

**ANAEROBIC CO-DIGESTION OF CHICKEN PROCESSING WASTEWATER  
AND CRUDE GLYCEROL FROM BIODIESEL**

A Thesis

by

LUCAS JOSE FOUCAULT

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE**

August 2011

Major Subject: Biological and Agricultural Engineering

Anaerobic Co-digestion of Chicken Processing Wastewater and Crude Glycerol from  
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## ABSTRACT

Anaerobic Co-digestion of Chicken Processing Wastewater and Crude Glycerol from Biodiesel. (August 2011)

Lucas Jose Foucault, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Cady R. Engler

The main objective of this thesis was to study the anaerobic digestion (AD) of wastewater from a chicken processing facility and of crude glycerol from local biodiesel operations. The AD of these substrates was conducted in bench-scale reactors operated in the batch mode at 35°C. The secondary objective was to evaluate two sources of glycerol as co-substrates for AD to determine if different processing methods for the glycerol had an effect on CH<sub>4</sub> production.

The biogas yields were higher for co-digestion than for digestion of wastewater alone, with average yields at 1 atmosphere and 0°C of 0.555 and 0.540 L (g VS added)<sup>-1</sup>, respectively. Another set of results showed that the glycerol from an on-farm biodiesel operation had a CH<sub>4</sub> yield of 0.702 L (g VS added)<sup>-1</sup>, and the glycerol from an industrial/commercial biodiesel operation had a CH<sub>4</sub> yield of 0.375 L (g VS added)<sup>-1</sup>. Therefore, the farm glycerol likely had more carbon content than industrial glycerol. It was believed that the farm glycerol had more impurities, such as free fatty acids, biodiesel and methanol. In conclusion, anaerobic co-digestion of chicken processing wastewater and crude glycerol was successfully applied to produce biogas rich in CH<sub>4</sub>.

## **DEDICATION**

To my mother, my father, and all my family for their love and support

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This thesis would not have been possible without the help from so many people including the faculty in the Agricultural Engineering Department, the colleagues working at the laboratory, the student workers at the laboratory, and the technicians and staff in the Agricultural Engineering workshop and laboratory. Also, I would like to thank all the people who supplied the materials for this research, including the personnel at the Wastewater Treatment Plants in Bryan and College Station, the personnel at Sanderson Farms, and the people at the biodiesel plants. I would like to thank the editors and the Writing Center staff for helping me in proofreading the thesis.

I am greatly thankful to all of them, and I am glad that during these last years I could encounter all of them in this meaningful experience. Furthermore, I believe that the people at this university provided me with a great environment for my studies. I would like to thank the committee members, Dr. Holtzapple and Dr. Mukhtar, I appreciate their support, and hope to learn from them and gain more experience for the rest of my career. I would like to specially thank Dr. Engler for his meaningful guidance and great assistance during my research and writing for the thesis. Finally, I learned much from this experience, and I would not have reached this far in this task without the guidance and support from the Agricultural Engineering faculty at Texas A&M University.

## TABLE OF CONTENTS

	Page
ABSTRACT .....	iii
DEDICATION .....	iv
ACKNOWLEDGEMENTS .....	v
TABLE OF CONTENTS .....	vi
LIST OF FIGURES.....	viii
LIST OF TABLES .....	x
 CHAPTER	
I INTRODUCTION AND LITERATURE REVIEW .....	1
Objectives.....	4
Anaerobic digestion characteristics.....	4
Factors affecting anaerobic digestion processes .....	6
Theoretical biogas composition and yield.....	9
Digestion of chicken processing wastewater .....	10
Co-digestion .....	11
Co-digestion using glycerol .....	13
II MATERIALS AND METHODS .....	16
Glycerol.....	16
Chicken processing wastewater.....	17
Inoculum sludge .....	17
Digesters and gas collector construction and set up.....	18
Digestion trials .....	21
Sampling techniques .....	23
Sample analyses .....	24
Total and volatile solids (TS and VS) .....	24
Chemical oxygen demand (COD) .....	25

CHAPTER	Page
pH.....	26
Alkalinity.....	26
Biogas analysis.....	26
III RESULTS.....	28
Characterization of substrates and inoculum.....	28
Biogas production.....	30
Biogas composition.....	34
Analysis of wastewater-only trials.....	39
Analysis of glycerol-only trials.....	43
Analysis of co-digestion trials.....	46
Degradability results.....	51
pH analysis.....	52
IV CONCLUSIONS.....	56
Recommendations for future studies.....	57
REFERENCES.....	59
APPENDIX A.....	63
APPENDIX B.....	65
APPENDIX C.....	78
APPENDIX D.....	83
VITA.....	85



