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**CO-DIGESTION OF SEWAGE SLUDGE AND PIG MANURE UNDER
MESOPHILIC CONDITIONS**

A Thesis in

Environmental Pollution Control

by

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ABSTRACT

The feasibility and kinetics of anaerobic co-digestion of pig manure (PM) and sewage sludge (SS) were investigated. In this study, bench scale batch reactors were setup under mesophilic conditions (35°C) with SS/PM volatile solid ratios at 7:1, 14:1, 21:1, and 2 controls of pure SS and PM. The ratio selection was based on the SS production of the University Park Wastewater Treatment Plant and PM production by the University Farm. Physical/chemical properties of sludge before and after digestion were analyzed. Daily methane production and cumulative methane yield were recorded, and the cumulative methane yields were fitted with both the first-order kinetic and the modified Gompertz model. All mixing ratios showed stable digestions as indicated by the pH, total ammonia nitrogen (TAN), and volatile fatty acid (VFA)/alkalinity ratios of the final digestates. PM produced more methane than SS and as a result, increasing PM in co-digestion also increased methane yield. The maximum methane yield occurred at SS/PM ratio of 7:1 with a 10% increase at 200 mL CH₄/g VS_{added} compared with SS alone at 182 mL CH₄/g VS_{added}. This is also the maximum co-digestion need from the University Farm in winter seasons. Although residual nitrogen increased in the co-digestion digestates, most of which were in solid form, resulting a decrease in soluble nitrogen. This implies a potential decrease in the nitrogen return which helps relief mainstream treatment. Both the first-order kinetic model and the modified Gompertz model showed good fit to the data produced. However, the modified Gompertz model proved to be the best choice that works for degradation with or without a lag-period. The first-order kinetic model indicated faster gas production kinetics by the 3 co-digestion treatments than the pure controls, which implies the existence of a positive synergistic effect by co-digestion. The modified Gompertz model also showed the highest maximum gas production rate, a decreased lag-period, and a shortened gas production period by co-digestion, which implies a facilitated hydrolysis has occurred from the mixture. This faster kinetics implies a

shorter digestion time than the original wastewater sludge digestion which can help accommodate the increased digestion materials from the co-digestion process.

TABLE OF CONTENTS

LIST OF FIGURES	vii
LIST OF TABLES	viii
ACKNOWLEDGEMENTS	ix
Chapter 1 Introduction and Objectives	1
1.1. Introduction	1
1.2. Objectives	3
Chapter 2 Literature Review	4
2.1 Anaerobic Digestion Overview	4
2.1.1 Microbiology of Anaerobic Digestion	5
2.1.1.1 Hydrolysis	5
2.1.1.2 Acidogenesis	6
2.1.1.3 Acetogenesis	7
2.1.1.4 Methanogenesis	8
2.1.2 Parameters Influencing Anaerobic Digestion	8
2.1.2.1 pH Value	8
2.1.2.2 Carbon to Nitrogen (C/N) Ratio	9
2.1.2.3 Retention Time	9
2.1.2.4 Organic Loading Rate	10
2.1.2.5 Ammonia Nitrogen	10
2.1.2.6 Temperature	11
2.2 Anaerobic Co-digestion	11
2.2.1 Background	11
2.2.2 Anaerobic Co-digestion with Sewage Sludge as the Main Substrate	12
2.2.3 Anaerobic Co-digestion with Manure as the Main Substrate	13
2.2.4 Anaerobic Co-digestion of Sewage Sludge and Manure	14
2.3 Kinetic Models	15
2.3.1 The First-order Kinetic Model	16
2.3.2 The Modified Gompertz Model	16
2.4 Summary of Literature Review	17
Chapter 3 Materials and Methods	18
3.1 Digestion Materials	18
3.2 Experimental Design and Set-up	20
3.3 Sample Analyses	21
3.3.1 Chemical Analysis	21
3.3.2 Volatile Fatty Acid Measurement	21
3.3.3 Biogas Measurement	22
3.3.4 Methane Content Measurement	23
3.4 Kinetic Model Analysis	23

3.5 Statistical Analysis.....	23
Chapter 4 Results and Discussion.....	24
4.1 Volatile Solid Removal Rate.....	24
4.2 Methane Production	25
4.3 Process Stability	28
4.3.1 pH.....	28
4.3.2 Volatile Fatty Acid/ Alkalinity Ratio	28
4.3.3 Total Ammonia Nitrogen	29
4.4 Kinetic Model	30
4.4.1 The First-order Kinetic Model	31
4.4.2 The Modified Gompertz Model	31
Chapter 5 Conclusion.....	35
References.....	37
Appendix.....	47
Experimental Reactors for 3 Co-digestion Treatments (Sewage Sludge/Pig Manure ratios of 21:1, 14:1, and 7:1) and 3 Pure Controls (Pure Sewage Sludge, Pure Pig Manure, and Inoculum) in Shaking Water Bath.....	47

LIST OF FIGURES

Figure 2-1. The metabolic degradation pathways for carbohydrates-rich, proteins-rich, lipids-rich organic materials in the anaerobic digestion process	5
Figure 3-1. The diagram for biogas volume measurement.....	22
Figure 4-1. Methane production rate and cumulative methane yield for sewage sludge (SS) only (A), pig manure (PM) only (B), SS to PM ratios at 21:1 (C), 14:1 (D), and 7:1 (E) incubated at 35°C. Circle represents methane production rate and square represents cumulative methane yield. The values are average of three replication bottles.....	27
Figure 4-2. Comparison among actual cumulative methane yield and predicted cumulative methane yield from the first-order kinetic model and the modified Gompertz model for sewage sludge (SS) only (A), pig manure (PM) only (B), SS to PM ratios at 21:1 (C), 14:1 (D), and 7:1 (E). Circle represents actual cumulative methane yield, dash line represents predicted cumulative methane yield from the first-order kinetic model, and solid line represents predicted cumulative methane yield from the modified Gompertz model.....	33

LIST OF TABLES

Table 3-1. Characteristics of pig manure, concentrated pig manure, sewage sludge, inoculum, and concentrated inoculum used in this study.....	19
Table 3-2. Substrate composition in different digesters.....	21
Table 4-1. Actual VS removal from co-digestion and estimated VS removal based on mono-digestion.....	24
Table 4-2. Physical-chemical properties of digestates.....	30
Table 4-3. Nitrogen balance calculation for each assay.....	30
Table 4-4. Calculated parameters for the first-order kinetic and the modified Gompertz models	34

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Chapter 1 Introduction and Objectives

1.1. Introduction

There are plenty of pig farms around the world, most of these farms are small farms. Generally, a pig weighing 21 to 100 kg is expected to generate 0.39 to 0.45kg dry waste materials per day and there were 1,140,000 head of pig in Pennsylvania by 2010 (Brumm, Sutton, & Jones, 1980; Kevin, 2010). So, the amount of pigs can produce a large amount of manure which can be significant problems, including increasing contamination of the soil, water, and air (Prats et al., 1995). Pig manure (PM) can be used as fertilizer; however, due to the lack of nutrient content information, it is hard to determine the appropriate application amount. This could cause a potential to surpass the soil's threshold, which would result in unrecoverable damage, such as nutrient imbalance (Hatfield, Brumm, & Melvin, 1998). High BOD value and pathogens in the manure is a big concern if leachate and runoff from manure application sites or storage sites enter the water body (Prats et al., 1995). Finally, pungent odor from PM can be a vexing problem for farmers (Al-Kanani, Akochi, MacKenzie, Alli, & Barrington, 1992).

The current disposal methods for manure include land application (Nicholson, Groves, & Chambers, 2005), composting (Huang, Wong, Wu, & Nagar, 2004), anaerobic digestion (Zhang et al., 2014), fish feed (Wohlfarth & Schroeder, 1979), organic fertilizer (Warman, 1986), and anaerobic lagoon (Chastain & Henry, 1999). Anaerobic digestion is one of the feasible options to deal with PM. It is a biological process which can digest organic matters to biogas in the absence of oxygen. Past research showed PM with great potential for biogas production (Astals, Nolla-Ardèvol, & Mata-Alvarez, 2012; Cuetos, Fernández, Gómez, & Morán, 2011; Wang, Yang, Feng, Ren, & Han, 2012).

Large amount of PM generated from Pennsylvania State University Park Pig Farm every day. The farm generates 2000 gpd of PM with a maximum of 3000 gpd. The current disposal method for manure is through fertilizer application; however, PM needs to be stored in a deep tank during winter, and as a result there is a need to look for an alternative outlet.

The wastewater treatment plant (WWTP) in State College has a two-stage anaerobic digestion process. The main purpose of anaerobic digestion in the plant is to treat sewage sludge (SS). The advantages of having this process are generating renewable energy (methane gas), and reducing odor issues, reducing sludge volumes, and inactivating pathogens. This plant is permitted to treat up to 4 million gallons per day of wastewater. The sludge from primary and secondary sedimentation tanks and scum from activated sludge tanks are sent to the sludge thickener building. The thickened sludge is then transferred to the primary anaerobic digester for anaerobic digestion. The primary digester is operated at 35°C with the hydraulic retention time (HRT) of 10 days. It can produce 105,200 ft³ CH₄/d. After digestion, the substrate goes through a belt filter press to separate sludge and filtrate. The filtrate in the sludge is then being pumped back to the primary settling tank for further treatment.

In order to increase methane production rate in the WWTP and provide a solution to PM winter storage, there is a potential to add PM into the anaerobic digester to co-digest with SS to produce more methane gas. This thesis aims to investigate the feasibility of using PM to co-digest with SS, obtaining information needed for potential future implementation.

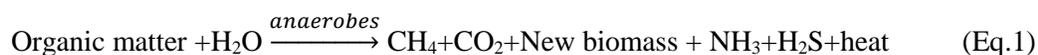
1.2. Objectives

In this study, the primary objective is to determine the maximum loading ratio of PM to SS for University Farm in winter time. The evaluation was based on four aspects, including methane gas production, percentage of methane in biogas, digester stability, and digestion efficiency (VS reduction and degradation kinetics). The second objective is to determine the level of total nitrogen (TN) increase in the mainstream as a result of PM co-digestion.

Chapter 2 Literature Review

2.1 Anaerobic Digestion Overview

Anaerobic digestion process plays a vital role in the exploration of renewable energy, since bioenergy from various biomass can be converted into methane-rich biogas in an oxygen-free environment. Biogas can be converted into heat and electricity through a Combined Heat and Power-unit (CHP) (Pilavachi, 2002). Therefore, anaerobic digestion provides an alternative green energy for the society. The general equation for anaerobic digestion process can be expressed in Eq.1 (Kelleher et al., 2002).



