage semi-continuous digester tests

BMP Test for Optimum Thermo-Chemical Pre-treatment Selection

Duplicate Samples	*Inoculum ADS (mL)*	*WAS (mL)*	*FOG (g)*	*S/I Ratio*	*Substrate Mixture pH*	*Temp (℃)*
ADS	65	0	0	0	--	Room
ADS+WAS	65	15	0	0.2	7.5	Room
ADS+WAS+FOG	65	15	0.3	0.48	6.5	Room
ADS+WAS+FOG 55 ℃	65	15	0.3	0.48	6.4	55 ℃
ADS+WAS+FOG 55 ℃ pH=8	65	15	0.3	0.48	8	55 ℃
ADS+WAS+FOG 55 ℃ pH=10	65	15	0.3	0.48	10	55 ℃
ADS+WAS+FOG 55 ℃ pH=12	65	15	0.3	0.48	12	55 ℃

Two-stage Thermophilic Semi-continuous Digestion System Tests (System I and II)

		Inlet Flow Rate (mL/day)	*FOG Rate (mL/d)*	*HRT (d)*	*Temp (℃)*	*OLR [a] (g TVS/L d)*	*pH Control*
System I	**PR0**	500	15	1 [c]	55±2	1.83±0.09 [d]	9.0-10
	PR1	500 from PR0	0 [b]	24	55±2	1.57±0.08 [e]	-- [f]
	PR2	500 from PR1	0 [b]	24	55±2	0.37±0.03 [e]	-- [f]
System II	**R1**	500	15	24	55±2	1.83±0.09 [d]	-- [f]
	R2	500 from R1	0 [b]	24	55±2	0.46±0.04 [e]	-- [f]

[a] $OLR = \frac{Inlet\ Organic\ Loading\ Mass\ (g\ TVS/d)}{Working\ Volume\ of\ the\ Reactor\ (L)}$

[b] Digesters PR1, PR2, and R2 were fed with the substrates from the preceding digesters.

[c] Batch reactor PR0 with working volume of 0.5 L was for 1 day thermo-chemical pre-treatment.

[d] OLRs of PR0 and R1 present both 2-stage digester systems' OLRs.

[e] These OLRs are average values as the specific OLR varies with time and operation conditions

[f] No pH control for these digesters.

Prior to mixing with the ADS inoculum, mixtures of WAS and FOG (15 mL WAS and 0.3 g FOG) were pre-treated to specified pH levels (8, 10 and 12) through the addition of sodium hydroxide (NaOH; Fisher Scientific) solutions as the alkaline agent and then conditioned at 55 ℃ for 1 hour. NaOH was employed since a number of studies have reported that, compared to other widely applied acidic and alkaline compounds (e.g. HCl, H_2SO_4, KOH, $Mg(OH)_2$ and $Ca(OH)_2$), NaOH showed the highest potential for thermo-chemical pre-treatment leading to enhanced anaerobic digestion performance (Kim et al., 2003; Patel et al., 1993). The pre-treated mixtures were then mixed with inocula in 250 mL septum top glass bottles as outlined in Table 7.2 and flushed with N_2 gas for 30 seconds prior to sealing to ensure all the bottles were under anaerobic conditions. All BMP bottles were incubated in a New Brunswick 4500 incubation shaker at 37 ℃ and 100 rpm. The experimental conditions are outlined in Table 7.2; all BMP experiments were conducted in duplicate.

7.2.2 Comparison of Biogas Production Performances in Two-stage Thermophilic Semi-continuous Co-digestion Systems with and without Thermo-chemical Pre-treatment

7.2.2.1 Inocula and Substrates

In order to simulate an actual anaerobic digestion process and to obtain fresh substrate for daily use, the thermophilic semi-continuous anaerobic digester experiments were conducted onsite at the Ravensview Water Pollution Control Plant (Kingston, Ontario, Canada). The anaerobic digester sludge (ADS) used as the inoculum to initiate the digestions throughout the testing was collected from the full-size anaerobic digesters at

Ravensview. Primary raw sludge (PRS), which was fed as the substrate to the digesters at the plant, was employed as the main substrate in the semi-continuous experiments. This sludge was collected and stored at 4 ℃ for no more than 2 days prior to use. Municipal FOG was collected from the waste oil receptacle of the Graduate Club, a restaurant located on the Queen's University main campus (Kingston, Ontario, Canada) and stored in a glass bottle at 4 ℃. Characteristics of the substrates are listed in Table 7.1. The characteristics of inocula and substrates (e.g., sludge moisture content) in BMP tests and semi-continuous flow digestion investigations varied seasonally. Hence, the S/I ratios employed in BMP tests and the OLRs explored in semi-continuous flow digesters were adjusted based on TVS.

7.2.2.2 Semi-continuous Anaerobic Digester Design

The semi-continuous experiments were conducted using four 15 L semi-continuous flow anaerobic digesters with 12 L working volumes. The heating of these digesters to 55 ℃ was accomplished with silicon heating blankets (silicone rubber heaters, National Plastic Heater Sensor & Control Inc., Toronto, Canada). The temperature was controlled using Love Controls® option model 986 (with 2500 Series temperature controller) portable thermo-control boxes combined with thermocouples.

Each digester was equipped with an electric motor mixer (RW 205DS1, IKA Works, Inc., USA). All mixers were held in place by stainless steel stands. It has been reported by Stroot et al. (2001), Kaparaju et al. (2008) and Sulaiman et al. (2009) that minimal mixing (or intermittent mixing) results in better improvements in biogas production when compared to continuous mixing. Similar observations were also reported by other researchers who used gas circulation to mix substrates (Karim et al., 2005; Rico et al., 2011). This phenomenon was attributed to a better consumption of digestion by-products by slower hydrolysis and fermentation without affecting the syntrophic association during minimal or intermittent mixing. Hence, after consideration of digester volume, mixing power input and practical operation, the mixers used in this study were adjusted to operate at approximately 200 rpm and all digesters were mixed for 2 hours daily. Substrate feed and waste sludge extraction were conducted during the mixing period.