## LIST OF FIGURES

FIGURE	Page
1 Basic pathways and microorganisms involved in anaerobic digestion .....	5
2 “Industrial” glycerol .....	16
3 “Farm” glycerol .....	16
4 Wastewater sample .....	18
5 Diluted sludge inoculum .....	18
6 Digester and gas collector .....	19
7 Reactors inside environmental chamber .....	20
8 Cumulative biogas production for first batch trial .....	31
9 Cumulative biogas production for second batch trial .....	32
10 Cumulative biogas production for third batch trial .....	33
11 CH <sub>4</sub> content of biogas produced during the first batch trials .....	36
12 Cumulative CH <sub>4</sub> production for first batch .....	37
13 Cumulative CH <sub>4</sub> production for second batch .....	38
14 Cumulative CH <sub>4</sub> production for second batch (glycerol-only trials) .....	39
15 Cumulative biogas volumes of wastewater-only trials .....	41
16 Biogas yield vs. volatile solids concentration in wastewater-only trials ....	42
17 Biogas yield vs. chemical-oxygen-demand concentration in wastewater-only trials .....	43
18 Cumulative biogas volumes of glycerol-only trials .....	44

FIGURE	Page
19 CH <sub>4</sub> yields on VS and COD basis for glycerol-only trials and theoretical yield for pure glycerol .....	45
20 Biogas cumulative volumes of all co-digestion trial .....	48
21 Biogas production vs. concentration of VS in co-digestion trials .....	49
22 Biogas yields for co-digestion and wastewater-only trials.....	50
23 Biogas yields from each glycerol .....	51
24 Profiles of reactor pH during first batch.....	54
25 Profiles of reactor pH during second batch .....	54
26 Profiles of reactor pH during third batch.....	55

**LIST OF TABLES**

TABLE		Page
1	Batch trials components .....	22
2	Gas composition of each standard mixture .....	27
3	Characterization of substrates and inoculum .....	28
4	Estimated compositions of the two types of glycerol along with data provided by the biodiesel plant .....	29
5	Biogas compositions for two reactors during the second batch trial.....	35
6	Feed characteristics of wastewater-only trials .....	40
7	Feed characteristics of glycerol-only trials .....	43
8	Feed characteristics of co-digestion trials .....	47
9	VS and COD removal efficiencies for all trials .....	53

# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

The problem of climate change and the current US policies regarding national energy security are situations that need to be addressed by university researchers and the whole population. Greenhouse gas (GHG) emissions such as CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub> from anthropogenic sources need to be controlled to diminish climate change. According to the Intergovernmental Panel on Climate Change (IPCC, 2001), CH<sub>4</sub> emissions are produced from human activities such as agriculture, natural gas production, and landfills. More than half of the current CH<sub>4</sub> emissions are anthropogenic, with CH<sub>4</sub> emissions rapidly increasing since the industrial revolution. The IPCC has reported an increase in atmospheric CH<sub>4</sub> concentrations from 700 ppb (10<sup>-9</sup>) in 1750 to 1745 ppb in 1998.

Recently, the US government enacted the Energy Security and Independence Act of 2007 to stimulate the usage of renewable energy and reduce petroleum imports (H.R. 6 (ENR), 2007). This US Congress act includes a requirement to reduce fossil fuel generated energy consumption by 100% in all federal buildings by 2030.

Renewable energy may involve the conversion of biomass material, which is converted to a gas or liquid fuel through processes such as gasification and pyrolysis. Additionally, biofuels such as biodiesel, ethanol, and biogas are produced through processes such as transesterification, fermentation, and anaerobic digestion, respectively. These technologies, among others such as wind and solar energy, would help supply energy to substitute for fossil fuels in the future and control climate change. The diminishing

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This thesis follows the style of *Transactions of the American Society of Agricultural and Biological Engineers*.

supply of fossil fuels encourages the use of renewable energy sources (Sung and Santha, 2003).

Methane is produced by the decomposition of organic matter by methanogenic bacteria in the absence of O<sub>2</sub> in a process called anaerobic digestion (AD). Large emissions of CH<sub>4</sub> are produced naturally in wetlands. The gas produced in the AD process is called biogas, and biogas reactors that treat waste material can produce gas containing approximately 60% CH<sub>4</sub> and 40% CO<sub>2</sub> with other trace gases. Biogas can be used to replace fossil fuels for heating or electricity generation purposes (Ward et al., 2008).

Biogas reactors are widely used, for example, in developing nations where energy sources are limited for rural populations. It is estimated that 1 m<sup>3</sup> of biogas can provide heat to cook three meals for a family of four. Furthermore, biogas is contained in the reactors and CH<sub>4</sub> is transformed to CO<sub>2</sub> when combusted, so a GHG that is less damaging to the atmosphere is emitted (Ward et al., 2008). Biogas reactors may have other benefits for biogas producers, like significant destruction of pathogens in the waste, reduction of bad odors, and production of biosolids that are useful for agricultural fertilization (Ward et al., 2008).

Biogas reactors are used to treat several types of organic substrates such as livestock waste (e.g., dairy manure) (Ahring et al., 1992; Burke, 2001), sewage sludge (Kayhanian and Rich, 1996; Rubia et al., 2006), wastewater from food processing industries (Angelidaki and Ahring, 1997; Ma et al., 2008; Marques et al., 1998), and grain or vegetable crop wastes (Baader, 1991; Stewart et al., 1984). Wastewater streams from chicken-processing plants generally have a high pollution load and may be treated by AD.