Three biomass, carbohydrate-rich organic materials, protein-rich organic materials, and fat-rich organic materials, are the predominant organic matters used in the anaerobic digestion process. Simple carbohydrates, such as simple sugar or disaccharides, can easily be decomposed by acidogenesis communities to form volatile fatty acids. Other complex carbohydrates, such as starch or cellulose, need to be hydrolyzed first before converting to volatile fatty acids. Protein-rich organic materials can be found in slaughterhouse waste, stillage from ethanol industry, and animal manure. It is demonstrated to be rich in energy, which indicates a high methane production potential (Zhen, Lu, Kato, Zhao, & Li, 2017). Fat-rich organic materials can be easily found in the food industry, including the edible oil industry and the dairy industry. Scholars has found fat possess a high methane production potential (Li, Champagne, & Anderson, 2011; Long, Aziz, Francis, & Ducoste, 2012; Wan, Zhou, Fu, & Li, 2011). The main application of anaerobic digestion in WWTP is to treat SS, which is considered as one of the sustainable options to manage SS. SS is considered to be the major cost of WWTP. Anaerobic digestion can reduce

sewage volume, odor issues, and pathogen. Therefore, it is one of the popular options in municipal wastewater treatment plants for SS treatment.

2.1.1 Microbiology of Anaerobic Digestion

The anaerobic digestion process is composed of four stages (Figure 2-1): hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each process involves different groups of microorganisms. The products generated from early stages can be used as reactants for subsequent stages.

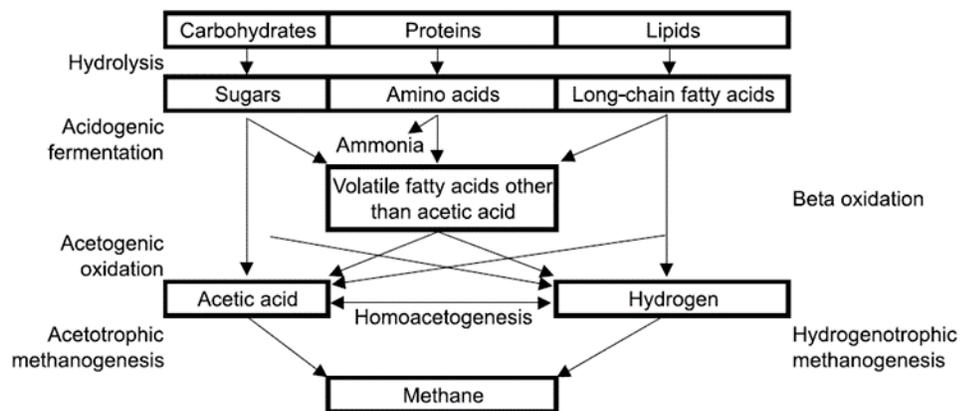


Figure 2-1. The metabolic degradation pathways for carbohydrates-rich, proteins-rich, lipids-rich organic materials in the anaerobic digestion process (Pavlostathis & Giraldo-Gomez, 1991).

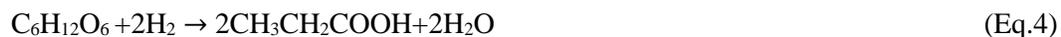
2.1.1.1 Hydrolysis

Hydrolysis is the step to break down complex materials into their corresponding simple component molecules by the addition of water. For example, carbohydrates like starch or polysaccharides are hydrolyzed into sugars (simple sugar or disaccharides); proteins are degraded into amino acids; lipids are converted into long chain fatty acids. All of these reactions are achievable under the assistance of extracellular enzymes. These resulting small soluble organics

can penetrate cell membranes for further degradation. The main microbes involved in the hydrolysis process include *Bacteroides*, *Clostridium*, *Cellulomonas*, *Acetovibrio*, *Succinivibrio*, *Prevotella*, *Microbispora*, *Fibrobacter*, *Firmicutes*, *Erwinia*, *Ruminococcus*, etc (Guo et al., 2015; Pavlostathis & Giraldo-Gomez, 1991).

2.1.1.2 Acidogenesis

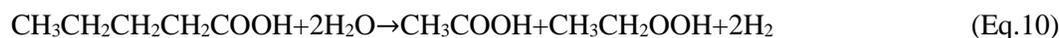
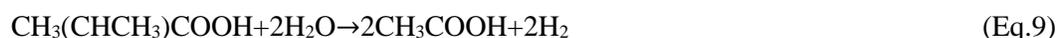
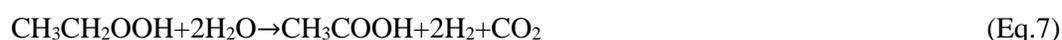
Acidogenesis is the second stage during the anaerobic digestion process. The hydrolyzed products from the first stage are fermented into volatile fatty acids (acetate, propionate, butyrate, and lactate) and minor by-products like ammonia, hydrogen gas, and carbon dioxide. Eq.2 to 4 are the example reactions showing how glucose is converted to acetate, ethanol, and propionate, respectively (Bilitewski et al., 1997; Ostrem & Themelis, 2004).



In this process, the main products are acetate, hydrogen gas, and carbon dioxide, which are the raw materials used for subsequent methanogenesis. The other products, like propionate, butyrate, and lactate, are going through acetogenesis to produce acetate used for methanogenesis. Key bacteria involved in this phase are *Peptococcus*, *Bacteroides*, *Geobacter*, *Sarcina*, *Desulfovibrio*, *Phodopseudomonas*, *Clostridium*, *Lactobacillus*, *Eubacterium*, *Desulobacter*, etc (Gonzalez-Fernandez, Sialve, & Molinuevo-Salces, 2015; Guo et al., 2015).

2.1.1.3 Acetogenesis

Acetogenesis is the third process in the anaerobic digestion process. In this process, higher organic acids, like propionic, butyric or other acids, are decomposed into acetate, hydrogen gas and carbon dioxide as demonstrated in Eq.5-12 (Angelidaki, Sorensen, & Schmidt, 2007; Pind, Angelidaki, & Ahring, 2003).



Two kinds of bacteria are involved in this process, including hydrogen-producing acetogenic bacteria and homoacetogenic bacteria (Lübken, Wichern, Schlattmann, Gronauer, & Horn, 2007; Sterling, Lacey, Engler, & Ricke, 2001). Hydrogen-producing acetogenic bacteria is the dominant bacteria in this process, which produce acetate, hydrogen gas and carbon dioxide from volatile fatty acids and alcohol. Homoacetogenic bacteria contribute to a small part of acetate from carbon dioxide and hydrogen gas. Typical acetogenic bacteria include *Syntrophus*, *Moorella*, *Syntrophomonas*, *Desulfovibrio*, *Pelotomaculum*, *Syntrophobacter*, and *Syntrophothermus* (Cai et al., 2016).

2.1.1.4 Methanogenesis

Methanogenesis is the last step in the anaerobic digestion process, which converts acetate into methane and carbon dioxide with *Methanosarcina* and *Methanosaeta* as the main archaea. Another part of methane is generated through the reduction of carbon dioxide by hydrogen and other electron donors with the help of *Methanobacterium* and *Methanoculleus* (Kugelman & McCarty, 1965; Lu, Zhen, Chen, Kubota, & Li, 2015; Okudoh, Trois, Workneh, & Schmidt, 2014). It has been agreed that approximately 70% methane production is converted from acetate and 30% methane production is generated from the redox reaction of hydrogen and carbon dioxide. Methanogenesis process can also be explained in reactions Eq.13-15.



2.1.2 Parameters Influencing Anaerobic Digestion

Several parameters are commonly monitored and maintained to assure stable digestion, which include pH value, carbon/nitrogen ratio, retention time, organic loading rate, ammonia nitrogen, and temperature.

2.1.2.1 pH Value

pH value changes during different digestion. Initially, the pH will decrease as organic materials convert into VFAs, but as the acids are consumed to generate biogas, pH will increase. Based on the reports, the fermentative bacteria require a pH higher than 5.0 to stay functioning and methanogenesis is required to be in an environment with an optimal range between 7.0 to 7.2

(Gerardi, 2003). Overall, the most suitable pH range for the anaerobic digestion process is from 6.5 to 8.2 (Dinamarca, Aroca, Chamy, & Guerrero, 2003). Any pH above or below this range can cause harmful effect to methanogenic organisms, which is more fragile to recover (Palatsi et al., 2009; Zonta, Alves, Flotats, & Palatsi, 2013).

pH can not only affect the growth of microorganisms, but also impact the composition of total ammonia nitrogen (Hashimoto, 1984). With an increase of pH, increasing fraction of free ammonia is shifted from ionized ammonia (NH_4^+), resulting in increased toxicity (Borja et al., 1996b). Meanwhile, process instability caused by free ammonia leads to VFA accumulation, resulting in pH reduction below ideal level.

2.1.2.2 Carbon to Nitrogen (C/N) Ratio

Carbon and nitrogen are two fundamental elements for cell growth. Carbon is used for both energy and both are used as the basic component of cell materials, but different organisms prefer different C/N ratio for growth. A high C/N ratio can cause N deficiency, while a low C/N ratio results in ammonia accumulation and pH increase. Based on the studies conducted by the researchers, the recommended C/N ratio range for anaerobic digestion is from 20/1 to 30/1 with an optimal ratio of 25/1 in the feed to meet the requirements of bacterial growth in the anaerobic digester (Wang et al., 2012).

2.1.2.3 Retention Time

The HRT is the theoretical time that liquid phase stays in the digester, while solid retention time (SRT) is the time that sludge stays in the digester. In the fully mixed digester or plug flow digester without recirculation, SRT is equal to HRT, while reactors with settling, SRT

is larger than HRT. The ideal reactor should have a short HRT but long SRT. Short HRT could ensure a small tank, while long SRT could ensure sufficient treatment time for the solids.

Generally, anaerobic digestion SRT ranges from 14 to 30 days depending on its configuration, the type of feedstocks, and temperature (Rafael Borja, González, Raposo, Millán, & Martín, 2002).