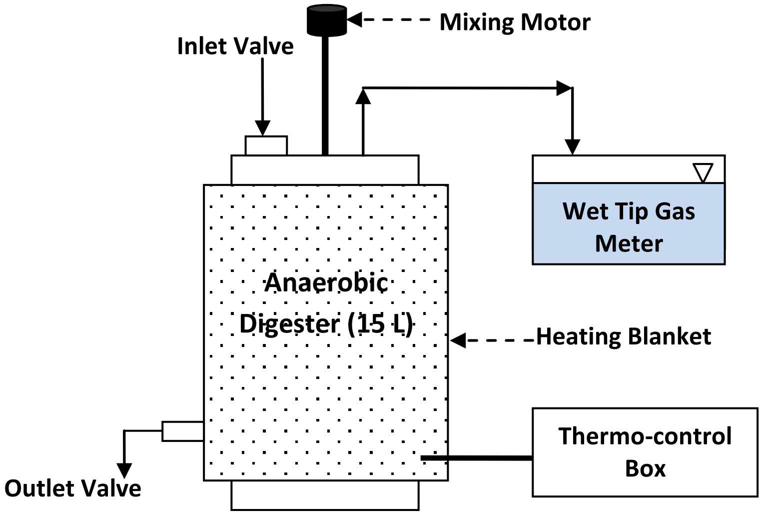


Figure 7.1 Schematic of bench scale (15L) semi-continuous flow anaerobic digester setup

For gas collection, characterization and quantification, wet tip gas meters were connected to the headspace of the digesters. The meters were refilled and recalibrated every two weeks due to water loss through evaporation. A feeding ball valve was fixed to the top of each digester and an effluent relief ball valve was located around the bottom-middle layer of the digester cylinder. Digesters were wasted and then fed manually at a 24 h interval. The feeding rate was equal to effluent removed. All digesters were installed and commissioned at Ravensview Water Pollution Control Plant, Kingston, Ontario. The digester setup is illustrated by the schematic in Figure 7.1.

7.2.2.3 Two-stage Thermophilic Semi-continuous Anaerobic Co-digestion System (with and without thermo-chemical pre-treatment) Experimental Procedure

In the two-stage co-digestion System I, three reactors PR0, PR1 and PR2 were operated sequentially. Thermo-chemical pre-treatment, with the optimum pre-treatment condition selected from Chapter 5, was incorporated into the system in the 1.5 L reactor (PR0) operated under batch mode. The pH value of the substrate mixtures were adjusted to around 10 and then the mixtures were pre-treated in the batch reactor at 55 ℃ for 1 day (24 hours). The pre-treated substrates were then fed into the first-stage semi-continuous digester PR1 for biogas production. In order to ensure sufficient digestion, effluent from PR1 was introduced into the second-stage semi-continuous digester PR2 for further methanogenesis and biogas production.

In the two-stage co-digestion System II without pre-treatment, only two sequential digesters R1 and R2 were operated. The substrate mixtures without pre-treatment were fed directly into the first-stage digester R1. The effluent from R1 was fed into the semi-continuous second-stage digester R2 for further methanogenesis and biogas production. The two-stage digestion experimental conditions for both Systems are summarized in Table 7.2.

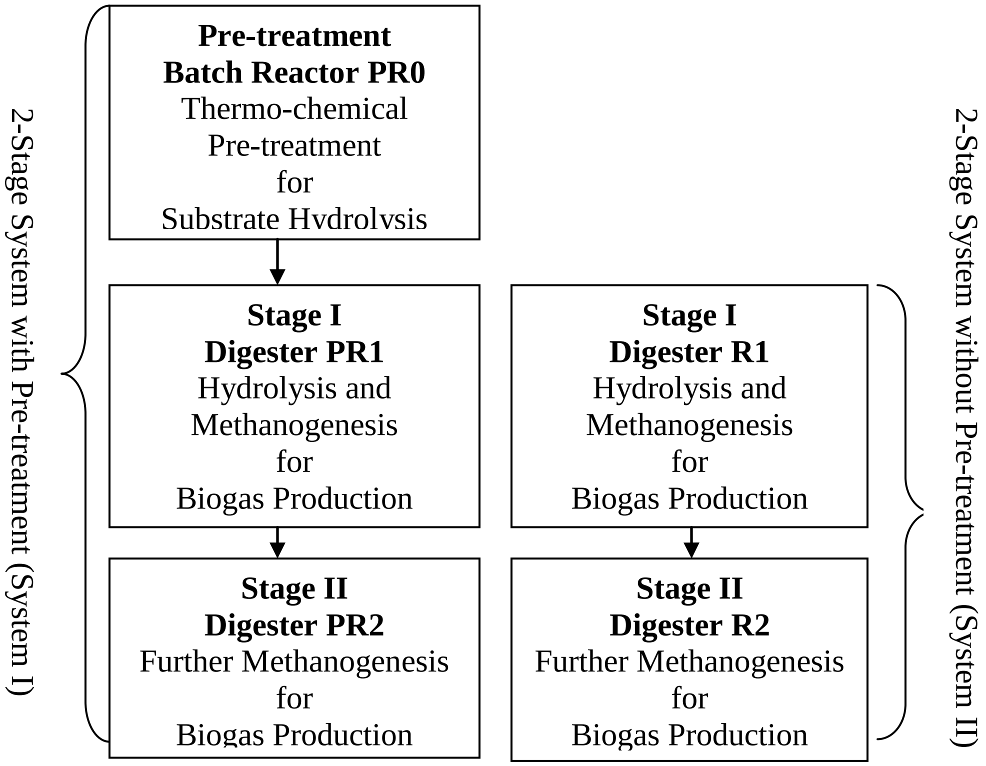


Figure 7.2 Flow diagram of the two-stage anaerobic digestion systems (System I with pre-treatment and System II without pre-treatment)

In a previous study within this research program (Chapter 6) and in other studies, it has been reported that thermophilic conditions can effectively improve biogas production and minimize digester content flotation when compared to mesophilic conditions (Goberna et al., 2010; Kabouris et al, 2009). Effective HRTs in the range 20-30 days and suitable OLR ranges (0-2.5g TVS/L d, where L represents digester working volume) were identified for continuous-flow anaerobic digestion in our previous work (Chapter 6), as well as in other studies (Callaghan et al., 2002; Fezzani and Cheikh, 2010). All semi-continuous flow digesters (PR1, PR2, R1 and R2) in each of the two-stage digestion systems were therefore operated at 55 ℃ with 24 day HRT and various OLRs within the ideal range (Table 7.2). At the beginning of the experiments, 12 L ADS were fed into the clean digesters as the inoculum and the digesters were initially operated for no less than 4-6 days. No substrates were fed into the digesters during the incubation period (for acclimation); and feeding was only initiated once the digesters had warmed to reach the required operational conditions and similar biogas production rates to each other were observed. PRS and FOG were then fed as the co-substrates into the digesters according to the flow diagrams and scheduled OLR and HRT as shown in Figure 7.2 and Table 7.2, respectively.

7.2.3 Sample Analysis

Parameters including pH, total solids (TS) and total volatile solids (TVS), and COD and soluble chemical oxygen demand (SCOD) among others were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Samples for SCOD, volatile fatty acids (VFA), ammonia-nitrogen (free ammonia NH_3-N) and total Kjeldahl nitrogen (TKN) analysis were extracted from the sample supernatant after centrifuging at 8000 rpm for 15 mins. VFA were analyzed using ion chromatography (Dionex ICS-3000, column IONPAC AS11). Free ammonia was analyzed using a flow Autoanalyzer System (SEAL AA3) and read colorimetrically at 630 nm. The TKN samples were prepared by acid digestion at 380 ℃ in the presence of a catalyst based on the standard method 4500-N (APHA, 2005) and analyzed with the SEAL Analytical ACCE 6.05 Autoanalyzer System by reading colorimetrically at 660 nm. Biogas samples were collected from the headspace of the digesters using Cole-

Parmer® Kynar dual-valve (0.3 L) gas sampling bags. The gas samples were analyzed for CH_4 and CO_2 contents by gas chromatography (Agilent 3000 Micro GC with Plot U backflush module, TCD detector). The results of the gas analysis were reported at standard temperature and pressure (STP, 101.325 kPa, 273.15 ° K). The gas sample hydrogen sulfide (H_2S) was measured using Dräger® tubes (0-200 ppm, Dräger, Germany).