Chicken processing wastewater has been previously studied as a substrate for biogas production (Del Nery et al., 2007; Harper et al., 1990; Salminen and Rintala, 1999).

These wastewaters provide the carbon substrate for conversion to CH<sub>4</sub> and the necessary nutrients (e.g., organic nitrogen) for bacteria to grow. Usually, chicken-processing wastewater is composed of blood, chicken fat, feathers, small meat parts, and other components (Del Nery et al., 2007). This wastewater is usually screened, which results in low suspended solids content entering the treatment process. Increasing CH<sub>4</sub> production from AD treatment of chicken processing wastewater could improve the economics for capturing and using the biogas produced. One means of increasing CH<sub>4</sub> production is to add a co-substrate such as glycerol from biodiesel production.

Crude glycerol (glycerine, glycerin) is a by-product of biodiesel production that separates from the biodiesel phase during production. Glycerol currently demands a low price because of excessive supplies (Yazdani and Gonzalez, 2007). Biodiesel producers do not wish to dispose of the crude glycerol (approximately 80% glycerol, C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>), but the cost of purifying the glycerol for entry into the commodity market is excessive. Glycerol (purified to approximately 99%) has thousands of applications such as soap, pharmaceuticals, and laboratory bacterial media (Britannica, 2010). Motivated by the low price and high availability of glycerol, recent studies (Dharmadi et al., 2006; Yazdani and Gonzalez, 2007) have investigated crude glycerol as a raw material for higher value products, e.g., ethanol via *E. coli* fermentation. Studies on biogas production have used crude glycerol as a secondary substrate with a primary substrate that provides necessary nutrients such as nitrogen and phosphorous because glycerol provides carbon only.

An increase in biogas production has been shown when using glycerol (pure, crude, or pretreated) with various primary substrates: potato processing wastewater (Ma et al., 2008), dairy manure (Chen et al., 2008a), blends of corn, silage and pig manure (Amon et al., 2006), municipal solid waste (Fountoulakis and Manios, 2009), blends of slaughterhouse and olive mill wastewater (Fountoulakis and Manios, 2009), and blends of pig manure and fish waste (Álvarez et al., 2010). However, no studies on co-digestion

of chicken processing wastewater and glycerol from biodiesel have been reported in the literature.

For this research, crude glycerol was obtained from two different biodiesel manufacturers, a commercial biodiesel operation (“industrial” glycerol) and an on-farm biodiesel operation (“farm” glycerol). The biodiesel from the farm operation was produced from waste restaurant oil (grease) using potassium hydroxide (KOH) as catalyst. The biodiesel from the industrial operation was produced from animal fat (tallow) using sodium hydroxide (NaOH) as catalyst. It was assumed that greater impurities, such as unreacted glycerides and free fatty acids, and methanol were likely present in the farm glycerol sample. For the industrial glycerol, it was assumed that the glycerol contained minimal amounts of impurities because of recovery units in the plant that remove methanol, catalyst, and unreacted glycerides from the biodiesel and glycerol layers.

## **Objectives**

The main objective of this research was to evaluate anaerobic co-digestion of chicken processing wastewater (wastewater) and crude glycerol from biodiesel production. Co-digestion of these two substrates was compared to digestion of the wastewater alone. The secondary objective was to evaluate two sources of glycerol as co-substrates for AD to determine if different processing methods for the glycerol had an effect on CH<sub>4</sub> production. Digestion was analyzed by determining the CH<sub>4</sub> yields and the degradability (organic matter consumed by the process) of each substrate.

## **Anaerobic digestion characteristics**

Anaerobic digestion is the natural degradation of organic matter by bacteria in the absence of O<sub>2</sub> with production of biogas, a mixture of primarily CH<sub>4</sub> and CO<sub>2</sub>. Numerous biological and chemical reactions occur in the AD process, which is mediated

by a large consortium of microorganisms. Organic macromolecules such as lipids, polysaccharides, and proteins are degraded through hydrolysis reactions, further degraded to organic acids, converted to acetic acid, and finally converted to CH<sub>4</sub> (biogas) as shown in Figure 1.

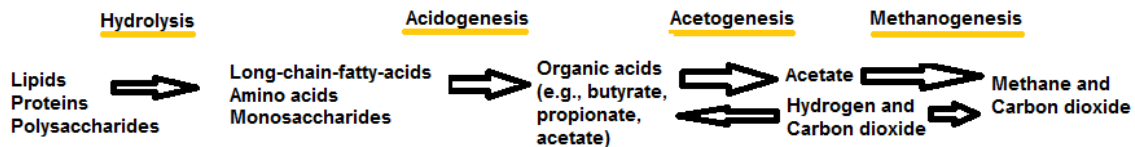


Figure 1. Basic pathways and microorganisms involved in anaerobic digestion (Gray, 2004).