2.1.2.4 Organic Loading Rate

Organic loading rate (OLR) refers to the amount of raw materials added into digester per volume per day. Typical values of OLR are in the range of 0.5 -1.6 kg VS/m³/d depending on the configurations (Jain, Jain, Wolf, Lee, & Tong, 2015; Luste & Luostarinen, 2010). Overloading of reactor can cause an increase in VFAs resulting in VFA accumulation. Excess VFA results in pH drop, which damages microbial activity and ultimately terminates reaction.

2.1.2.5 Ammonia Nitrogen

Ammonia is produced by the biological degradation of nitrogenous matter. Two forms of inorganic ammonia nitrogen are present in aqueous solution, which includes free ammonia and ammonium. The composition of these two types of ammonia nitrogen is dependent on pH and temperature in the solution (Chen, Cheng, & Creamer, 2008). The equilibrium constant (pKa) for ammonium ion is about 9.23. If the pH is higher than 9.23, free ammonia is the predominant form, while ammonium dominates at pH below 9.23. Free ammonia has been agreed to be the main inhibitor to the functioning bacteria since it can penetrate cell membranes, resulting in toxicity (Chen et al., 2008). Studies showed that the most vulnerable digester microorganisms are methanogenic archaea (Kayhanian, 1994). Kostor (1998) found that acidogenic population remained the same while methanogens lost 56.5% of its activity when ammonia concentration

increased from 4051 to 5734 mg NH₃-N/L. McCarty (1964) explained that total ammonia nitrogen (TAN) concentration at 1500 to 3000 mg/L in high pH conditions could become an inhibitor for digester, and free ammonia itself could cause severe toxicity when its concentration exceeds 150 mg/L (McCarty & McKinney, 1961).

2.1.2.6 Temperature

Temperature is related to bacterial enzyme activity. It was found that 35°C is the optimal temperature for mesophilic digestion, while 55°C is the optimum for thermophilic digestion (Hartmann & Ahring, 2006; M. Kim, Ahn, & Speece, 2002). Temperature affects microbial growth rate. Increasing temperature encourages faster microbial growth, and consequently results in faster biodegradation. Currently, most wastewater treatment plants implemented the mesophilic anaerobic digester, while limited numbers of large facilities adopted the thermophilic anaerobic digester. Although thermophilic digestion may require more heating energy, it possesses advantages such as acceleration of digestion process, reduction of pathogen in the digestate, and increased dewatering ability, etc (Zhen et al., 2017).

2.2 Anaerobic Co-digestion

2.2.1 Background

Anaerobic co-digestion (AcoD) is a process in which two or more substrates are digested together in a single anaerobic digester to enhance biogas production. AcoD is a feasible option to improve methane production. AcoD has already been applied to deal with organic fraction of municipal solid waste (OFMSW) (Sosnowski et al., 2008), fruit and vegetable waste (Bouallagui

et al., 2009), fatty, oil, and grease (FOG) (Davidsson, Lövestedt, la Cour Jansen, Gruvberger, & Aspegren, 2008), and agricultural waste (Converti, Drago, Ghiazza, Borghi, & Macchiavello, 1997), etc. In addition to increasing methane production, AcoD also improves digestion efficiency by balancing macro- and micronutrient (Wan et al., 2011) and reducing toxicity impacts through dilution (Campos et al., 2008).

In addition to investigating co-digestion materials, many researchers also focused on pretreatment of digestion materials to enhance digestion efficiency. Since SS contains mainly intact cells from secondary aeration, pretreatment facilitates the rate-limiting hydrolysis process by rupturing cell walls, releasing intracellular substrates for subsequent microbial degradation. Mechanical, thermal, and chemical pretreatments are the three prevailing pretreatment methods commonly used these days (Carrère et al., 2010).

2.2.2 Anaerobic Co-digestion with Sewage Sludge as the Main Substrate

Based on wastewater treatment plant financial report, up to 60% of the total operating cost is used for SS processes (Baawain, Al-Jabri, & Choudri, 2014). SS is characterized as having low C/N ratio and high buffering capacity. Therefore, it is best to co-digest with other substrates rich in easily biodegradable organic materials, high C/N ratio, and poor buffering capacity, such as, food waste (S.-H. Kim, Han, & Shin, 2004), OFMSW (Sosnowski et al., 2008), fruit and vegetable waste (Bouallagui et al., 2009), FOG (Davidsson, Lövestedt, la Cour Jansen, Gruvberger, & Aspegren, 2008), algae (Olsson et al., 2014), agricultural waste (Converti, Drago, Ghiazza, Borghi, & Macchiavello, 1997), and glycerol (Fountoulakis, Petousi, & Manios, 2010), etc.

Kim et al. (2004) conducted an anaerobic co-digestion study of food waste and SS and discovered that hydrogen production increased with the increasing SS fraction, with a maximum

ratio of 87:13 (food waste: SS) at 3.0%. The maximum specific hydrogen production rate was 111 mL H₂/g VSS/h. Sosnowski et al. (2008) investigated the feasibility of co-digestion SS and OFMSW. The cumulative biogas yield for SS (209 ml/g VS) was lower than that for co-digestion of SS and OFMSW at the mixing ratio of 75:25 volume based (273 ml/g VS) or OFMSW (233 ml/g VS). Bouallagui et al. (2009) studied the feasibility of co-digestion of SS and fruit and vegetable wastes. The results showed that the addition of wasted activated sludge enhanced the biogas yield by 43.8%. Davidsson et al. (2008) conducted the research of co-digestion of SS and grease trap sludge and showed that by adding the grease trap sludge at 10-30% VS to SS, methane yield increased 9-27% without increasing the sludge production. Olsson et al. (2014) did the co-digestion of cultivated microalgae and SS under thermophilic and mesophilic conditions. The methane yield at the mixing ratio of 63:37 (SS: microalgae) based on VS is 23% higher than that of SS alone. Co-digestion of SS and pre-hydrolyzed woody agricultural wastes was conducted by Converti et al. (1997). The OLR from 0.8 to 6.1 g COD/dm³/d with corresponding volatile solids loading rate of 0.6 to 4.5 g COD/dm³/d was studied in this project. The maximum methane production rate was found to be 5.6 mmol/dm³/d at OLR of 4.6 g COD/dm³/d. Fountoulakis et al. (2010) tested the feasibility of co-digestion of crude glycerol and SS in both batch and continuous experiment at 35°C. Only 1% (v/v) glycerol addition could boost methane production from 1106 ± 36 mL CH₄/d to 2353 ± 94 mL CH₄/d. Any further increase of glycerol caused a low pH and VFA accumulation in the digester.

2.2.3 Anaerobic Co-digestion with Manure as the Main Substrate

A large amount of manure is produced every year mainly including PM and cow manure (CM) and several successful demonstrations were performed for AcoD. Manures are characterized as low C/N ratio, high buffering capacity, and high biodegradability. The suitable

co-substrates for manure are rich in carbon, macro and micronutrients, and poor buffering capacity, such as agro-industrial waste (Kaparaju & Rintala, 2005), OFMSW (Macias-Corral et al., 2008), crude glycerol (Astals et al., 2012), and cheese whey (Comino, Riggio, & Rosso, 2012)

Kaparaju (2005) reported that it is possible to treat potato waste with PM in a farm-scale level. The maximum methane yield was observed at mixing ratio of PM to potato waste at 80:20 (VS) with an overall 0.30 to $0.33 \text{ m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$. Maclas-Corral et al. (2008) did the co-digestion of cow manure (CM) and OFMSW. He found that co-digestion of CM and OFMSW produced synergistic effects, which means co-digestion can produce more methane yield than any of the pure controls. At the mixing ratio of 97:3 (dry weight) for OFMSW to CM, 172 m^3 methane per ton of dry waste can be achieved, while only 62 and 37 m^3 methane per ton of dry waste was achieved for OFMSW and CM alone. Astals et al. (2012) indicated that around 400% biogas increased if PM co-digested with 4% of glycerol. Comino et al. (2012) studied the feasibility of co-digestion of cheese whey and cattle slurry and found that at the mixing ratio of 1:1, 621 L/kg VS biogas production could be achieved at an HRT of 42 days.

2.2.4 Anaerobic Co-digestion of Sewage Sludge and Manure

For the anaerobic co-digestion of SS and manure, only limited work were performed since both materials contain low C/N ratios and thus is not the optimal combination for co-digestion. Zhang et al. (2014) did co-digestion of PM and dewatered SS at various ratios under mesophilic conditions. At the mixing ratio of 2 to 1 (PM to dewatered SS), the maximum cumulative methane yield of $316 \text{ mL/g VS}_{\text{added}}$ was obtained, which was 82.4% higher than digesting only dewatered SS.

Borowski and Weatherley (2013) investigated the feasibility of anaerobic co-digestion of solid poultry manure and SS. The results showed no ammonia inhibition and pathogen

inactivation when operating at mesophilic conditions. With the addition of 30% poultry manure, methane production rate did not increase, while carbon dioxide did increase 50% over SS.

Another research (Borowski, et al., 2014) showed that with the addition of 30% poultry manure and 70% SS, 400 dm³/kgVS of biogas was achieved, whereas only 336 dm³/kgVS of biogas was generated from ternary mixture of 70% SS, 20% swine manure, and 10% poultry manure.

Marañón et al. (2012) evaluated co-digestion of cattle manure to food waste and SS under ultrasound pretreatment. With the mixture of 70% manure, 20% food waste and 10% SS (total solid concentration around 4%), the maximum methane production was 603 LCH₄/kg VS_{feed} under the OLR of 1.2 g VS/L day. With the increase of OLR and decrease of HRT, the specific methane production was decreased.

2.3 Kinetic Models

Two models, first-order and modified Gompertz, were commonly used to evaluate methane production kinetics by researchers (Vavilin, Fernandez, Palatsi, & Flotats, 2008; Xie, Wickham, & Nghiem, 2017; Zhang et al., 2014). The difference between these two models are that the modified Gompertz model has two more parameters which are maximum methane production rate (R_{max}) and length of lag-phase (λ). Maximum methane production rate can provide methane production peak value. Lag-phase considers the initial adaptation and hydrolysis period needed by the microorganisms.

2.3.1 The First-order Kinetic Model

Cumulative methane yield can be fitted by the first-order kinetic model to evaluate gas production rate constant. Since biogas production is the result of substrate degradation, this also infers degradation rate constant.