In this experiment the digestion was considered to have achieved steady state when the biogas production rate varied by less than 5% for five consecutive days. Gas and liquid samples were collected 2-3 times weekly with duplicate or triplicate analysis during the steady state period.

7.3 Results and Discussion

7.3.1 BMP Tests for Optimum Pre-treatment Selection

Figure 7.3 and Table 7.3 present the results obtained for the FOG co-digestions with various thermo-chemical pre-treatment conditions during the BMP experiments.

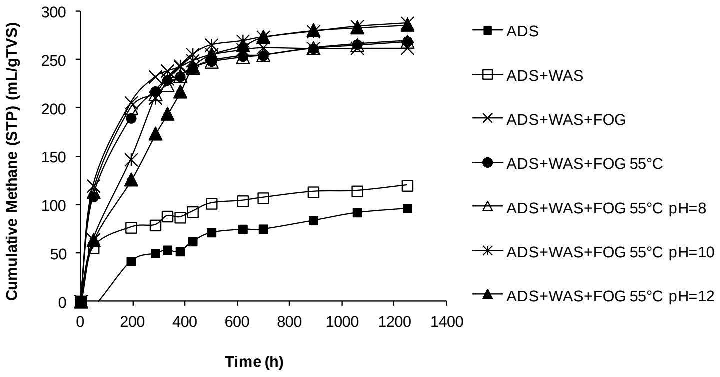


Figure 7.3 Cumulative methane production (STP, mL/g TVS) from FOG co-digestions with thermo-chemical pre-treatments in BMP testing

Table 7.3 Characteristics (acetic acid, TVS, %SCOD) of samples (mixture of inoculum and thermo-chemically pre-treated co-substrate) and the ultimate methane productions of various samples in BMP tests

Duplicate Samples	*Acetic Acid (mg/L)*	*SCOD/COD (%)*	*Experimental CH_4 (mL/g TVS)*
ADS	--	--	96.4 ±3.39
ADS+WAS	--	--	120 ±6.14
ADS+WAS +FOG	< 5	47.7 ±2.65	262 ±3.23
ADS+WAS+FOG 55 ℃	18.8	47.5 ±1.17	269 ±1.80
ADS+WAS+FOG 55 ℃ pH=8	26.2	48.7 ±0.76	269 ±1.00
ADS+WAS+FOG 55 ℃ pH=10	25.7	51.0 ±1.79	288 ±0.85
ADS+WAS+FOG 55 ℃ pH=12	9.31	68.7 ±1.19	287 ±1.98

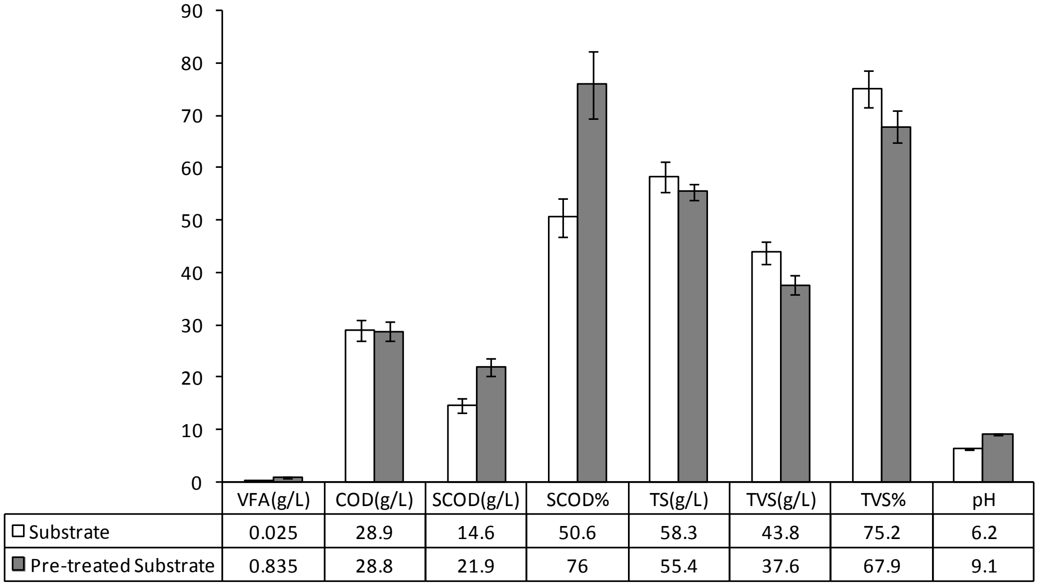
Compared to the FOG co-digestion with thermal pre-treatment at 55 ℃ alone, all FOG co-digestions with thermo-chemical pre-treatment yielded higher ultimate methane production, which is consistent with the results reported by other researchers that indicated that thermo-chemical pre-treatment had a positive effect on methane production compared to thermal pre-treatment alone (Rafique et al., 2010; Valo et al., 2004). The ultimate methane production from FOG co-digestions also increased with increasing alkaline dosage and corresponding pH. The pre-treated FOG co-digestions at pH=10 (55 ℃) and pH=12 (55 ℃) achieved the highest ultimate methane productions with values of 288 ±0.85 mL/g TVS and 287 ±1.98 mL/g TVS, respectively. Although FOG co-digestions with pH=10 and pH=12 pre-treatments had similar ultimate methane production, the thermo-chemical pre-treatment at pH=10 would represent a better operational choice, if the chemical dosage to achieve target pre-treatment pH levels were considered. The FOG co-digestion with pH=10 at 55 ℃ thermo-chemical pre-treatment exhibited an increase of 9.92% (P=0.01) in ultimate methane production compared to the FOG co-digestion without pre-treatment. This could be attributed to the increase in VFA (acetic acids) concentrations after pre-treatment, which can accelerate methanogenesis and, consequently, methane production potential (Gerardi, 2003; Luste et al., 2009). This was also supported by the results of the acetic acid analysis for the thermo-chemically pre-treated FOG co-substrates in Table 7.3. As a result of the pre-treatment, the increase in COD solubilization (SCOD/COD %) could also enhance the biogas production (Fdez-Güelfo et al., 2011; Vlyssides and Karlis, 2004). More detailed results and discussion have previously been presented in Chapter 5. Therefore, to ensure sufficient biodegradation of the organics and efficient methane production enhancement in FOG co-digestions with thermo-chemical pre-treatments, a pre-treatment at pH=10 and at 55 ℃ is recommended for application in the pre-treatment of the co-substrates.