The first step in the process involves hydrolysis of complex organic molecules, such as lipids, polysaccharides and proteins, through the action of enzymes produced by hydrolytic bacteria. The hydrolysis reactions produce simple organics such as fatty acids and glycerol, mono- and oligosaccharides, and amino acids (Angelidaki and Sanders, 2004).

These simple organic substrates are then used by both the hydrolytic bacteria and the acidogenic microorganisms as a source of food and energy resulting in the production of organic acids such as acetic, butyric, and propionic acids. The next step in the process involves converting the higher organic acids into acetic acid via action of the acetogens. There are two kinds of acetogens: hydrogen-producing and hydrogen-consuming (Archer and Kirsop, 1990).

The end of the process is reached with conversion of the end products of the acetogens (acetic acid, CO<sub>2</sub>, and H<sub>2</sub>) into CH<sub>4</sub> by the methanogens (Archer and Kirsop, 1990). There are two kinds of methanogens: (1) acetoclastic methanogens, which consume



acetic acid; and (2) hydrogenotrophic methanogens, which consume  $H_2$  and  $CO_2$  (Mladenovska et al., 2003).

Competition for substrates by other organisms present in ADs occurs, for example, sulfate-reducing bacteria (SRB) which produce hydrogen sulfide ( $H_2S$ ) and  $CO_2$ . SRB compete for carbon substrate with methanogens and acetogens (Chen et al., 2008b).

### **Factors affecting anaerobic digestion processes**

Many factors have to be considered to operate a successful AD process. These factors include dilution of feedstocks to avoid inhibition of bacteria, control of pH and temperature, appropriate feed-to-inoculum (F:I) ratio, suitable retention times to avoid washout of bacteria, and sufficient micronutrients and macronutrients to support bacterial activity.

The goals for a successful AD process are to produce the maximum volume of  $CH_4$ , to have a constant and high organic loading rate that is sustainable and tolerable by the bacteria, and to minimize reactor volume by using a short hydraulic retention time (HRT) (Ward et al., 2008). A minimum reactor volume is desired for economic purposes.

Dilution of feedstocks is a common factor in AD processes. A digester is called a “wet” digester when it contains less than 16% total solids content and a “dry” digester if the content is above this value. Most digesters are of the wet type because of the amount of water used in collection of the waste (e.g., dairy manure) or to activate the AD process. However, municipal solid wastes (MSW), which generally have high solids content, are normally treated in landfills which often results in a “dry” digestion process (Ward et al., 2008).

The pH of a digester needs to be kept constant to avoid disturbing the microbial populations. The pH range in digesters is commonly 6.8–7.2 (the ideal pH for methanogens) (Ward et al., 2008). However, the optimum pH for hydrolysis reactions is lower than that for methanogens. If the pH is too low, it is usually adjusted by adding NaOH or other alkaline solution or by decreasing the organic loading rate. The alkalinity is also an important factor; sufficient alkalinity in the reactor helps the performance because it acts as a pH buffer. The alkalinity range for optimum AD operation is generally 2000–4000 of calcium carbonate ( $\text{mg CaCO}_3 \text{L}^{-1}$ ) (supernatant alkalinity) (APHA, 2005).

Anaerobic digesters can operate over a wide variety of temperature ranges and can be classified accordingly. Mesophilic digesters operate around ambient summer temperatures with an optimum around  $35^\circ\text{C}$  ( $95^\circ\text{F}$ ), and thermophilic digesters operate at higher temperatures, usually around  $55^\circ\text{C}$  ( $131^\circ\text{F}$ ). Digester operations in these two temperature ranges involve very different microbial populations. The AD process also can occur at temperatures below  $20^\circ\text{C}$  ( $68^\circ\text{F}$ ), which is called psychrophilic operation; however, the rate is quite slow.

Solid retention time (SRT) and HRT should be optimized in a continuous AD process to obtain the best reactor performance. Retention times affect the population of methanogens and the composition of volatile fatty acids (VFAs) present in a digester (Rubia et al., 2006). A SRT of 15 days in a thermophilic reactor treating sludge gave higher degradability than reactors having SRTs of 20, 27, and 40 days (Rubia et al., 2006).

Inoculum is added to the reactor to provide an active source of bacteria during start-up, and feed is added to supply substrates that are degradable. Digested sewage sludge and digested manure from established anaerobic reactors are the most common inoculum sources (Ward et al., 2008). A feed-to-inoculum ratio of 1:1 is generally thought

appropriate (1 g VS of feed per 1 g VS of inoculum) for efficient start-up of a reactor (Luostarinen et al, 2009).

If a substrate lacks a proper nutrient balance, then other substrates or nutrient supplements have to be added to the reactor. The primary nutrients required are C and N. Other important nutrients include P, K, Ca, Fe, Ni, and Co (Ward et al., 2008). The carbon-to-nitrogen (C:N) ratio generally recommended in the literature is 20–25 g carbon per g nitrogen. One method of estimating carbon in the substrate is by measuring the chemical oxygen demand (COD) which is measured in mg O<sub>2</sub> equivalent. A chemical oxygen demand-to-nitrogen ratio (COD:N) of 70 g COD per g nitrogen is typical for ADs (Álvarez et al., 2010). In addition, the Total Kjeldahl Nitrogen can be used to measure the nitrogen in the substrate.