First-order kinetic model

$$M(t) = M_{max} \times (1 - e^{-kt})$$

Where: $M(t)$ is the cumulative methane yield at time t (mL/g VS)

M_{max} is the potential maximum methane yield (mL/g VS)

K is degradation rate constant (d^{-1})

t is duration of the assay (d)

2.3.2 The Modified Gompertz Model

Cumulative methane yield can also be fitted by the modified Gompertz model, which is especially useful when the digestion exhibits a significant lag-period at early stage.

The modified Gompertz model

$$M(t) = M_{max} \exp \left\{ -\exp \left[\frac{R_{max} e}{M_{max}} (\lambda - t) + 1 \right] \right\}$$

Where: $M(t)$ is the cumulative methane yield at time t (mL/g VS)

M_{max} is the potential maximum methane yield (mL/g VS)

R_{max} is the maximum methane production rate (mL/g VS-d)

λ is the length of lag-phase (d)

t is the duration of the assay (d)

2.4 Summary of Literature Review

Co-digestion is a promising technology that not only produces additional biogas but also serves as an alternative outlet for waste treatment. For wastewater treatment utilities, co-digestion can produce additional revenue through both increased gas production and fees from accepting the wastes. Although PM presents a great potential for gas production, its low C/N ratio can be a problem to co-digest with SS. Therefore, a feasibility study needs to be conducted and verified, especially on the potential nitrogen increase in the digestates.

Chapter 3 Materials and Methods

3.1 Digestion Materials

SS was collected from Penn State University WWTP. It was the combination of primary sludge and secondary sludge with the mixing ratio of 1:1. PM was obtained from Penn State University Pig Farm. The mesophilic inoculum used in this study was obtained from the primary anaerobic digester of the Penn State University WWTP.

All the samples were obtained and transported in a cooler with ice packs from the WWTP and pig farm to the laboratory within 3 hours. Samples were homogenized with a blender (Robeson 12 speed, USA) for 5 min to reduce error. PM was then sieved through 0.5 mm sieve to remove coarse materials. All the parameters were measured within 24 hours, including pH, COD, SCOD, TAN, TN, VFA, and Alkalinity. SS and PM samples were frozen at -20°C to prevent biological decomposition and inoculum was put into the shaking water bath (18L Shaking bath, VWR) under 35°C to reduce residual organic materials in the inoculum until there was no biogas production. Before setting up this study, all frozen samples, including PM and SS, were transferred to a refrigerator at 4°C for 1 day. Due to the low VS concentration in PM and inoculum, both PM and inoculum were concentrated to 2.24% and 1.65% in order to meet target solid concentration in the experimental digesters.

Table 3-1. Characteristics of pig manure, concentrated pig manure, sewage sludge, inoculum, and concentrated inoculum used in this study

Characteristics	Unit	PM	SS	Inoculum	Conc. PM	Conc. Inoculum
TS	% FM	1.57 (0)	2.16 (0)	1.29 (0)	3.40 (0)	2.41 (0)
VS	% FM	1.06 (0)	1.87 (0)	0.97 (0)	2.24 (0)	1.65 (0)
VS/TS	%	67.3 (0)	86.6 (0)	75.2 (0)	65.8 (0)	68.5 (1)
SCOD	mg/L	14510 (82)	408 (8)	255 (4)	14700 (183)	276 (9)
COD	mg/L	27200 (624)	21800 (458)	11300 (276)	28100 (681)	14400 (218)
TAN	mg/L	660 (20)	70.0 (8)	202 (3)	627 (12)	215 (5)
VFA	mg/L as acetic	1550 (68)	254 (10)	--	1500(65)	--
TN	mg/L	1600 (80)	1050 (107)	442 (3)	1820(20)	597(10)
pH		6.44 (0)	6.53 (0)	6.92 (0)	6.52(0)	6.52(0)
Alkalinity	mg/L as CaCO ₃	4920 (120)	300 (4)	2030 (14)	3610(6)	1420(18)

FM: fresh material; PM: pig manure; SS: sewage sludge; Conc. PM: concentrated pig manure; Conc. Inoculum: concentrated inoculum. The values in the table are expressed as mean and one standard deviation in parentheses (n=3). Did not do the VFA test for inoculum and Con. inoculum.

3.2 Experimental Design and Set-up

In this experiment, co-digestion of SS and PM in the batch reactor was carried out with the ratio of 21:1, 14:1, and 7:1 based on VS, plus the pure SS, PM, and inoculum controls. SS/PM ratios of 21:1, 14:1, and 7:1 are based on below averaging PM production, averaging PM production, and the maximum PM production, respectively. All of the batch experiments were performed in triplicate. Each 160 mL serum bottles with the working volume of 100 mL and 60 mL headspace was used as a digester. Each reactor contained 40 mL of inoculum and 60 mL substrates with $16.5 \text{ gVS}_{\text{added}}/\text{L}$. The feed to inoculum (F/I) ratio is the initial ratio of VS in the feedstock to the VS in the inoculum at the beginning of each batch reactor. In this experiment, F/I was controlled at 1.5 which was in the range of 0.1-2 as recommended by Braguglia et al. (2006). Before seeding the mesophilic digesters, inoculum was placed in an incubator for 20 d until biogas production ceased to minimize the contribution from residual organic materials contained in the inoculum. After adding the required amounts of inoculum and substrate listed in Table 3-2, each bottle was filled with distilled water to reach the desired volume. The initial pH of the mixture in each digester was adjusted to 7.0 ± 0.1 by using 1 M HCl or 1 M NaOH. The digesters were flushed with 100% pure nitrogen for approximately 2-3 min to ensure anaerobic conditions. Then the bottles were capped with natural rubber sleeve stoppers and aluminum crimp cap. Bottles were incubated at 35°C in a temperature-controlled shaking water bath (VWR, USA) with 90 rpm to provide continual homogeneous mixing of the mixtures. Gas production and methane percent were monitored daily. After methane production ceased, the final digestates were analyzed for TS, VS, pH, TAN, VFA, and alkalinity. The experimental set up diagram is in the appendix.

Table 3-2. Substrate composition in different digesters

Substrate type	SS/PM ratio				
	1:0	21:1	14:1	7:1	0:1
PM (g)	-	0.05	0.07	0.12	1.00
SS (g)	1.00	0.95	0.92	0.87	-

3.3 Sample Analyses

3.3.1 Chemical Analysis

TS and VS were measured based on Standard method 2540 (APHA). COD (COD 200-15000mg/L kit, Cat. 2415915), SCOD (COD 20-1500mg/L kit, Cat. 2125925), TAN (high range ammonia reagent 0-50mg/L N kit, Cat. 2606945), and TN (high range total nitrogen hydroxide reagent 10-150mg/L N kit, Cat. 271405) were tested by HACH analysis kit. pH was measured by a pH meter (symphony™, Benchtop meters, VWR). Alkalinity was tested using Standard method 2320 (APHA). Filtration samples were used for SCOD, alkalinity, TAN, and VFA measurement. All the samples were centrifuged first by using the Centrifuge 5810R (Eppendorf centrifuge, USA) at 3500 xg for 20 min at 4°C, and then supernatant was filtered through a 0.45µm cellulose filter.

3.3.2 Volatile Fatty Acid Measurement

The procedure used for the determination of total VFA in this study was based on the Nordmann-titration method (Kafle & Kim, 2011). In short, VFA can be calculated by recording how much 0.1 N H₂SO₄ is being used to reach pH 5.0 and 4.4, and using the following equation to calculate VFA.

$$\text{VFA} = [((\text{Con B} - \text{Con A}) \times 20 \text{ mL/EF} \times 1.66) - 0.15] \times 500 \text{ [mg L}^{-1} \text{ acetate]}$$

Where

Con A = mL of 0.1 N H₂SO₄ consumed by the sample to reach a 5.0 pH value

Con B = mL of 0.1 N H₂SO₄ consumed by the sample to reach a 4.4 pH value

EF = extracted fluid volume (sample volume) (mL)

3.3.3 Biogas Measurement

The method to measure biogas was by puncturing the rubber septum with a syringe connected to a burette. Biogas volume for each digester was measured every day until there was no biogas production. The biogas from the blank digester (inoculum only) was subtracted from the biogas produced from each digester (inoculum and substrates) to get the biogas generated just from substrates. Then all the biogas produced from the digesters was corrected to standard temperature and pressure (STP) conditions (0°C and 1 atm). Figure 3-1 showed the diagram of biogas volume measurement.

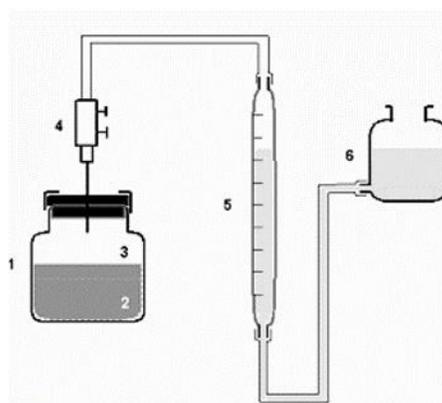


Figure 3-1. The diagram for biogas volume measurement.

3.3.4 Methane Content Measurement

The methane content in biogas was measured using Hewlett Packard 6890 gas chromatography (GC, Agilent Technology, USA), equipped with a flame ionization detector (VWR, USA) using a $30\text{ m} \times 0.53\text{ mm} \times 20\text{ }\mu\text{m}$ Rt-QS-BOND column (VWR, USA). 20%-80% methane standard biogas (80% methane calibration gas, GASCO, USA) was carried out for the methane standard curve. Helium was used as the carrier gas with a flow rate of 30 mL/min. The inlet and detector temperature was set at 240°C. The oven temperature was initially held at 40°C for 2 min, followed by a 20°C/min ramp until 225°C, and a 5-min final hold.

3.4 Kinetic Model Analysis

SigmaPlot 13.0 was used for the estimation of cumulative methane yield and degradation rate constant for the first-order kinetic model, as well as the cumulative methane yield, maximum methane production rate, and length of lag-phase for the modified Gompertz model.

3.5 Statistical Analysis

ANOVA (95% confidence interval) and Student's t-test (95% confidence interval) were calculated by Microsoft Excel 2010 in this study to distinguish whether there is difference between two means or among three or more means.