7.3.2 Comparison of Biogas Production in Two-stage Thermophilic Semi-continuous Co-digestions with and without Thermo-chemical Pre-treatment

7.3.2.1 Effect of thermo-chemical pre-treatment on substrate characteristics in two-stage co-digestion tests

In order to comprehensively and accurately assess pre-treatment efficiency in the two-stage semi-continuous co-digestion system, the effects of a 1-day thermo-chemical pre-treatment (PR0 in System I) on 0.5 L of substrates were assessed. The results are shown in Figure 7.4. Thermo-chemical pre-treatment (pH=10 at 55 ℃ in batch-mode reactor PR0) enhanced substrate hydrolysis by affecting the substrate characteristics including

TVS, VFA, and COD solubilization (SCOD/COD, %) and could thereby increase biogas production in the subsequent digestion process.



	VFA(g/L)	COD(g/L)	SCOD(g/L)	SCOD%	TS(g/L)	TVS(g/L)	TVS%	pH
□ Substrate	0.025	28.9	14.6	50.6	58.3	43.8	75.2	6.2
■ Pre-treated Substrate	0.835	28.8	21.9	76	55.4	37.6	67.9	9.1

Figure 7.4 Comparison of the characteristics of substrates with and without thermo-chemical pre-treatment (pH=10, 55 ℃, 24 hours) for feeding to two-stage semi-continuous digestion systems.

It can be noted from Figure 7.4 that the TVS concentrations in the pre-treated substrate decreased 16.5% ($P=1.7x10^{-7}$) from 43.8±2.16 g/L to 37.6±1.83 g/L. This is consistent with the observations of Luste et al. (2009) and Valo et al. (2004), who indicated that the TVS reduction was likely due to the substrate solid organic solubilization after pre-treatment. The results obtained by Vlyssides and Karlis (2004) also supported this observation. In their study, the VSS decreased by approximately 20% with a thermo-chemical pre-treatment of pH=10 at 55 ℃ for 10 hours. Although thermo-chemical pre-treatment resulted in a reduction in TVS, the concentrations of soluble organics, which are the intermediate products from efficient hydrolysis for biogas production, usually increased as a result of pre-treatment. The increase of COD solubilization (SCOD/COD%) and VFA concentration after thermo-chemical pre-treatment (PR0) in the System I tests (Figure 7.4) can therefore be taken to indicate an increase in soluble organics with pre-treatment.

Figure 7.4 illustrates that, although the total COD concentrations did not significantly change during the thermo-chemical pre-treatment, the SCOD concentrations increased by approximately 50% ($P=9.6\text{x}10^{-6}$), from 14.6±1.30 g/L to 21.9±1.64 g/L. The corresponding COD solubilization (SCOD/COD %) in the substrate increased from 50.6±3.63% to 76.0±6.41%. This is consistent with the results obtained during the previous BMP tests (Table 7.3). COD solubilization has been widely reported as the reliable indicator of pre-treatment efficiency for different substrates, and an increase in COD solubilization represents one of the main predictors of anaerobic digestion efficiency (Park et al., 2005; Valo et al., 2004; Zhang et al., 2009).

It can be also noted from Figure 7.4 that, although the initial substrate mixture was adjusted to pH=10 at the beginning of the pre-treatment process, the pH value of the substrate mixture after one day of pre-treatment decreased slightly and reached an average of approximately 9.1±0.1, which was still higher than the original pH of the substrates mixtures (pH=6.1±0.1). This phenomenon was likely due to the increase of the VFA concentrations after pre-treatment. In all the substrate mixtures, acetic and propionic acids were the predominant constituents in VFA (acetic acid, propionic acid, butyric acid and iso-butyric acid) analysis. This observation is consistent with the results of Luste et al. (2009), where it was noted that the VFA consisted primarily of acetic and propionic acids after the effective pre-treatment of lipid-rich substrates. Hence, the VFA concentrations presented in Figure 7.4 are a combination of acetic and propionic acids for both the pre-treated and original substrate samples. The VFA concentration in the pre-treated co-substrate mixtures increased substantially from 25.1±2.85 mg/L to 835±221 mg/L as a result of pre-treatment, which would consequently decrease the pH of the pre-treated substrate mixture.

Overall the parameters analyzed (including TVS, VFA and COD solubilization) before and after thermo-chemical pre-treatment prior to the two-stage semi-continuous co-digestion tests indicated that the batch-mode thermo-chemical pre-treatment reactor PR0 achieved an efficient hydrolysis. As such, the two-stage anaerobic co-digestion system (System I) with a thermo-chemical pre-treatment process might be expected to yield a higher biogas production than the two-stage system (System II) without pre-treatment.

7.3.2.2 Biogas Production and Digester Performance in Two-stage Thermophilic Co-digestion Systems with and without Thermo-chemical Pre-treatment

Figure 7.5 and Table 7.4 present the biogas production and digester performance of the two-stage co-digestion system with pre-treatment (System I) and without pre-treatment (System II). It can be noted from Figure 7.5(a) and (b) that the first-stage digester R1 and the second-stage digester R2 in System II reached a steady biogas production after 15 days. First-stage digester R1 achieved an average biogas production rate of 16.0±0.55 L/d at steady state with a constant methane content of 67.6±2.52%, which would imply that a 24 day HRT and OLR of 1.83±0.09 g TVS/L·d, which was within the target OLR range of 0 to 2.5 g TVS/L·d as suggested in Chapter 6, would be appropriate to ensure a consistent biogas production from a semi-continuous co-digestion system with the addition of FOG.

