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Improving Energy Recovery From Anaerobic Co-Digestion Of Potato Peel And Pig Manure

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IMPROVING ENERGY RECOVERY FROM ANAEROBIC CO-DIGESTION OF POTATO PEEL AND PIG MANURE

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ABSTRACT

The influence of mixing ratio, thermal pre-treatments and biological-acidification pre-treatment on methane yield was evaluated for anaerobic co-digestion of potato peel and pig manure, in a batch study at mesophilic conditions. The biological-acidification pre-treatment was conducted at mesophilic (37 $^{\circ}$C) and thermophilic (55 $^{\circ}$C) condition at a retention time of 6 days, with a food-microorganism ratio of 0.5. The thermal pre-treatment was performed at 100 $^{\circ}$C for 1 hour, using a reflux heating method. A food-microorganism ratio of 0.5 was applied in a biochemical methane potential test for the untreated and pre-treated substrate, at a mesophilic temperature. Results showed that the highest experimental methane yield for the untreated substrate was 231 ml $CH_4/gTCOD_{added}$, which was attained at 50:50 mix of potato peel to pig manure. The pre-treated substrates had the highest methane yield of 285 ml $CH_4/gTCOD_{added}$ and 283 ml $CH_4/gTCOD_{added}$, for the thermally treated and biological-thermophilic treated substrate, respectively, at 50:50 mix of potato peel to pig manure. This was 23% higher than the methane yield from untreated substrate. However, the biological-acidification at mesophilic temperature attained the highest experimental methane yield of 255 ml $CH_4/gTCOD_{added}$ at 75:25 mix of PP to PM. The reaction kinetics showed that the biological acidification pre-treatment had the highest methane production rate. However, the thermal pre-treatment produced 95% of the cumulative methane in less than 15 days due to the longer pre-treatment time in biological acidification. Hence, biological-acidification and thermal pre-treatment enhanced methane yield and reaction kinetics from co-digestion of potato peel and pig manure.

DEDICATION

To God almighty, families, teachers and mentors who have inspired me and supported my inquisitiveness and academic quests.

I would also like to dedicate this work to all the countries with a great dependency on fossil fuel, may the results of this thesis help empower those countries with alternative energy source that is renewable and environmentally friendly.

♦♦♦

"The time is past, when humankind thought it could selfishly draw on exhaustible resources. We know now the world is not a commodity".

~ Francois Hollande

Ex-president of French Republic

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- Greenfield Specialty Alcohol

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LIST OF ABBREVIATIONS/SYMBOLS

AD	Anaerobic digestion
PP	Potato peel
PM	Pig manure
PT	Pre-treatment
VFAs	Volatile fatty acids
TCOD	Total chemical oxygen demand
SCOD	Soluble chemical oxygen demand
VS	Volatile solids
TS	Total solids
GC-FID	Gas chromatograph-flame ionization detector
GC-TCD	Gas chromatograph-thermal conductivity detector
USEIA	United states energy information administration
OECD	Organization for economic co-operation and development
EMY	Experimental methane yield
TMY	Theoretical methane yield
BMP	Biochemical methane potential
IPCC	Intergovernmental panel on climate change
GHG	Greenhouse gas
OMOECC	Ontario ministry of environment on climate change
ECCC	Environment and climate change canada
ADS	Anaerobic digested sludge
CH_4	Methane gas
H_2	Hydrogen gas

$C_6H_{12}0_6$	Glucose
CH_3CH_2COOH	Propionic acid
CH_3COOH	Acetic acid
CH_3CH_2OH	Ethanol
CO_2	Carbon dioxide
NH_3	Ammonia
S.D.	Standard deviation

CHAPTER 1:

INTRODUCTION TO THE STUDY

General Introduction

The rising world population has led to an increase in the energy demand and volume of waste generated. World's energy consumption is projected to further increase by 28% between 2015 and 2040. 77% of the energy demand is met by fossil fuel (USEIA, 2017). Energy sector plays a significant role in greenhouse gas (GHG) emission in Canada, accounting for about 80% of total emission sources. The GHG emission accrues from a variety of sources, including fossil fuel extraction, processing and usage of fossil fuel products. Waste also accounts for 10% GHG emissions in Canada (ECCC, 2017). For instance, in Ontario, 32% of the waste generated in Ontario are food and organic waste, which represents 3.7 million tonnes of waste. About 60% of the waste generated in 2015 was sent to the landfill (OMOECC, 2015). GHGs, in the form of methane gas, are generated from the disposal of organic waste in the landfill. Researchers are constantly investigating sustainable means of meeting the world's growing energy demands, to offset the dependency on fossil fuel and address the volume of waste sent to the landfill. This can be achieved through energy recovery from anaerobic digestion (AD) of organic waste.

Organic Wastes for Anaerobic Digestion

Two major sources of organic waste in Canada are from agricultural waste and animal manure. For this thesis, the focus is on pig manure (PM) and potato peel (PP).

Pig manure

The increase in income and a changing nutritional requirement that favors the presence of animal protein in diets has led to a continuous increase in meat demand. Meat production is capital intensive with a high potential for environmental pollution. The most consumed animals in the global meat industry are pig, sheep, beef, poultry and veal (OECD, 2019). Pork is the most widely consumed meat, especially in Europe, America and Asia, accounting for 36.3% of production, closely followed by poultry meat (34.4%) and beef (21.2%). Pig production increased from twenty million tons to one hundred and eight million tons, between 1960 to 2011 (Statistics Canada, 2015). Despite the low consumption of pork in Canada, Canada plays a significant role in the pork production market, exporting more than one million tons of pork in 2011 and ranks as the fifth major exporter of pork worldwide (Statistics Canada, 2015).

The growth in the pig industry has led to an increase in volume of PM generated. PM is characterized as having good buffering capacity, high nitrogen content and high presence of other essential nutrients for anaerobic digestion, making it an excellent co-substrate for AD (Weiland, 2000; Moral et al, 2008). AD of PM has the potential of reducing pathogen levels, greenhouse gas emission and producing renewable fuel in form of methane gas.

Potato Peel

PP on the other hand is a major source of organic-rich agricultural and industrial waste that is unavoidable. Potato ranks as the fourth most consumed crop worldwide, behind only rice, wheat and maize (FAO, 2008). In Canada, potato is the largest grown vegetable crop, representing 27.3% of all vegetable receipt in 2017. About 5 million tons were produced in 2017 (Agriculture and Agri-food Canada, 2018). A large volume of potato peel is

generated as by-products in the processing sector since peel removal is an inevitable stage in potato processing (Liang and McDonald, 2014). Improper disposal of PP results in emission of GHGs from its decomposition.

Due to the diverse chemical composition of PP, several studies have investigated the extraction of the chemicals. Lactic acid was extracted by Liang et al (2014), phenolic acid was extracted by Maldonado et al (2014) and steroidal alkaloids was extracted by Hossain et al (2014). However, a more promising application is the use of potato peel as a co-substrate in anaerobic digestion which involves less operational and capital cost, little technical capability and the added potential for energy recovery.

Hence, the complimentary characteristics of PP and PM is indicative of its potential as a co-substrate for anaerobic co-digestion. Anaerobic co-digestion has the benefit of balancing the C-N ratio; reducing the potential for ammonia inhibition from mono-digestion of animal manure, reducing the risk of acidification from the mono-digestion of organic waste, diluting of toxic materials in a substrate, supplying essential nutrients for microbial growth and increasing the biogas production from anaerobic digestion (Mata-Alvarez et al., 2011).

Anaerobic Digestion

AD is a complex biological process that produces biogas through the decomposition of organic waste, under the action of a consortia of anaerobic microbes that operate in the absence of oxygen. This biogas is made up of mainly methane, carbon dioxide and minor traces of other gases such as water vapor, hydrogen sulphide and hydrogen gas (Ward et al., 2008). AD for disposal of organic waste is an attractive option because of its numerous environmental and economic benefits. AD cuts the volume of waste going to the landfill

and reduces the potential for groundwater or soil pollution, from improper waste management. It also acts as a renewable energy source as opposed to the fossil fuel alternative. Electricity and heat can be generated from the biogas. More income source can be obtained from the sale of the semi-solid digested by-products, popularly called digestate which serves as a nutrient-rich source that can be used as a soil conditioner or fertilizer (Tambone et al., 2009).

Stages of Anaerobic Digestion

AD process is made up of four successive stages, as shown in Figure 1.1. This include hydrolysis, acidogenesis, acetogenesis and methanogenesis. These stages are carried out by the action of different groups of microbial community, usually co-existing in one digester (single stage reactors). The stages occur in parallel with the action of different anaerobic and facultative microbes that have a syntrophic relationship with one another.

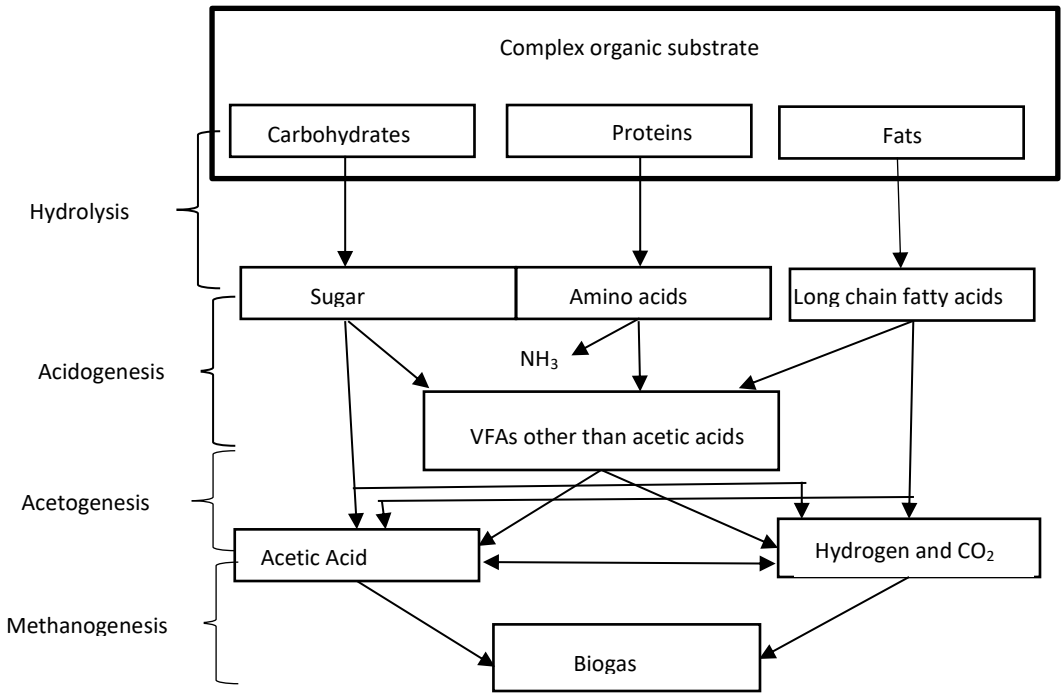

Figure 1.1: *Stages of anaerobic digestion*

Source: Adapted from Gujer and Zehnder, 1983

Hydrolysis

Hydrolysis stage is the breakdown of complex organic materials (such as protein, carbohydrates, lipids and celluloses) into monomers (such as fatty acids, amino acids, pyrimidines, sugar and purines) by the action of hydrolytic bacteria that secretes exoenzymes (such as cellulase, cellulosome, lipase, xylanase, protease, amylase) (Li et al., 2011). The extracellular enzymes break down the complex macromolecules to generate products that can diffuse through the cell membrane of acidogenic bacteria (Van lier et al., 2008). Hydrolytic bacteria have an optimum temperature range of 30 ^{0}C to 60 ^{0}C, with a pH range of 5 to 7 (Azman, 2016). These bacteria belong to the phylum Firmicutes, Bacteroidetes, Thermotogae, Fibrobacter and Spirochaetes (Azman et al., 2015). This reaction is summarized by equation (1)

$$\{C_6H_{12}O_6\}_n + 2n\ H_2O + Enzymes \rightarrow n\ C_6H_{12}O_6 + 2n\ H_2 \quad (1)$$

Acidogenesis

The monomers from the hydrolysis stage are absorbed through the cell membranes of the acidogenic bacteria and are further converted to short chain VFAs (such as acetate, propionate, iso-butyrate, butyrate, isovalerate, valerate, iso-caproate, hexanoate, heptanoate) and other product (such as hydrogen, carbon dioxide and alcohols) (Van Lier et al., 2008). The acidogenic bacteria have an optimum pH range of 4.5 to 5.5. Some of the bacteria genera linked to hydrolysis and acidification are Peptococcus, Lactobacillus, Clostridium, Actinomyces, Escherichia coli, Corynebacterium, and Bifidobacterium (Christy et al., 2014). The formation of the acetate and butyrate is accompanied by hydrogen gas production while propionate formation consumes hydrogen gas. The partial pressure of the hydrogen formed in this stage have a major influence on the products from

the acidification stage. High partial pressure inhibits acetogenic bacteria (Meltcalf, 2003). The formation of propionate and ethanol from glucose is summarized by equation 2 and 3 below.

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (2)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (3)$$

Acetogenesis

The acetogenic bacteria converts the higher VFAs from the acidogenic phase into acetic acid, carbon dioxide and hydrogen. These bacteria are strict anaerobes that utilize the acetyl coenzyme A pathway. Examples of acetogenic bacteria include; Syntrophobacter wolinii and Syntrophomonos wolfei (Christy et al., 2014). Acetic acid production is accompanied by hydrogen gas production which results in an increase in the hydrogen partial pressure. However, the hydrogen consuming methanogens continuously consume the hydrogen to keep the partial pressure low. Hence, there is an interspecies hydrogen transfer between the acetogens and methanogens (Stams and Plugge, 2009). Acetogenesis stage is summarized by equation 4.

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (4)$$

Methanogenesis

In the methanogenic phase, the methane is produced by the action of acetoclastic and hydrogenotrophic methanogens that consumes acetic acid and hydrogen gas, respectively. Methanogens are strict obligate anaerobic bacteria with a high sensitivity to oxygen. Methanogens have an optimum pH range of 6.5 to 7.5. Examples of the hydrogen consuming methanogens are Methanobacterium, Methanobaccillus and Methanococcus

while Methanosacrina and Methanothrix are acetoclastic methanogens (Meltcalf, 2003; Ferry, 2010). Methanogenesis reaction is summarized by equation 5 and 6.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (5)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (6)$$

Factors affecting Anaerobic Digestion

Several factors influence the performance of anaerobic digestion system. These include; chemical properties of the substrate and operational parameters (such as temperature, pH, hydraulic retention time).

Temperature

Temperature is an important factor that affects the stability and performance of an AD process. AD can occur in psychrophilic, mesophilic and thermophilic temperature regime. The temperature range for psychrophilic, mesophilic and thermophilic are 20-25^{0}C, 30-40^{0}C and 50-60^{0}C, respectively. Very few studies exist on AD at psychrophilic temperature (Lettinga et al., 1999). AD at mesophilic temperature has a higher stability due to the presence of more microbial community that favors the mesophilic temperature range (Yang et al., 2018). However, AD at thermophilic temperature has a faster reaction rate due to the increased rate of hydrolysis, with an added benefit of greater pathogen destruction. The increase in temperature also causes a decrease in CO_2 solubility, resulting in an increase in CO_2 content of the biogas at thermophilic temperature (Siddique et al., 2018). Hence, temperature influences both biogas generation and microbial growth.

pH Level

pH is an important parameter that influence the performance of AD process. pH affects the enzymatic reaction of different anaerobic bacteria involved in AD. These bacteria are very sensitive to pH change. The optimum pH for AD process occurring in a single stage is 6.8 to 7.2 (Lemmer et al, 2017). However, the optimum pH range for acidogenic and hydrolytic bacteria is between 5.5 and 6.5 (Kusch et al., 2011). Methanogens represent the most sensitive AD bacteria. Hence, the optimum pH range of methanogens is adopted for a single stage AD. pH below 6.8 inhibits the growth of methanogenic bacteria.

Alkalinity

The capacity of an aqueous solution to neutralize an acid is referred to as alkalinity. For a stable AD performance, alkalinity is a very important factor. Since AD process performs best in the neutral range, keeping the pH neutral is very vital. Alkalinity ensures the pH level is within the neutral range, despite the production of carbonic acids (from CO_2) and volatile fatty acids (VFAs from acidification). Hence, alkaline medium acts a buffer to stabilize the pH in AD. Animal manures have a high alkalinity, making it an excellent co-substrate for AD (Neshat et al., 2017)

Carbon-Nitrogen Ratio

Carbon and nitrogen are important for microbial growth and optimal performance of an AD system. The carbon serves as the organic matter source while the nitrogen acts as the nutrient source. The optimal C/N ratio provides enough nutrient and organic matter required for microbial growth. Reports suggest the optimum C-N ratio for proper functioning of the AD is between 20:1 to 30:1 (Wang et al., 2012). AD process is affected by a deviation from these optimum range of C-N ratio. A high C-N ratio causes nitrogen

deficiency resulting in VFAs accumulation while a low C-N ratio leads to a high concentration of ammonia in the reactor. The high ammonia content or VFAs inhibits the activity of the methane producing bacteria and possibly leads to reactor failure (Giuliano et al., 2013). Usually, plant-based materials have a high C-N ratio with a complex lignocellulose structure that makes their degradation difficult and slow while animal manures have a low C-N ratio, which is inhibitory to AD set up (Risberg et al., 2013). A common approach of managing these two different waste streams is co-digestion, which helps to solve the imbalance that exist between the carbon and nitrogen.

Volatile Fatty Acids

VFA is an intermediate product that is formed from the acidogenic stage of AD process. These organic acids include, acetic acid, butyric acid, propionic acid, valeric acid, caproic acid, iso-valeric acid and iso-butyric acid. The most dominant of the acids are acetic acid, butyric acid and propionic acid. However, acetic acid and butyric acid are the most favorable to AD. Accumulation of these acids have a high tendency of lowering the pH of the system below 6, which is detrimental to methane-forming microbes. This causes the formation of undesirable products and possibly reactor failure (Bah et al., 2014). In the absence of enough buffering medium, a high concentration of VFAs in the range of 1500 to 2000 mg/L can inhibit AD system (McCarthy, 1964). Addition of enough buffering medium is important for stabilizing the pH within the optimal range in an AD system with high VFAs. Animal manure serves as an excellent buffering medium.

Hydraulic Retention Time

The HRT is an important parameter in AD that represents the average length of time it takes for anaerobic microbial culture to consume the organic matter present in the substrate

and synthesize the by-product. Too short or too long HRT can result in the washout of the microbial community and death of microorganism, respectively. This is due to the shortage of nutrient or organic matter at long HRT or insufficient time for cell generation, at short HRT. Hence, the HRT should be kept at a time greater than the generation time of the microorganisms, as well as the time required for the consumption of organic matter and synthesis of the by-products (Metcalf, 2003).

Mixing

Agitation in a digester helps to increase the contact between the microbes and the organic matter. The mixing ensures a homogenous mixture in the digester and prevents temperature variation and scum layer or inactive regions formation (Lucas, 2014). A smooth agitation will ensure the concentration of nutrients and degradation products are uniform for different groups of bacteria. However, a significant level of agitation is necessary to destroy layers of hydrogen gas that might form around the bacteria (Deublein and Steinhauser, 2011). However, excessive agitation has a tendency of destroying microbial cells, resulting in an instability in the AD process (Lucas, 2014). Hence, mixing acts to improve the reactions taking place in the digesters

Chemical Properties of Substrate

Selection of suitable substrates is vital in the performance of AD. The chemical property of any substrate is dependent on the source of the substrate. Classification of different substrates by its chemical composition is useful in assessing the substrate's digestibility and availability.

Generally, there are 3 common classes of substrates used in AD; carbohydrates, proteins and lipids. Based on Buswell's equation, the theoretical methane yield from carbohydrate, lipids and proteins are 370, 415, 1014 and 496 l/KgVS, respectively. This was based on an estimation of the average composition of carbohydrate ($C_6H_{10}O_5$), lipids ($C_{57}H_{104}O_6$) and proteins ($C_5H_7O_2N$) (Moller et al., 2004).

Another way of identifying the properties of a substrate is by the lignocellulose contents. Lignocellulose are complex structural carbohydrates that is made up of lignin, cellulose and hemicellulose. Lignocellulose materials have a high potential for biogas production but are limited by their slow and difficult degradation (Khanal et al., 2011). Hence, introducing suitable pre-treatment (PT) on a lignocellulose-rich substrate could potentially improve its performance as a substrate in AD.

Pre-treatment of Organic Waste

The energy recovery from complex substrate are limited by hydrolysis. This can be addressed by introducing a suitable PT to enhance the substrates biodegradability. For this thesis, biological PT and low thermal PT are under consideration.

Biological Pre-treatment

Biological PT involves the addition of certain enzymes (such as amylase, carbohydrolase, peptidase and/or lipase) to enhance the digestion of different substrates (carbohydrates, proteins, lipids) in AD system. Recently, the addition of enzymes is not very common with complex substrate because it is a slow process (Ariunbaatar et a., 2014). Another common technique used as a biological PT is the physical separation of the hydrolysis/acidification and acetogenesis/methanogenesis stage to form two stage AD, which allows for the

enrichment of the microbial community required for each stage. Some of the potential benefits of two stage AD over the conventional single stage AD include; higher stability with improved pH control, higher activities of microbial communities for each stage and higher loading rate (Bouallagui et al., 2005). For this study, the separation of the acidification and methanogenesis stage is utilized as a biological PT technique.

Thermal Pre-treatment

Thermal PT results in the solubilization of organic matter through the disintegration of its cell membrane by the application of heat. It has the advantages of removing pathogens, improving the dewatering performance of digestate, reducing the digestate viscosity and thereby enhancing the digestate quality (Bougrier et al., 2006a). However, thermal PT could result in loss of volatile organic matter at high temperature. Thermal PT at high temperature could also results in process inhibition due to formation of complex substrates, resulting from the agglomeration of particles and formation of chemical bonds. This reaction between protein and carbohydrate at temperature above 150^0C results in a phenomenon known as Maillard reaction (Bougrier et al., 2006b). Hence, for this thesis, only thermal PT at low temperature is under consideration.

Thesis Objectives

The main objective of this thesis was to improve the energy recovery from anaerobic co-digestion of PP and PM. In order to examine the energy recovery potential of PP and PM, five different mix ratios of PP and PM were investigated for the untreated substrate in a batch study. Also, the effects of thermal PT, biological-mesophilic PT and biological-thermophilic PT on the five different mixes of PP and PM were investigated in a batch study. The aim was to compare the energy recovery and reaction kinetics of five mix ratio

of untreated PP and PM with the five mix ratios of pre-treated (thermal PT, biological-mesophilic PT and biological-thermophilic PT) PP and PM.