Chapter 4 Results and Discussion

4.1 Volatile Solid Removal Rate

The percent VS removal of SS and PM were 78.2% and 56.6%, while the percent VS removal increased with an increasing percentage of SS in the mixing substrates (Table 4-1). The percent VS removals were 71.4%, 69.5%, and 56.3% for the mixing ratios of 21, 14, and 7, respectively. Percent VS removal of PM was lower than that of SS alone. This might be due to larger fraction of unbiodegradable organic matters presented in PM than SS. Similar results were also reported by other researchers (Panichnumsin, Nopharatana, Ahring, & Chairasert, 2010). For residual solids, co-digestion of PM and SS increased residual solid by 0.27%, 0.65%, and 9.27% for 21:1, 14:1, and 7:1 SS/PM ratio respectively when comparing with pure SS. These are the increased biosolids that need to be disposed of or further managed. When comparing the actual VS reduction from the co-digestion reactors and those estimated based on mono-digestion of SS and PM, it is apparent that co-digestion significantly enhanced VS reduction (Table 4-1). This shows that co-digestion exhibits a synergistic effect with better efficiency over mono-digestion.

Table 4-1. Actual VS removal from co-digestion and estimated VS removal based on mono-digestion

SS/PM ratio	SS	PM	21	14	7
VS removal (%)	78.2	56.6	71.4	69.5	56.3
Actual VS removed (g)	1.29	0.93	1.18	1.15	0.93
VS removed per mono-digestion (g)			0.76 ^a	0.76 ^a	0.75 ^a
VS removed from SS (g)			0.74	0.72	0.68
VS removed from PM (g)			0.03	0.04	0.07

SS: sewage sludge; PM: pig manure

^a Significantly different from actual VS removed (p<0.05)

4.2 Methane Production

Figure 4-1 showed the methane production rate and cumulative methane yield for all the digesters. As shown in Figure 4-1 A and B, there was no lag-phase during the operation of the anaerobic digestion process, except for a short lag-phase for PM. This was due to the inoculum used in this study being collected from the WWTP that is actively digesting SS. In addition, almost no industrial wastewater stream discharged to Penn State University WWTP, which minimized the chance of toxic heavy metals or other toxic compounds to inhibit. Other researchers also pointed out no lag-phase at the start part of the digestion (Koupaie, Leiva, Eskicioglu, & Dutil, 2014; Zhang et al., 2014). The lag-phase showed by PM indicated that a short adaptation or hydrolysis time was needed before gas production.

Figure 4-1 A and B also showed two peak days (day 5 and 11) for PM, while only one peak day (day 5) for SS. High SCOD concentration (14700mg/L) in PM might be the reason for the first peak, since hydrolyzed SCOD can be easily assessed by microorganisms for degradation. Particulate organic matters will need extra time to hydrolyze before degradation (Kafle, Kim, & Sung, 2013), and thus result in the delayed 2nd peak. Gas production with two distinct peaks were also observed by other researchers (Xie, Lawlor, Frost, Hu, & Zhan, 2011; Zhang et al., 2014).

At the end of this study, CMY of SS (182 mL/g VS_{added}) was significantly lower (24%) than that for PM (239 mL/g VS_{added}) ($p < 0.05$). This indicated that substrate type has significant effect on methane yield. Due to the poorly biodegradable cell structure in most of the organics present in SS, SS is regarded as a material that is uneasily biodegradable and has poor biogas production (Yang, Wang, & Wang, 2010), while PM has been demonstrated to be a great substrate for anaerobic digestion (Chae, Jang, Yim, & Kim, 2008; Wu, Yao, Zhu, & Miller, 2010).

Figure 4-1 C, D, and E, showed that only one peak of daily methane production was observed when SS and PM were mixed for co-digestion at ratios of 21:1, 14:1, and 7:1, which

occurred on day 5 (24.2 mL/g VS_{added-d}), day 6 (23.4 mL/g VS_{added-d}), and day 5 (22.5 mL/g VS_{added-d}), respectively. Xie et al. (2011) in his research also pointed out that only one peak of the daily methane production was observed during the co-digestion of concentrated PM and grass silage. Compared with PM (day 5 and 11), co-digestion assays removed the 2nd peak, which implied an accelerated hydrolysis. This phenomenon was also reported by Zhang et al. (2014), who indicated that this is contributed to the accelerated hydrolysis from balanced nutrients in the mixtures.

The CMYs of the co-digestion mixtures increased with the increasing percentage of PM. The CMYs in the co-digestion of SS and PM at the mixing ratios of 21:1, 14:1, and 7:1 were 190, 191, and 200 mL/g VS_{added}, respectively, which were 4%, 5%, and 10% larger than methane yield from digesting SS alone. However, the CMYs for co-digestion were 16% to 20% less than that of PM, which was 239 mL/g VS_{added}. This work only tested up to 7:1 co-digestion ratio based on the need for the University Farm. Further increase of PM may continue to raise CMY in the mixture. The methane percentage in all the samples were above 50%, while co-digestion samples (around 55%) had a lower methane percentage than pure SS and PM (around 65%). When comparing the actual methane production from co-digestion and those estimated from mono-digestion, it appeared that additional 50%, 33%, and 36% of methane can be generated from co-digestion of PM and SS at the mixing ratio of 21, 14, and 7, respectively, which again demonstrated a positive synergistic effect of co-digestion in enhancing biogas production.

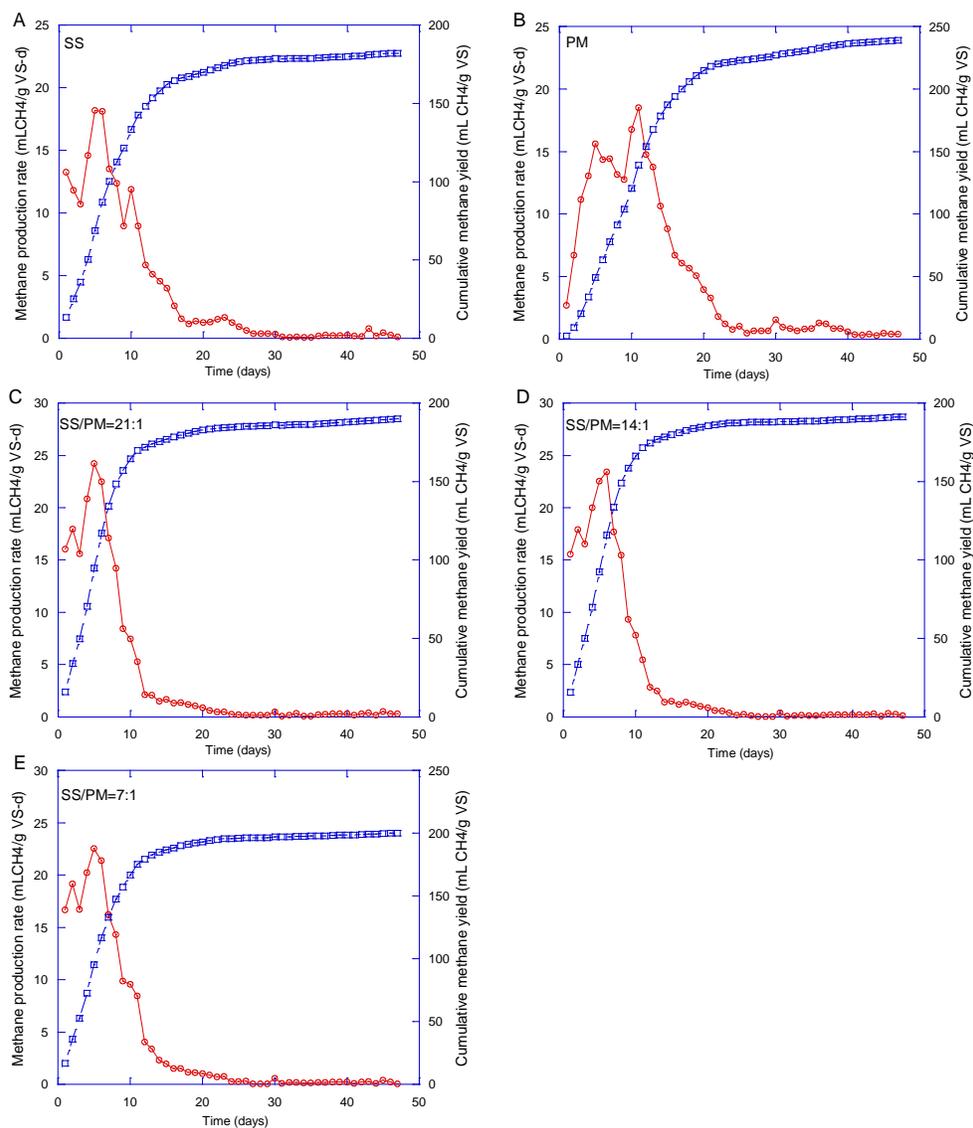


Figure 4-1. Methane production rate and cumulative methane yield for sewage sludge (SS) only (A), pig manure (PM) only (B), SS to PM ratios at 21:1 (C), 14:1 (D), and 7:1 (E) incubated at 35°C. Circle represents methane production rate and square represents cumulative methane yield. The values are average of three replication bottles.

4.3 Process Stability

4.3.1 pH

pH is a crucial parameter in the anaerobic digestion process. pH can not only affect the growth of microorganisms, but also impact the composition of total ammonia nitrogen (Hansen, Angelidaki, & Ahring, 1999; Kroecker, Schulte, Sparling, & Lapp, 1979). An increase in pH could result in an increase in free ammonia, which can penetrate cell wall and cause toxicity to the microorganisms (Kroecker et al., 1979). A decrease of pH indicates the accumulation of VFA, which can damage microorganisms. So, the optimal pH range for anaerobic digestion is between 6.5 to 8.2 (Dinamarca, Aroca, Chamy, & Guerrero, 2003). In this study, after digestion all the pH from the digesters were around 7, except for SS and PM, which were 7.29 and 7.56 respectively. The slight increase of pH could be due to the degradation of VFAs, or the increase of free ammonia released from the organic nitrogen. Since VFA and TAN were only measured on pure SS and PM prior to digestion, change of VFA and TAN cannot be verified. However, the final VFAs in the digestates were only 186 and 228 mg/L for SS and PM which assure no accumulation (Table 4-2). Zhang et al. (2014) also reported a slight pH increased from 7.00 to 7.69 at the end of digestion for PM.