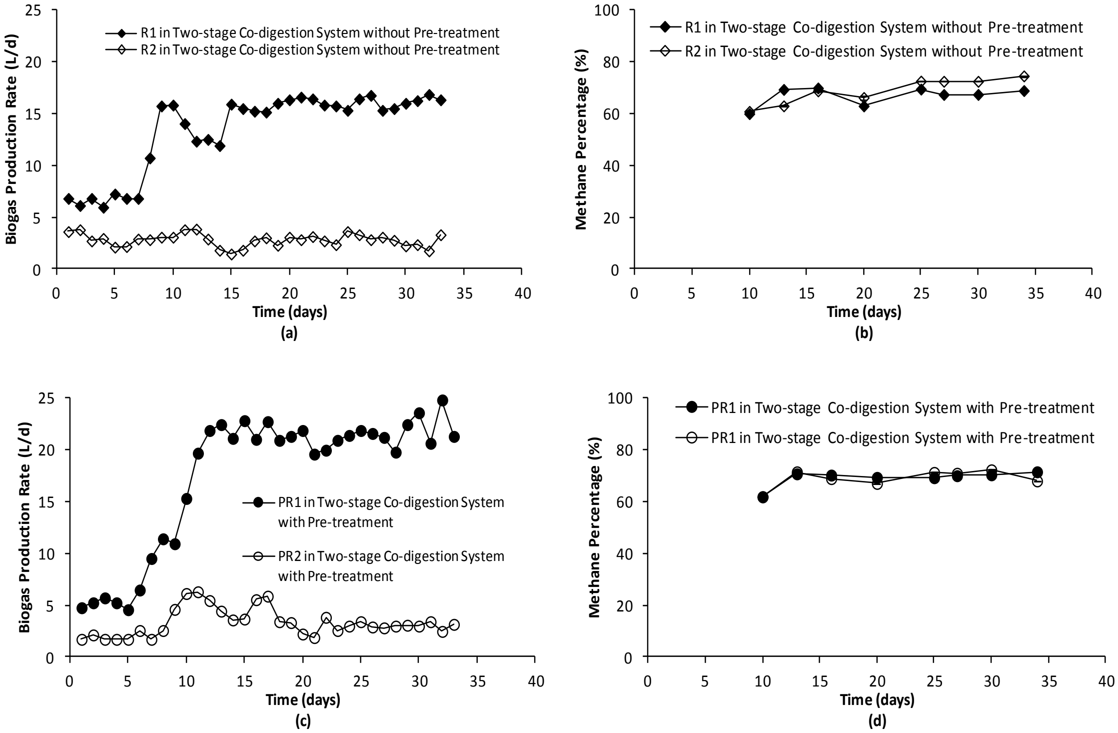


Figure 7.5 Biogas production rates (L/day) of (a) digesters R1 and R2 in two-stage semi-continuous anaerobic co-digestion system (System II) without pre-treatment and (c) digesters PR1 and PR2 in two-stage semi-continuous anaerobic co-digestion system (System I) with thermo-chemical pre-treatment; and methane content (%) of the biogas from digesters in (b) two-stage semi-continuous anaerobic co-digestion system (System II) without pre-treatment and (d) two-stage semi-continuous anaerobic co-digestion system (System I) with thermo-chemical pre-treatment.

However, the second-stage digester R2 in System II did not appear to achieve biogas production as effectively as the first-stage digesters R1 at steady state. Similarly, as can be seen from Figure 7.5(c) and (d), the first-stage digester PR1 in the two-stage co-digestion System I receiving the thermo-chemically pre-treated substrate achieved biogas production at steady state that yielded approximately 21.7 ±1.18 L/d with 70.2 ±0.79%

methane content, which was higher than that obtained from the second-stage digester PR2 in System I at steady state (3.44±0.96 L/d with 69.9±2.00 % methane content).

Table 7.4 Characteristics of influents and effluents (within steady state) of digesters (PR0, PR1, and PR2) in two-stage co-digestion system (System I) with pre-treatment and those of digesters (R1 and R2) in two-stage co-digestion system (System II) without pre-treatment.

	Two-stage System I with Pre-treatment			**Two-stage System II without Pre-treatment**	
	PR0	***PR1***	***PR2***	***R1***	***R2***
HRT (day)	1	24	24	24	24
OLR (gTVS/L d)	1.83±0.09	1.57±0.08	0.37±0.03	1.83±0.09	0.46±0.04
Influent COD (g/L)	28.9±2.08	28.8±1.77	13.5±1.83	28.9±2.08	16.5±1.83
Influent SCOD (g/L)	14.6±1.30	21.9±1.64	1.89±0.37	14.6±1.30	4.68±1.69
Influent SCOD (%)	50.6±3.63	76.0±6.41	14.2±3.86	50.6±3.63	24.8±9.40
Influent TS (g/L)	58.3±2.78	55.4±1.49	17.6±1.01	58.3±2.78	20.0±2.77
Influent TVS (g/L)	43.8±2.16	37.6±1.83	8.92±0.70	43.8±2.16	10.9±0.91
Biogas Rate (L/d)	--[a]	21.7±1.18	3.44±0.96	16.0±0.55	2.73±0.56
Methane Content (%)	--[a]	70.2±0.79	69.9±2.00	67.6±2.52	70.1±3.07
H_2S (ppm)	--[a]	20-25	15-20	15-30	15-20
Effluent pH	9.1±0.1	7.30±0.11	7.35±0.13	7.15±0.05	7.25±0.18
Effluent COD (g/L)	28.8±1.77	13.5±1.83	14.7±1.04	16.5±1.83	13.8±0.66
Effluent SCOD (g/L)	21.9±1.64	1.89±0.37	1.68±0.36	4.68±1.69	2.41±0.81
Effluent SCOD (%)	76.0±6.41	14.2±3.86	10.9±2.86	24.8±9.40	16.3±5.16
Effluent TS (g/L)	55.4±1.49	17.6±1.01	14.2±0.78	20.0±2.77	19.1±2.79
Effluent TVS (g/L)	37.6±1.83	8.92±0.70	7.69±0.69	10.9±0.91	9.87±1.20
Effluent Acetic Acid (mg/L)	835±221	50-90	<1.00	100-120	<1.00
Effluent NH_3-N (g/L)	--[b]	0.55±0.06	0.68±0.05	0.66±0.05	0.68±0.04
Effluent TKN (g/L)	--[b]	0.66±0.06	0.76±0.09	0.67±0.02	0.77±0.05
COD Removal (%)	0	53.1±0.03	-8.89±0.43	42.9±0.12	16.4±0.64
SCOD Removal (%)	-50±0.26	91.4±0.77	11.1±0.03	67.9±0.30	48.5±0.52
TS Removal (%)	4.97±0.46	68.2±0.32	19.3±0.23	65.7±0.01	4.50±0.003
TVS Removal (%)	14.2±0.15	76.3±0.62	13.8±0.14	75.1±0.58	9.45±0.32

[a] Batch-mode reactor PR0 was operated for pre-treatment process and the gases were not measured.

[b] Batch-mode reactor PR0 was operated for pre-treatment process and the parameters were not measured.