The objective of Chapter 2 was to investigate the performance of anaerobic co-digestion of different mix ratios of PP and PM in a batch study at mesophilic temperature and to predict the reaction kinetics using the modified Gompertz equation. A comprehensive review of relevant literature was conducted to identify the gap that exist on anaerobic co-digestion of PM with other organic waste. The first objective was to investigate the mix ratio of PP and PM with the highest methane yield and energy recovery potential. Five different mix ratios of PP and PM were prepared (based on the VS ratio) and subjected to a biochemical methane potential (BMP) batch test. The second objective was to predict the reaction kinetics by fitting the experimental data to the modified Gompertz equation. In this study, the synergistic effects of simultaneous digestion of PP and PM were also analyzed. The synergistic effect compares the methane yield from co-digestion of PP and PM to mono-digestion of each waste. Then, the organic matter removal efficiency was quantified by determining the VS removal and COD removal. Correlations were developed between methane yield, energy yield, kinetic parameters and organic matter removal efficiencies of the different mixes.

In Chapter 3, the primary objective was to investigate the effects of thermal PT, biological-mesophilic PT and biological-thermophilic PT on different mix ratios of PP and PM. The focus was on the energy recovery and reaction kinetics in a batch study. In this study, different mixes of PP and PM were prepared and subjected to thermal PT and biological acidification (at mesophilic and thermophilic temperature). The effluents from the different treatments were analyzed and used in a BMP batch study to compare the effects of different

treatments on methane yield and reaction kinetics. Correlations were made between the pre-treated substrates and the untreated substrates.

Finally, Chapter 4 provides recommendation on potential future works, based on the results from this thesis. To have a better understanding of the results from this thesis, the implications and applications of the result obtained from chapter 2 and 3 were discussed in this chapter.

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CHAPTER 2:

INFLUENCE OF MIX RATIO ON ENERGY RECOVERY FROM ANAEROBIC CO-DIGESTION OF POTATO PEEL AND PIG MANURE

Introduction

The increase in world population and economic growth has led to a corresponding increase in energy consumption and organic waste generation. Energy demand is projected to further increase by 28% between 2015 and 2040, with 77% of the energy coming from fossil fuel sources (USEIA, 2017). The depletion of fossil fuel reserve and the significant environmental pollution associated with its extraction, processing and use phase makes it an unsustainable energy source. Researchers have been exploring alternative means of generating renewable energy in an environmentally friendly way. A popular technique is by anaerobic digestion (AD) which is an established waste management strategy that serves to recover energy from organic waste and divert potential greenhouse gas sources away from the landfill (Fernandez et al., 2001). Hence, recovering energy from organic waste will contribute to the circular economy by addressing the challenges of environmental pollution, resulting from improper management of organic waste and providing a renewable energy source that can offset the extreme dependency on fossil fuel.

The two major sources of organic waste are from animal manure and agricultural residues. Globally, pork is the most consumed meat in Asia, Europe and America. Between 1960 to 2011, pork production rose from 20 million tonnes to 108 million tonnes. In Canada, pork exportation represents a significant source of income, with Canada ranking as the fifth major exporter of pork on a global scale. More than 1 million tonnes of pork (worth 2.9 billion dollars) was exported in 2011 (Statistics Canada, 2015). The intensive pork

production has resulted in the generation of a large volume of nutrient rich pig manure (PM) that is potentially harmful to the environment, if not properly managed.

Historically, the common method of disposal of PM is by land application. However, due to the increase in pig production and the concentration of pig farms in certain regions, it is impossible to use all the generated manure as fertilizer. Regulations, such as the EU Nitrates Directive, has moved to limit the application rate of PM due to the rising amount of nitrogen in the soil and the potential for soil, water and air contamination (S.I. No. 610, 2010). This has led to the exploration of an alternative approach of managing PM. One of such approach is by using PM as a substrate in AD. PM as a substrate in AD has the potential benefits of providing a renewable energy source, displacing the use of fossil fuel, improving fertilizer value of the digested PM, reducing pathogens, decreasing greenhouse gas emission and odour generation (Ward et al., 2008; Dennehy et al., 2017; Chae et al., 2008). Despite the numerous benefits, PM as a mono-substrate in AD has a high tendency to be limited by the high ammonia nitrogen level, resulting from protein degradation and hence, inhibiting the methanogens (Hansen et al., 1998; Kaparaju and Rintala, 2005; Strik et al., 2006). A popular technique for addressing the high ammonia nitrogen content resulting from PM degradation is by simultaneous digestion with a complimentary waste. This technique is called anaerobic co-digestion.

A potential co-substrate for anaerobic co-digestion with PM is PP. PP is an unavoidable agricultural waste from potato consumption, which currently ranks as the fourth most consumed food in the world, behind, rice wheat and maize ((FAO, 2008). In Canada, it is the most consumed vegetable crop, accounting for 27.3% of total vegetable receipt, worth $1.19 billion in 2017. About 65% of the total produced potato in Canada, is used in the

processing sector. (Agriculture and Agri-food Canada, 2018). Globally, about 70-140 thousand tonnes of PP are generated worldwide from industrial processing (Chang, 2011).

Some of the application of PP are in the production of animal feeds and recovery of the diverse chemical content in the peel (such as antibacterial, anti-inflammatory, antioxidant, chemo-preventive and apoptotic chemicals). The limitation of the use of PP as animal feed is the low quality of feed that is produced while the capital, energy and operational cost of chemical recovery is high, making it unsustainable (Nelson, 2010; Liang and McDonald, 2014). However, energy recovery from PP through AD serves as a more beneficial application of PP due to its less operational requirements, energy cost, capital cost with little technical complexity and an added benefit of energy production. The study conducted by Stewart et al., (1984) showed that a higher energy conversion efficiency (95%) was attainable when potato was used as a substrate in AD compared to its use for ethanol production (with a conversion efficiency of 60%). Despite the potential benefits of PP as a substrate in AD, it poses a high tendency for rapid volatile fatty acids (VFAs) accumulation from organic matter degradation which occurs in the absence of enough buffering (Kaparaju and Rintala, 2005). This can result in the acidification of the reactors, and consequently, inhibition of the methane producing bacteria.

AD of organic waste requires the addition of significant volume of external sources of buffering chemicals and nutrients sources, to counteract the potential accumulation of VFAs from the breakdown of the organic matter (Demirel and Scherer, 2008). Animal manure is a representative of a nutrient-rich substrate, with high buffering capacity. Hence, a co-digestion of organic waste with animal manure may represent a more effective and less expensive treatment of these two waste streams. The organic waste acts as the carbon

source and the animal manure acts as a nutrient/buffer source. The benefits of co-digestion of two complimentary waste streams include; balancing of the carbon to nitrogen ratio (C-N ratio), reducing the potential for ammonia inhibition from the animal manure, reducing the risk of acidification from the organic waste and increasing the biogas production in the reactor (Hashimoto, 1983; Hills and Roberts, 1981, El-Mashad and Zhang, 2007). Therefore, the use of PM and PP as a co-substrate in AD could be a more effective treatment solution for both wastes, with a potential for higher energy recovery.

The energy recovered in an anaerobic co-digestion is greatly influenced by the mixing proportion of the different waste streams and the substrate composition. Selection of a suitable co-substrate in the right proportion is paramount in the performance of anaerobic co-digestion. Past work on anaerobic co-digestion of PP and PM was limited to a mix ratio of 0-20% PP to 80-100% PM, with 20:80 (PP:PM, mixing on a VS basis) representing the mix ratio with the highest methane yield (0.30–0.33 m^3 CH_4 kg^{-1} VS) (Kaparaju and Rintala, 2005). A lack of information exists on methane yield from higher mixes of PP and PM. However, other studies investigating co-digestion of PM with other organic waste, have explored higher mixes of both substrates, ranging from 0-100%. In the study conducted by Kafle et al., (2013), apple waste and PM co-digestion produced the highest methane yield at a mix ratio of 33:67. Also, Xie et al., (2011) reported the co-digestion of grass silage and PM, was optimal at 50:50 mix. Similarly, mixture of 75% kitchen waste with 25% cattle manure was concluded to be the best performing mix ratio during anaerobic co-digestion (Li et al., 2009). These literatures indicate the optimal mix ratio of co-substrate can not be unequivocally determined but requires investigation for different substrate. Hence, susbtrate characteristics determines the optimal mixing ratio in anaerobic

co-digestion and it varies for different substrates as shown in past literature. Therefore, in this study, the effects of five different mix ratio of PM and PP on energy recovery and reaction kinetics was investigated in a BMP test.

The objective of this study was to investigate the energy recovery from anaerobic co-digestion of PP and PM. The specific objectives were;

1. To evaluate and compare the effects of mix ratio on methane yield from five different mix ratios of PP and PM in a batch study at mesophilic temperature.
2. To model and compare the effects of mix ratios of PP and PM on reaction kinetics, using the modified Gompertz equation.

Materials and Methods

Materials

Fresh PM was obtained from a local pig farm in Windsor, Canada. Visible coarse materials were removed from the PM. A concentrated PM was prepared by blending and homogenising about 1kg of moist PM for a duration of 5 minutes. This was done using an electric blender, Ninja model (Model number NJ 600WMC). The concentrated PM had a TS of 198 g/l and VS of 167 g/l. The prepared PM was stored in an airtight 20 litre gallon and kept in a cold storage room at 4°C, to prevent further degradation. Prior to the preparation of the mixes, it was placed at room temperature 24 hours before use. Also, potato was obtained from a local store in Windsor. The peels were extracted manually, using a knife. About 1kg of the extracted peels were blended and homogenised in an electric blender for 10 minutes. The blended PP had a TS of 115 g/l and VS of 112 g/l. After preparation, the PP was stored in an airtight 20 litre gallon and stored in a cold storage

room at 4°C, to prevent further degradation. Similarly, the PP was placed at room temperature, 24 hours before preparation of mixes.

Anaerobically digested sludge (ADS), which is the source of the microbial community, required for AD, was obtained from the Chatham-Kent municipal wastewater treatment plant (WWTP) in Chatham, Ontario, Canada. The sludge was stored in an airtight 20 litre gallon, inside the cold storage room, at 4°C, to prevent further degradation. The sludge had a TS of 43 g/l and VS of 18 g/l. Also, the sludge was taken out of the cold storage room (4°C) and placed at room temperature 24 hours before use, while undergoing continuous stirring. Table 2.1 shows the characteristics of the PM, PP and ADS.

Table 2.1: *Characterization of PP, PM and ADS (values are the average ± standard deviation of triplicates set of data)*

	PP	**PM**	**ADS**
VFAs, mg/l	1770±27	440±13	44±2
TCOD, g/l	102±4	196±7	25.8±0.2
SCOD, g/l	34.4±1	22.5±0.5	0.496±0.4
Ammonia Nitrogen, mg/l	275.4±6	986.1±4	561.6±3
Alkalinity, g/l as $CaCO_3$	2.89±0.1	14.0±0.3	5.06±0.2
TS, g/l	121.8±5.8	197.9±8.6	42.7±0.5
VS, g/l	114.5±5.3	166.5±9.3	18.2±0.4
TSS, g/l	101.6±2.0	162.5±9.6	40.2±0.7
VSS, g/l	97.5±1.5	139.2±7.5	16.3±0.2
pH	6.31	6.86	7.42
VS/TS, %	94	84	41

Preparation of Mixes

Five different mixes of PP and PM were prepared based on the volatile solids ratio as shown in Table 2.2. The composition of PP to PM in individual mixes were; 100:0 (mix A), 75:25

(mix B), 50:50 (mix C), 25:75 (mix D) and 0:100 (mix E). Total mass of volatile solids of PP and PM in each mix was set to 40 gVS. The ratio of VS of PP to VS of PM for mix A, B, C, D and E were respectively 40g:0g, 30g:10g, 20g:20g, 10g:30g and 0g:40g. Distilled water was added to each mix to make up a total volume of 1 litre.

Table 2.2: *Preparation of different mixes of PP and PM*

PP:PM	Volume of PP, ml	Mass of VS of PP, gVS	Volume of PM, ml	Mass of VS of PM, gVS	Volume of DIW, ml	Total Volume, ml
Mix A, 100:0	351	40	0	0	649	1000
Mix B, 75:25	263	30	60	10	677	1000
Mix C, 50:50	175	20	120	20	705	1000
Mix D, 25:75	88	10	180	30	733	1000
Mix E, 0:100	0	0	240	40	760	1000

Biochemical Methane Production Potential Tests

The biochemical methane potential (BMP) test of the different mixes of PM and PP were investigated for all mix ratio in a 150 ml digester glass bottles, with a 50 ml headspace. The ADS in each bottle was set to the same volume and different mixes of PP and PM were added as substrates. The substrates volume required to attain a FM ratio of 0.5 in the BMP test was calculated based on gram total chemical oxygen demand per gram volatile suspended solids (gTCOD/gVSS), as shown in equation (7). Distilled water was added to each prepared mix to make a total volume of 150 ml. Each digester bottles were filled with 100 ml of the prepared sample and the remaining 50 ml was kept in the refrigerator for

characterisation. The calculations of each reactor set up is shown in Table 2.3 and the experimental design of each reactor bottle is shown in Figure 2.1.

$$FM\ (\frac{gTCOD}{gVSS}) = \frac{V_{substrate}\ (L) * TCOD_{substrate}(\frac{g}{L})}{V_{seed}\ (L) * VSS_{seed}\ (\frac{g}{L})} \quad (7)$$

Where FM is food to microorganism ratio, $V_{substrate}$ is volume of substrate, V_{seed} is volume of seed, $TCOD_{substrate}$ is TCOD of substrate and VSS_{seed} is VSS of anaerobic digested sludge.

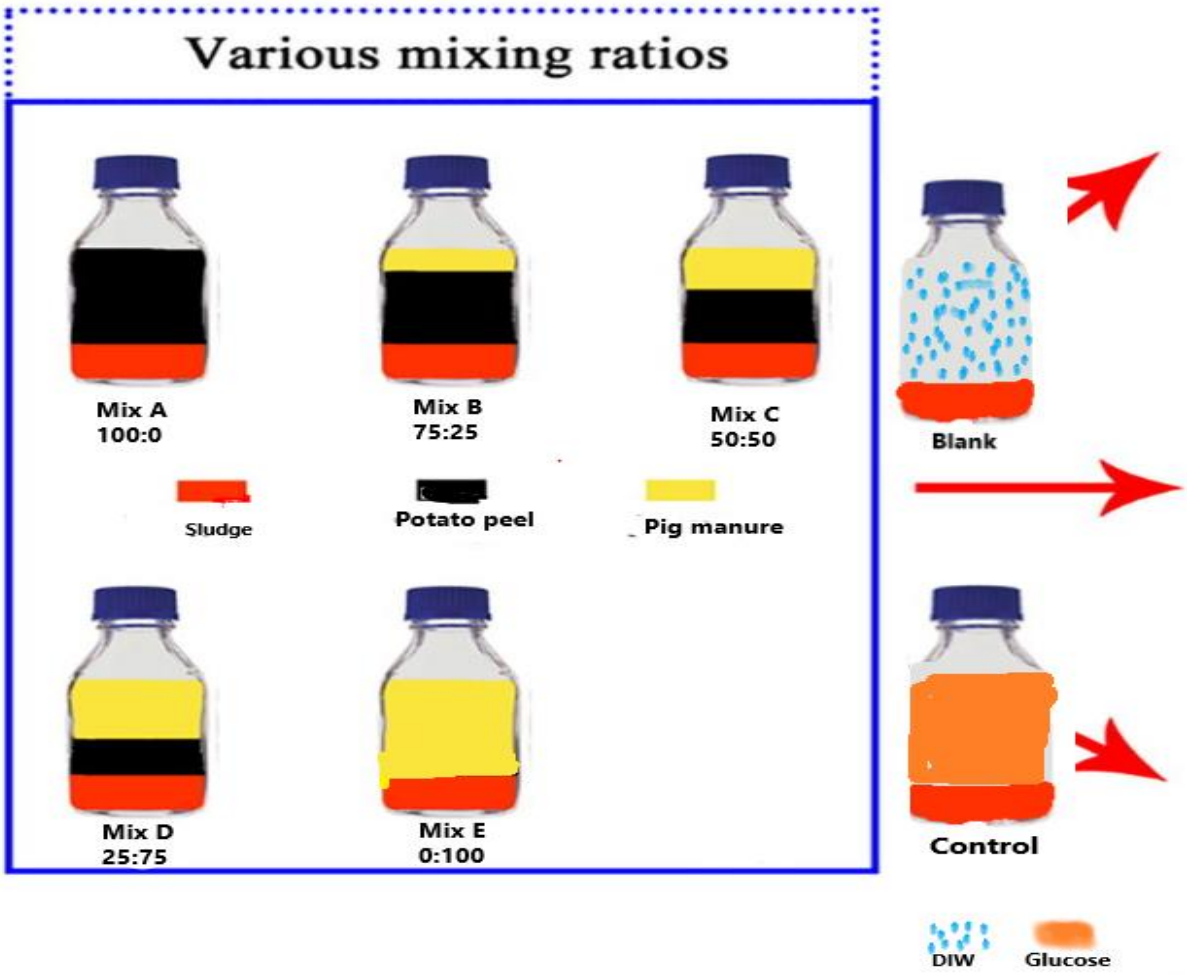

Figure 2.1: *Experimental set-up of each reactors*

Table 2.3: *Set-up of reactors for BMP study*

Sample label	$V_{substrate}$, ml	V_{seed}, ml	DIW, ml	Total, ml	TCOD (g/L)
A11	26.6	120.0	3.4	150.0	39.3
A22	26.6	120.0	3.4	150.0	39.3
B33	27.7	120.0	2.3	150.0	37.7
B44	27.7	120.0	2.3	150.0	37.7
C55	28.7	120.0	1.3	150.0	36.4
C66	28.7	120.0	1.3	150.0	36.4
D77	27.0	120.0	3.0	150.0	38.7
D88	27.0	120.0	3.0	150.0	38.7
E99	21.6	120.0	8.4	150.0	48.3
E1010	21.6	120.0	8.4	150.0	48.3
Control	0.9787	120.0	30.0	150.0	1.0667
Blank	0.0	120.0	30.0	150.0	0

The digester bottles were covered with an air-tight plastic cap with a rubber septum centred at the top. Each bottle was purged with Praxair, containing 99.9% nitrogen gas at 5 Psi, for three minutes to ensure the absence of oxygen. The pH of each digester bottle was adjusted to 7.3$\pm$ 0.2 by the addition of 4.5N NaOH or 4.0N HCl. There was no external alkalinity or trace elements addition to each digester bottles. It was assumed that the nutritional requirements and alkalinity from the PM and anaerobic digested sludge were enough.

The digester bottles were kept in an enclosed mechanical shaker, operating at 38$\pm$1 $^{\circ}$C and 150 rpm, for a retention time of 27 days. Each BMP test was carried out in two replicates. However, the glucose control and the blank were done in single replicate. The blank was carried out to account for endogenous methane production from the ADS and the glucose control was used to ensure proper operational set up.

Measurements and Data Analysis

Influent and effluent of the BMP study was characterized. Solids test was determined according to standard methods (APHA, 1995). The SCOD, TCOD, $NH^{4+}N$ and VFAS were measured by using the Hach methods (Hafez et al., 2010). pH of every sample was measured with the Hach pH meter (Benchtop meter, Hach company, Loveland Company, USA).

The biogas produced in the headspace of each digester bottles was measured with a 10 to 50 ml air-tight glass syringe using the plunger displacement method (Owen et al., 1979). The methane content of the biogas was determined by using a gas chromatograph (SRI 8610C, SRI instruments, Torrance, CA, USA), equipped with a thermal conductivity detector, TCD. The temperature of the column oven was 60 ^{0}C. Nitrogen gas was used as a carrier gas, with a flow rate of 20 ml/min. The biogas volume in the headspace was measured every 12 hours for the first day, everyday for day 2 to day 20 and every other day for day 20 to day 27. This was done to avoid unnecessary gas pressure build-up in the headspace, which might result in a leakage. After each biogas volume measurements, 0.5 ml of the produced biogas was injected into the GC-TCD, through the injection port, using a 1 ml air-tight syringe. The BMP test was assumed to be complete when the methane production was less than 1% of the cumulative methane (Elbeshbishy et al., 2012).

Calculations

The headspace methane volume produced at each time interval was calculated as shown in equation (8).

$$V_{CH4,i} = V_{CH4,i-1} + C_{CH4,i} * V_{T,i} + V_{H,i}(C_{CH4,i} - C_{CH4,i-1}) \quad (8)$$

Where $V_{CH4,i}$ and $V_{CH4,i-1}$ are current cumulative methane volume and preceding cumulative gas volume, respectively, $C_{CH4,i}$ and $C_{CH4,i-1}$ are current fraction of methane in the headspace and preceding fraction of methane in the headspace, $V_{T,i}$ and $V_{H,i}$ are total gas volumes accumulated between the preceding and present interval, and the total volume of the reactor headspace in the present interval (Gomez-Flores et al., 2015).

The experimental methane yield (EMY) in each mix was calculated with equation (9).

$$EMY = \frac{CM_{mix} - \mathrm{CM}_{blank}\,(ml)}{gTCOD_{influent} - gTCOD_{blank}\ \ (Net\ gTCOD_{added})} \tag{9}$$

Where CM_{blank} is the cumulative methane produced in the blank, CM_{mix} is the cumulative methane produced in each mix of PP:PM, $gTCOD_{influent}$ is the mass of TCOD in the influent and $gTCOD_{blank}$ is the mass of TCOD in the blank.

The experimental methane yield, EMY, was converted to a specific energy recovered (SER) per unit mass of TCOD added to the system, as shown in equation (10). The specific energy recovery was calculated based on the lower heating value of methane, LHV (35.16 MJ/Sm^3), as suggested by Schievano et al., (2012) and Schievano et al., (2014).

$$SER\left(\frac{kJ}{TCOD_{added}}\right) = EMY\ \left(\frac{\mathrm{ml}}{\mathrm{g}TCOD_{added}}\right) * \frac{1Sm^3}{1000000ml} * \frac{35.16\frac{\mathrm{MJ}}{Sm^3} * 1000kJ}{1MJ} \tag{10}$$

The synergistic effect was quantified by using a technique that involves the mass balance approach that compares the experimental methane yield from co-digestion of PP and PM to the experimental methane yield from mono-digestion of PP and PM, as shown in equation (11). To allow for better interpretation of results, this was done in a reverse order

as compared to the study conducted by Xie et al., (2017a); Xie et al., (2017b) and Xie et al., (2017c).

$$\alpha = \frac{(A+B).EMY_{co}}{EMY_{pm}.A + EMY_{pp}.B} \quad (11)$$

Where EMY_{pm}, EMY_{pp} and EMY_{co} are experimental methane yield of the pig manure, experimental methane yield of potato peel and experimental methane yield of co-digestion mix, respectively. $A\ and\ B\ are\ the$ mass of TCOD fraction of PM and mass of TCOD of PP in the mix, respectively. α is the synergistic effect. Where less than 1 denotes antagonistic effects and greater than 1 denotes synergistic effect.