4.3.2 Volatile Fatty Acid/ Alkalinity Ratio

Anaerobic digestion stability is also associated with VFA and alkalinity. The first phase of anaerobic digestion is hydrolyzing and degrading the complex organic matter to volatile fatty acids. The accumulation of VFA can cause the pH to decrease, resulting in unstable digestion. Alkalinity represents buffering capacity in the digester, which resist the change of pH. VFA/Alkalinity ratio, therefore, serves as an early warning of digester performance before the

actual pH drop. In this experiment, the final VFA/ALK ratios of all the digesters were in the range of 0.05-0.07, which is far from the critical limitation. As reported, the anaerobic digestion process was stable without an acidification risk when the VFA/ALK ratio was less than 0.3-0.4 (R Borja et al., 2004). The reason why the resulting ratio is far less than the limit is likely due to the fact that the reactors were operated under batch condition and were measured once gas production terminated, i.e. most VFAs were converted to methane/CO₂.

4.3.3 Total Ammonia Nitrogen

The biggest concern in using PM as the co-digestion material for SS is its low C/N ratio which may generate high residual nitrogen in digestates which then return to the mainstream creating nitrogen burden to its treatment. Interestingly, TANs for co-digestion of PM and SS at the mixing ratios of 21:1 (287 mg/L), 14:1 (320 mg/L), and 7:1 (367mg/L) were significantly lower than those of SS (440mg/L) and PM (580 mg/L) mono-digestion ($p < 0.05$). Based on the nitrogen balance, about 61-66% nitrogen were in the solid form for co-digestion while only 44-45% for mono-digestion (Table 4-3). This phenomenon might be due to struvite precipitation in co-digestion assays (Taddeo & Lepistö, 2015; Marti et al., 2008; Yilmazel et al., 2011). Therefore, the possibility of increased nitrogen return to the mainstream from codigesting PM may not be a concern since struvite can be removed with dewatering process. Further studies, however, is needed to verify if they are indeed struvite.

The highest TAN (580mg/L) was from the PM digester which was still lower than the reported critical concentration (3000mg/L) to inhibit digestion (McCarthy, 1964). Therefore, co-digestion of PM and SS should not result in ammonia inhibition. Marañón et al. (2012) also reported no ammonia inhibition when co-digesting cattle manure with food waste and SS.

Table 4-2. Physical-chemical properties of digestates

SS/PM	SS	PM	21:1	14:1	7:1
TAN (mg/L)	440 (32.7)	580 (51.6)	287 (30.1)	320 (25.3)	367 (30.1)
pH	7.29 (0.09)	7.56 (0.23)	6.96 (0.03)	6.96 (0.01)	7.08 (0.01)
Alkalinity (mg CaCO ₃ /L)	4010 (60.0)	4470 (34.6)	2500 (52.8)	2630 (37.2)	2890 (30.3)
VFA (mg CH ₃ COOH/L)	186 (8.49)	228 (18.0)	143 (14.5)	169 (7.07)	224 (11.7)
VFA/Alk	0.045	0.051	0.057	0.064	0.077
VS _{removal} (%)	78.2%	56.6%	71.4%	69.4%	56.3%

The values in the table are expressed as mean and one standard deviation in parentheses (n=3)

Table 4-3. Nitrogen balance calculation for each assay

SS/PM	SS	PM	21:1	14:1	7:1
TN in the feed (mg/l)	802	1048	853	878	945
TN in the digestate (mg/l)	786	1054	844	871	935
Percent recovery	98%	100%	99%	99%	99%
TN in the supernatant (mg/l)	440	580	287	320	367
TN in the solid (mg/l)	346	474	557	551	568
Solid nitrogen percentage	44%	45%	66%	63%	61%

4.4 Kinetic Model

The CMYs are the results of biological degradation of volatile solids and therefore, its production corresponds to degradation of substrates. Degradation kinetics of digestion, therefore, is commonly derived through the analyses of biogas production kinetics. Two models, the first-order and the modified Gompertz, were commonly used by researchers for digester kinetic analysis (Xie, Wickham, & Nghiem, 2017; Zhang et al., 2014). For better comparisons, the CMYs obtained from all digesters were fitted with both models to further analyze the impact of co-digestion.

4.4.1 The First-order Kinetic Model

K, degradation rate constant, for pure SS, pure PM, and SS/PM ratio of 21:1, 14:1, and 7:1 were 0.119, 0.074, 0.162, 0.160, and 0.152 d⁻¹, respectively (Table 4-4). All co-digestion mixtures exhibited a faster degradation rate constant than the two pure controls, indicating co-digestion can facilitate the degradation process, elucidating the synergistic effect. This synergistic effect was also confirmed by Xie et al. (2017) when co-digesting food waste and paper pulp reject, and contributed it to the possibly enhanced nutrient balance and reduced toxicity through dilution. Neither PM or SS used in this study were unlikely to contain toxic materials, and the C/N ratio in the mixtures were actually less optimal than the pure controls. Therefore, other reasons may have been in play which need further verification. Between SS and PM, the K value for SS (0.117 d⁻¹) was 1.7 times higher than that of PM (0.074 d⁻¹). This is likely due to pig manure having a lag-phase at the early stage of the digestion and a later second peak corresponding to slowly degradable organics, and thus slowed down the overall degradation rate constant. The obtained rate constants are all comparable to the other researches, with values in the range of 0.003-0.22 d for SS and, 0.02-0.56 for PM (Xie et al., 2011; Parameswaran & Rittmann, 2012). All predicted and measured CMY were less than 1.5% difference except for PM (6.69%). This is likely due to PM alone having a lag-phase that interferes with the first-order kinetic model. Although the overall R-squares are between 0.98-0.99 for our tested reactors, it is anticipated that the R-square will drop as the lag-phase increases.

4.4.2 The Modified Gompertz Model

The maximum methane production rate (R_m) were 15.3, 16.6, 22.3, 22.5, and 21.1 mL CH₄/g VS_{added}/d for pure SS, pure PM and SS/PM ratio of 21:1, 14:1, and 7:1, indicating all co-

digestion mixtures are larger than the pure controls (Table 4-4). The R_m was improved up to 47% in co-digestion compared to pure SS, and 36% compared to PM. The higher R_m values for the 3-co-digestion treatments were likely due to the merge of the two distinct degradation peaks for PM, which resulted in a much larger maximum degradation rate. λ means the length of lag-phase. Only pure PM had a lag-time (λ) of 2.36 day, while the rest of the mixtures and controls were all less than 1 day. This means co-digestion can reduce adaptation time for PM or the rate-limiting hydrolysis period, and thus achieve an overall shortened degradation period. T_{90} in Table 4-4 is defined as the time to achieve 90% of CMY in the digestion. T_{90} for the 3 co-digestions were only 12-13 d which are shorter than the SS and PM controls (16 and 21 d). This indicates that the time to achieve 90% degradation is shortened in co-digestion mixtures. Similarly, T_{ef} is the term used to identify effective methane production time, which was calculated by T_{90} subtract λ . In this study, T_{ef} for co-digestion mixtures were 11.3 -12.5 d, again shorter than SS and PM controls (15.5 and 18.6 d). Both parameters also showed that PM requires the longest time to complete gas production, again likely the cause of the needed adaptation lag-phase and the existence of slowly biodegradable materials. Co-digestion removed this lag-phase and facilitated the rate-limiting hydrolysis process, and thus completed gas production within the shortest time, consequently resulted in the largest maximum gas production rate.

The R-squares for all reactors were all close to 1, which indicates the modified Gompertz model is better fit for this type of experiment than the first-order kinetic model. Furthermore, since lag-phase is included in this model, the suitability for this model is even more apparent for the PM which exhibits a lag-phase (Figure 4-2). Overall, the modified Gompertz model works well for both reactors with or without a lag-phase and thus is a better choice for digestion model. However, the first-order kinetic model allows interpretations to the overall degradation rate constant, and thus also provides values, but should be used with caution especially when the lag-phase is apparent.

Based on the kinetic analysis, synergistic effect in term of length of lag-phase, maximum methane production rate, effective methane production time, and degradation rate constant can be achieved when co-digesting PM and SS.

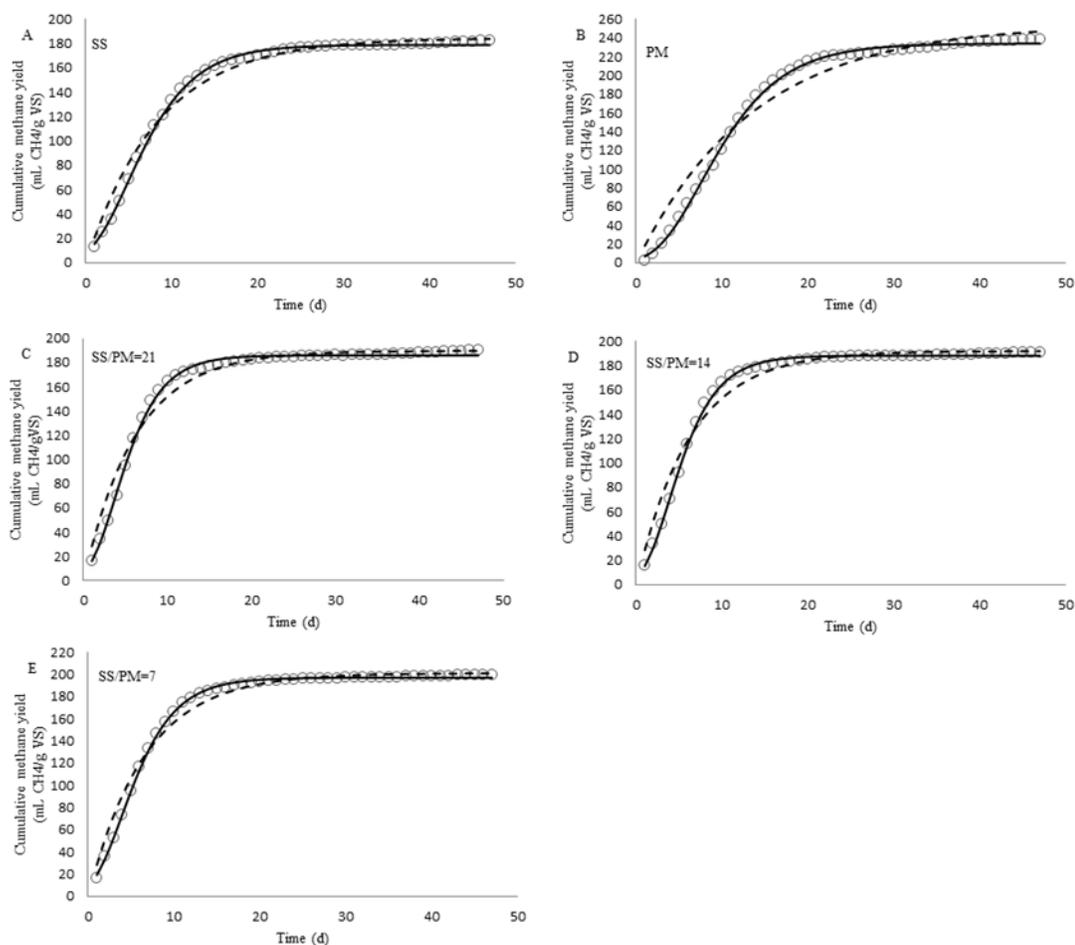


Figure 4-2. Comparison among actual cumulative methane yield and predicted cumulative methane yield from the first-order kinetic model and the modified Gompertz model for sewage sludge (SS) only (A), pig manure (PM) only (B), SS to PM ratios at 21:1 (C), 14:1 (D), and 7:1 (E). Circle represents actual cumulative methane yield, dash line represents predicted cumulative methane yield from the first-order kinetic model, and solid line represents predicted cumulative methane yield from the modified Gompertz model.