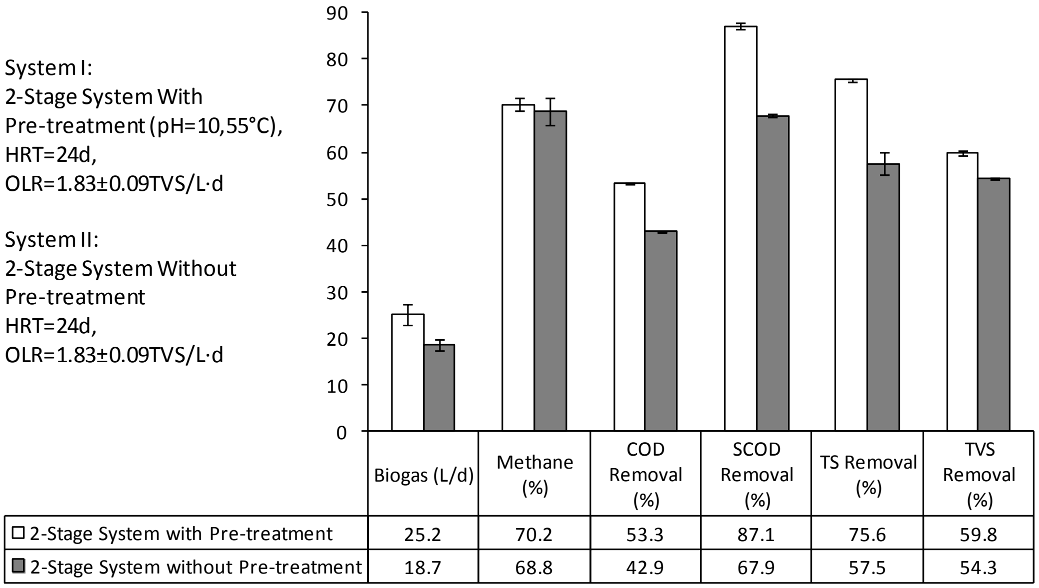
In comparing the two first-stage digesters PR1 (HRT=24 d and OLR=1.57g TVS/L·d) and R1 (HRT=24 d and OLR=1.83g TVS/L·d) in System I and System II, it is apparent that the performance of PR1 in terms of biogas production and organic removal was higher than that of R1. Although the influent pH to PR1 was 9.1±0.1, the average pH value at steady state decreased to 7.30±0.11, which is within the optimum pH range of 7.0-7.6 for biogas production (Fezaani and Cheikh, 2010; Vergara-Fernández et al., 2008). The average biogas production from PR1 was 21.7±1.18 L/d, which represented a 35.6% (P=7.3x10^{-9}) higher yield than the biogas production in R1. The methane content (70.2±0.79%) of the biogas yielded by PR1 was also higher than that from R1 (67.6±2.52%). COD, SCOD, TS, and TVS removal efficiencies in PR1 were also higher than those obtained in R1 (Table 7.4). Specifically, the SCOD removal achieved in PR1 was 91.4±0.77%, which was 34.6% (P=5.2x10^{-6}) higher than that obtained in R1. As previously discussed, the higher biogas production and organic constituent removal observed in PR1 could be attributed to the pre-treatment and hydrolysis process in the thermo-chemical pre-treatment reactor PR0 of System I, which provided a substrate with high VFA (835±221 mg/L), high SCOD concentration (21.9±1.64 g/L), and high COD solubilization (SCOD/COD %= 76.0±6.41%) to PR1 leading to an increase in biogas and methane production (Park et al., 2005).

The lower biogas production observed in both second-stage digesters (PR2 in System I and R2 in System II) would be due to low OLR, SCOD and TVS inputs to these digesters. As can be seen from Table 7.4, in comparison with the OLRs and influent COD, SCOD and TVS of the first-stage digesters PR1 and R1, the concentrations and loading rates of these parameters to the second-stage digesters PR2 and R2 were all much lower.

Consistent with observations during the BMP experiment and Chapter 5, COD solubilization (SCOD/COD %) and SCOD concentrations in the substrate would significantly affect the biogas production. From Table 7.4, it can be noted that COD solubilization (SCOD/COD %) in the influents to PR2 and R2 were 14.2±3.86 % and 24.8±9.40 %, respectively. These were much lower than the COD solubilizations in the influents to PR1 (76.0±6.41 %) and R1 (50.6±3.63 %), respectively. In addition, the organic (COD, SCOD, TS and TVS) removal efficiencies in the second-stage digesters PR2 and R2 in both systems were much lower than those achieved by the first-stage digesters PR1 and R1. These observations suggest that the performances of the anaerobic digestion in the second-stage digesters of both systems were not as high as those achieved in the first-stage digesters. Hence, the lower influent organic concentrations and anaerobic degradation activity resulted in the reduced biogas production observed in the second-stage digesters.

The VFA, TKN, and NH_3-N concentrations in the effluents from PR2 and R2 would also support this observation. During the steady state of an efficient methanogenesis stage, acetic acid has been reported as the predominant constituent in the total VFA that influences the biogas production rates and methane contents (Cavaleiro et al., 2008; Fezzani and Cheikh, 2010; Gerardi, 2003; Martín-González et al., 2010). In our VFA analysis of the effluent from digesters PR1, PR2, R1 and R2, consistent with the literature reported, acetic acid was always the major product (> 50%) in the total VFA detected. Other VFAs (propionic acid and butyric acid) were seldom detected during steady state in these digesters. Hence, only acetic acid is shown in Table 7.4 as representative of the VFA in the effluents from the different digesters. As can be seen from Table 7.4, compared to the second-stage digesters, apparently higher concentrations of acetic acids were detected in both first-stage digesters PR1 and R1. Acetic acid was seldom detected in the effluents from the second-stage digesters PR2 and R2. This would indicate a more efficient anaerobic microbial acclimation and that most anaerobic degradable organics and anaerobic digestion intermediate products including long chain fatty acids (LCFAs) and VFAs had been converted to biogas in the first-stage digesters PR1 and R1, which resulted in lower acetic acid concentrations and biogas production rates in the second-stage digesters (Martín-González et al., 2010; Martín-González et al., 2011). Furthermore, as indicated in Table 7.4, compared to the first-stage digester effluents, the increase of both TKN and NH_3-N in the second-stage digesters would suggest that digesters PR2 and R2 were more resistant to VFA instability and prevented the risk of nutrient shortage in methanogenesis (Martín-González et al., 2010; Nakakubo et al., 2008). Hence, although methane production was not as active in the second-stage digesters due to the low concentrations of organics and VFA in the influent, the existing nitrogen-sourced organics allowed for the second-stage digesters to achieve stable biogas productions.

Based on the findings of this study, it can be argued that, in each of the two-stage digestion systems (System I and System II), the primary biogas production and organic constituent removal was achieved in the first-stage digesters (PR1 in System I and R1 in System II). In addition, as PR1 achieved a higher biogas production and organic constituent removal than R1, the two-stage system (System I) with a thermo-chemical pre-treatment of pH=10 at 55 °C would be expected to achieve a higher biogas production and organic constituent removal than System II. To demonstrate this, the performance of each system, including biogas production, methane content and organic removal, was analyzed and these are shown in Figure 7.6.



	Biogas (L/d)	Methane (%)	COD Removal (%)	SCOD Removal (%)	TS Removal (%)	TVS Removal (%)
☐ 2-Stage System with Pre-treatment	25.2	70.2	53.3	87.1	75.6	59.8
■ 2-Stage System without Pre-treatment	18.7	68.8	42.9	67.9	57.5	54.3

Figure 7.6 Comparison of the biogas production and treatment efficiency of the two-stage anaerobic digestion systems with and without pre-treatment.

As anticipated, the two-stage semi-continuous digestion system with thermo-chemical pre-treatment (System I) outperformed the system without pre-treatment overall. Under the same OLR (1.83±0.09 g TVS/L d) to both systems, System I obtained a biogas production rate 34.8% ($P=3.1x10^{-7}$) higher than System II with the similar methane percentage. COD, SCOD, TVS. TS removals in System I were also more efficient than those observed in the conventional two-stage digestion configuration without pre-treatment.