TCOD removal and VS removal were calculated using the mass balance approach described in equation (12) and equation (13). The net mass TCOD in the influent and effluent was obtained by accounting for the blank mass TCOD in the influent and effluent. Similarly, the net mass VS in the influent and net mass VS in the effluent was obtained by deducting the blank mass VS in the influent and effluent mass VS.

$$TCOD_{removal} = \frac{Net\ gTCOD_{Influent} - Net\ gTCOD_{effluent}}{Net\ gTCOD_{influent}} \quad (12)$$

$$VS_{removal} = \frac{Net\ gVS_{Influent} - Net\ gVS_{effluent}}{Net\ gVS_{influent}} \quad (13)$$

Kinetic Modelling

Modified Gompertz equation is one of the most common equation used to fit experimental data to predict kinetic parameters, with goodness of fit ranging from 0.910 to 1.00 in different waste (Donoso-Bravo et al., 2010; Nielfa et al., 2015). The modified Gompertz equation described by Xie et al., (2011) was utilized to estimate the maximum methane production, lag phase and production rate in different mixes of PP and PM. The parameters

were estimated by using the sigmoid-type modified Gompertz function in Sigmaplot, version 12 and Microsoft Excel 2016. The sum of squared residual between the experimental data and theoretical data was minimized by varying the values of lag phase, methane production rate and cumulative methane production, using a fit algorithm in the sigmaplot and solver function in excel. The modified Gompertz equation is shown in equation (14)

$$T(t) = T_{max}\ exp\left\{-exp\left[\left[\frac{R_m.e}{T_{max}}(\lambda - t) + 1\right]\right]\right. \quad (14)$$

Where $T(t)\ is$ the cumulative methane production at a time, t, in mL, (t), T_{max} is maximum methane production in mL, e is the value of exponential 1 (2.718281828), R_m is methane production rate in mL/d, t is time in days and λ is the lag phase in days. These values were normalized by dividing by $gTCOD_{added}$, after accounting for the contribution of the ADS.

An additional kinetic parameter, t_{95}, which is the estimated time required to attain 95% of the total cumulative methane production from the BMP test was calculated using equation (15) (Lavagnolo et al., 2018). The effective methane production period, t_{eff} was obtained by subtracting the lag phase from the t_{95}.

$$t_{95} = \frac{T_{max}}{R_m}\{1 - \ln(-0.95)\} + \lambda \quad (15)$$

Results and Discussions

Characterization of influent and effluent

The influent to the BMP study were characterised. Results of the parameters analyzed are presented in Table 2.4. The results show that the pH level to each reactor were within the

range that favours the growth of methane producing bacteria. There was also increasing ammonia nitrogen and alkalinity in all mixes, as the PM proportion increased from A to E. The use of ADS with high concentration of ammonia nitrogen and alkalinity also contributed to the ammonia nitrogen and alkalinity. The ammonia nitrogen and alkalinity serve as nutrient for bacteria growth and buffering medium that helps to the keep the pH stable. Also, the starting VFAs and SCOD in all mixes were low and the starting TCOD in each reactor bottles were similar.

Table 2.4: *Influent to methanogenesis of untreated substrates (values are average ± range of duplicate set of reactors)*

PP:PM	A (100:0)	B (75:25)	C (50:50)	D (25:75)	E (0:100)
TCOD, g/l	34.15±0.8	34.57±1.1	34.30±0.7	33.27±1.2	32.36±1.4
SCOD, g/l	1.44±0.1	1.22±0.3	1.17±0.2	0.99±0.1	0.92±0.2
pH	7.37	7.30	7.35	7.27	7.28
NH_3 Nitrogen, mg/l	448±12	488±8	526±15	550±8	572±7
TA (g/l as $CaCO_3$)	5.3±0.3	5.7±0.2	6.0±0.3	6.3±0.1	6.6±0.2
VFAs (mg/l)	240±5	269±4	224±6	204±3	335±5
VS, g/l	39.3±1.4	38.55±0.8	39.2±0.9	41.4±1.6	45.9±1.4
TS, g/l	50.9±2.3	53.8±1.9	55.9±1.6	56.8±3.4	60.7±3.0

At the end of the BMP study after biogas production, the characteristics of the effluent from each reactor were analyzed. Table 2.5 shows the results of the characterization of the digested mix A to mix E. There was a slight drop in the pH of the digested mixes, but it was still within 7.2±0.1. This shows that the alkalinity present in the sludge and PM was enough to stabilize the pH in all mixes. Also, the increase in ammonia nitrogen shows that protein degradation occurred and the ammonia nitrogen level in all mixes were below the concentration that has been reported to be inhibitory in previous literature. This shows that there was no ammonia toxicity in this study. The reduction in the TCOD and VS shows

that the degradable part of the organic matter was consumed during the AD. The low value of SCOD and VFAs in the effluent further confirms the absence of inhibition and the completion of the digestion, as the soluble organic matter and organic acids were consumed.

Table 2.5: *Effluent from methanogenesis of untreated substrates (values are average ± range of duplicate set of reactors)*

PP:PM	A (100:0)	B (75:25)	C (50:50)	D (25:75)	E (0:100)
TCOD, g/l	24.05±1.3	23.97±1.5	23.23±0.7	23.75±1.2	24.06±1.4
SCOD, g/l	0.44±0.02	0.46±0.03	0.56±0.01	0.64±0.02	0.66±0.01
pH	7.11	7.21	7.25	7.20	7.21
NH_3 Nitrogen, mg/l	734±15	775±11	815±13	880±8	952±12
TA (g/l as $CaCO_3$)	4.7±0.2	5.0±0.1	5.2±0.2	5.3±0.4	5.5±0.2
VFAs (mg/l)	55.6±2	58.6±3	65.6±2	69.8±3	70.9±2
VS, g/l	16.8±1.0	16.8±0.8	15.5±0.6	15.9±0.3	17.6±0.5
TS, g/l	42.1±1.3	42.8±0.6	44.9±1.1	44.1±0.8	43.8±1.2

Methane Yield from Different PP and PM Mixtures

The cumulative methane produced from mono-digestion and co-digestion of PP and PM was observed in duplicate set of reactors for 27 days. The time series of the methane production is reported in Table 2.15 to Table 2.24, Appendix A. Figure 2.2 shows the cumulative methane produced against time over the duration of the BMP test. The average cumulative methane produced in each reactor is reported in Table 2.6. Apart from mix D, the co-digested mixes produced a higher cumulative methane volume than the mono-digested substrate of PP and PM. The lower cumulative methane volume observed in mix D can be linked to the presence of lower mass of TCOD in the influent to reactor D, shown in Table 2.4. Due to the slightly varying mass of TCOD in the influent of some of the reactor bottles, it is impossible to make a direct comparison of the cumulative methane

produced from different mixes. A better approach is the normalization of the cumulative methane produced in each bottle per gTCOD added to individual reactor bottles.

Table 2.6: *Cumulative methane produced in each mix (Values are average range of duplicate set of reactors)*

Sample	Cumulative methane, ml
A	273
B	294
C	300
D	258
E	213

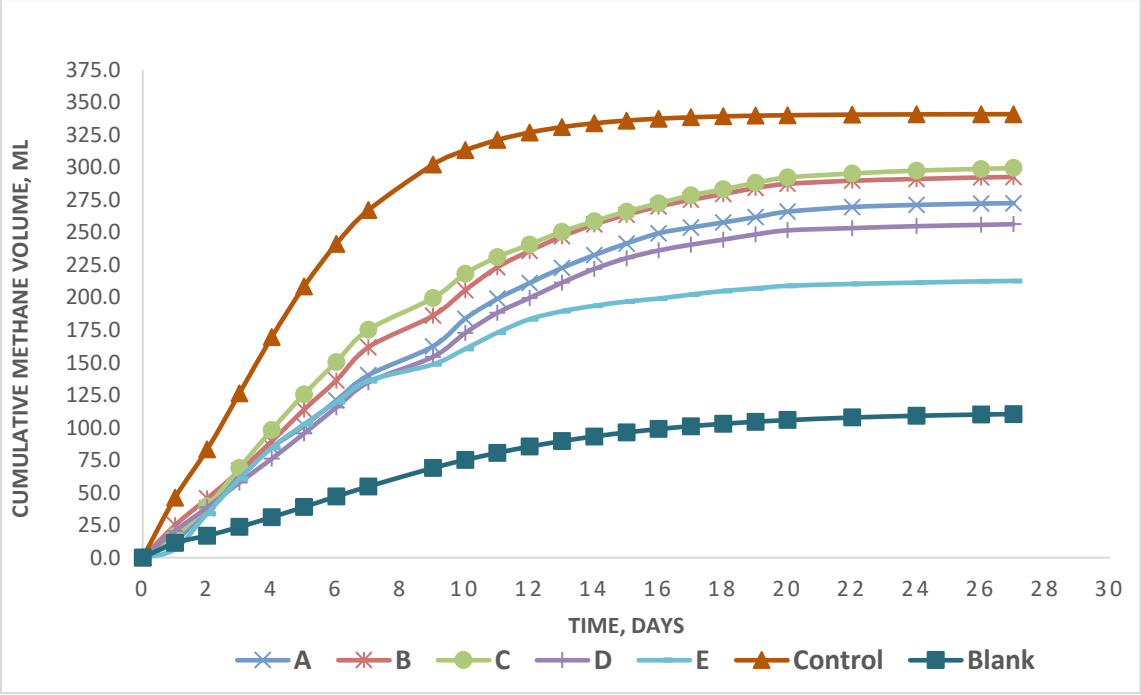

Figure 2.2: *Cumulative methane production against time*

The cumulative methane produced in each mix was corrected for the contribution of the ADS present in the PP and PM mix. For better comparison of the methane produced in

different mixes, the corrected cumulative methane volume was normalized per gTCOD added to give the experimental methane yield, EMY. The results of the EMY is shown in Table 2.7. The EMY increased as the VS proportion of PM increased in the mix, up to 50% PM (mix C). Above 50% PM, the methane yield reduced slightly. However, the three co-digested mixes produced a higher methane yield than the mono-digested PP or PM. In this study, mix C produced the highest methane yield. This shows that the simultaneous digestion of PP and PM at 50:50 mix was able to improve methane yield from AD, as compared to mono-substrate digestion.

Table 2.7: *Experimental methane yield from co-digestion of PP and PM (values are average ± range of duplicate set of reactors)*

SAMPLE NAME (PP:PM)	Yield, $ml/gTCOD_{added}$	Yield, ml/gVS_{added}	Yield, ml/ gTCOD $_{removed}$
A (100:0)	202 ± 2	328 ± 3	316±3
B (75:25)	217 ± 1	356 ± 2	325±2
C (50:50)	231 ± 3	380 ± 3	349±2
D (25:75)	206 ± 1	334 ± 2	315±1
E (0:100)	164 ± 2	274 ± 4	307±2

Synergistics Effects of Co-digestion of PP and PM

For better comparison of the methane yield from co-digestion and mono-digestion, the synergistic coefficient, α, was calculated based on mass of the TCOD from PM and PP and the methane yield from mono-digestion and co-digestion of PP and PM. The results of the synergistic coefficient are reported in Table 2.8. The result shows that positive synergistic effect was observed in all co-digested mix. The synergistic coefficient increased as the VS contribution from the PM increased from 25% to 50%. This was followed by a slight decrease, as the VS contribution from PM increased beyond 50%. The highest synergistic effect was observed in mix C, which was 25% higher than mono-digestion of PP or PM.

Similarly, mix B and D had higher synergistic effects, compared to mono-digestion of PP and PM. This further confirms the benefits of co-digesting PP and PM in 50:50 mix. Previous studies co-digesting PM with other organic waste have suggested that the positive synergism that occurs at a certain mix ratio of co-substrate AD is due to the presence of a better balance between the nutrients and organic matter (Panichnumsin et al., 2010, Xie et al., 2017a; Xie et al., 2017b; Xie et al., 2017c). Also, the PM also provides buffering capacity and serves as a source of trace elements, that improves methane yield from anaerobic co-digestion process.

Table 2.8: *Synergistic effects of PP and PM mixes (values are average ± range of duplicate set of reactors)*

Sample name (PP:PM)	Synergistic effects
B (75:25)	1.17 ± 0.08
C (50:50)	1.25 ± 0.05
D (25:75)	1.11 ± 0.03

Previous studies have shown that substrate composition is an important factor in the performance of a substrate in AD (Lethomaki et al., 2007, Amon et al., 2007, Neves et al., 2008). For example, carbohydrate, protein and lipid have a theoretical methane yield of 415 ml/gVS, 496 ml/gVS and 1014 ml/gVS, respectively (Moller et al., 2004). PP is a representative of carbohydrate rich waste while PM represents a protein rich waste. Despite the higher theoretical methane potential of protein rich waste, there is also a high tendency of ammonia inhibition. Ammonia nitrogen have been reported to be toxic to methane producing bacteria at a concentration of 1.5 g/l (Angelidaki and Ahring, 1993). In this study, the ammonia nitrogen level in all reactors were below the level that has been reported to be inhibitory, in both the influent and effluent. This is due to the use of large

volume of ADS, that makes up 80% of the total volume in the reactor. The abundance of alkalinity and nutrient in the sludge also aided the mono-digestion of PP. Mono-digestion of PP and other organic waste has been reported to be inhibited by VFAs accumulation in previous literature (McCarthy, 1964). This shows that VFAs accumulation can be addressed by alkalinity supplementation in AD.

The EMY observed in this study was compared with the EMY from co-digestion of PM with other substrates in past studies. Table 2.9 shows the comparison of highest methane yield observed from similar studies at different mix ratio. For better comparison, the yield (in volume of methane produced per gVS added) from this study was used. The result shows that 15% higher methane yield was obtained by increasing the PP in the mix from 20% (in the study by Kaparaju and Rintala, (2005)) to 50% (in this study). A comparable result was obtained in this study and the study co-digesting cassava pulp and PM (Panichnumsin et al., 2010). However, while the highest methane yield was obtained at 50:50 mix in this study, the co-digestion of PM and cassava pulp at 20:80 mix produced the highest methane yield in the study by Panichnumsin et al., (2010). This further confirms that the methane yield from AD of co-substrate is greatly influenced by the mix ratio.

Table 2.9: *Comparison of methane yield from previous studies*

Substrates	Highest yield	Reference
Potato peel:PM Ratios of 100:0, 75:25, 50:50, 25:75, 0:100	231 ml $CH_4/gTCOD_{added}$ (380 ml CH_4/gVS_{added}) at 50:50 mix	In this study
Potato peel:PM Ratios of 0:100, 10:90, 15:85, 20:80	300-330 ml CH_4/g VS at 20:80 mix	Kaparaju and Rintala, 2005
Grass silage:PM Ratios of 0:100, 100:0, 75:25, 50:50 and 25:75	302.8 ml CH_4/g VS at 50:50 mix	Xie et al, 2011
Glycerine:PM Ratios of 20:80, 40:60, 50:50, 60:40 and 80:20	215 mL CH_4/gCOD at 20:80 mix	Astals et al., 2011
Apple waste:PM 0:100, 25:75, 33:67, 50:50, 100:0	276 mL CH_4/g TCOD added at 33:67 mix	Kafle et al., 2012
Cassava pulp:PM Ratios of 0:100, 20:80, 40:60, 50:50, 60:40, 80:20 and 100:0	391 CH_4/gVS at 20:80 mix	Panichnumsin et al., 2010

Energy Recovered

The methane yield from each mix was converted to the energy recovered per mass of TCOD added to each reactor bottles using the lower heating value of methane. The results of the energy recovered are presented in Table 2.10. Similar to the methane yield, the energy recovered from the mono-digestion of PP and PM was lower than in co-digested mixes. Mix C had the highest energy recovered. In the co-digested mixes, the energy recovered increased from mix B to C. This indicates the co-digested mix was able to increase the energy recovered per unit mass of TCOD added, as the proportion of PM increased up to 50%. However, the presence of more than 50% PM in the mix resulted in a decrease in the energy recovered, which was still more than the mono-digested substrate.

Hence, this result shows that more energy can be recovered by co-digesting PM with PP. Also, increasing the PP and PM to 50:50 proportion will produce the highest amount of energy per mass of TCOD added.

Table 2.10: *Energy recovered from different mixes (values are average ± range of duplicate set of reactors)*

SAMPLE (PP:PM)	ER (kJ/gTCOD added)
A (100:0)	7.09 $\pm$ 0.07
B (75:25)	7.61 $\pm$ 0.07
C (50:50)	8.12 $\pm$ 0.05
D (25:75)	7.23 $\pm$ 0.06
E (0:100)	5.75 $\pm$ 0.04

Organic Matter Removal Efficiency

VS and COD are representative of organic matter present in each mix. The VS removal and COD removal in the influent and effluent were corrected for the ADS. The organic matter removal efficiency observed in this study are reported in Table 2.11. The results show that the VS removal of mono-digestion of PM and PP was 53% and 63%, respectively. In the co-digested mix, the VS removal increased as the proportion of PM increased in the mix, up to a 50% PM proportion. A similar trend was observed in the COD removal of mono-digested and co-digested substrate. In this study, mix C had the highest VS and COD removal. This follows a similar trend with the methane yield. This indicates the presence of PP and PM in the right proportion resulted in a higher VS and COD removal in mix C. The results of the VS reduction observed in this study is comparable with past work co-digesting PM with other organic waste, where 50-67% VS reduction was reported

when fruit and vegetable waste was co-digested with a mixture of cattle manure and PM (Alvarez and Liden, 2008).

Table 2.11: *Organic matter removal efficiency (values are average ± range of duplicate set of reactors)*

SAMPLE NAME (PP:PM)	COD REMOVAL	VS REMOVAL
A (100:0)	63% ± 2	63% ± 1
B (75:25)	66% ± 2	66% ± 1
C (50:50)	74% ± 1	73% ± 2
D (25:75)	63% ± 2	64% ± 2
E (0:100)	52% ± 1	53% ± 1

Kinetic Modelling

The results of the cumulative methane produced in each mix was fitted into the modified Gompertz equation, to obtain the kinetic parameters in each reactor bottles. The curve fitting is shown in Figure 2.3 to Figure 2.7. The results of the kinetic parameters obtained were normalized to mass of TCOD added, after correcting for the contribution of the ADS. The results show that the modified Gompertz equation was able to model the experimental data with good correlation coefficients. The R^2 value obtained was in the range of 0.991 to 0.994. Table 2.12 shows the results of the estimated kinetic parameters for different mixes of PP and PM. The maximum methane production rate and the maximum methane produced per gTCOD added was attained at mix C. Hence, this shows that the 50:50 mix of PP and PM attained a faster substrate utilization and better degradability.

The lag phase in all mixes were below 5 hours. This is probably due to the use of fresh sludge (less than a week old) and the present of large volume of sludge in each reactor which resulted in quicker availability and enrichment of methane producing bacteria

culture. Also, the shortest time required to produce 95% of the maximum methane potential was attained in mix C. This is due to the faster production rate that was observed in mix C.

Table 2.12: *Results of modified Gompertz kinetic model (values are average ± range of duplicate set of reactors)*

SAMPLE	Lag phase, hr	Rm, ml CH4/gTCOD.d	Tmax, ml/gTCOD	t95, day	teff, days
A	3.0 ± 0.4	25.1 ± 0.2	209.1 ± 2	19.2 ± 0.2	19.1
B	3.6 ± 0.2	27.0 ± 0.1	221.0 ± 4	18.4 ± 0.3	18.3
C	4.2 ± 0.1	30.0 ± 0.3	234.1 ± 3	17.9 ± 0.2	17.7
D	3.5 ± 0.2	23.2 ± 0.1	213.2 ± 5	20.3 ± 0.1	20.2
E	4.8 ± 0.3	22.2 ± 0.2	168.0 ± 4	20.4 ± 0.2	20.2