Table 4-4. Calculated parameters for the first-order and the modified Gompertz models

Models	SS/PM	R ²	K (d ⁻¹)	R _m (ml/gVS-d)	λ(d)	T ₉₀ (d)	T _{ef} (d)	Cumulative methane yield (ml/gVS _{added})		
								Predicted	Measured	Difference (%)
First-order kinetic Model	SS	0.990	0.119	-	-	-	-	184	182	1.30
	PM	0.980	0.074	-	-	-	-	255	239	6.69
	21:1	0.986	0.162	-	-	-	-	190	190	0.24
	14:1	0.985	0.160	-	-	-	-	192	191	0.42
	7:1	0.989	0.152	-	-	-	-	201	200	0.61
Modified Gompertz Model	SS	0.999	-	15.3	0.55	16.0	15.5	179	182	1.78
	PM	0.999	-	16.6	2.36	21.0	18.6	234	239	2.01
	21:1	0.998	-	22.3	0.68	12.0	11.3	186	190	2.19
	14:1	0.999	-	22.5	0.75	12.0	11.3	188	191	1.55
	7:1	0.999	-	21.1	0.47	13.0	12.5	197	200	1.40

Chapter 5 Conclusion

1. PM produced more methane than SS and as a result, increasing PM in co-digestion also increased methane yield. The maximum methane yield occurred at SS/PM ratio of 7:1 with a 10% increase at 200 mL CH₄/g VS_{added} compared with SS alone at 182 mL CH₄/g VS_{added}. This is also the maximum co-digestion need from the University Farm in winter seasons. So, the optimal SS/PM ratio is 7:1.
2. All mixing ratios showed stable digestions as indicated by the pH, TAN, and VFA/ALK ratios of the final digestates.
3. Based on the nitrogen balance calculation, TN in the solid of co-digestion were higher than those of pure SS and PM, which might due to struvite precipitation. The liquid fraction of the digestates contain less TN in co-digestion treatments than the pure controls, indicating a potential reduction of nitrogen in the return stream.
4. The first-order kinetic model showed faster gas production kinetics by the 3 co-digestion treatments than the pure controls, which implied the existence of a positive synergistic effect by co-digestion.
5. The modified Gompertz model showed the highest maximum gas production rate, the reduction of lag-period, and a shortened gas production period by co-digestion, which implies a shortened adaptation period or facilitated hydrolysis had occurred in the mixtures. This faster kinetics implies a shorter digestion time than the original wastewater sludge digestion which can help accommodate the increased digestion materials from the co-digestion process.
6. Both the first-order kinetic model and the modified Gompertz model showed good fit to the data produced, but the first-order kinetic model is limited to degradation without a

lag-period. Overall, the modified Gompertz model proved to be a better fit for digester kinetic analysis than the first-order kinetic model.

References

- Al-Kanani, T., Akochi, E., MacKenzie, A., Alli, I., & Barrington, S. (1992). Odor control in liquid hog manure by added amendments and aeration. *Journal of environmental quality*, 21(4), 704-708.
- Angelidaki, I., Sorensen, A., & Schmidt, J. (2007). Notes for the course 12133: environmental biotechnology, technical university of Denmark. *Lyngby, Denmark*.
- APHA, A. WEF, 1998. Standard methods for the examination of water and wastewater 20th ed. *Persulfate Method. APHA, AWWA & WEF, Washington*.
- Astals, S., Nolla-Ardèvol, V., & Mata-Alvarez, J. (2012). Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate. *Bioresource Technology*, 110, 63-70.
- Baawain, M. S., Al-Jabri, M., & Choudri, B. (2014). Characterization of Domestic Wastewater Sludge in Oman from Three Different Regions and Recommendations for Alternative Reuse Applications. *Iranian journal of public health*, 43(2), 168-177.
- Bilitewski, B., Härdtle, G., Marek, K., Bilitewski, B., Härdtle, G., & Marek, K. (1997). Waste Disposal. *Waste management*, 259-338.
- Borja, R., González, E., Raposo, F., Millán, F., & Martín, A. (2002). Kinetic analysis of the psychrophilic anaerobic digestion of wastewater derived from the production of proteins from extracted sunflower flour. *Journal of agricultural and food chemistry*, 50(16), 4628-4633.
- Borja, R., Rincón, B., Raposo, F., Dominguez, J., Millán, F., & Martín, A. (2004). Mesophilic anaerobic digestion in a fluidised-bed reactor of wastewater from the production of protein isolates from chickpea flour. *Process Biochemistry*, 39(12), 1913-1921.

- Borja, R., Sánchez, E., & Weiland, P. (1996). Influence of ammonia concentration on thermophilic anaerobic digestion of cattle manure in upflow anaerobic sludge blanket (UASB) reactors. *Process Biochemistry*, *31*(5), 477-483.
- Borowski, S., Domański, J., & Weatherley, L. (2014). Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. *Waste management*, *34*(2), 513-521.
- Borowski, S., & Weatherley, L. (2013). Co-digestion of solid poultry manure with municipal sewage sludge. *Bioresource Technology*, *142*, 345-352.
- Bouallagui, H., Lahdheb, H., Romdan, E. B., Rachdi, B., & Hamdi, M. (2009). Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition. *Journal of Environmental Management*, *90*(5), 1844-1849.
- Braguglia, C. M., Mininni, G., Tomei, M. C., Rolle, E., & Snyman, H. G. (2006). Effect of feed/inoculum ratio on anaerobic digestion of sonicated sludge. *Water Science & Technology A Journal of the International Association on Water Pollution Research*, *54*(5), 77-84.
- Brumm, M., Sutton, A., & Jones, D. (1980). Effect of season and pig size on swine waste production. *Transactions of the ASAE*, *23*(1), 165-0168.
- Cai, M., Wilkins, D., Chen, J., Ng, S.-K., Lu, H., Jia, Y., & Lee, P. K. (2016). Metagenomic reconstruction of key anaerobic digestion pathways in municipal sludge and industrial wastewater biogas-producing systems. *Frontiers in microbiology*, *7*.
- Campos, E., Almirall, M., Mtnes-Almela, J., Palatsi, J., & Flotats, X. (2008). Feasibility study of the anaerobic digestion of dewatered pig slurry by means of polyacrylamide. *Bioresource Technology*, *99*(2), 387-395.
- Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenès, J. P., Steyer, J. P., & Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: a review. *Journal of hazardous materials*, *183*(1), 1-15.

- Chae, K., Jang, A., Yim, S., & Kim, I. S. (2008). The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology*, *99*(1), 1-6.
- Chastain, J., & Henry, S. (1999). Management of Lagoons and Storage Structures for Swine Manure: Swine Version II. chapter 4 in. *Confined Animal Manure Managers Certification Program Manual: Swine Version II*, 4-1.
- Chen, Y., Cheng, J. J., & Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. *Bioresource Technology*, *99*(10), 4044-4064.
- Comino, E., Riggio, V. A., & Rosso, M. (2012). Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. *Bioresource Technology*, *114*, 46-53.
- Converti, A., Drago, F., Ghiazza, G., Borghi, M. D., & Macchiavello, A. (1997). Co-digestion of municipal sewage sludges and pre-hydrolysed woody agricultural wastes. *Journal of Chemical Technology and Biotechnology*, *69*(2), 231-239.
- Cuetos, M. J., Fernández, C., Gómez, X., & Morán, A. (2011). Anaerobic co-digestion of swine manure with energy crop residues. *Biotechnology and Bioprocess Engineering*, *16*(5), 1044-1052.
- Davidsson, Å., Löfstedt, C., la Cour Jansen, J., Gruvberger, C., & Aspegren, H. (2008). Co-digestion of grease trap sludge and sewage sludge. *Waste management*, *28*(6), 986-992.
- Dinamarca, S., Aroca, G., Chamy, R., & Guerrero, L. (2003). The influence of pH in the hydrolytic stage of anaerobic digestion of the organic fraction of urban solid waste. *Water Science and Technology*, *48*(6), 249-254.
- Fountoulakis, M., Petousi, I., & Manios, T. (2010). Co-digestion of sewage sludge with glycerol to boost biogas production. *Waste management*, *30*(10), 1849-1853.
- Gerardi, M. H. (2003). *The microbiology of anaerobic digesters*: John Wiley & Sons.

- Gonzalez-Fernandez, C., Sialve, B., & Molinuevo-Salces, B. (2015). Anaerobic digestion of microalgal biomass: challenges, opportunities and research needs. *Bioresource Technology*, *198*, 896-906.
- Guo, J., Peng, Y., Ni, B.-J., Han, X., Fan, L., & Yuan, Z. (2015). Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing. *Microbial cell factories*, *14*(1), 33.
- Hansen, K. H., Angelidaki, I., & Ahring, B. K. (1999). Improving thermophilic anaerobic digestion of swine manure. *Water research*, *33*(8), 1805-1810.
- Hartmann, H., & Ahring, B. K. (2006). Strategies for the anaerobic digestion of the organic fraction of municipal solid waste: an overview. *Water Science and Technology*, *53*(8), 7-22.
- Hashimoto, A. G. (1983). Thermophilic and mesophilic anaerobic fermentation of swine manure. *Agricultural wastes*, *6*(3), 175-191.
- Hatfield, J., Brumm, M., & Melvin, S. (1998). Swine manure management. *Agricultural uses of municipal, animal, and industrial byproducts*, *44*, 78-90.
- Huang, G., Wong, J., Wu, Q., & Nagar, B. (2004). Effect of C/N on composting of pig manure with sawdust. *Waste management*, *24*(8), 805-813.
- Jain, S., Jain, S., Wolf, I. T., Lee, J., & Tong, Y. W. (2015). A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. *Renewable and Sustainable Energy Reviews*, *52*, 142-154.
- Kafle, G. K., & Kim, S. H. (2011). Sludge exchange process on two serial cstrs anaerobic digestions: process failure and recovery. *Bioresource Technology*, *102*(13), 6815-6822.