7.4 Conclusions

According to the results presented here, BMP tests can be utilized as an economical and practical method to provide information for continuous-flow digestion testing. Through BMP testing, a thermo-chemical pre-treatment of pH=10 and 55 °C was identified as the best pre-treatment process option to enhance the biogas production from FOG co-digestions. In the two-stage FOG co-digestion system experiments, the thermo-chemical pre-treatment effectively promoted the hydrolysis of the substrates by increasing the COD solubilization (SCOD/COD %) and intermediate product VFA concentrations. With subsequent feeding of this pre-treated substrate to a semi-continuous two-stage digester system operating with HRT=24 days and OLR=1.83 ± 0.09 g TVS/L d, the first-stage digesters yielded the highest biogas production and organic constituent reduction of the two-stage systems. As expected, the two-stage digestion system with thermo-chemical pre-treatment presented various advantages with respect to improving biogas production performance and enhancing the treatment of organics in the co-substrate. Biogas production increased from 18.7 ± 1.11 L/d to 25.1 ± 2.14 L/d with 70.2 ± 1.40% methane content with pre-treatment. COD and SCOD removal in the two-stage digestion system with thermo-chemical pre-treatment reached 53.3 ± 0.12% and 87.1 ± 0.72%, respectively.

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Chapter 8

Conclusions and Recommendations

8.1 Conclusions from Experimental Results

According to the literature reviewed in Chapters 2 and 3, to enhance biogas production and assist in wastewater treatment and municipal organic waste management, anaerobic co-digestion has been widely recognized as an effective, low-cost and commercially viable approach. Municipal organic wastes including kitchen waste (KW) and fat, oil and grease (FOG), which could be collected in close proximity to the wastewater treatment plant, hence, imparting lower transportation costs, should be considered as reasonable and economical co-substrates. Pre-treating substrates using various methods has also been reported as a potential approach to improve methane production. However, few studies have reported on the use of pre-treatments on the co-digestion of a range of municipal organic substrates, particularly FOG, to enhance biogas production at municipal wastewater treatment plants. Hence, the primary objectives of this research were to achieve enhanced biogas production from co-digestion with addition of municipal organic wastes, to investigate thermo-chemical and ultrasonic pre-treatment that might be expected to enhance biogas production from co-digestions, and to explore a two-stage co-digestion system combined with an effective pre-treatment method. To meet these objectives, a step-by-step project was conducted and important results were obtained.

The feasibility of using synthetic KW and FOG as co-substrates in the anaerobic co-digestion of wastewater treatment plant sludge (WAS) was investigated in Chapter 4. Results indicated that co-digestions with FOG and KW all enhanced methane production, from 117 ±2.02 mL/g TVS (with WAS alone) to 418 ±13.7 mL/g TVS and 324 ±4.11 mL/g TVS, respectively. KW and FOG positively affected methane production with ideal estimated substrate/inoculum (S/I) ratios of 1.20 and 0.46, respectively. Combined linear and non-linear regression models indicated that co-substrate addition shortened the lag phases of organic biodegradation. Comprehensively, the results and discussions in Chapter 4 suggest that, when compared to KW, FOG would be the preferred co-substrate since it required less mass loading per unit methane production, presented better methane production potential, achieved higher ultimate methane production and exhibited a shorter estimated exponential time within the ideal S/I ratio ranges.

The effects of ultrasonic and thermo-chemical pre-treatments on the biogas production potential of anaerobic co-digestion with KW or FOG were investigated using BMP tests in Chapter 5. Ultrasonic pre-treatment was not found to be a effective approach to significantly improve methane production from FOG or KW co-digestions. Thermo-chemical pre-treatment did increase methane production yields from both FOG and KW co-digestion. A comprehensive evaluation indicated that the thermo-chemical pre-treatments of pH=10, 55 ℃ and pH=8, 55 ℃ provided the best conditions to increase biogas production from FOG and KW co-digestions, respectively. The most effective biogas enhancement was achieved from thermo-chemically pre-treated FOG co-digestion, which had a biogas production that was 9.9 ±1.5% higher than FOG co-digestion without thermo-chemical pre-treatment. Comparing the improvement in ultimate methane production, non-linearly estimated lag phase periods and estimated maximum methane production rates of all co-digestions, thermo-chemical pre-treatment could be proposed to be the best pre-treatment method to enhance methane production from co-digestions with municipal organic wastes particularly with FOG. In addition, as FOG was recommended as the preferred co-substrate in Chapter 4, the results obtained from Chapters 4 and 5 indicated that FOG co-digestion with thermo-chemical pre-treatment of pH=10, 55 °C could be expected to yield optimum biogas production using semi-continuous flow anaerobic digesters with reasonable operational conditions.

In semi-continuous flow digestion, operational parameters such as temperature, hydraulic retention time (HRT) and organic loading rate (OLR) can affect biogas production. Hence in Chapter 6, anaerobic co-digestions with FOG were tested in semi-continuous flow digesters under various operating conditions. The effects of HRT of 12 and 24 days, OLR between 1.19 and 8.97 g TVS/L·d, and digestion temperature of 37 °C (mesophilic) and 55 °C (thermophilic) on biogas production were investigated. Compared to anaerobic digestion with wastewater treatment plant sludge (primary sludge), semi-continuous flow anaerobic co-digestion with FOG effectively enhanced biogas and methane production under ideal conditions. Thermophilic (55 °C) co-digestions exhibited higher biogas production, with lower hydrogen sulfide emissions and higher organic conversion than mesophilic co-digestions. The best biogas production rate of 17.4 ± 0.86 L/d and methane content 67.9 ± 1.46% was obtained in a thermophilic co-digestion at HRT=24 days and OLR=2.43 ± 0.15 g TVS/L·d; these were 32.8% and 7.10% higher than the respective values for mesophilic co-digestion under similar operating conditions.

Chapter 7 investigated FOG co-digestions in two-stage thermophilic (55 ℃) semi-continuous flow co-digestion systems. The two-stage co-digestion system was modified to incorporate thermo-chemical pre-treatment of pH=10 at 55 ℃, which was the best pre-treatment condition for FOG co-digestion identified during testing in Chapter 5. The other two-stage co-digestion system was operated without a pre-treatment process. As anticipated it was found that the co-digestion system with pre-treatment effectively enhanced biogas production since the thermo-chemical pre-treatment effectively promoted the hydrolysis of the substrates and increased the COD solubilisation (SCOD/COD %) and intermediate product VFA concentrations. Overall, the modified two-stage co-digestion system with pre-treatment yielded a 25.1±2.14 L/d biogas production rate, which was higher than the 18.7±1.11 L/d obtained in the two-stage system without pre-treatment. COD and SCOD removal in the two-stage digestion system with thermo-chemical pre-treatment reached 53.3±0.12% and 87.1±0.72%, which were higher than the 42.9% and 67.9% yielded by the conventional two-stage digestion system without pre-treatment, respectively.