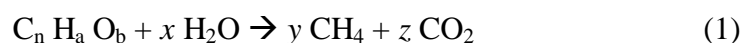
Nutrient ratios reported in the literature include COD:N ratios of 18:1 for potato processing wastewater (Ma et al., 2008), 162:1 for a blend of olive oil and slaughterhouse wastewaters (Fountoulakis and Manios, 2009), 58:1 for organic fraction of municipal solid waste (Fountoulakis and Manios, 2009), 27:1 for raw sludge (Alatríste-Mondragon et al., 2006), 8.9:1 for pig manure (Álvarez et al., 2010), and 7315:1 for crude glycerol from biodiesel production (Álvarez et al., 2010). A feedstock with a low COD:N ratio is needed to obtain the recommended nutrient ratio when glycerol is added to the reactor. The crude glycerol from biodiesel production contains negligible amounts of N, e.g., Thompson and He (2005) reported glycerol from biodiesel produced from waste vegetable oil contained 1.2 mg L<sup>-1</sup> of N.

The VFA intermediate products (butyrate, propionate, acetate) in AD can accumulate if any type of inhibition occurs in the AD process resulting in low gas production. In addition, VFAs in high concentration inhibit the methanogens (Ward et al., 2008). During inhibition, a sharp decrease in pH is seen as VFAs accumulate. This low pH, in turn, causes inhibition of methanogens, further slowing down the AD process. In

addition, H<sub>2</sub>S, NH<sub>3</sub>, and light and heavy metals can inhibit the AD process at high concentrations (Chen et al., 2008b). VFA inhibitory limits are hard to determine in general; the amount of inhibition by VFAs highly depends on the type of AD process or system.

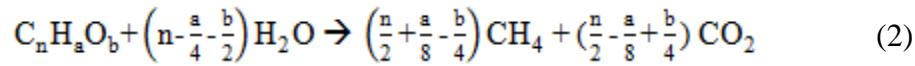
### **Theoretical biogas composition and yield**

Buswell and Neave (1930) determined the volume (or mol) ratio of CH<sub>4</sub>:CO<sub>2</sub> produced by AD based on complete conversion of the organic molecule used as feed. That work involved trials with propionate or other acids used as feed and sludge or synthetic medium as inoculum providing nutrients. In that work, data for CH<sub>4</sub> and CO<sub>2</sub> closely followed the stoichiometric ratios given by Equation 1 (known as Buswell's equation) with  $x$ ,  $y$ , and  $z$  determined from elemental balances on C, H, and O.



In the reaction described by Equation 1, water acts as an oxidizing agent. The carbon of the organic molecule is either oxidized or reduced to gaseous products CO<sub>2</sub> and CH<sub>4</sub>, respectively. This equation does not account for carbon utilized in the production of cell matter (anabolic reactions) or remaining in recalcitrant solid matter. Hydrogen emissions were negligible in the AD studies of Buswell and Neave (1930), suggesting that the H<sub>2</sub> produced by anabolic and degradation reactions was transferred to hydrogen acceptors which ultimately were the carbon atoms of the acid.

The Buswell equation (Eq. 1) along with the ideal gas law can be used to obtain theoretical CH<sub>4</sub> and CO<sub>2</sub> yields. In theory, any organic molecule that is easily biodegradable, such as glycerol, containing only C, H and O, can be used in the Buswell equation (Eq. 1) (Angelidaki and Sanders, 2004; Fountoulakis and Manios, 2009; Ma et al., 2008). The stoichiometric coefficients of Equation 1 ( $x$ ,  $y$ , and  $z$ ) obtained from elemental balances are given by Equation 2:



These stoichiometric coefficients do not assume a specific methanogenic population because both types of methanogens generally occur in stable AD processes. The stoichiometric equation for conversion of pure glycerol, which is completely biodegradable, is given by Equation 3:



These stoichiometric coefficients can be used to determine the theoretical yields for biogas and CH<sub>4</sub> from glycerol assuming ideal gas behavior at standard temperature and pressure (STP) conditions (0°C and 1 atm). The theoretical volumetric yield of CH<sub>4</sub> is 3.92x10<sup>-2</sup> m<sup>3</sup> CH<sub>4</sub> (mol glycerol)<sup>-1</sup>.