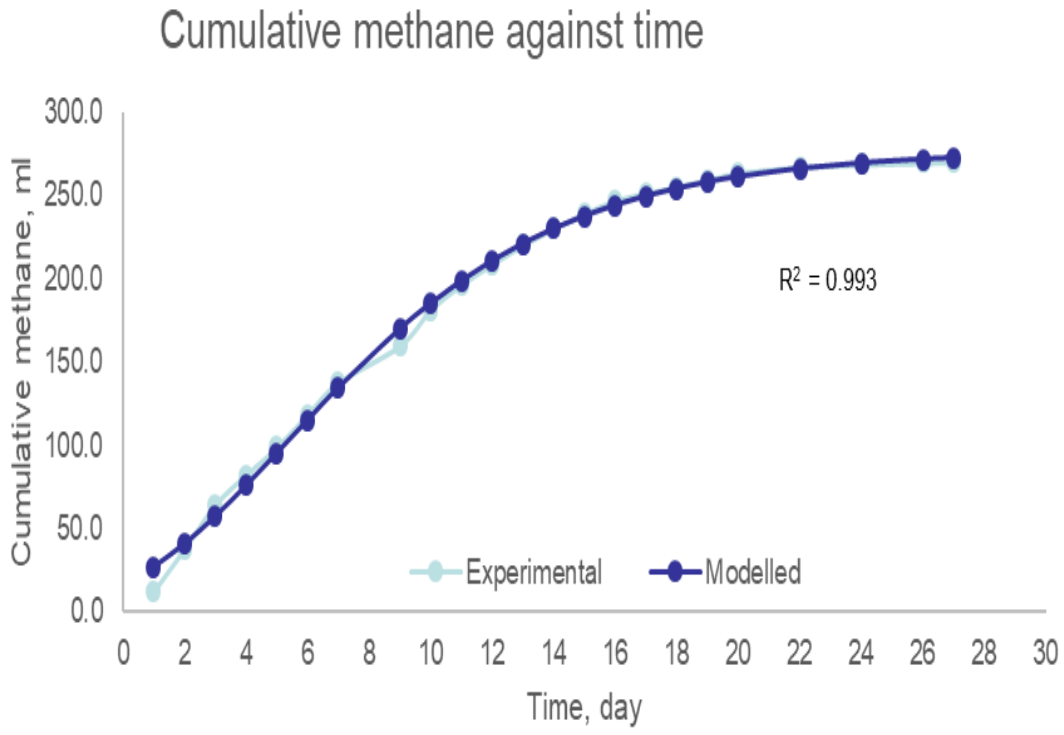

Figure 2.3: *Curve fitting for Mix A*

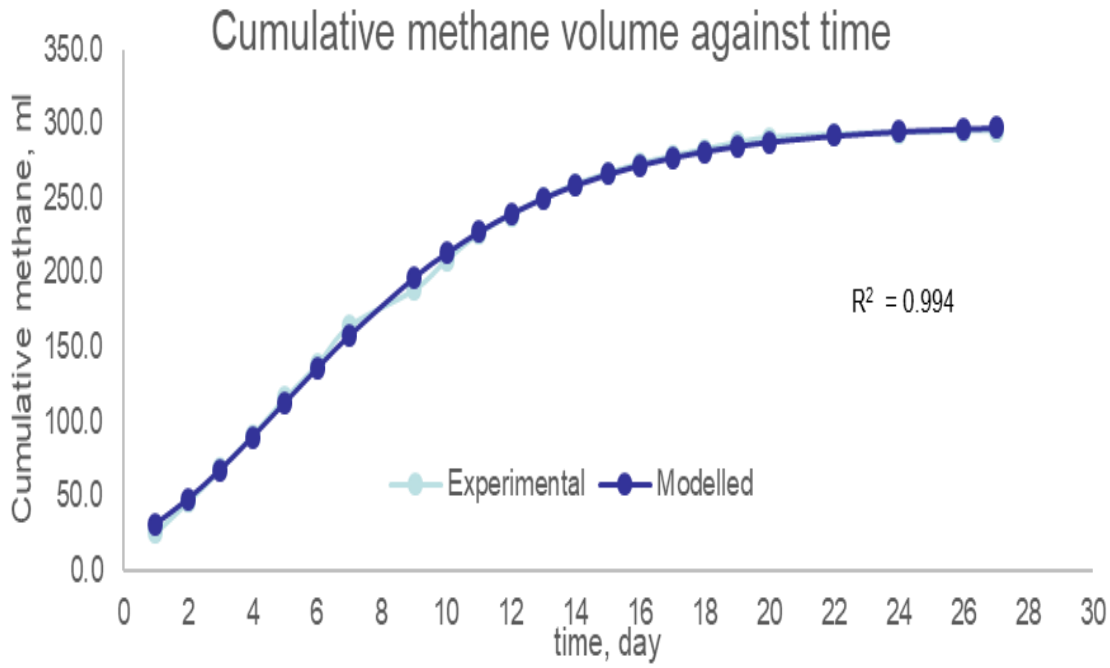

Figure 2.4: *Curve fitting for mix B*

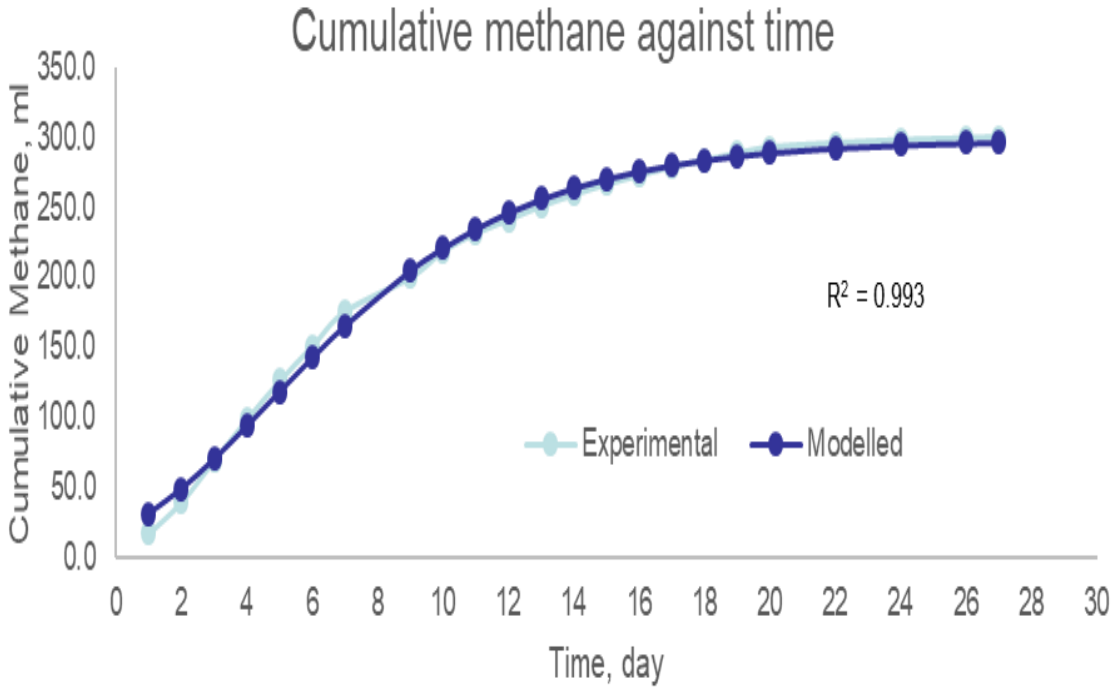

Figure 2.5: *Curve fitting for mix C*

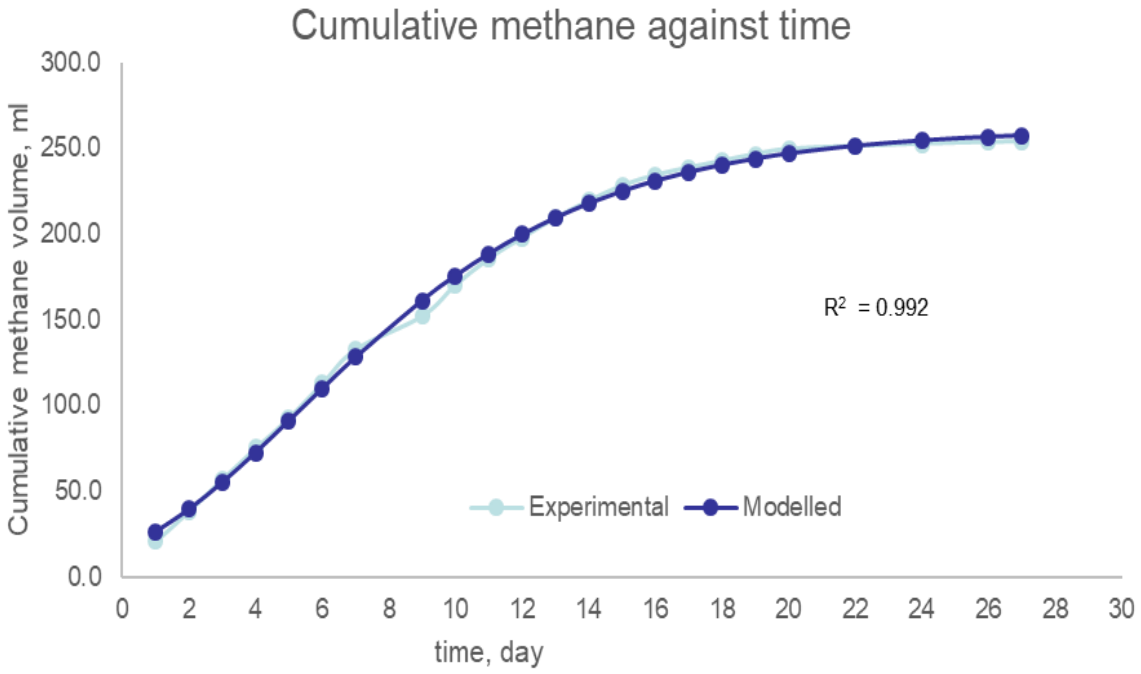

Figure 2.6: *Curve fitting for mix D*

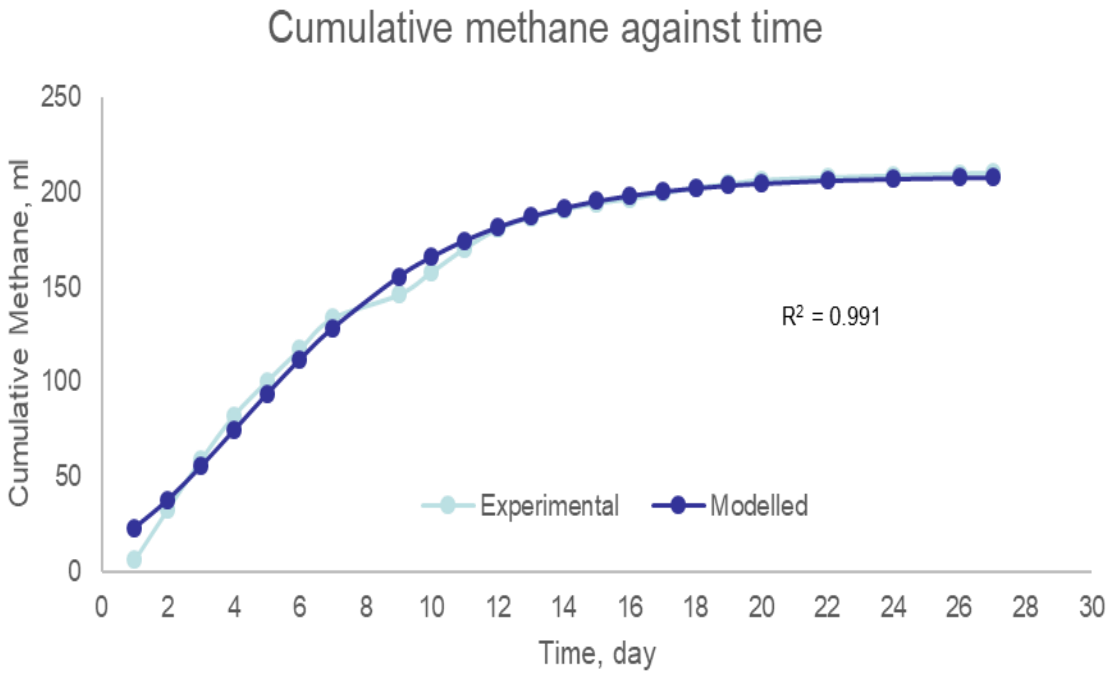

Figure 2.7: *Curve fitting for mix E*

Conclusions

The current study investigated the effects of mix ratio on the methane yield and reaction kinetics during the anaerobic co-digestion of PP and PM in a batch study. The results obtained show that:

1. The co-digestion of PP and PM synergistically improved the methane yield for all mix ratio. Enhancement of methane yield varying from 11 - 25% was observed in all the co-digested mixes. The highest increment in methane yield was observed in the 50:50 mix of PP and PM, which was 25% higher than mono-digestion of PP or PM.
2. The methane production rate was increased by co-digesting different mixes of PP and PM. Faster methane production rate was observed in the co-digested mixes, compared to the mono-substrate. The highest methane production rate was observed in the 50:50 mix of PP and PM. A decrease in the methane production rate was observed when the PM proportion exceeded 50%.
3. The t95, which shows the technical digestion time required to consume 95% of the degradable organic matter, was reduced by co-digesting PP and PM at different mix ratio. The shortest digestion time was observed in the 50:50 mix of PP and PM, which had the highest methane production rate.

Thus, co-digestion of PP and PM at 50:50 mix ratio is an optimal mix to maximize methane production rate and methane yield in this study. Co-digestion of PP and PM at 50:50 mix ensures better substrate degradability and faster rate of methane production.

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CHAPTER 3:

INFLUENCE OF DIFFERENT PRE-TREATMENTS ON ENERGY RECOVERY FROM ANAEROBIC CO-DIGESTION OF POTATO PEEL AND PIG MANURE

Introduction

AD is a widely used technique for generating energy from organic waste, with the aim of reducing the dependency on fossil fuels. However, energy recovery from the AD process is greatly influenced by the substrate characteristics. Researchers have reported that complex substrates are limited by the hydrolysis stage, due to the formation of either complex heterocyclic compounds that are slow to degrade or VFAs that are undesirable (Neves et al., 2006). PM has been reported to have a high biofiber content which is made up of lignocellulose materials and as low as 30 – 50% of PM biogas potential is utilized due to the slow and difficult degradation of lignocellulose materials (Moller et al., 2004). PP as a substrate for AD has also been reported to be limited by hydrolysis, due to its rigid structure and the enriched lignin content in the cell wall, that shields the intracellular components from microorganisms (Liang and McDonald, 2014; Lucas, 2014).

Due to the adverse effects of recalcitrant lignocellulose materials in anaerobic co-digestion, different studies have explored the possibility of introducing diverse pre-treatments to overcome the hydrolysis limitation of complex organic waste. This is achieved by improving the solubility and degradability of the lignocellulose contents in the substrate (Nizami et al., 2010; Speece et al., 1985). The study conducted by Rafique et al., (2010) showed that thermal PT of PM at 100 $^{\circ}$C for one hour improves methane yield by 28%. Similarly, thermal PT of PP at 100 $^{\circ}$C for one hour was shown to improve COD and carbohydrate solubility, with a corresponding 30% increase in methane yield

(Shahabazuddin et al, 2019). Despite the added energy cost of the thermal PT, the current regulation that requires organic waste to be sterilized, makes thermal PT a reasonable PT, due to its potential for pathogen destruction (Gray, 2004).

Another potential PT is the biological PT which involves the physical separation of the AD process into two different reactors where the acid fermentation and methanogenesis occurs in two separate reactors. This technique allows for the optimization of process parameters (such as FM ratio, pH and temperature of each stage) to further improve hydrolysis of complex substrates in the first stage and consequently, methane production in the second stage. Previous studies have also shown that degradation kinetics, pathogen destruction and gas production rates were improved under thermophilic fermentation (Shin et al., 2004; Cheong and Hansen, 2007; Sompong et al., 2011). Similarly, Parawira et al., (2007) investigated the effects of mesophilic-thermophilic, thermophilic-thermophilic and mesophilic-mesophilic temperature variation in the acid fermentation and methanogenic reactor, respectively. It was concluded that the thermophilic temperature range could treat more waste at a shorter time. However, the mesophilic temperature produced biogas with higher methane content.

Despite the potential benefits of temperature variation as a PT in a two-stage anaerobic co-digestion, limited literature exists on its effects on anaerobic co-digestion, with contradicting results. Schievano et al., (2012) investigated the energy recovery from PM and fruit and vegetable waste (FVW) in a two stage and single stage reactors, operating at thermophilic temperature. It was concluded that there was no improvement in the energy recovered from a two stage AD, as compared to a single stage reactor set up. However, this was disputed by Schievano et al., (2014), where 15-30% higher energy was recovered from

two stage AD of PM and FVW, as compared to a single stage. Similarly, Ren et al. (2014) investigated the effects of different mixes of PM and cassava dregs (CD) in a two stage AD and it was concluded that PM:CD mix of 4:6 had the highest methane yield, indicating the mix proportion of both substrates could also influence the methane recovery in an anaerobic co-digestion. However, there was no comparison with a single stage anaerobic co-digestion. Therefore, biological PT (at mesophilic or thermophilic temperature) and thermal PT at different mixing proportion of PP and PM could potentially influence the energy recovery or reaction kinetics from anaerobic co-digestion of PP and PM.

Hence, the objective of this study was to investigate the effects of three different pre-treatments, at five mix ratios on energy recovery and reaction kinetics during the anaerobic co-digestion of PP and PM. The specific objectives were;

1. To evaluate and compare the effects of different pre-treatments on methane yield and energy recovered from anaerobic co-digestion of PP and PM in a batch study at mesophilic temperature.
2. To model and compare the effects of different pre-treatments on reaction kinetics, using the modified Gompertz equation

Materials and Methods

Materials

Fresh PM was collected from a local pig farm in Windsor, Canada. Visible coarse materials were removed from the PM. The PM was blended and homogenized by using an electric blender (Model number NJ 600WMC, Ninja) for 5 minutes. Deionized water was added to improve blending. The prepared PM was stored in an airtight 20 litre gallon and kept in a cold storage room at 4°C. Prior to use, the prepared PM was placed at room temperature

overnight. Potato was obtained from a local grocery store in Windsor. The peels were manually extracted. Deionized water was added to about 1kg of the extracted peels. The extracted peel was blended and homogenized in an electric blender (Model number NJ 600WMC, Ninja) for 10 minutes. The blended PP had a TS of 115 g/l and VS of 112 g/l. The blended PP was poured in an airtight 20 litre gallon and stored in a cold storage room operating at 4°C. Prior to the preparation of mixes, the PP was kept at ambient temperature for 24 hours.

Mesophilic anaerobic digested sludge (ADS) was obtained from the Chatham-Kent municipal wastewater treatment plant (WWTP) in Chatham, Ontario, Canada. The sludge was stored in an airtight 20 litre gallon inside the cold storage room (4°C), to prevent further degradation. The sludge had a TS of 43 g/l and VS of 18 g/l. Also, the sludge was taken out of the cold storage room and kept at room temperature, 24 hours before use. Table 3.1 shows the characteristics of the PM, PP and ADS.

Table 3.1: *Characteristics of the PP, PM and ADS (values are average ± standard deviation of triplicates set of data)*

	PP	**PM**	**ADS**
VFAs, mg/l	1770±27	440±13	44±2
TCOD, g/l	102±4	196±7	25.8±0.2
SCOD, g/l	34.4±1	22.5±0.5	0.496±0.4
Ammonia Nitrogen, mg/l	275.4±6	986.1±4	561.6±3
Alkalinity, g/l as CaCO3	2.89±0.1	14.0±0.3	5.06±0.2
TS, g/l	121.8±5.8	197.9±8.6	42.7±0.5
VS, g/l	114.5±5.3	166.5±9.3	18.2±0.4
TSS, g/l	101.6±2.0	162.5±9.6	40.2±0.7
VSS, g/l	97.5±1.5	139.2±7.5	16.3±0.2
pH	6.31	6.86	7.42
VS/TS, %	94	84	41

Preparation of Mixes

Different mixes of PP and PM were prepared based on the volatile solid ratio, as shown in Table 3.2. The composition of individual mixes was; Mix A (PP:PM – 100:0), Mix B (PP:PM – 75:25), Mix C (PP:PM – 50:50), Mix D (PP:PM – 25:75) and Mix E (PP:PM – 0:100). Total mass of volatile solids of PP and PM in each mix was set to 40 gVS. The ratio of VS of PP to VS of PM for mix A, B, C, D and E were respectively 40g:0g, 30g:10g, 20g:20g, 10g:30g and 0g:40g. Distilled water was added to each mix to make up a total prepared volume of 1 liter.

Table 3.2: *Preparation of different mixes*

PP:PM	Volume of PP, ml	Mass of VS of PP, gVS	Volume of PM, ml	Mass of VS of PM, gVS	Volume of DIW, ml	Total Volume, ml
Mix A, 100:0	351	40	0	0	649	1000
Mix B, 75:25	263	30	60	10	677	1000
Mix C, 50:50	175	20	120	20	705	1000
Mix D, 25:75	88	10	180	30	733	1000
Mix E, 0:100	0	0	240	40	760	1000

Biological and Thermal Pre-treatments

The different mixes of PP and PM were used in the three pre-treatments, namely; thermophilic acid fermentation (biological), mesophilic acid fermentation (biological) and thermal pre-treatment. The thermal PT of the different mixes was carried out, using the reflux heating method at 100°C for 1 hour. To ensure uniform heating, 300 ml of each mixes was continually stirred and subjected to heating. The vapor generated during boiling was condensed and returned to the boiling mixes, to reduce loss of vapor from evaporation.

For the biological PT, the mesophilic ADS was used in thermophilic conditions ($55\pm1^{\circ}C$) for the thermophilic acid fermentation, without prior acclimatization. Similarly, the mesophilic ADS was used for mesophilic acid fermentation at $38\pm1^{\circ}C$. To inhibit methane producing bacteria in the acid fermentation stage, the ADS was thermally treated at $70^{\circ}C$ for 30 minutes (Nasr et al., 2012) and the pH of the ADS was adjusted to 5.12 by the addition of 4.0N HCl.

The biological PT was carried out in 150 ml Wheaton bottles with a working volume of 100 ml and headspace of 50 ml. The setup of mesophilic and thermophilic biological fermentation is shown in Table 3.3. Each mix was carried out in duplicate, with exception of the glucose and blank, that were done in one replicate. The blank was done to account for endogenous respiration from the bacteria culture and the glucose control was set up to ensure proper functioning of the reactors. A food to microorganisms' ratio of 0.5 g TCOD/g VSS was adopted to determine the volume of substrate to be added to each bottle, using equation 16. Distilled water was added to each prepared mix to make a total volume of 150 ml. Each digester bottle was filled with 100 ml of the prepared sample and the remaining 50 ml was kept in the refrigerator for characterization.

The pH of the prepared mixes for thermophilic and mesophilic acid fermentation were adjusted to 5.51 ± 0.12 and 5.44 ± 0.13, respectively, by the addition of 4.5N NaOH or 4.0N HCl. The ideal pH for acidogenic bacteria has been reported to be 5.50 and methane producing bacteria are also inhibited in that range (Nath and Das, 2011). There was no external nutrient or buffer addition, as it was assumed that the alkalinity and nutrient from the sludge and PM were enough.

$$FM\,\frac{(gTCOD)}{(gVSS)} = \frac{V_{substrate}\,(L) * TCOD_{substrate}(\frac{g}{L})}{V_{seed}\,(L) * VSS_{seed}\,(\frac{g}{L})} \quad (16)$$

Where $V_{substrate}$ is volume of substrate, V_{seed} is volume of ADS, $TCOD_{substrate}$ is TCOD of substrate and is VSS_{seed} volatile suspended solid of ADS

Table 3.3: *Experimental set-up of mesophilic and thermophilic acid fermentation*

	Bottle #		$V_{substrate}$, ml	V_{seed}, ml	DIW, ml	Total, ml
Mesophilic acid fermentation	1	A	26.3	120	3.7	150
	2		26.3	120	3.7	150
	3	B	27.4	120	2.6	150
	4		27.4	120	2.6	150
	5	C	28.4	120	1.6	150
	6		28.4	120	1.6	150
	7	D	26.7	120	3.3	150
	8		26.7	120	3.3	150
	9	E	21.4	120	8.6	150
	10		21.4	120	8.6	150
	11	CONTROL	0.967	120	30	150
	12	BLANK	0	120	30	150
Thermophilic acid fermentation	14	A	26.3	120	3.7	150
	15		26.3	120	3.7	150
	16	B	27.4	120	2.6	150
	17		27.4	120	2.6	150
	18	C	28.4	120	1.6	150
	19		28.4	120	1.6	150
	20	D	26.7	120	3.3	150
	21		26.7	120	3.3	150
	22	E	21.4	120	8.6	150
	23		21.4	120	8.6	150
	24	CONTROL	0.967	120	30	150
	25	BLANK	0	120	30	150

The digester bottles were covered with an air-tight plastic cap with a rubber septum. Each bottle was purged with Praxair, containing 99.9% nitrogen gas at 5 Psi, for three minutes to ensure the absence of oxygen. The digester bottles were kept in two separate enclosed mechanical shakers, both operating at 150 rpm. The temperature was set to $38 \pm 1°C$ and

55 ±1°C, for the mesophilic acid fermentation and thermophilic acid fermentation, respectively and at a retention time of 6 days. The experimental design is shown in Figure 3.1.