- Kafle, G. K., Kim, S. H., & Sung, K. I. (2013). Ensiling of fish industry waste for biogas production: a lab scale evaluation of biochemical methane potential (BMP) and kinetics. *Bioresource Technology*, 127, 326-336.
- Kaparaju, P., & Rintala, J. (2005). Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. *Resources, Conservation and Recycling*, 43(2), 175-188.
- Kayhanian, M. (1994). Performance of a high - solids anaerobic digestion process under various ammonia concentrations. *Journal of Chemical Technology and Biotechnology*, 59(4), 349-352.
- Kelleher, B., Leahy, J., Henihan, A., O'dwyer, T., Sutton, D., & Leahy, M. (2002). Advances in poultry litter disposal technology—a review. *Bioresource Technology*, 83(1), 27-36.
- Kevin, G. P. D., F.C. (2010). Pennsylvania agricultural statistics 2009-2010.
- Kim, M., Ahn, Y.-H., & Speece, R. (2002). Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water research*, 36(17), 4369-4385.
- Kim, S.-H., Han, S.-K., & Shin, H.-S. (2004). Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. *International Journal of Hydrogen Energy*, 29(15), 1607-1616.
- Koster, I., & Lettinga, G. (1988). Anaerobic digestion at extreme ammonia concentrations. *Biological Wastes*, 25(1), 51-59.
- Koupaie, E. H., Leiva, M. B., Eskicioglu, C., & Dutil, C. (2014). Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: Process and cost-benefit analysis. *Bioresource Technology*, 152, 66-73.
- Kroeker, E., Schulte, D., Sparling, A., & Lapp, H. (1979). Anaerobic treatment process stability. *Journal (Water Pollution Control Federation)*, 718-727.
- Kugelman, I. J., & McCarty, P. L. (1965). Cation toxicity and stimulation in anaerobic waste treatment. *Journal (Water Pollution Control Federation)*, 97-116.

- Li, C., Champagne, P., & Anderson, B. C. (2011). Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions. *Bioresource Technology*, *102*(20), 9471-9480.
- Long, J. H., Aziz, T. N., Francis, L., & Ducoste, J. J. (2012). Anaerobic co-digestion of fat, oil, and grease (FOG): a review of gas production and process limitations. *Process Safety and Environmental Protection*, *90*(3), 231-245.
- Lu, X., Zhen, G., Chen, M., Kubota, K., & Li, Y.-Y. (2015). Biocatalysis conversion of methanol to methane in an upflow anaerobic sludge blanket (UASB) reactor: Long-term performance and inherent deficiencies. *Bioresource Technology*, *198*, 691-700.
- Lübken, M., Wichern, M., Schlattmann, M., Gronauer, A., & Horn, H. (2007). Modelling the energy balance of an anaerobic digester fed with cattle manure and renewable energy crops. *Water research*, *41*(18), 4085-4096.
- Luste, S., & Luostarinen, S. (2010). Anaerobic co-digestion of meat-processing by-products and sewage sludge—Effect of hygienization and organic loading rate. *Bioresource Technology*, *101*(8), 2657-2664.
- Macias-Corral, M., Samani, Z., Hanson, A., Smith, G., Funk, P., Yu, H., & Longworth, J. (2008). Anaerobic digestion of municipal solid waste and agricultural waste and the effect of co-digestion with dairy cow manure. *Bioresource Technology*, *99*(17), 8288-8293.
- Manni, G., & Caron, F. (1995). Calibration and determination of volatile fatty acids in waste leachates by gas chromatography. *Journal of Chromatography A*, *690*(2), 237-242.
- Marañón, E., Castrillón, L., Quiroga, G., Fernández-Nava, Y., Gómez, L., & García, M. (2012). Co-digestion of cattle manure with food waste and sludge to increase biogas production. *Waste management*, *32*(10), 1821-1825.
- Marti, N., Bouzas, A., Seco, A., & Ferrer, J. (2008). Struvite precipitation assessment in anaerobic digestion processes. *Chemical Engineering Journal*, *141*(1–3), 67-74.

- McCarthy, P. (1964). Anaerobic waste treatment fundamentals. Part III: toxic materials and their control. *Public works*, 95(11), 91-94.
- McCarty, P. L., & McKinney, R. E. (1961). Salt toxicity in anaerobic digestion. *Journal (Water Pollution Control Federation)*, 399-415.
- Nicholson, F. A., Groves, S. J., & Chambers, B. J. (2005). Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, 96(2), 135-143.
- Okudoh, V., Trois, C., Workneh, T., & Schmidt, S. (2014). The potential of cassava biomass and applicable technologies for sustainable biogas production in South Africa: A review. *Renewable and Sustainable Energy Reviews*, 39, 1035-1052.
- Olsson, J., Feng, X. M., Ascue, J., Gentili, F. G., Shabiimam, M., Nehrenheim, E., & Thorin, E. (2014). Co-digestion of cultivated microalgae and sewage sludge from municipal waste water treatment. *Bioresource Technology*, 171, 203-210.
- Ostrem, K., & Themelis, N. J. (2004). Greening waste: Anaerobic digestion for treating the organic fraction of municipal solid wastes. *Earth Engineering Center Columbia University*, 6-9.
- Palatsi, J., Laurenzi, M., Andrés, M., Flotats, X., Nielsen, H. B., & Angelidaki, I. (2009). Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. *Bioresource Technology*, 100(20), 4588-4596.
- Panichnumsin, P., Nopharatana, A., Ahring, B., & Chaiprasert, P. (2010). Production of methane by co-digestion of cassava pulp with various concentrations of pig manure. *Biomass and bioenergy*, 34(8), 1117-1124.
- Parameswaran, P., & Rittmann, B. E. (2012). Feasibility of anaerobic co-digestion of pig waste and paper sludge. *Bioresource technology*, 124, 163-168.
- Pavlostathis, S., & Giraldo - Gomez, E. (1991). Kinetics of anaerobic treatment: a critical review. *Critical Reviews in Environmental Science and Technology*, 21(5-6), 411-490.

- Pilavachi, P. (2002). Mini-and micro-gas turbines for combined heat and power. *Applied thermal engineering*, 22(18), 2003-2014.
- Pind, P. F., Angelidaki, I., & Ahring, B. K. (2003). Dynamics of the anaerobic process: effects of volatile fatty acids. *Biotechnology and bioengineering*, 82(7), 791-801.
- Prats, I., Danes, R., Molina, V., Alamos, M., Boixadera, J., & Torres, E. (1995). Manual de gestió dels purins i de la seva reutilització agrícola. *Junta de Residus del Departament de Medi Ambient i Departament d'Agricultura, Ramaderia i Pesca. Generalitat de Catalunya, Barcelona, Spain.*
- Sosnowski, P., Klepacz-Smolka, A., Kaczorek, K., & Ledakowicz, S. (2008). Kinetic investigations of methane co-fermentation of sewage sludge and organic fraction of municipal solid wastes. *Bioresource technology*, 99(13), 5731-5737.
- Sterling, M., Lacey, R., Engler, C., & Ricke, S. (2001). Effects of ammonia nitrogen on H₂ and CH₄ production during anaerobic digestion of dairy cattle manure. *Bioresource Technology*, 77(1), 9-18.
- Taddeo, R., & Lepistö, R. (2015). Struvite precipitation in raw and co-digested swine slurries for nutrients recovery in batch reactors. *Water Science & Technology*, 71(6), 892-897.
- Vavilin, V., Fernandez, B., Palatsi, J., & Flotats, X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. *Waste management*, 28(6), 939-951.
- Wan, C., Zhou, Q., Fu, G., & Li, Y. (2011). Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. *Waste management*, 31(8), 1752-1758.
- Wang, X., Yang, G., Feng, Y., Ren, G., & Han, X. (2012). Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. *Bioresource Technology*, 120, 78-83.

- Warman, P. (1986). Effects of fertilizer, pig manure, and sewage sludge on timothy and soils. *Journal of environmental quality*, 15(2), 95-100.
- Wohlfarth, G., & Schroeder, G. (1979). Use of manure in fish farming—a review. *Agricultural wastes*, 1(4), 279-299.
- Wu, X., Yao, W., Zhu, J., & Miller, C. (2010). Biogas and CH₄ productivity by co-digesting swine manure with three crop residues as an external carbon source. *Bioresource Technology*, 101(11), 4042-4047.
- Xie, S., Lawlor, P. G., Frost, J. P., Hu, Z., & Zhan, X. (2011). Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresource Technology*, 102(10), 5728-5733.
- Xie, S., Wickham, R., & Nghiem, L. D. (2017). Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. *International Biodeterioration & Biodegradation*, 116, 191-197.
- Yang, X., Wang, X., & Wang, L. (2010). Transferring of components and energy output in industrial sewage sludge disposal by thermal pretreatment and two-phase anaerobic process. *Bioresource Technology*, 101(8), 2580-2584.
- Yilmazel, Y. D., Uysal, A., & Demirel, G. N. (2011). Recovery of nutrients from anaerobic co-digestion effluents of poultry manure and sewage sludge as struvite. *Proceedings of the Water Environment Federation*, 594-616(23).
- Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z., & Dong, R. (2014). Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. *Applied energy*, 128, 175-183.
- Zhen, G., Lu, X., Kato, H., Zhao, Y., & Li, Y.-Y. (2017). Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: Current

advances, full-scale application and future perspectives. *Renewable and Sustainable Energy Reviews*, 69, 559-577.

Zonta, Ž., Alves, M., Flotats, X., & Palatsi, J. (2013). Modelling inhibitory effects of long chain fatty acids in the anaerobic digestion process. *Water research*, 47(3), 1369-1380.

Appendix

Experimental Reactors for 3 Co-digestion Treatments (Sewage Sludge/Pig Manure ratios of 21:1, 14:1, and 7:1) and 3 Pure Controls (Pure Sewage Sludge, Pure Pig Manure, and Inoculum) in Shaking Water Bath