Assuming one gram of glycerol equals one gram of VS, the theoretical volumetric yield of CH<sub>4</sub> per gram of VS added (L CH<sub>4</sub> (g VS added)<sup>-1</sup>) is 0.426 L CH<sub>4</sub> (g VS)<sup>-1</sup>. The theoretical yield also can be determined on the bases of COD of the substrate. During COD analysis, organic matter consisting of only C, H, and O is fully oxidized to CO<sub>2</sub> and H<sub>2</sub>O; if the organic matter contains S and N, the oxidized end-products include NH<sub>3</sub> or HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> (Angelidaki and Sanders, 2004). The theoretical COD for pure glycerol found from the stoichiometric equation for complete oxidation of glycerol is 1.22 g COD (g glycerol)<sup>-1</sup> (1.22 g COD (g VS)<sup>-1</sup>). The theoretical CH<sub>4</sub> yield from glycerol on COD basis is then 0.350 L CH<sub>4</sub> (g COD)<sup>-1</sup>. Finally, the theoretical biogas yield from glycerol is 0.730 L (g VS)<sup>-1</sup> or 0.598 L (g COD)<sup>-1</sup>.

### **Digestion of chicken processing wastewater**

Anaerobic treatment of chicken processing wastewater was reviewed to investigate the potential for use of this wastewater as a primary substrate in co-digestion

with glycerol. The wastewater is usually treated with grit chambers, screens, settling tanks, and dissolved-air flotation systems to remove the oil, grease, and suspended solids (Del Nery et al., 2007). Biological treatment of the wastewater usually includes activated sludge, stabilization ponds, and anaerobic reactors. The wastewater includes mainly residual blood, skin fat, grease, feces, and feathers among other components (Del Nery et al., 2007).

Salminen and Rintala (1999) reported a  $\text{CH}_4$  yield of  $0.330 \text{ L (g VS)}^{-1}$  and VS removal of 68% for chicken processing waste, including food packing waste and crushed feathers, diluted to 1% VS content. Del Nery et al. (2007) reported a COD removal of 67% for treatment of wastewater using two full-scale upflow anaerobic sludge blanket (UASB) reactors over a four-year period.

Harper et al. (1990) reported a biogas yield of  $0.143 \text{ L (g COD)}^{-1}$  and COD removal efficiency of 66% for treatment of poultry processing wastewater. Treatment of this wastewater was with a pilot-scale anaerobic filter reactor in continuous operation. The composition of the gas was 75%  $\text{CH}_4$ , 16%  $\text{CO}_2$ , 8%  $\text{N}_2$ , and 2000 ppm of  $\text{H}_2\text{S}$ . Loss of buffer control (using  $\text{NaHCO}_3$  solution) and consequently low pH was observed to cause a lower gas production rate. The high concentration of  $\text{H}_2\text{S}$  was also thought to have inhibited the bacteria and lowered the reactor efficiency.

### **Co-digestion**

An AD reactor that uses two or more substrates as feed is called a co-digestion reactor (Alatriscite-Mondragon et al., 2006). A number of studies have found many benefits for co-digestion treatment compared to digestion without co-substrate(s): increase in  $\text{CH}_4$  production, optimization of nutrient balance, improvement of degradability (e.g., % VS or % COD removed), dilution of toxic compounds, and cost efficiency by using one plant to treat more than one waste.

Luostarinen et al. (2009) investigated co-digestion of sewage sludge with grease trap sludge. The grease trap sludge was a high-lipid-content waste from processing of cow and swine meat. The study showed that co-digestion was feasible with grease trap sludge comprising up to 46% of the VS in the feed. The CH<sub>4</sub> yield of this optimum mixture of grease trap sludge with sewage sludge was 0.463 L (g VS)<sup>-1</sup>. When feeding above this amount, the reactor became unstable with decreasing CH<sub>4</sub> production and increasing VS, COD and VFAs in the effluent. This inhibition by high concentrations of grease trap sludge was believed to be caused by long chain fatty acids produced during degradation of the lipid-rich substrate.

Kayhanian and Rich (1996) investigated AD of two substrates: the biodegradable organic fraction of municipal solid waste (BOF/MSW) and sewage sludge. Municipal solid waste is by nature a heterogeneous waste with high solids content, but when co-digested with a highly nutritious and diluted waste such as sewage sludge, CH<sub>4</sub> production was stabilized. The CH<sub>4</sub> yield of the co-digestion reactor was 0.360 L (g VS)<sup>-1</sup> (day)<sup>-1</sup>.

Alvarez and Lidén (2008) evaluated the co-digestion of fruit and vegetable waste (FVW) with solid cattle and swine manure (SCSM) and solid cattle and swine slaughterhouse waste (SCSSW). This study showed that FVW substrates (low N and P), when mixed with the higher N- and P-containing wastes (SCSSW and SCSM) improved digestion and CH<sub>4</sub> yields. In addition, digestion trials using only FVW or SCSSW were inhibited. This inhibition was believed to be caused by accumulation of acetic, propionic, and butyric acids, which suggested the nutrient balance was inappropriate to support efficient conversion of acids to CH<sub>4</sub> by the bacterial populations in the failing digesters.