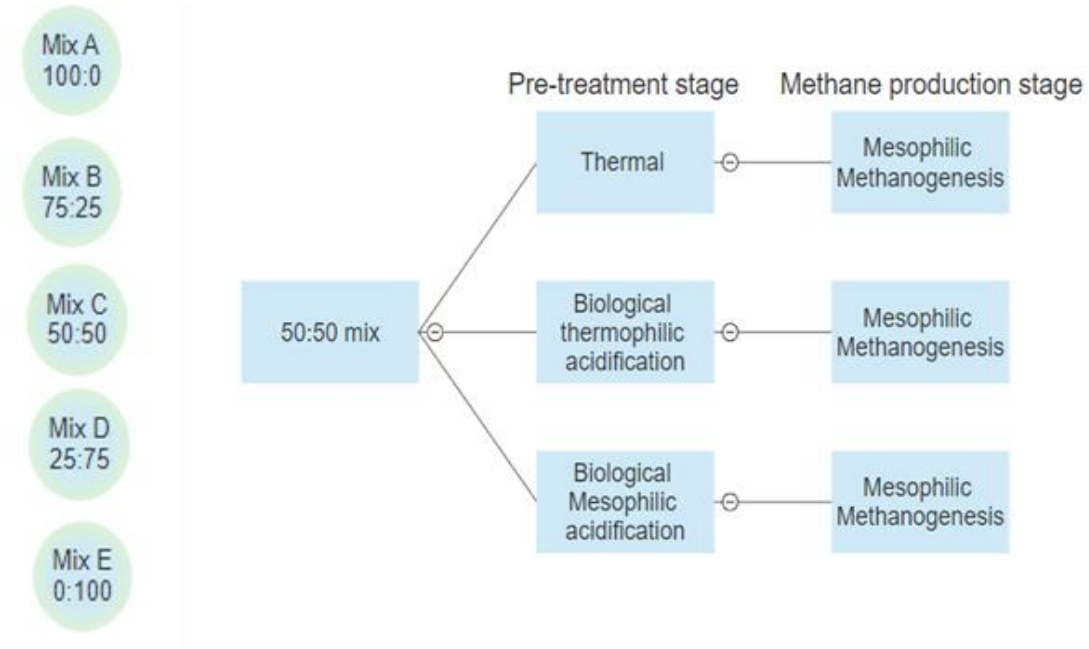

Figure 3.1: *Experimental design of pre-treated substrates*

Biochemical Methane Potential Set-up for Pre-treated Substrates

The thermally treated substrates and the effluent of the biologically treated substrates from mesophilic and thermophilic acid fermentation were used in a BMP study at mesophilic temperature (38±1 °C). The BMPs test were investigated in 100 ml digester glass bottles, with a 50 ml headspace. Each test was conducted in duplicate. The volume of substrates required to attain the FM ratio of 0.5 in the BMP test was calculated based on gTCOD/gVSS, as shown in equation 16. The set up for the BMP studies for thermo-acidified, meso-acidified and thermally treated substrates is shown in Table 3.4, Table 3.5 and Table 3.6, respectively.

Table 3.4: *BMP set-up for thermo-acidified effluents*

Bottle label	$V_{substrate}$, ml	V_{seed}, ml	DIW, ml	Total, ml	TCOD (g/L)
A14	36.8	100	13.2	150	24.8
A15	38.8	100	11.2	150	23.4
B16	38.8	100	11.2	150	23.4
B17	38.3	100	11.7	150	23.7
C18	39.3	100	10.7	150	23.1
C19	38.1	100	11.9	150	23.9
D20	39.3	100	10.7	150	23.2
D21	41.8	100	8.2	150	21.8
E22	42.6	100	7.4	150	21.4
E23	43.0	100	7.0	150	21.2
New control	0.8531	100	50.0	150	1.0667
Old control	42.9	100	7.1	150	21.2
New blank	0.0	100	50.0	150	0.0
Old blank	43.8	100	6.3	150	20.8

Table 3.5: *BMP set-up for meso-acidified effluents*

	$V_{substrate}$, ml	V_{seed}, ml	DIW, ml	Total, ml	TCOD (g/L)
A1	30.0	100.0	20.0	150.0	29.0
A2	30.1	100.0	19.9	150.0	28.9
B3	29.8	100.0	20.2	150.0	29.2
B4	29.9	100.0	20.1	150.0	29.1
C5	30.2	100.0	19.8	150.0	28.8
C6	30.1	100.0	19.9	150.0	28.9
D7	30.0	100.0	20.0	150.0	29.0
D8	30.6	100.0	19.4	150.0	28.4
E9	30.4	100.0	19.6	150.0	28.6
E10	30.5	100.0	19.5	150.0	28.5
New control	0.8156	100.0	50.0	150.0	1.0667
Old control	30.3	100.0	19.7	150.0	28.7
New blank	0.0	100.0	50.0	150.0	0.0
Old blank	34.9	100.0	15.1	150.0	24.9

Table 3.6: *BMP set-up for thermal treated Mixes*

	$V_{substrate}$, ml	V_{seed}, ml	DIW, ml	Total, ml	TCOD (g/L)
A10	14.6	120	15.4	150	74.6
A20	14.6	120	15.4	150	74.6
B30	19.1	120	10.9	150	57.3
B40	19.1	120	10.9	150	57.3
C50	20.7	120	9.3	150	52.8
C60	20.7	120	9.3	150	52.8
D70	23.7	120	6.3	150	46.0
D80	23.7	120	6.3	150	46.0
E90	20.3	120	9.7	150	53.9
E100	20.3	120	9.7	150	53.9
Control	1.024	120	30.0	150	1.0667
Blank	0.0	120	30.0	150	0.0

Distilled water was added to each prepared mix to make a total volume of 150 ml. Each digester bottles were filled with 100 ml of the prepared sample and the remaining 50 ml was kept in the refrigerator for characterization. The digester bottles were covered with an air-tight plastic cap with a rubber septum. Each bottle was purged with Praxair, containing 99.9% nitrogen gas at 5 Psi, for three minutes to ensure the absence of oxygen.

The pH adjustment was done by the addition of 4.5N NaOH or 4.0N HCl. The final pH of the digester bottles was 7.30 ± 0.15, 7.23 ± 0.11 and 7.26 ± 0.10 for the methanogenesis of the thermal treated substrate, methanogenesis of meso-acidified substrate and methanogenesis of thermo-acidified substrate, respectively. There was no external alkalinity or trace elements addition to any digester bottles. It was assumed that the nutritional requirements and alkalinity from the PM and anaerobic digested sludge were enough.

The digester bottles were kept in an enclosed mechanical shaker, operating at 38 $\pm$1°C and 150 rpm, for a retention time of 24 days and 30 days, for the methanogenesis of the acidified substrates and thermal treated substrate, respectively. Each BMP test was carried out in two replicates. However, the glucose control and the blank were done in a single replicate. The blank was carried out to account for endogenous methane production from the ADS and the glucose control was used to ensure proper operational set up. Also, the blank and glucose control from the acid fermentation stage was used in the BMP test in single replicate. This was done to account for the old ADS contribution to the mixes during the methane production stage.

Measurements and Data Analysis

The VFAs in the effluent of the meso-acidified and thermo-acidified was analyzed using gas chromatograph equipped with a flame ionization detector (SRI 8610C, SRI instruments, Torrance, CA, USA). This was done to determine the concentration of different VFAs (acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, hexanoate, iso-hexanoates and heptanoate). The samples were first centrifuged and then filtered with a 0.45 µm nylon membrane syringe filter, followed by filtration with a 0.2 µm nylon membrane syringe filter. The filtered samples were acidified with Phosphoric acid (H_3PO_4). 0.5 ml of the acidified samples was injected into the GC-FID. The initial set point of the oven temperature was 80 $^{\circ}$C (1-minute holding time), followed by a ramp of 8°C/min up to 190 $^{\circ}$C (5 minutes holding time). Helium was used as a carrier gas with a flow rate of 20ml/min.

The biogas produced in the headspace of each digester bottles was measured with a 10 to 50 ml air-tight glass syringe using the plunger displacement method (Owen et al., 1979).

The methane composition of the biogas was determined by using a gas chromatograph, equipped with a thermal conductivity detector, TCD (SRI 8610C, SRI instruments, Torrance, CA, USA). The temperature of the column oven was 60 °C. Nitrogen gas was used as a carrier gas, with a flow rate of 20 ml/min. The biogas volume in the headspace were measured every 12 hours for the first day, every day from the second day to the twentieth day and every other day from the twentieth day to the twenty fourth day and thirtieth day for the methanogenesis of biological pre-treated and thermal treated substrates, respectively. This was done to avoid unnecessary gas pressure build-up in the headspace, that might result in a leakage. The BMP test was assumed to be complete when the methane production was less than 1% of the cumulative methane (Elbeshbishy et al., 2012).

The influent and effluent of the BMP studies were characterized. Solids tests were analyzed in accordance to the Standard methods (APHA, 1995). SCOD, TCOD, $NH^{4+}N$ and VFAS were measured by using the Hach methods (Hafez et al., 2010). Alkalinity of each sample was determined by titration method, using 1N sulphuric acid, H_2SO_4. pH of every sample was measured with the Hach pH meter (Benchtop meter, Hach company, Loveland Company, USA).

Calculations

The cumulative methane volumes in the produced biogas at each time interval was calculated with equation (17).

$$V_{CH4,i} = V_{CH4,i-1} + C_{CH4,i} * V_{T,i} + V_{H,i}(C_{CH4,i} - C_{CH4,i-1}) \quad (17)$$

Where $V_{CH4,i}$ and $V_{CH4,i-1}$ are current cumulative methane volume and preceding cumulative gas volume, respectively, $C_{CH4,i}$ and $C_{CH4,i-1}$ are current fraction of methane in the headspace and preceding fraction of methane in the headspace, $V_{T,i}$ and $V_{H,i}$ are total gas volumes accumulated between the preceding and present interval, and the total volume of the reactor headspace in the present interval (Gomez-Flores et al., 2015).

The experimental methane yield (EMY) in each mix was calculated with equation (18).

$$EMY = \frac{CM_{mix} - \mathrm{CM}_{blank}\,(ml)}{gTCOD_{influent} - gTCOD_{blank}\ \ (Net\ gTCOD_{added})} \qquad (18)$$

Where CM_{blank} is the cumulative methane produced in the blank, CM_{mix} is the cumulative methane produced in each mix of PP:PM, $gTCOD_{influent}$ is the mass of TCOD in the influent and $gTCOD_{blank}$ is the mass of TCOD in the blank.

The experimental methane yield, EMY, was converted to energy recovered (ER) per unit mass of TCOD added to each mix, as shown in equation (19). The energy recovery was calculated based on the lower heating value of methane, LHV (35.16 MJ/Sm^3), as suggested by Schievano et al., (2012) and Schievano et al., (2014).

$$ER\left(\frac{kJ}{TCOD_{added}}\right) = EMY\ (\mathrm{ml/g}TCOD_{added} * \frac{1Sm^3}{1000000ml} * \frac{35.16\frac{\mathrm{MJ}}{Sm^3} * 1000kJ}{1MJ} \qquad (19)$$

Kinetic Modelling

Different models are used for fitting experimental data to predict the kinetic performance of AD process. A common method is the Modified Gompertz equation which has been reported to predict kinetic parameters, with goodness of fit ranging from 0.910 to 1.00 in AD of different waste (Donoso-Bravo et al., 2010; Nielfa et al., 2015). The modified Gompertz equation described by Lay et al., (1999) was utilized to estimate the lag phase,

cumulative methane production and methane production rate in different mixes. These parameters were estimated by using the sigmoid-type modified Gompertz function on sigmaplot, version 12 and Microsoft Excel 2016. The sum of squared residual between the experimental data and theoretical data was minimized by a fit algorithm in the sigmaplot. The modified Gompertz equation is shown in equation (20)

$$T(t) = T_{max}\ exp\left\{-exp\left[\left[\frac{R_m.e}{T_{max}}(\lambda - t) + 1\right]\right]\right. \quad (20)$$

Where $T(t)\ is$ the cumulative methane production at time in mL, (t), T_{max} is maximum methane production in mL, e is the value of exponential 1 (2.718281828), R_m is methane production rate in mL/d, t is time in days and λ is the lag phase in days. These values were normalized by dividing by $gTCOD_{added,}$ after accounting for the contribution of ADS. Hence, the new unit of T_{max} and R_m is $ml/\mathrm{gTCOD_{added}}$ and $\mathrm{mL/gTCOD_{added}.d}$, respectively.

An additional kinetic parameter, t95, which is the estimated time required to attain 95% of the total cumulative methane production from the BMP test was calculated using equation (21) (Lavagnolo et al., 2018).

$$t_{95} = \frac{T_{max}}{R_m}\{1 - \ln(-0.95)\} + \lambda \quad (21)$$

Results and Discussions

Effects of Different Pretreatments

Different mixes of PP and PM were subjected to thermal and biological PT at mesophilic and thermophilic temperature. The characteristics of the influent and effluent at the end of the biological treatment is shown in Table 3.19, Appendix B. The results show that the

biological PT increased the VFAs and SCOD of the mixes, after the 6 days acid fermentation. For the meso-acidified substrates, the VFAs of different mixes increased by varying amount, as shown in Table 3.7. As the proportion of PP in the mix increased from 0 to 100%, a 2 to 12-fold increment in VFAs was observed in the different mixes. Interestingly, the highest increment occurred in mix B. Similar increment were observed in the study conducted by Ren et al., (2014), where 4:6 mix of PM and CD had the highest VFAS increment (9 fold) after 4 days acidification. In this study, a longer duration of 6 days in the acidification reactor could have contributed to the higher VFAs increment. Also, there was a reduction in the SCOD as the PM proportion increased in the co-substrate. The highest SCOD increment was observed in mix A. This indicates the PP was more easily solubilized during the acid fermentation, as compared to the PM. Similarly, Ren et al., (2014) attained the highest SCOD increment of 2.9-fold at 100% CD.

Table 3.7: *Fold increment of VFAs and SCOD in meso-acidified substrates (values are average ± range for duplicate reactors)*

SAMPLE (PP:PM)	SCOD	VFAs
A (100:0)	3.18±0.03	9.62±0.3
B (75:25)	2.35±0.05	12.04±0.1
C (50:50)	1.93±0.04	7.01±0.3
D (25:75)	1.66±0.02	4.86±0.5
E (0:100)	1.42±0.01	2.50±0.2

The results of the VFAs distribution in all mixes is shown in Figure 3.2. In the meso-acidified PT, the dominant VFAs in the effluent were acetate and butyrate, representing

77%±2, 85%±1, 76%±2, 67%±1 and 39%±3 of the TVFAs for mix A, B, C, D and E, respectively. The raw data for the VFAs distribution in each mix is presented in Table 3.21, Appendix B.

Despite the acidification of the substrates, the pH was relatively stable. The initial pH of all mixes ranged from 5.4 to 5.6, shown in Table 3.19, Appendix B. At the end of the fermentation, the average effluent pH for all mixes had slightly reduced to 5.2 ± 0.1. This indicates that the buffering capacity in the mixes were enough to stabilize the pH, despite the production of VFAs. A contrasting result was observed in the study conducted by Ren et al., (2014), where a sharp pH drop was observed in different mixes of PM and CD, from an initial pH 7.08-7.35 to below pH 5. In this study, the use of ADS with high alkalinity contributed to the stability in pH.

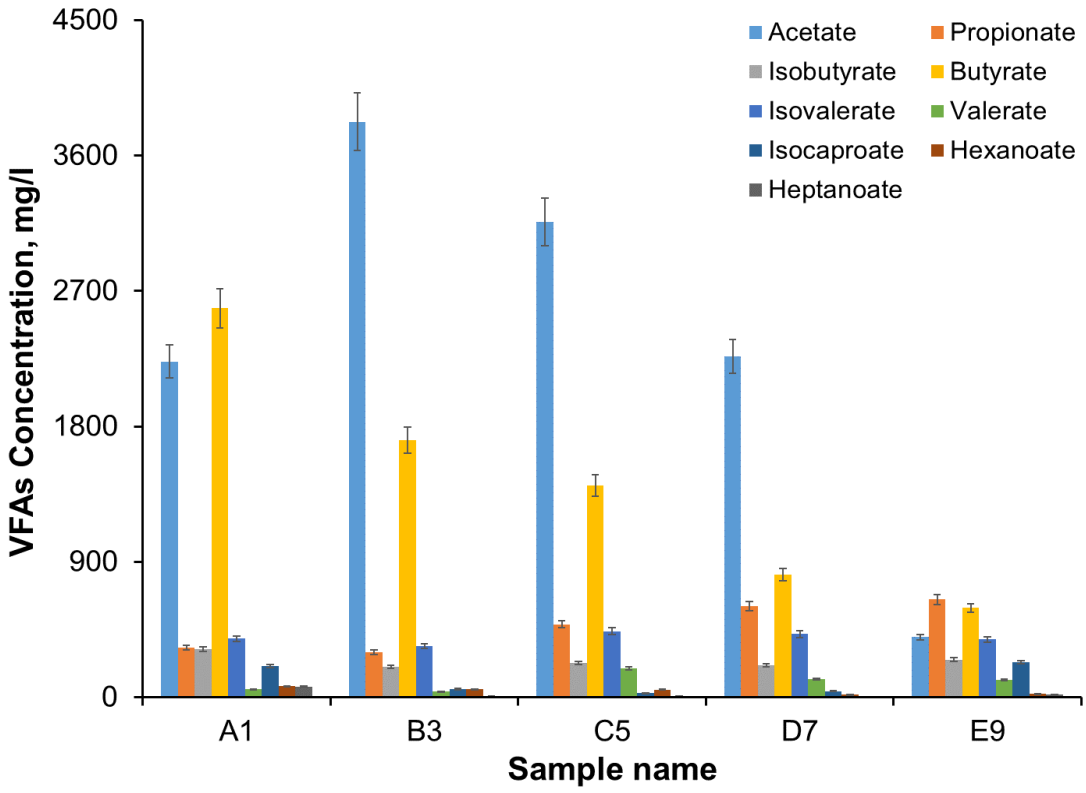

Figure 3.2: *VFAs distribution of meso-acidified effluent*

In the thermo-acidified effluents, SCOD and VFAs were also increased, as shown in Table 3.8. The increment showed a similar trend to the meso-acidified effluents. However, the increment was to a lesser extent to the meso-acidified effluents. This is in contrast with literature where thermophilic temperature has been reported to have a higher degree of organic matter degradation, as compared to mesophilic temperature (Kim et al., 2002; Ahn and Forster, 2002). The contrasting result is due to the use of unacclimated mesophilic sludge as a thermophilic sludge, indicating the 6 days acid fermentation was not enough for a microbial culture shift from mesophilic to thermophilic culture.

The maximum SCOD increment was observed at mix A. However, the SCOD reduced as the PM proportion increased in the mix. This shows the PP was more easily solubilized, as compared to the PM. Also, the VFAs increased as the proportion of PM increased in the mix, up to 50% PM. Above 50%, there was a decrease in the VFAs. The maximum VFAs increment occurred at mix C. This indicates the thermophilic temperature was able to improve the acidification of PM at mix C, as opposed to mesophilic temperature where mix B produced the highest VFAs.

Similar to the meso-acidified substrates, the acetate and butyrate were the dominant VFAs in the mixes, accounting for 87% $\pm$ 4, 96% $\pm$ 1, 94%$\pm$2, 71% $\pm$ 1 and 68% $\pm$ 3 of the TVFAs in mixes A, B, C, D and E, respectively. Figure 3.3 shows the VFAs distribution of the effluent of the thermophilic biological PT. The raw data for the VFAs distribution in each mix is presented in Table 3.20, Appendix B.

Table 3.8: *Fold increment of VFAs and SCOD in thermo-acidified Substrate (values are average ± range for duplicate sets of reactors)*

SAMPLE	SCOD	VFAs
A (100:0)	2.94±0.01	4.53±0.2
B (75:25)	2.04±0.02	5.05±0.2
C (50:50)	1.53±0.04	7.34±0.1
D (25:75)	1.31±0.02	1.63±0.1
E (0:100)	1.23±0.01	1.44±0.2

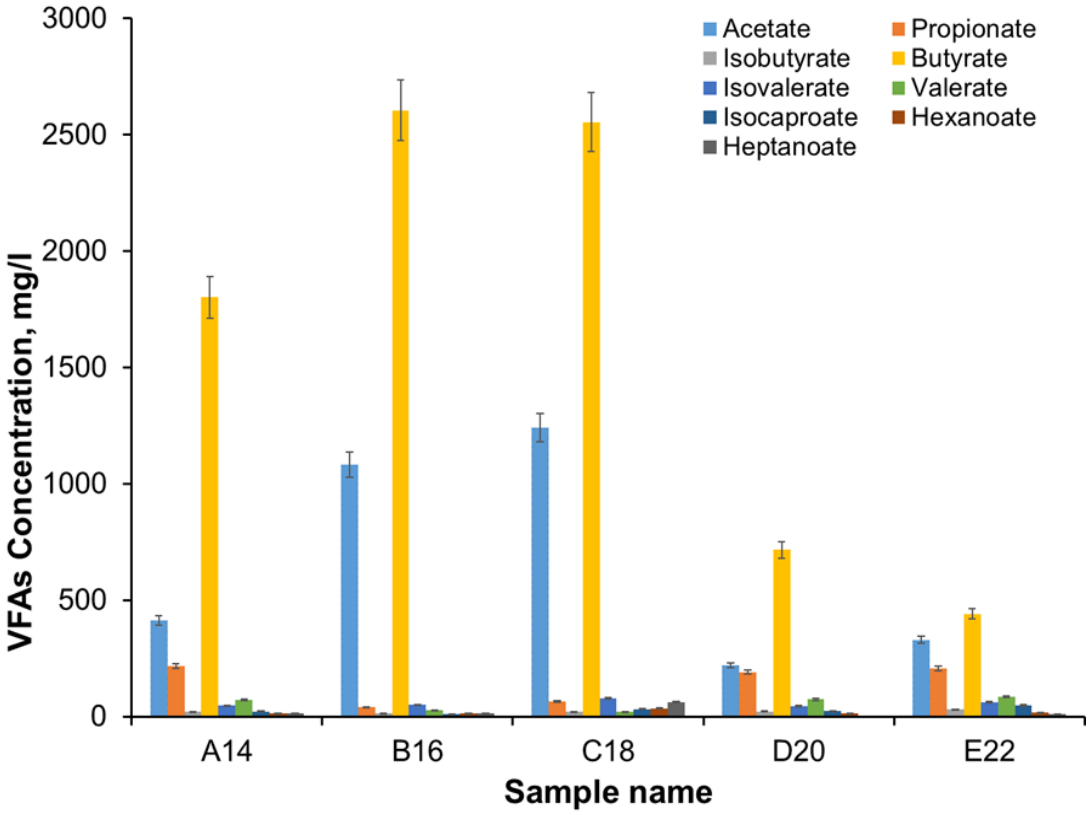

Figure 3.3: *VFAs distribution of thermo-acidified effluent*

The thermal PT of PP and PM resulted in the improvement in the SCOD of different mixes of PP and PM, as shown in Table 3.9. The thermal PT resulted in more than 2-fold increment in the SCOD of all mixes. The highest improvement was attained at mix C. This indicates that thermal PT of different mixes at 100° C for 1 hour was able to improve

solubility of the complex organic materials present in PP and PM. This is probably due to the disruption of the strong chemical bonds in the lignin-rich cell wall of PP and breakdown of complex organic materials in the PM. Thermal PT also has the added benefit of pathogen destruction (Skiadas et al., 2005). This agrees with previous literature where thermal PT has been shown to improve protein solubility and particulate carbohydrate removal (Neyens and Baeyens, 2003).