8.2 Original Contributions

The results of this project have not only demonstrated that wastewater treatment sludge can be successfully stabilized with effective organic removal efficiencies, but it can also produce quantities of biogas with high methane content using anaerobic co-digestion with addition of municipal organic wastes. Synergistic effects between wastewater treatment plant sludge and municipal organic waste (KW and FOG) were investigated through BMP and semi-continuous flow digester testing. Through the comparison of the ultimate methane production potentials from the KW and FOG co-digestions, the ideal co-substrates, optimum mixing ratios and feeding regime were identified, which can provide important information for potential application in full-scale facilities. The non-linear regression modeling of the BMP test results provided an accurate mathematical approach to simulate and predict the biogas production.

FOG, particularly yellow grease, is a low-cost municipal organic waste that has attracted much research interests during last decade, but it has seldom been applied in anaerobic co-digestion at wastewater treatment plants. FOG was employed and co-digested with wastewater treatment plant sludge in this research, and was found to represent an effective and economical approach for wastewater treatment plants to enhance on-site energy production through full-sized anaerobic digesters. This project not only successfully enhanced the biogas production from wastewater treatment plant sludge, but also proposed a new concept for municipal organic waste (e.g. FOG) management and utilization.

Many existing studies have reported on the utilization of pre-treatment methods for organic waste solubilization and biogas production improvement. However, few have reported on the use of pre-treatment for the co-digestion of a range of municipal organic substrates, particularly FOG and KW, to enhance biogas production at municipal wastewater treatment plants. This project investigated and compared ultrasonic and thermo-chemical pre-treatments and found that thermo-chemical pre-treatment could be utilized as a reliable and stable approach for biogas production enhancement.

In addition, a novel two-stage bench-scale semi-continuous flow anaerobic digestion system combined with a thermo-chemical pre-treatment process was designed and operated at Ravensview Water Pollution Control Plant, Kingston, Ontario under the appropriate operational conditions of suitable temperature, co-substrate feeding regime, OLR and HRT. The results provided valuable information for scaling up of the process and for future industrial applications. Moreover, the optimized biogas production process advanced from this research will contribute to an on-going series of research efforts concentrating on the creation of an integrated wastewater treatment plant in the City of Kingston, which will also be relevant to other Canadian municipalities looking for guidance on renewable energy development. Details on experimental execution, advanced chemical analytical instrument training and utilization, and the specific process parameter analysis will also provide beneficial support for future research in the area of anaerobic co-digestion.

8.3 Recommendations for Future Work

FOG has been found to yield higher biogas production than KW, since the degradable fraction of the lipids is higher than that of the typical carbohydrates and proteins found in most municipal solid wastes. However, it has still been less studied than municipal solid wastes (e.g. KW or food processing wastes) in co-digestion applications with wastewater treatment plant sludge due to the practical operational and application challenges including sludge flotation, digester foaming and blockages of pipes and pumps. Consequently, co-digestion of FOG with wastewater treatment plant sludge has not been as widely reported as other co-digestions during last few decades, and it has really only gained more attention in the last 5 years. This thesis research provided valuable fundamental information on the anaerobic co-digestion with the addition of FOG.

However, compared to co-digestion of wastewater treatment plant sludge with the addition of other municipal organic wastes (e.g. KW), FOG co-digestion still requires a better understanding and more specific research in the future.

Economical, practical and reliable laboratory-scale batch-mode BMP tests are highly recommended, not only by this project but also by other researchers, to be conducted prior to bench-scale or pilot-scale anaerobic investigations. Constituent characterization and co-substrate mixing ratios (S/I ratio) could be easily demonstrated through the tests. The fundamental information gathered can effectively minimize the conditions leading to inhibition and failure of digestions in large-scale investigations. In addition, as discussed in Chapters 4 and 5, non-linear regressions can more accurately assess and compare the biogas production progress than conventional linear regressions. Hence in future work, various modified non-linear regressions are highly recommended to achieve representative biogas production simulations and predictions within BMP tests.

According to the literature review presented in Chapter 3, various pre-treatments including mechanical, thermal, chemical, thermo-chemical, ultrasonic, ozonation, microwave and biological methods have been well researched during the last two decades for the improvement of anaerobic digestion with wastewater treatment plant sludge alone. However, less attention has been paid by researchers on pre-treatment applications in anaerobic co-digestion, particularly co-digestion with FOG. Although thermo-chemical and ultrasonic pre-treatment methods have been investigated in Chapter 5 and thermo-chemical pre-treatment (pH=10 at 55 ° C) has been demonstrated as the preferred approach in Chapter 7, it is still necessary to conduct further research to assess other thermo-chemical pre-treatment conditions with various chemical reagent addition and temperatures to provide optimum operational parameters for their application in anaerobic co-digestion.

Various operational parameters including OLR, HRT and temperature (mesophilic and thermophilic) that affected the bench-scale semi-continuous flow co-digestion have been identified and investigated in Chapter 6. However, for full-scale application, further and more comprehensive pilot-scale evaluations need to be conducted to obtain more specific operational information for process scale-up. Generally, based on the results obtained from the study discussed in Chapter 6 and from the literature review in Chapter 4, the thermophilic operating temperature regime can be expected to be a better condition for most anaerobic co-digestion than mesophilic operating conditions. Hence, it would be worthwhile to test the effects of higher operating temperatures (>55 ℃) on biogas production in future research. For this, digesters made of heat resistant materials (e.g. glass and stainless steel) with stable heating systems would be necessary. A comprehensive evaluation of the energy consumption should be also considered.

In the research presented in this thesis, based on the suitable HRT and OLR obtained from one-stage semi-continuous flow tests discussed in Chapter 6, the two-stage semi-continuous flow co-digestion with thermo-chemical pre-treatment process investigated in Chapter 7 achieved a higher biogas production than conventional two-stage co-digestion without pre-treatment. Most biogas production in the two-stage co-digestions was yielded by the first-stage digester, as it is well recognized that in two-stage digestion systems hydrolysis, acetogenesis and methanogenesis occur simultaneously and continuously in the first-stage digester and sludge thickening and further organic matter reduction take place in the second-stage digester. Recently, since the overall HRT has been considered lower and the organic removal efficiency has been considered higher in two-phase digestion than those in conventional two-stage digestions, interest in evaluating anaerobic digestion in two-phase configurations is increasing. In two-phase digestion systems, hydrolysis and acetogenesis occur in the first phase and methanogenesis takes place in the second phase. Although the two-phase strategy has been shown to be a better configuration for performance consideration, it is not as widely used in full-scale applications due to its operational complexity. Hence, it is recommended that two-phase anaerobic co-digestion, which allows for the separation and optimization of the HRT and OLR in hydrolysis/acetogenesis and methanogenesis, needs to be conducted and investigated for optimizing FOG co-digestions in future work.

In this research, a substantial number of experiments were conducted with valuable results achieved. However, in order to correlate experimental data and modify future scale-up applications, models for simulating the continuous-flow digestion mode need to be developed in the future. In addition, as anaerobic digestion is a time dependent process and usually requires long experimental periods (usually more than 40 days) to achieve sufficient experimental data and results for precise kinetic evaluation and modelling, future experiments would be benefit from automatic substrate feeding and sampling systems and accurate analytical facilities .