Mladenovska et al. (2003) evaluated the co-digestion of cattle manure with a synthetic lipid, glycerol trioleate (GTO) added at 2% (w.b.). The co-digestion reactor had a CH<sub>4</sub> yield of 0.382 L (g VS)<sup>-1</sup>, whereas a manure-only reactor had a yield of 0.224 L (g VS)<sup>-1</sup>. Also, the degradability of the co-digestion reactor was higher than in the manure-only

reactor, 51% and 37% VS removed, respectively. Microbial analysis showed that sludge from the co-digestion reactor had a larger microbial population and greater methanogenic activity than sludge from the manure-only reactor.

Ahring et al. (1992) performed successful co-digestion studies at large scale using cattle manure supplemented with up to 6% of bentonite-bound oil (BBO). BBO is a waste produced during edible oil production after cleanup and de-colorization of oil. The BBO in that study contained rape seed oil (30%–35% lipid) and bentonite clay. The CH<sub>4</sub> yield from the BBO substrate was reported to be 0.875 L (g VS)<sup>-1</sup>.

### **Co-digestion using glycerol**

Glycerol as co-substrate in AD has been the subject of numerous studies. Several types of primary substrates have been used with either pure, crude, or pretreated glycerol as co-substrate. Glycerol concentrations were generally around 1% of feed (v v<sup>-1</sup>) or 1 to 5 g L<sup>-1</sup> of reactor. Because of inhibition caused by high VFA concentrations, low concentrations of glycerol in the feed were necessary. Advantages of using glycerol as a co-substrate were higher biogas yields and higher degradability.

Ma et al. (2008) evaluated addition of crude, pure and high conductivity (HC) glycerol as co-substrates with potato processing wastewater in an UASB reactor. The CH<sub>4</sub> production of the co-digestion reactor (crude glycerol) was higher than the wastewater-only reactor by a factor of 1.5 (v v<sup>-1</sup> feed). The CH<sub>4</sub> yield for the crude glycerol alone was determined to be 0.393 L (g COD)<sup>-1</sup>. Also, the COD removal efficiency was around 85% for both the glycerol-supplemented tests (three types) and the non-supplemented tests. The study also found CH<sub>4</sub> production to be 102%, 100% and 80% of the theoretical yield for pure, crude, and HC glycerol, respectively. Although the potato processing wastewater feedstock COD varied from 2 to 14 (g COD) L<sup>-1</sup> during the study because of the natural variation of the source, the reactor effluent COD was low and constant.



Crude glycerol had a COD of  $918 \text{ mg COD (g glycerol)}^{-1}$  (w.b.) and a density of  $1.22 \text{ kg L}^{-1}$ .

Fountoulakis and Manios (2009) evaluated crude glycerol (pH 5) as a co-substrate with BOF/MSW and a blend of olive mill (OMW) and slaughterhouse (SW) wastewaters. After adding glycerol, the daily  $\text{CH}_4$  production increased by 1.5 and 2.5 times when added to BOF/MSW and to the OMW:SW blend, respectively. The OMW:SW blend had a C:N ratio of 167, which required the addition of urea to provide adequate nitrogen for the microbial population. In contrast, the BOF/MSW had a C:N:P ratio of 100:1.7:0.2 and no addition of nutrients was needed. Glycerol concentration in the reactor was  $0.52 \text{ g L}^{-1}$ .

Holm-Nielsen et al. (2008) investigated addition of pure glycerol to swine and cow manure in a thermophilic AD bioreactor using a near-infrared sensor to analyze the effect of glycerol concentration on reactor performance. They concluded that 3 to  $5 \text{ g L}^{-1}$  of glycerol was the maximum concentration possible. At higher concentrations, accumulation of VFAs in the bioreactor caused instability.

Chen et al. (2008a) evaluated co-digestion of crude glycerol with dairy manure as the primary substrate in both batch and continuous reactors. Mixtures containing 60% ( $2.22 \text{ g L}^{-1}$ ) and 45% ( $1.67 \text{ g L}^{-1}$ ) crude glycerol on a VS basis were evaluated. The study also included a control reactor with dairy manure and another reactor with glycerol as the only substrate ( $3.71 \text{ g L}^{-1}$ ). The mixture with 60% glycerol had a C:N ratio of 20 and produced a  $\text{CH}_4$  yield of  $0.310 \text{ L (g VS)}^{-1}$ . The second mixture had a C:N ratio of 15 and produced a  $\text{CH}_4$  yield of  $0.220 \text{ L (g VS)}^{-1}$ . However, the  $\text{CH}_4$  yield of the manure-only reactor (control) and the glycerol-only reactor was  $0.140 \text{ L (g VS)}^{-1}$  and  $0.360 \text{ L (g VS)}^{-1}$ , respectively. In addition, the VS removal efficiencies were 100% for glycerol, 38% for manure (control), 95% for the first mixture and 60% for the second mixture. A second peak in daily gas production in the batches with crude glycerol was believed to indicate

re-establishment of the methanogenic population. Batches lasted only 14 days in this study.