Table 3.9: *Fold increment of thermally treated Substrates (values are average ± range for duplicate sets of reactors)*

SAMPLE (PP:PM)	SCOD
A (100:0)	2.29±0.01
B (75:25)	2.55±0.03
C (50:50)	2.78±0.03
D (25:75)	2.47±0.04
E (0:100)	2.62±0.01

Overall, in this study, the biological PT resulted in an increase in VFAs and SCOD, while thermal PT resulted in SCOD improvement. The acidified and solubilized effluents from the biological PT and thermal PT has a potential to be more beneficial in the methanogenesis stage. Despite the low hydrogen gas production that was observed during the acid fermentation, the reactors were not optimized for hydrogen gas production as the purpose of the biological fermentation was to act as a PT to improve the fermentation process and consequently improve the methane production from the methanogenesis of the acidified substrate. A similar approach was adopted in the study by Voelklein et al., (2016) and Grimberg et al., (2015) where the fermentation stage was utilized as a PT for food

waste and hydrogen gas production was not optimized. Moreover, previous studies have also shown that the energy recovered from hydrogen gas in a two stage AD is less than 6% (Zhu et al., 2008; Luo et al., 2011).

Characteristics of Influent and Effluent of BMP Study

The characteristics of the influent and effluent of the BMP study for thermally treated, thermo-acidified and meso-acidified substrates are summarized in Table 3.10, Table 3.11 and Table 3.12, respectively. The results of the characterization of the influents to the BMP study from the thermal, meso-acidified and thermo-acidified treated substrates showed that the pH in all the mixes were within 7.30±0.25 range, which is favorable for methane production stage. The ammonia nitrogen and alkalinity increased as PM proportion increased in the mix. Part of the ammonia nitrogen and alkalinity were from the sludge. The ammonia nitrogen and alkalinity serve as an important nutrient source for bacteria growth and a buffering medium for stabilizing pH, respectively.

The characteristics of the effluent from each BMP study shows a reduction in the TCOD and VS. This is indicative of the organic matter consumption by the microbial culture. The pH of all the digested mixes was close to the initial value. This shows that the alkalinity present in the sludge and PM was enough to stabilize the pH in all mixes. The ammonia nitrogen concentration increased in all mixes, which confirms protein degradation. Also, the ammonia nitrogen level in all mixes were below the concentration that has been reported to be inhibitory in previous literature. This shows that there was no ammonia toxicity in this study. The low value of SCOD and VFAs in the effluent further confirms the absence of inhibition and the completion of the digestion, as the soluble organic matter and organic acids were consumed.

Table 3.10: *Characteristics of influent and effluent of thermal-treated substrates in BMP study (values are average ± range for duplicate sets of reactors)*

	Influent to methanogenesis, thermal PT					Effluent from methanogenesis, thermal PT				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
TCOD, g/l	30.71±1.4	30.31±1.1	30.62±0.7	30.40±1.2	30.43±0.6	22.45±1.0	21.85±1.2	21.84±0.5	22.71±1.1	23.01±0.4
SCOD, g/l	3.81±0.2	2.59±0.1	2.24±0.3	1.93±0.1	1.54±0.2	0.39±0.1	0.42±0.1	0.52±0.2	0.57±0.1	0.55±0.1
pH	7.46	7.34	7.45	7.47	7.50	7.36	7.23	7.37	7.41	7.43
NH_3 Nitrogen, mg/l	445±6	479±8	515±4	550±8	583±6	764±6	804±4	860±5	893±7	1068±14
TA (g/l as $CaCO_3$)	5.2±0.2	5.7±0.2	5.9±0.3	6.3±0.2	6.6±0.1	4.8±0.1	5.0±0.2	5.1±0.2	5.3±0.3	5.7±0.2
VFAS (mg/l)	457.5±5	586.5±7	687.5±5	351.5±4	244.5±6	55.2±6	55.0±2	59.3±3	79.8±4	61.1±5
VS, g/l	20.21±0.8	22.50±1.1	21.08±1.5	22.64±1.0	23.14±1.1	15.30±1.3	15.53±1.3	14.99±1.2	15.87±0.8	16.89±1.2
TS, g/l	46.22±0.6	48.65±1.7	47.18±1.1	48.19±0.8	46.59±1.3	41.99±1.2	42.04±0.6	42.79±0.9	43.53±0.4	42.64±1.0

Table 3.11: *Characteristics of influent and effluent of thermo-acidified substrates in BMP study (values are average ± range for duplicate sets of reactors)*

	Influent to methanogenesis, thermo-acidified					Effluent from methanogenesis, thermo-acidified				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
TCOD, g/l	21.93±1.2	21.96±0.4	21.93±0.3	21.76±0.2	22.13±0.7	16.46±1.0	16.37±0.8	16.00±0.4	16.36±1.1	16.53±0.8
SCOD, g/l	1.20±0.2	0.94±0.3	0.71±0.1	0.73±0.1	0.71±0.2	0.35±0.1	0.35±0.2	0.33±0.2	0.37±0.1	0.33±0.1
pH	7.17	7.11	7.14	7.13	7.09	7.02	7.04	7.03	7.06	7.02
NH_3 Nitrogen, mg/l	484.2±6	513.0±8	536.4±5	558.0±6	581.4±3	619.2±8	640.8±7	668.7±9	792.1±11	891.8±13
TA (g/l as $CaCO_3$)	5.4±0.1	5.8±0.2	6.1±0.1	6.4±0.2	6.7±0.3	4.8±0.2	5.1±0.2	5.2±0.3	5.4±0.1	5.6±0.2
VFAS (mg/l)	415.0±7	263.5±9	185.0±5	203.5±	227.0±9	39.4±5	38.0±8	37.5±7	37.2±4	35.0±8
VS, g/l	17.50±1.2	18.78±1.1	18.82±0.7	17.86±1.1	17.27±0.6	11.48±1.3	11.42±0.7	11.22±1.3	11.50±0.7	12.18±1.0
TS, g/l	33.38±0.8	33.53±1.4	34.05±1.2	33.82±0.2	35.74±0.7	31.56±1.1	31.50±0.9	31.51±1.1	31.33±0.9	33.49±1.2

Table 3.12: *Characteristics of influent and effluent of meso-acidified substrates in BMP study (values are average ± range for duplicate sets of reactors)*

	Influent to methanogenesis, meso-acidified					Effluent from methanogenesis, meso-acidified				
	A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
TCOD, g/l	28.64±0.7	28.76±0.8	28.73±0.5	28.61±0.6	28.30±0.7	19.95±1.0	19.69±0.2	20.13±0.6	20.30±0.6	20.37±0.4
SCOD, g/l	1.35±0.2	1.21±0.3	1.08±0.1	0.90±0.2	0.87±0.1	0.43±0.2	0.41±0.1	0.43±0.2	0.43±0.1	0.46±0.2
pH	7.33	7.27	7.28	7.32	7.35	7.10	7.21	7.19	7.15	7.18
NH_3 Nitrogen, mg/l	489.6±7	519.3±5	546.3±6	569.7±4	589.5±5	590.4±4	626.4±3	657.0±8	732.6±7	951.5±6
TA (g/l as $CaCO_3$)	5.4±0.1	5.8±0.2	6.1±0.1	6.4±0.2	6.7±0.3	4.7±0.2	5.0±0.1	5.2±0.2	5.3±0.4	5.5±0.2
VFAS (mg/l)	334.5±8	442.5±10	140.5±7	94.8±6	104.0±6	63.3±4	64.0±3	65.8±5	63.4±3	61.1±2
VS, g/l	18.30±0.5	18.41±1.0	18.53±1.2	18.17±1.1	17.76±0.7	11.40±0.4	11.15±1.0	11.41±1.2	11.66±0.6	11.91±0.8
TS, g/l	35.33±0.9	34.91±0.6	35.78±1.0	35.25±0.7	35.37±0.5	32.25±0.8	32.27±1.2	33.41±1.0	33.34±0.5	33.62±0.8

Methane Yield and Energy Recovered

The biologically treated and thermally treated substrates were used as feedstock in a BMP study. The BMP study were conducted at mesophilic temperature at a constant FM ratio of 0.5. The contribution of the ADS to methane yield were accounted for in all mixes. Results of the methane yield in $ml/gTCOD_{added}$ and $ml/gTCOD_{removed}$ from different mixes of pre-treated PP and PM is shown in Table 3.13 and Table 3.14. For better comparison, the result of the methane yield from untreated mixes (from previous study in chapter 2) is also included in Table 3.13 and Table 3.14.

The result shows that all the pre-treated mixes produced higher methane yield than the untreated, with the highest experimental methane yield observed at mix C, in the methanogenesis of the thermally treated and thermo-acidified substrate. Mix B had the highest yield for the meso-acidified substrate. The higher methane yield observed in the pre-treated substrates shows that the separation of the acidification and thermal pre-treatment of the substrate was able to improve the methane yield in the methane production stage. Also, the similarity in the highest methane yield observed in the two different treatments that involves application of higher heat shows that the thermal PT or thermophilic acidification were both suitable for improving the biodegradability of the PP and PM either at low temperature (55˚C) for 6 days or higher temperature (100˚C) for 1 hour.

Table 3.13: *Results for methane yield from pre-treated mixes of PP and PM (values are average ± range for duplicate sets of reactors in ml/gTCOD$_{added}$)*

SAMPLE NAME (PP:PM)	Thermal treated ($ml/gTCOD_{added}$)	Thermo-acidified ($ml/gTCOD_{added}$)	Meso-acidified substrate ($ml/gTCOD_{added}$)	Untreated ($ml/gTCOD_{added}$)
A (100:0)	236±3	226±4	220±3	202 ± 2
B (75:25)	274±6	250±3	255±5	217 ± 1
C (50:50)	285±3	283±5	246±4	231 ± 3
D (25:75)	242±4	235±4	228±2	206 ± 1
E (0:100)	197±4	198±3	183±3	164 ± 2

Table 3.14: *Results for methane yield in from pre-treated mixes of PP and PM (values are average ± range for duplicate sets of reactors ml/gTCOD$_{removed}$)*

Sample Label (PP:PM)	Thermal treated ($ml/gTCOD_{removed}$)	Thermo-acidified ($ml/gTCOD_{removed}$)	Meso-acidified ($ml/gTCOD_{removed}$)	Untreated ($ml/gTCOD_{removed}$)
A (100:0)	316 ± 5	333±5	305±1	316±3
B (75:25)	327 ± 4	359±7	326±5	325±2
C (50:50)	333 ± 2	361±2	346±3	349±2
D (25:75)	351 ± 3	330±5	336±5	315±1
E (0:100)	310 ± 6	327±6	308±3	307±2

The BMP study showed that the thermal PT, thermo-acidified PT and meso-acidified PT resulted in a 23%, 23% and 10% improvement in methane yield, respectively, as compared to the mix ratio with highest yield in the single stage co-digestion of PP and PM (Chapter 2). This agrees with literature where 11 to 23% higher methane yield have been reported to be recovered from separation of acid fermentation and methanogenesis (Voelklein et al, 2016; Luo et al., 2011; Grimberg et al, 2015). Also, 28% higher methane yield was obtained when dewatered PM was subjected to thermal PT at 100^0C for a duration of 1 hour and 30% higher methane yield was obtained from thermal PT of PP (Rafique et al., 2010; Shahabazuddin et al, 2019).

The experimental methane yield observed in all mixes were converted to energy recovered, by using the lower heating value of methane. The results of the energy recovered is shown in Table 3.15. The highest energy recovered from the three different PT were observed in the thermal treated substrates and thermo-acidified substrates. In both treatments, mix C had the highest energy recovered. However, the maximum energy recovered in the meso-acidified substrate was observed mix B. This follows a similar trend to the experimental methane yield observed in the PT.

The energy recovered in the thermal treated, thermo-acidified and meso acidified substrates were 23%, 23% and 10% higher than the highest energy recovered from a single stage AD, respectively, in the previous study (Chapter 2). Despite the potential benefits of hydrogen gas recovery from the acidification stage, previous studies have shown that hydrogen gas contribution to energy recovered in a two stage AD is lower than 6% of the total energy recovered (Zhu et al., 2008; Luo et al., 2011). This indicates the higher energy recovered from the separation of fermentation stage and methanogenesis stage, compared to a single stage is due to the efficient methanogenesis of fermented effluent from the acidification. However, the thermal treatment was able to achieve similar results, in terms of methane yield and energy recovered, without the separation of the fermentation and methanogenesis stage. This shows that low thermal treatment at 100°C for 1 hour was able to improve the methane yield and energy recovered from PP and PM mix in anaerobic co-digestion.

Table 3.15: *Energy recovered from different pre-treatments (values are average ± range of duplicate set of reactors)*

Sample Label (PP:PM)	Thermal treated (kJ/gTCOD added)	Thermo-acidified (kJ/gTCOD added)	Meso-acidified (kJ/gTCOD added)	Untreated (kJ/gTCOD added)
A (100:0)	8.30±0.05	7.93±0.04	7.72±0.06	7.09 ± 0.07
B (75:25)	9.61±0.03	8.78±0.08	8.84±0.03	7.61 ± 0.07
C (50:50)	10.00±0.02	9.94±0.11	8.64±0.06	8.12 ± 0.05
D (25:75)	8.51±0.05	8.27±0.05	8.02±0.10	7.23 ± 0.06
E (0:100)	6.93±0.10	6.95±0.03	6.44±0.01	5.75 ± 0.04

Kinetic Modelling

The results of the experimental cumulative methane production for the methanogenesis stage of the thermal treated, meso-acidified and thermo-acidified substrates were fitted into the modified Gompertz equation as shown in Figure 3.4 to Figure 3.8. The results of the kinetic parameters were normalized to mass of TCOD added, after correcting for the contribution of the ADS from the acid fermentation stage and the ADS in the methanogenesis stage. The results of the kinetic parameter for the thermal treated, thermo-acidified and meso-acidified substrates are shown in Table 3.16, Table 3.17 and Table 3.18, respectively.

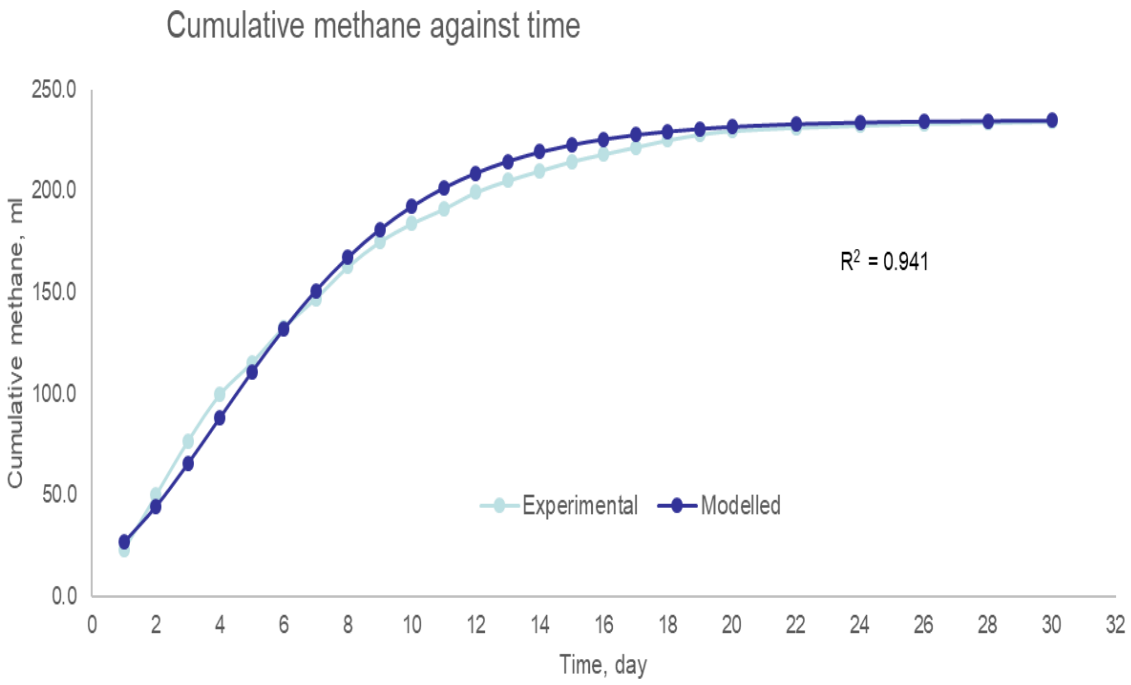

Figure 3.4: *Curve fitting for mix A*

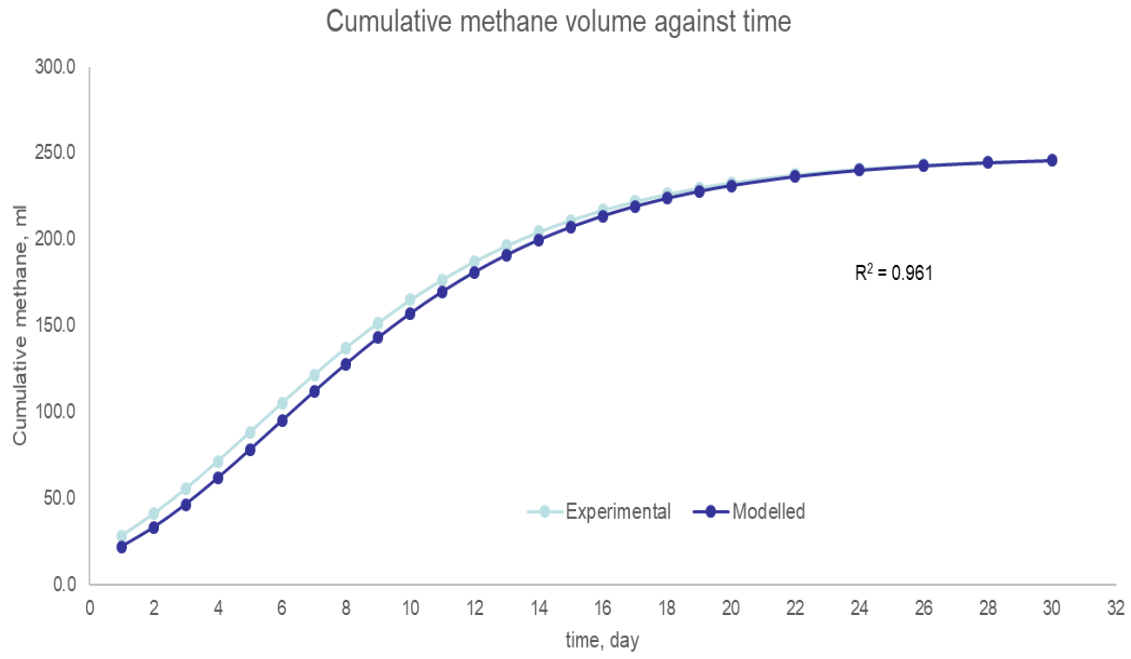

Figure 3.5: *Curve fitting for mix B*

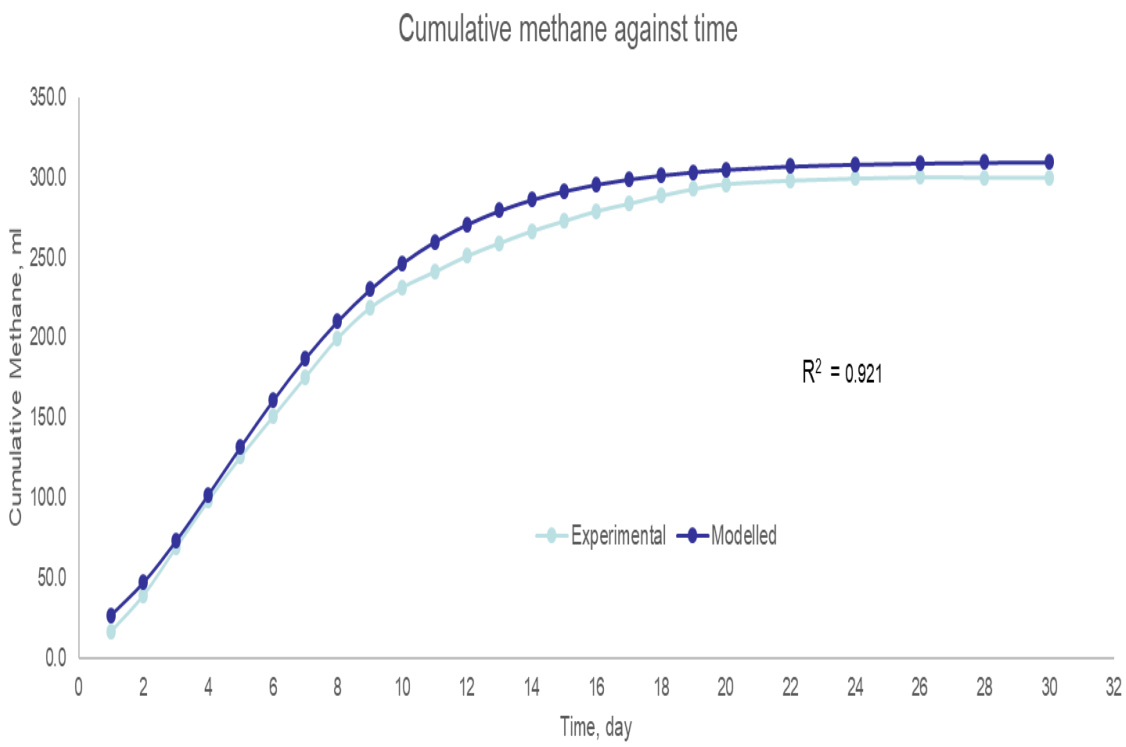

Figure 3.6: *Curve fitting for mix C*

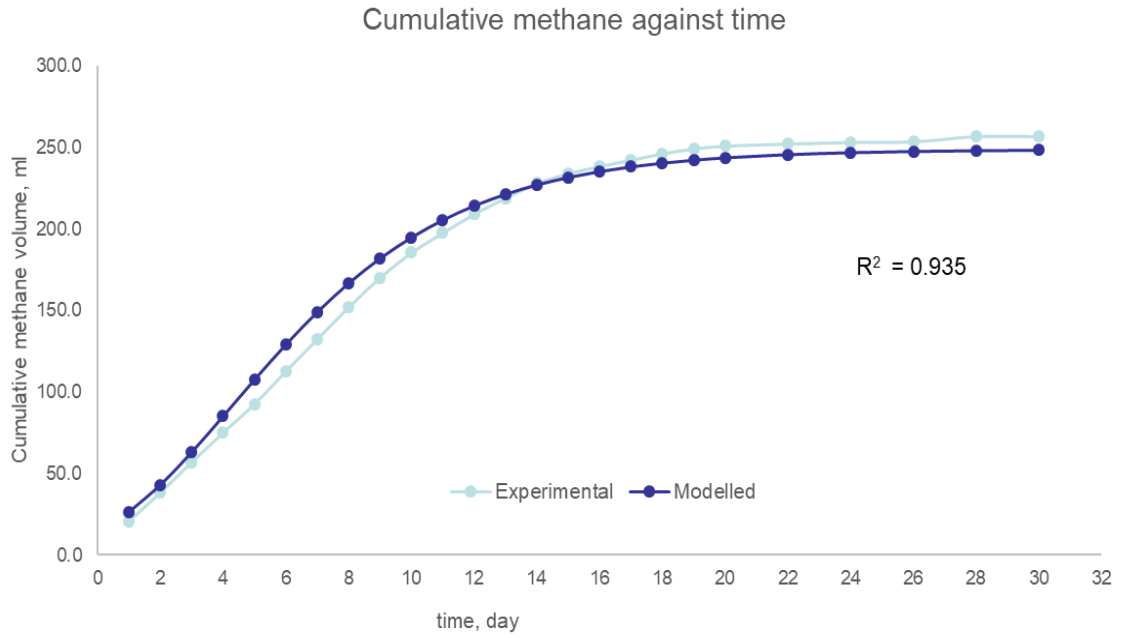

Figure 3.7: *Curve fitting for mix D*

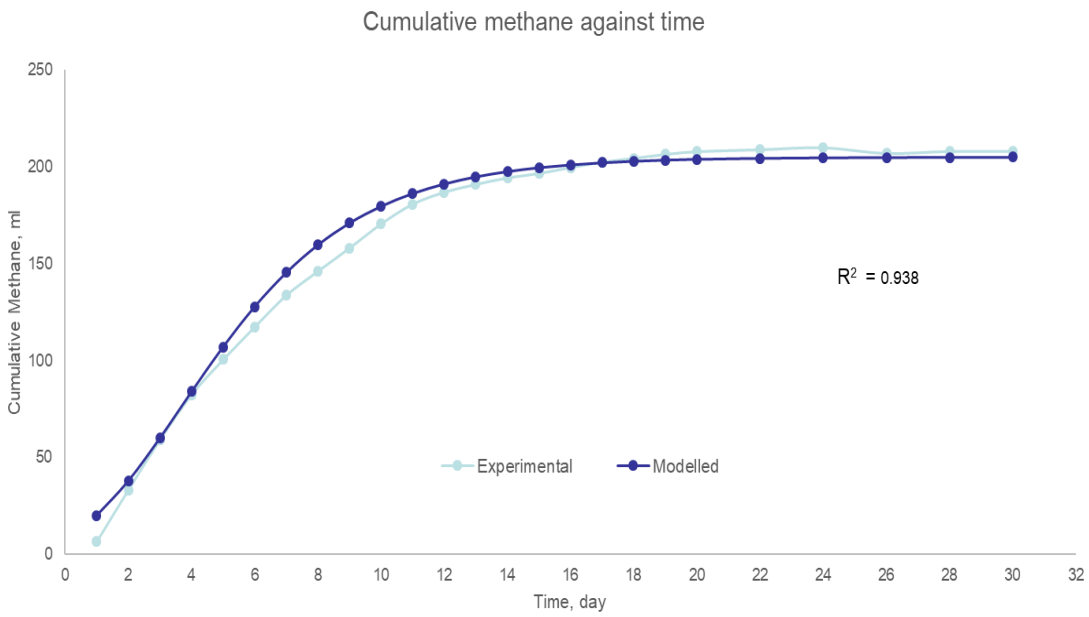

Figure 3.8: *Curve fitting for mix E*

The results showed that methanogenesis of the biologically treated substrates showed a higher methane production rate, as compared to the thermal treated substrates. The highest methane production rate was observed in mix C and mix B, for the methanogenesis of the thermo-acidified and meso-acidified substrates, respectively. The slightly lower methane production rate observed in the thermo-acidified substrates was due to the availability of lesser amount of VFAs and SCOD in the pre-treated mixes. This was due to the use of unacclimated mesophilic culture in a thermophilic temperature, during the acid fermentation. However, the organic matter that were not solubilized or acidified during the thermophilic acid fermentation stage, were able to undergo further degradation in the methanogenesis stage.

On the other hand, the thermal treated substrates showed the lowest methane production rate among all the PT, but the highest cumulative methane production per $gTCOD_{added}$ was

observed in this treatment due to a longer production period. The slower production rate compared to the biological treated substrate can be explained by the availability of high VFAs concentration in the influent to the methanogenesis of the biological treated substrates, as opposed to the availability of solubilized carbohydrate, protein and other organic matter in the influent to the thermal treated. The solubilized organic matter in the thermal treated substrates undergoes further acidogenesis stage prior to methane production while the VFAs in biological treated substrates are readily available for immediate methane production. This contributed to the shorter lag phase in the biologically treated substrates, as compared to the thermal treated substrates.

The results of the t95 shows that the time required for 95% of the degradable materials to be consumed by the microbial community in the methanogenesis stage of the biological pre-treated substrates was shorter, as compared to the methanogenesis of the thermal treated substrates. However, if the initial 6 days acid fermentation in the biological PT was considered, the time required in the thermal treated substrate was shorter than the biological treated substrate. This shows that more waste can be treated within a shorter time by using the thermal PT.

Table 3.16: *Results for kinetic modelling of thermal treated substrates (values are average ± range of duplicate set of reactors)*

SAMPLE (PP:PM)	Lag phase, hr	Rm, ml CH_4/gTCOD.d	Tmax, ml/gTCOD	t95, day	teff, days
A (100:0)	3.3±0.3	35.7±3	240.7±4	15.0±0.2	14.9
B (75:25)	3.8±0.2	36.4±4	276.9±3	14.5±0.6	14.3
C (50:50)	6.5±0.5	38.8±3	287.6±5	13.4±0.3	13.1
D (25:75)	5.5±0.5	33.4±4	246.6±3	15.5±0.2	15.3
E (0:100)	8.5±0.4	32.7±2	200.6±4	15.9±0.4	15.5

Table 3.17: *Results for kinetic modelling of thermo-acidified Substrates (values are average ± range of duplicate set of reactors)*

SAMPLE (PP:PM)	Lag phase, hr	Rm, ml CH_4/gTCOD.d	Tmax, ml/gTCOD	t95, day	teff, days
A (100:0)	1.0±0.2	38.8±2	228.4±5	11.8±0.1	11.8
B (75:25)	1.0±0.1	46.6±2	255.3±5	11.5±0.3	11.5
C (50:50)	1.0±0.1	48.8±3	284.9±3	10.9±0.4	10.9
D (25:75)	2.0±0.2	39.5±2	237.7±4	12.7±0.2	12.6
E (0:100)	2.0±0.1	37.8±1	200.1±2	12.9±0.3	12.8

Table 3.18: *Results for kinetic modelling of meso-acidified substrates (values are average ± range of duplicate set of reactors)*

SAMPLE (PP:PM)	Lag phase, hr	Rm, ml CH_4/gTCOD.d	Tmax, ml/gTCOD	t95, day	teff, day
A (100:0)	1.0±0.1	39.6±1	221.3±6	11.2±0.1	11.2
B (75:25)	1.0±0.1	51.1±2	254.6±4	10.1±0.2	10.1
C (50:50)	1.0±0.2	48.5±2	247.5±5	10.6±0.2	10.6
D (25:75)	2.0±0.1	38.9±3	230.1±2	11.9±0.1	11.8
E (0:100)	2.0±0.3	37.5±1	184.5±3	12.3±0.3	12.2

Conclusions

The current study investigated the effects of thermal PT, thermophilic biological acidification and mesophilic biological acidification on energy recovery and reaction kinetics from anaerobic co-digestion of PP and PM in a batch study. The results obtained show that:

1. Thermal pre-treatments increased the solubility of the organic matter in the PP and PM while the biological pre-treatments contributed to both increment in solubility of organic material and formation of readily available organic acids for the consumption of methane producing microbial community.
2. The thermal pre-treatment and biological pre-treatment at different temperature enhanced methane yield during the anaerobic co-digestion process of different mixes of PP and PM. In comparison to the highest methane yield in untreated mixes of PP and PM (Chapter 2), thermal PT and thermophilic biological acidification improved methane yield by 23% at 50:50 mix of PP and PM while the mesophilic biological acidification improved methane yield by 10% at 75:25 mix of PP and PM.
3. The maximum methane production rate was improved by the biological PT and thermal PT. The highest improvement was observed in the biological PT at mesophilic temperature. Compared to the untreated substrate, the methane production rate was higher in all pre-treated substrates. The highest methane production rate in the mesophilic biological PT was almost two times faster than the untreated, which was observed in 75:25 mix and 50:50 mix of PP and PM, respectively.

4. The technical digestion time required to produce 95% of the methane from the pre-treated substrates is shorter than the untreated substrates. The shortest time was observed in the methanogenesis of the biological treated substrates. However, by considering the pre-treatment time, the overall time required for the thermal PT to produce 95% of its methane potential is shortest.

Hence, the application of heat (thermal PT) and the separation of acidification stage (biological PT) improved methane yield and reaction kinetics in the second stage methanogenesis. Despite the slower methane production rate observed in the thermal treated substrates, the longer methane production time ensures a higher methane yield. Therefore, thermal PT is very attractive because it is simple and requires lesser treatment time, with the added benefit of pathogen destruction. On the other hand, biological PT takes longer time in the PT stage and requires additional cost for the separate reactor in the acidification stage.

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CHAPTER 4: Engineering Significance

This thesis focused on improving energy recovered from organic waste, such as PP and PM, by varying the mix proportion and by introducing three different PT. For the first study (Chapter two), the effects of different mix ratio of PP and PM was investigated for PP and PM. The PP represents a carbon rich waste while PM represents a nutrient rich waste. The results show that methane recovery and reaction kinetics were enhanced by the co-digestion of PP and PM. Hence, co-digestion rather than mono-digestion should be actively considered when similar carbon rich or nutrient rich wastes are available within reasonable proximity. Also, co-digestion of two similar waste streams has the added benefits of sharing resources by using a single reactor for two different wastes at the same time, which could potentially lead to cost saving in the operation and capital cost.

The focus of the second study (Chapter 3) was on the effects of thermal PT and biological PT (at mesophilic and thermophilic temperature) on energy recovery and reaction kinetics from anaerobic co-digestion of PP and PM. Results show that higher methane yield and faster reaction kinetics was observed by introducing the three PT in anaerobic co-digestion of PP and PM. The efficiency of a PT is dependent on the substrate characteristics. PP and PM are carbon rich and nutrient rich waste, respectively. Many agricultural waste and energy crops produced in large volume are also rich in carbon and nutrient. Hence, thermal and biological PT should be considered for enhancing the degradability of such waste stream. Also, the use of thermal and biological PT has the added advantage of cutting down the cost of post-treatment of the digested materials (digestate), due to the pathogen destruction and its improvement in dewaterability.

Overall, the variation in the mix ratio and the use of thermal and biological PT was able to improve methane yield and reaction kinetics. The mix ratio comes at no added cost, but the use of PT comes at an added cost. The additional energy cost required in thermal PT and the additional reactor cost in biological PT needs to be justifiable before application in the real world. Hence, a detailed economic feasibility and energy balance needs to be conducted.

Recommendations for Future Work

Future works should be done in a continuous or semi-continuous reactor, to have a more comprehensive understanding of real-life performance of different set-up (pre-treated and untreated), as the BMP study is only a preliminary test. Also, a variation of process parameters, such as FM ratio, should also be investigated for the acidification and methanogenesis stage, to assess its effects on the performance of the different mixes and pre-treatments.

Hydrogen gas, being the cleanest form of energy with a higher heating value than methane, is a potential gas that can be recovered from the acidification stage. Future work should explore the possibility of optimizing and recovering hydrogen gas from the mesophilic biological and thermophilic biological PT of PP and PM. Finally, the effects of acclimated and unacclimated thermophilic culture in the thermophilic-biological PT should also be investigated.

APPENDICES

Appendix A: Supplementary Information for Chapter 2

Table 2.13: *Calculation of yield and COD removal*

	initial TCOD, g/l	g TCOD, initial	Final TCOD, g/l	g TCOD, final	CH4 VOL	g COD of CH4	net initial cod	net final cod	net CH4 VOL	COD REMOVAL	NET g TCOD added	ml/gTCOD added	ml CH4/gCODremoved
A11	34.09	3.41	24.00	2.40	270	0.677	0.800	0.297	160	63%	0.800	200	318
A22	34.22	3.42	24.10	2.41	275	0.689	0.812	0.307	165	62%	0.812	203	313
B33	34.70	3.47	23.95	2.40	295	0.739	0.860	0.292	185	66%	0.860	215	326
B44	34.44	3.44	23.99	2.40	292	0.732	0.835	0.296	182	65%	0.835	218	323
C55	34.28	3.43	23.20	2.32	300	0.752	0.819	0.217	190	74%	0.819	232	347
C66	34.31	3.43	23.25	2.33	299	0.749	0.822	0.222	189	73%	0.822	230	351
D77	33.13	3.31	23.80	2.38	255	0.639	0.704	0.277	145	61%	0.704	206	316
D88	33.41	3.34	23.70	2.37	260	0.652	0.732	0.267	150	64%	0.732	205	314
E99	32.15	3.22	24.04	2.40	210	0.526	0.606	0.301	100	50%	0.606	165	308
E1010	32.57	3.26	24.07	2.41	215	0.539	0.648	0.304	105	53%	0.648	162	305
CTRL	32.92	3.29	21.23	2.12	340	0.852	0.683	0.020	230	97%	0.683	337	347
BLK	26.09	2.61	21.03	2.10	110	0.276	0.000	0.000	0	19%			

Table 2.14: *Synergistic effects calculation*

Sample	methane yield from co-digestion	methane yield from PP	methane yield from PM	gTCOD from PM	gTCOD from PP	Synergistic effects
B33	215	200	165	0.606	0.800	1.1624
B44	218	200	165	0.606	0.800	1.1786
C55	232	200	165	0.606	0.800	1.2543
C66	230	200	165	0.606	0.800	1.2435
D77	206	200	165	0.606	0.800	1.1138
D88	205	200	165	0.606	0.800	1.1084

Table 2.15: *Time series of methane production for A11*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1356	21%	20	0.000974	60	12.6	0.1	12.6	0.1
2	2207	34%	13	0.000633	75	25.6	0.0	38.1	0.1
3	2645	41%	29	0.001412	65	26.5	0.1	64.7	0.2
4	2567	40%	23	0.00112	45	17.8	0.1	82.5	0.2
5	2381	37%	32	0.001558	45	16.5	0.1	99.0	0.3
6	2897	45%	25	0.001217	44	19.7	0.1	118.7	0.4
7	2834	44%	33	0.001606	44	19.3	0.1	138.0	0.4
9	3246	50%	24	0.001168	44	22.1	0.1	160.0	0.5
10	3374	52%	26	0.001266	41	21.4	0.1	181.4	0.5
11	2610	40%	27	0.001314	38	15.3	0.0	196.7	0.6
12	2811	43%	16	0.000779	28	12.2	0.0	208.9	0.6
13	2678	41%	30	0.00146	28	11.6	0.0	220.4	0.7
14	2456	38%	34	0.001655	26	9.9	0.0	230.3	0.7
15	2893	45%	34	0.001655	20	8.9	0.0	239.2	0.7
16	2811	43%	22	0.001071	18	7.8	0.0	247.0	0.8
17	2590	40%	26	0.001266	11	4.4	0.0	251.4	0.8
18	2512	39%	22	0.001071	10	3.9	0.0	255.3	0.8
19	2578	40%	20	0.000974	10	4.0	0.0	259.3	0.8
20	2712	42%	19	0.000925	10	4.2	0.0	263.5	0.8
22	2584	40%	18	0.000876	9	3.6	0.0	267.1	0.8
24	2567	40%	27	0.001314	4	1.6	0.0	268.7	0.8
26	2177	34%	25	0.001217	3	1.0	0.0	269.7	0.8
27	2257	35%	29	0.001412	1	0.3	0.0	270.0	0.8

Table 2.16: *Time series of methane production for A22*

Day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1781	28%	21	0.001022	75	20.6	0.1	20.6	0.1
2	2291	35%	21	0.001022	80	28.3	0.1	48.9	0.2
3	2418	37%	24	0.001168	70	26.1	0.1	75.1	0.2
4	2371	37%	22	0.001071	65	23.8	0.1	98.9	0.3
5	2511	39%	29	0.001412	61	23.7	0.1	122.5	0.4
6	2681	41%	25	0.001217	60	24.8	0.1	147.3	0.5
7	2812	43%	0	0	49	21.3	0.0	168.6	0.5
9	2369	37%	21	0.001022	42	15.4	0.0	184.0	0.5
10	2489	38%	21	0.001022	40	15.4	0.0	199.4	0.6
11	2321	36%	25	0.001217	35	12.5	0.0	211.9	0.6
12	2413	37%	17	0.000828	30	11.2	0.0	223.1	0.6
13	2761	43%	0	0	25	10.7	0.0	233.7	0.6
14	2675	41%	35	0.001704	20	8.3	0.0	242.0	0.7
15	2216	34%	30	0.00146	16	5.5	0.0	247.5	0.7
16	2478	38%	32	0.001558	15	5.7	0.0	253.2	0.7
17	2816	43%	31	0.001509	12	5.2	0.0	258.4	0.7
18	2209	34%	12	0.000584	10	3.4	0.0	261.8	0.7
19	2379	37%	19	0.000925	7	2.6	0.0	264.4	0.7
20	2500	39%	22	0.001071	5	1.9	0.0	266.3	0.7
22	2624	41%	26	0.001266	5	2.0	0.0	268.4	0.7
24	2156	33%	21	0.001022	3	1.0	0.0	269.4	0.7
26	2103	32%	22	0.001071	2	0.6	0.0	270.0	0.7
27	2291	35%	19	0.000925	1	0.4	0.0	270.4	0.7

Table 2.17: *Time series of methane production for B33*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
0	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1515	23%	20	0.000974	110	25.7	0.1	25.7	0.1
2	2457	38%	13	0.000633	55	20.9	0.0	46.6	0.1
3	2630	41%	29	0.001412	53	21.5	0.1	68.1	0.2
4	2921	45%	23	0.00112	51	23.0	0.1	91.1	0.3
5	3019	47%	32	0.001558	53	24.7	0.1	115.8	0.4
6	2920	45%	25	0.001217	50	22.5	0.1	138.4	0.4
7	3484	54%	33	0.001606	48	25.8	0.1	164.2	0.5
9	3428	53%	24	0.001168	46	24.3	0.1	188.5	0.5
10	3213	50%	26	0.001266	40	19.8	0.1	208.4	0.6
11	3044	47%	27	0.001314	38	17.9	0.0	226.2	0.6
12	2905	45%	16	0.000779	28	12.6	0.0	238.8	0.7
13	3028	47%	30	0.00146	24	11.2	0.0	250.0	0.7
14	2850	44%	34	0.001655	21	9.2	0.0	259.3	0.7
15	3046	47%	34	0.001655	16	7.5	0.0	266.8	0.8
16	2969	46%	22	0.001071	14	6.4	0.0	273.2	0.8
17	2915	45%	26	0.001266	11	5.0	0.0	278.2	0.8
18	2933	45%	22	0.001071	10	4.5	0.0	282.7	0.8
19	2873	44%	20	0.000974	10	4.4	0.0	287.1	0.8
20	3006	46%	19	0.000925	7	3.2	0.0	290.4	0.8
22	2852	44%	18	0.000876	5	2.2	0.0	292.6	0.8
24	2173	34%	27	0.001314	4	1.3	0.0	293.9	0.8
26	2177	34%	25	0.001217	3	1.0	0.0	294.9	0.8
27	2257	35%	29	0.001412	1	0.3	0.0	295.3	0.8

Table 2.18: *Time series of methane production for B44*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
0	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1410	22%	20	0.000974	110	23.9	0.1	23.9	0.1
2	2419	37%	13	0.000633	55	20.5	0.0	44.5	0.1
3	2531	39%	29	0.001412	53	20.7	0.1	65.2	0.2
4	2879	44%	23	0.00112	51	22.7	0.1	87.9	0.3
5	2917	45%	32	0.001558	53	23.9	0.1	111.7	0.4
6	2888	45%	25	0.001217	50	22.3	0.1	134.0	0.4
7	3419	53%	33	0.001606	48	25.3	0.1	159.4	0.5
9	3394	52%	24	0.001168	46	24.1	0.1	183.5	0.5
10	3124	48%	26	0.001266	40	19.3	0.1	202.8	0.6
11	2999	46%	27	0.001314	38	17.6	0.0	220.4	0.6
12	2871	44%	16	0.000779	28	12.4	0.0	232.8	0.7
13	2993	46%	30	0.00146	24	11.1	0.0	243.9	0.7
14	2816	43%	34	0.001655	21	9.1	0.0	253.0	0.7
15	2959	46%	34	0.001655	16	7.3	0.0	260.3	0.8
16	2912	45%	22	0.001071	14	6.3	0.0	266.6	0.8
17	2891	45%	26	0.001266	12	5.4	0.0	272.0	0.8
18	2844	44%	22	0.001071	10	4.4	0.0	276.4	0.8
19	2817	43%	20	0.000974	11	4.8	0.0	281.2	0.8
20	2991	46%	19	0.000925	7	3.2	0.0	284.4	0.8
22	2815	43%	18	0.000876	6	2.6	0.0	287.0	0.8
24	2098	32%	27	0.001314	4	1.3	0.0	288.3	0.8
26	2154	33%	25	0.001217	4	1.3	0.0	289.6	0.8
27	2212	34%	29	0.001412	1	0.3	0.0	290.0	0.8

Table 2.19: *Time series of methane production for C55*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1375	21%	18	0.000876	80	17.0	0.1	17.0	0.1
2	2520	39%	20	0.000974	58	22.6	0.1	39.6	0.1
3	3315	51%	30	0.00146	58	29.7	0.1	69.2	0.2
4	3385	52%	25	0.001217	56	29.3	0.1	98.5	0.3
5	3488	54%	34	0.001655	51	27.5	0.1	126.0	0.4
6	3219	50%	30	0.00146	50	24.9	0.1	150.8	0.4
7	3343	52%	36	0.001752	48	24.8	0.1	175.6	0.5
9	3497	54%	0	0	45	24.3	0.0	199.9	0.5
10	3210	50%	24	0.001168	38	18.8	0.0	218.7	0.6
11	3460	53%	19	0.000925	24	12.8	0.0	231.6	0.6
12	3118	48%	31	0.001509	20	9.6	0.0	241.2	0.6
13	3365	52%	31	0.001509	19	9.9	0.0	251.1	0.6
14	3463	53%	0	0	15	8.0	0.0	259.1	0.6
15	3481	54%	27	0.001314	14	7.5	0.0	266.6	0.7
16	3356	52%	29	0.001412	12	6.2	0.0	272.8	0.7
17	3353	52%	23	0.00112	12	6.2	0.0	279.0	0.7
18	2977	46%	25	0.001217	10	4.6	0.0	283.6	0.7
19	3210	50%	27	0.001314	10	5.0	0.0	288.6	0.7
20	3433	53%	23	0.00112	8	4.2	0.0	292.8	0.7
22	3258	50%	23	0.00112	6	3.0	0.0	295.9	0.7
24	2895	45%	26	0.001266	5	2.2	0.0	298.1	0.7
26	2798	43%	24	0.001168	3	1.3	0.0	299.4	0.7
27	2538	39%	24	0.001168	2	0.8	0.0	300.2	0.7

Table 2.20: *Time series of methane production for C66*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1377	21%	20	0.000974	78	16.6	0.1	16.6	0.1
2	2516	39%	20	0.000974	58	22.5	0.1	39.1	0.1
3	3310	51%	31	0.001509	58	29.6	0.1	68.8	0.2
4	3377	52%	25	0.001217	56	29.2	0.1	98.0	0.3
5	3469	54%	36	0.001752	51	27.3	0.1	125.3	0.4
6	3212	50%	30	0.00146	50	24.8	0.1	150.1	0.5
7	3339	52%	36	0.001752	48	24.7	0.1	174.8	0.5
9	3494	54%	0	0	45	24.3	0.0	199.1	0.5
10	3208	50%	24	0.001168	38	18.8	0.0	217.9	0.6
11	3456	53%	19	0.000925	24	12.8	0.0	230.7	0.6
12	3109	48%	31	0.001509	20	9.6	0.0	240.3	0.6
13	3351	52%	0	0	19	9.8	0.0	250.2	0.6
14	3449	53%	30	0.00146	15	8.0	0.0	258.2	0.7
15	3478	54%	0	0	14	7.5	0.0	265.7	0.7
16	3342	52%	22	0.001071	12	6.2	0.0	271.9	0.7
17	3345	52%	23	0.00112	12	6.2	0.0	278.1	0.7
18	2945	45%	32	0.001558	10	4.5	0.0	282.6	0.7
19	3201	49%	12	0.000584	10	4.9	0.0	287.5	0.7
20	3426	53%	34	0.001655	8	4.2	0.0	291.8	0.7
22	3248	50%	22	0.001071	6	3.0	0.0	294.8	0.7
24	2893	45%	34	0.001655	5	2.2	0.0	297.0	0.7
26	2782	43%	17	0.000828	3	1.3	0.0	298.3	0.7
27	2537	39%	18	0.000876	2	0.8	0.0	299.1	0.7

Table 2.21: *Time series of methane production for D77*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1356	21%	20	0.000974	99	20.7	0.1	20.7	0.1
2	1905	29%	13	0.000633	60	17.6	0.0	38.4	0.1
3	2367	37%	29	0.001412	51	18.6	0.1	57.0	0.2
4	2345	36%	23	0.00112	50	18.1	0.1	75.1	0.3
5	2381	37%	32	0.001558	48	17.6	0.1	92.8	0.3
6	2897	45%	25	0.001217	45	20.1	0.1	112.9	0.4
7	2834	44%	33	0.001606	45	19.7	0.1	132.6	0.5
9	2810	43%	24	0.001168	45	19.5	0.1	152.1	0.5
10	2871	44%	26	0.001266	41	18.2	0.1	170.3	0.6
11	2610	40%	27	0.001314	38	15.3	0.0	185.6	0.6
12	2811	43%	16	0.000779	28	12.2	0.0	197.8	0.6
13	2678	41%	30	0.00146	28	11.6	0.0	209.3	0.7
14	2456	38%	34	0.001655	26	9.9	0.0	219.2	0.7
15	2893	45%	34	0.001655	20	8.9	0.0	228.1	0.8
16	2811	43%	22	0.001071	14	6.1	0.0	234.2	0.8
17	2590	40%	26	0.001266	11	4.4	0.0	238.6	0.8
18	2512	39%	22	0.001071	10	3.9	0.0	242.5	0.8
19	2578	40%	20	0.000974	10	4.0	0.0	246.5	0.8
20	2712	42%	19	0.000925	7	2.9	0.0	249.4	0.8
22	2210	34%	18	0.000876	5	1.7	0.0	251.1	0.8
24	2120	33%	27	0.001314	4	1.3	0.0	252.4	0.8
26	2177	34%	25	0.001217	3	1.0	0.0	253.4	0.8
27	2257	35%	29	0.001412	1	0.3	0.0	253.8	0.8

Table 2.22: *Time series of methane production for D88*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
0	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1366	21%	16	0.000779	96	20.2	0.1	20.2	0.1
2	1971	30%	22	0.001071	61	18.6	0.1	38.8	0.1
3	2404	37%	30	0.00146	53	19.7	0.1	58.5	0.2
4	2385	37%	26	0.001266	50	18.4	0.1	76.9	0.3
5	2920	45%	37	0.001801	47	21.2	0.1	98.1	0.4
6	2853	44%	27	0.001314	45	19.8	0.1	117.9	0.4
7	2810	43%	37	0.001801	44	19.1	0.1	137.0	0.5
9	2893	45%	0	0	44	19.7	0.0	156.7	0.5
10	2844	44%	25	0.001217	42	18.4	0.1	175.1	0.6
11	2682	41%	23	0.00112	38	15.7	0.0	190.8	0.6
12	2500	39%	0	0	28	10.8	0.0	201.6	0.6
13	2908	45%	31	0.001509	26	11.7	0.0	213.3	0.6
14	2843	44%	29	0.001412	26	11.4	0.0	224.7	0.7
15	2581	40%	0	0	19	7.6	0.0	232.3	0.7
16	2520	39%	23	0.00112	15	5.8	0.0	238.1	0.7
17	2631	41%	20	0.000974	11	4.5	0.0	242.6	0.7
18	2510	39%	28	0.001363	10	3.9	0.0	246.5	0.7
19	2588	40%	18	0.000876	10	4.0	0.0	250.5	0.7
20	2736	42%	16	0.000779	8	3.4	0.0	253.9	0.7
22	2232	34%	23	0.00112	5	1.7	0.0	255.6	0.7
24	2142	33%	22	0.001071	5	1.7	0.0	257.2	0.7
26	2180	34%	24	0.001168	3	1.0	0.0	258.3	0.7
27	2264	35%	22	0.001071	2	0.7	0.0	259.0	0.7

Table 2.23: *Time series of methane production for E99*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1735	27%	17	0.000828	25	6.7	0.0	6.7	0.0
2	1801	28%	21	0.001022	96	26.7	0.1	33.4	0.1
3	2089	32%	15	0.00073	80	25.8	0.1	59.2	0.2
4	2011	31%	27	0.001314	75	23.3	0.1	82.5	0.3
5	2018	31%	40	0.001947	58	18.1	0.1	100.6	0.4
6	2100	32%	32	0.001558	52	16.9	0.1	117.4	0.5
7	2201	34%	36	0.001752	48	16.3	0.1	133.7	0.6
9	1869	29%	22	0.001071	43	12.4	0.0	146.1	0.6
10	2034	31%	27	0.001314	38	11.9	0.0	158.1	0.6
11	2247	35%	18	0.000876	36	12.5	0.0	170.6	0.7
12	2276	35%	31	0.001509	29	10.2	0.0	180.8	0.7
13	2215	34%	25	0.001217	18	6.2	0.0	186.9	0.7
14	2198	34%	24	0.001168	12	4.1	0.0	191.0	0.8
15	2420	37%	23	0.00112	9	3.4	0.0	194.4	0.8
16	2109	33%	18	0.000876	7	2.3	0.0	196.6	0.8
17	2490	38%	21	0.001022	8	3.1	0.0	199.7	0.8
18	2210	34%	18	0.000876	8	2.7	0.0	202.4	0.8
19	2160	33%	36	0.001752	6	2.0	0.0	204.4	0.8
20	2156	33%	25	0.001217	6	2.0	0.0	206.4	0.8
22	2309	36%	21	0.001022	4	1.4	0.0	207.9	0.8
24	2218	34%	12	0.000584	3	1.0	0.0	208.9	0.8
26	2160	33%	18	0.000876	3	1.0	0.0	209.9	0.8
27	2010	31%	12	0.000584	1	0.3	0.0	210.2	0.8

Table 2.24: *Time series of methane production for E1010*

day	CH4 peak area	CH4 cont.	H_2 peak area	H_2 cont.	gas vol	CH4 VOL	H_2 vol	CUM CH4	cum. H_2
	0	0	0	0	0	0.0	0.0	0.0	0.0
1	1712	26%	19	0.000925	25	6.6	0.0	6.6	0.0
2	1819	28%	24	0.001168	99	27.8	0.1	34.4	0.1
3	2100	32%	28	0.001363	85	27.6	0.1	62.0	0.3
4	2119	33%	25	0.001217	75	24.5	0.1	86.5	0.3
5	2018	31%	40	0.001947	58	18.1	0.1	104.6	0.5
6	2125	33%	0	0	52	17.1	0.0	121.7	0.5
7	2234	34%	38	0.00185	48	16.6	0.1	138.2	0.5
9	1935	30%	18	0.000876	43	12.8	0.0	151.1	0.6
10	2034	31%	25	0.001217	38	11.9	0.0	163.0	0.6
11	2238	35%	21	0.001022	36	12.4	0.0	175.4	0.7
12	2348	36%	29	0.001412	29	10.5	0.0	185.9	0.7
13	2178	34%	32	0.001558	18	6.1	0.0	192.0	0.7
14	2278	35%	31	0.001509	12	4.2	0.0	196.2	0.8
15	2348	36%	0	0	9	3.3	0.0	199.5	0.8
16	2128	33%	23	0.00112	7	2.3	0.0	201.8	0.8
17	2488	38%	25	0.001217	8	3.1	0.0	204.9	0.8
18	2158	33%	19	0.000925	8	2.7	0.0	207.5	0.8
19	2148	33%	20	0.000974	6	2.0	0.0	209.5	0.8
20	2178	34%	22	0.001071	6	2.0	0.0	211.5	0.8
22	2348	36%	18	0.000876	4	1.5	0.0	213.0	0.8
24	2182	34%	22	0.001071	3	1.0	0.0	214.0	0.8
26	2153	33%	19	0.000925	3	1.0	0.0	215.0	0.8
27	2014	31%	21	0.001022	1	0.3	0.0	215.3	0.8

Appendix B: Supplementary Information for Chapter 3

Table 3.19: *Characteristics of influent and effluent of acid fermentation (values are average ± range of two set of duplicates reactors)*

	INFLUENT, thermophilic					EFFLUENT, thermophilic				
	A	B	C	D	E	A	B	C	D	E
TCOD, g/l	29.3±0.3	29.5±0.2	29.3±0.4	29.4±0.1	29.2±0.2	24.1±0.3	23.6±0.1	23.5±0.2	22.5±0.1	21.3±0.2
SCOD, g/l	2.9±0.2	3.6±0.3	4.1±0.2	3.9±0.2	3.9±0.1	8.5±0.2	7.3±0.3	6.2±0.1	5.0±0.2	4.7±0.1
pH	5.6	5.5	5.4	5.5	5.6	5.1	5.2	5.2	5.3	5.4
VFAS (mg/l)	589.5±6	765.0±4	552.5±6	834.5±8	817.5±5	2663.9±7	3862.1±7	4056.5±11	1356.3±9	1181.2±8
	INFLUENT, mesophilic					**EFFLUENT, mesophilic**				
	A	B	C	D	E	A	B	C	D	E
TCOD, g/l	29.3±0.2	29.5±0.1	29.3±0.1	29.4±0.3	29.2±0.4	29.0±0.2	29.2±0.1	28.8±0.2	28.7±0.2	28.6±0.3
SCOD, g/l	2.9±0.2	3.6±0.2	4.1±0.1	3.9±0.2	3.9±0.1	9.2±0.3	8.4±0.3	7.8±0.2	6.4±0.1	5.5±0.1
pH	5.5	5.6	5.5	5.6	5.5	5.0	5.1	5.1	5.2	5.3
VFAS (mg/l)	648.0±7	516.5±5	859.5±8	908.0±4	1150.5±5	6227.5±6	6553.8±8	6009.0±5	4378.9±6	2848.4±7

Table 3.20: *VFAs distribution for effluent of thermophilic acidification*

EFFLUENT OF THERMOPHILIC ACIDIFICATION										
Sample name	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Isocaproate	Hexanoate	Heptanoate	Total
A14	413	216	20	1802	46	71	21	14	13	2616
A15	400	114	19	2035	44	65	17	9	11	2712
B16	1083	40	13	2605	50	26	9	13	14	3852
B17	1029	45	10	2680	47	31	10	11	8	3872
C18	1241	63	20	2555	78	21	32	36	62	4107
C19	1193	55	16	2631	70	5	6	11	19	4006
D20	221	191	22	717	44	73	23	13	0	1304
D21	294	209	26	689	51	86	26	17	10	1409
E22	330	207	30	441	61	85	48	17	11	1229
E23	437	92	29	388	55	80	42	12	0	1134

Table 3.21: *VFAs distribution for effluent of mesophilic acidification*

EFFLUENT OF MESOPHILIC ACIDIFICATION

Sample name	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Isocaproate	Hexanoate	Heptanoate	Total
A1	2231	331	321	2585	389	52	208	73	69	6259
A2	2313	322	325	2469	368	35	225	67	73	6196
B3	3824	298	203	1710	339	37	54	54	7	6526
B4	3854	304	190	1811	346	30	35	9	4	6582
C5	3161	485	227	1406	439	191	29	51	6	5995
C6	3179	467	224	1425	445	192	21	64	5	6023
D7	2266	607	212	815	419	120	39	20	4	4502
D8	2013	621	215	813	431	108	37	19	0	4256
E9	399	650	250	593	383	114	233	23	16	2661
E10	589	662	259	677	398	139	268	25	17	3036

Table 3.22: *SCOD comparison of effluent of thermal Treated, thermo-acidified, meso-acidified and untreated Substrates*

SAMPLE NAME	Biological-mesophilic, g/l	Biological thermophilic, g/l	Thermal treated, g/l	Untreated, g/l
A14, A1, A10, A11	9.08	8.35	9.11	3.96
A15, A2, A20, A22	9.30	8.64	9.16	4.01
B16, B3, B30, B33	8.30	7.45	9.82	3.81
B17, B4, B40, B44	8.50	7.15	9.79	3.89
C18, C5, C50, C55	7.89	6.30	10.18	3.62
C19, C6, C60, C66	7.75	6.07	10.21	3.71
D20, D7, D70, D77	6.45	5.03	7.85	3.13
D21, D8, D80, D88	6.36	5.07	7.81	3.21
E22, E9, E90, E99	5.36	4.71	7.61	2.91
E23, E10, E100, E1010	5.60	4.75	7.72	2.94

Table 3.23: *Energy Recovered from Different Pretreatments*

Sample Label	Thermal treated	Meso-acidified	Thermo-acidified
A10, A1, A14	8.26	7.67	7.96
A20, A2, A15	8.33	7.76	7.91
B30, B3, B16	9.60	8.82	8.72
B40, B4, B17	9.63	8.86	8.84
C50, C5, C18	10.02	8.68	10.01
C60, C6, C19	9.99	8.59	9.86
D70, D7, D20	8.47	7.95	8.24
D80, D8, D21	8.54	8.09	8.30
E90, E9, E22	7.00	6.45	6.97
E100, E10, E23	6.86	6.43	6.92

Table 3.24: *Calculation of experimental methane yield for methanogenesis of thermal treated substrate*

	initial TCOD, g/l	g TCOD, initial	Final TCOD, g/l	g TCOD, final	CH4 VOL	g COD of CH4	net initial cod	net final cod	net CH4 VOL	COD BAL	COD REMOVAL	NET g TCOD added	ml/gTCOD added	ml CH4/gCODremoved
A10	30.60	3.06	22.50	2.25	234.0	0.586	0.567	0.15	133.3	93%	74%	0.567	235	320
A20	30.81	3.08	22.40	2.24	240.0	0.602	0.588	0.14	139.3	92%	76%	0.588	237	311
B30	30.25	3.03	21.90	2.19	245.9	0.616	0.532	0.09	145.2	93%	83%	0.532	273	328
B40	30.37	3.04	21.80	2.18	249.7	0.626	0.544	0.08	149.0	92%	85%	0.544	274	321
C50	30.52	3.05	21.77	2.18	260.0	0.652	0.559	0.08	159.3	93%	86%	0.559	285	331
C60	30.71	3.07	21.90	2.19	265.0	0.664	0.578	0.09	164.3	93%	84%	0.578	284	336
D70	30.56	3.06	22.80	2.28	236.4	0.593	0.563	0.18	135.7	94%	68%	0.563	241	354
D80	30.25	3.02	22.61	2.26	230.0	0.576	0.532	0.16	129.3	94%	70%	0.532	243	348
E90	30.32	3.03	23.00	2.30	208.0	0.521	0.539	0.20	107.3	93%	63%	0.539	199	316
E100	30.53	3.05	23.01	2.30	210.0	0.526	0.560	0.20	109.3	93%	64%	0.560	195	304
CONTROL	31.40	3.14	21.40	2.14	300.0	0.752	0.647	0.04	199.3	92%	94%	0.647	308	328
BLANK	24.93	2.49	21.00	2.10	100.7	0.252	0.000	0.00	0.0	94%	16%	0.000		

Table 3.25: *Calculation of experimental methane yield for methanogenesis of thermo-acidified substrate*

	initial TCOD, g/l	g TCOD, initial	Final TCOD, g/l	g TCOD, final	CH4 VOL	g COD of CH4	net initial cod	Net Net initial gTCOD	net final cod	Net Net Final COD	net CH4 VOL	Net Net CH4 vol	NET g TCOD added	ml/gTCOD added	ml CH4/gCODremoved
A14	28.55	2.86	16.51	1.65	150.0	0.376	0.901	0.225	0.201	0.040	94.8	51.0	0.225	226	326
A15	28.79	2.88	16.41	1.64	151.0	0.378	0.925	0.231	0.191	0.038	95.8	52.0	0.231	225	339
B16	28.73	2.87	16.40	1.64	156.0	0.391	0.919	0.230	0.190	0.038	100.8	57.0	0.230	248	367
B17	28.45	2.85	16.33	1.63	155.0	0.388	0.891	0.223	0.183	0.037	99.8	56.0	0.223	251	351
C18	28.81	2.88	16.10	1.61	165.0	0.414	0.927	0.232	0.160	0.032	109.8	66.0	0.232	285	358
C19	28.67	2.87	15.90	1.59	163.0	0.409	0.913	0.228	0.140	0.028	107.8	64.0	0.228	280	363
D20	28.59	2.86	16.31	1.63	152.0	0.381	0.905	0.226	0.181	0.036	96.8	53.0	0.226	234	339
D21	28.18	2.82	16.40	1.64	150.0	0.376	0.864	0.216	0.190	0.038	94.8	51.0	0.216	236	321
E22	28.62	2.86	16.50	1.65	144.0	0.361	0.908	0.227	0.200	0.040	88.8	45.0	0.227	198	333
E23	28.68	2.87	16.55	1.66	144.0	0.361	0.914	0.229	0.205	0.041	88.8	45.0	0.229	197	331
NEW CTL	28.01	2.80	14.98	1.50	300.0	0.752	0.847		0.048		244.8	244.8	0.847	289	306
OLD CTL	28.15	2.82	16.82	1.68	150.0	0.376	0.861	0.215	0.232	0.058	94.8	51.0	0.215	237	324
NEW BLK	19.54	1.95	14.50	1.45	55.2	0.138	0.000		0.000		0.0	0.0			
OLD BLK	28.45	2.85	17.42	1.74	99.0	0.248	0.891	0.223	0.292		43.8	0.0			

Table 3.26: *Calculation of experimental methane yield for methanogenesis of meso-acidified substrate*

	initial TCOD, g/l	g TCOD, initial	Final TCOD, g/l	g TCOD, final	CH4 VOL	g COD of CH4	net initial cod	Net Net initial gTCOD	net final cod	Net Net Final COD	net CH4 VOL	Net Net CH4 vol	NET g TCOD added	ml/gTCOD added	ml CH4/gCODremoved
A1	28.45	2.85	20.00	2.00	148.0	0.371	0.550	0.137	0.197	0.039	72.5	30.0	0.137	218	306
A2	28.39	2.84	19.89	1.99	148.0	0.371	0.544	0.136	0.186	0.037	72.5	30.0	0.136	221	304
B3	28.85	2.89	19.65	1.97	155.0	0.388	0.590	0.148	0.162	0.032	79.5	37.0	0.148	251	321
B4	28.67	2.87	19.73	1.97	154.0	0.386	0.572	0.143	0.170	0.034	78.5	36.0	0.143	252	330
C5	28.78	2.88	20.16	2.02	154.0	0.386	0.583	0.146	0.213	0.043	78.5	36.0	0.146	247	349
C6	28.68	2.87	20.10	2.01	153.0	0.383	0.573	0.143	0.207	0.041	77.5	35.0	0.143	244	344
D7	28.79	2.88	20.35	2.04	151.0	0.378	0.584	0.146	0.232	0.046	75.5	33.0	0.146	226	331
D8	28.43	2.84	20.25	2.03	149.5	0.375	0.548	0.137	0.222	0.044	74.0	31.5	0.137	230	340
E9	28.19	2.82	20.36	2.04	142.0	0.356	0.524	0.131	0.233	0.047	66.5	24.0	0.131	183	311
E10	28.42	2.84	20.38	2.04	143.0	0.358	0.547	0.137	0.235	0.047	67.5	25.0	0.137	183	305
NEW CTL	28.89	2.89	18.35	1.83	289.0	0.724	0.594		0.031		213.5		0.594	359	379
OLD CTL	28.56	2.86	20.79	2.08	165.0	0.414	0.561	0.140	0.276	0.055	89.5	47.0	0.140	335	313
NEW BLK	22.95	2.30	18.03	1.80	75.5	0.189	0.000		0.000						
OLD BLK	28.51	2.85	21.75	2.18	118.0	0.296	0.556	0.139	0.372	0.074	42.5	0.0			

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