Alvarez et al. (2010) investigated co-digestion of swine manure, fish waste, and crude glycerol. The blend components were determined using linear programming and the theoretical CH<sub>4</sub> production was based on lipid, carbohydrate and protein content of each component (Neves et al., 2008). The blends that produced the greatest amounts of CH<sub>4</sub> were mixtures composed of 5% (w.b.) fish waste, 11% to 16% glycerol and the remainder was swine manure. The highest CH<sub>4</sub> yield was 0.321 L (g COD)<sup>-1</sup>. The optimal blends had a COD:N ratio between 45:1 and 60:1.

Kaprzak et al. (2009) investigated co-digestion of cheese whey, corn silage, and crude glycerol. Their results showed that biogas yields and COD removal efficiency (degradability) were improved when glycerol was added to the feed. It was reported that VFA concentrations increased and gas production decreased in failing digesters. The glycerol concentration in the semi-continuous reactor was 2.18 (g TS) L<sup>-1</sup> d<sup>-1</sup>.

Siles Lopez et al. (2009) studied glycerol as feed (up to 3 g L<sup>-1</sup>) with two types of sludge (granular sludge from brewery wastewater and non-granular sludge from urban wastewater) as inoculum. Also, different types of glycerol were tested: an acidified glycerol (with phosphoric acid to recover KOH), glycerol distilled to nearly pure quality, and a pure (commercial) glycerol. The CH<sub>4</sub> yield of the best-performing reactor containing acidified glycerol and granular sludge was 0.302 L (g COD)<sup>-1</sup>, and the COD removal was nearly 100%. This study also showed that large additions of glycerol caused severe inhibition because of organic overload. This study used only glycerol as feed (no co-digestion); however, the necessary nutrient supplements were added and the digested sludge supplied the inoculum.

## CHAPTER II

### MATERIALS AND METHODS

#### Glycerol

Two sources of crude glycerol were used in this study (Figs. 2 and 3). The first source was a commercial biodiesel production facility in Galena Park, Texas, operated by Green Earth Fuels. The biodiesel in this plant was produced from animal fat (tallow) using NaOH as catalyst. The second was an on-farm biodiesel plant operated by Caleb Tonn near Giddings, Texas, which produces biodiesel from waste restaurant oil (grease) using KOH as catalyst.



Figure 2. “Industrial” glycerol.

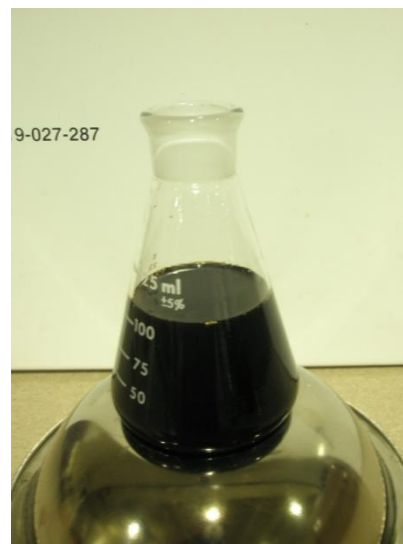


Figure 3. “Farm” glycerol.

The crude glycerol samples were kept in closed containers and left in storage at room temperature. The glycerol samples were characterized for solids content, COD, pH, and density. Also, the crude glycerol samples were significantly different in appearance.

Because pure glycerol is clear and colorless, the appearance of the samples suggests significant levels of impurities present, particularly for the farm glycerol.

### **Chicken processing wastewater**

The primary substrate used in this study was chicken processing wastewater (wastewater) obtained from the Sanderson Farms chicken processing plant in Bryan, Texas. Samples were collected at the inflow channel to an anaerobic lagoon, which is the first step in the wastewater treatment process at the plant. Fresh samples of approximately 55 L were obtained for each batch experiment.

The wastewater included small amounts of chicken solids and feathers, and had a light yellowish brown color (Fig. 4). The wastewater passed through two screens prior to collection; therefore, most of the suspended solids had been removed. Once samples were obtained, they were kept in buckets and immediately used in reactors or put in the freezer (-20°C) until the reactors were started. The wastewater samples were characterized for solids content, COD, pH, and density.

The processing plant treated the wastewater in a covered anaerobic lagoon with a retention time of 7 days. The biogas produced was harvested and flared. Following anaerobic treatment, the wastewater was sent to an aerobic basin for additional treatment before being discharged to a nearby creek.

### **Inoculum sludge**

The inoculum used in this study was sludge from the Burton Creek wastewater treatment facility in Bryan, Texas. Sludge was provided as dewatered sludge (high moisture solids) from the wastewater treatment plant, which has an AD treatment. One 25-L bucket of dewatered sludge was obtained from the plant and characterized for solids content.

To start up the reactors, the sludge was diluted with tap water at a volume ratio of 1:1 (Fig. 5), and the reactors were filled with the diluted sludge to approximately 4.8 L. The reactors, containing only diluted sludge, were operated for approximately two weeks to stabilize the methanogenic population and allow it to grow. During this time, organic matter in the sludge was consumed and biogas production began decreasing gradually. The diluted sludge was characterized for solids content, COD, pH, and density.



Figure 4. Wastewater sample.

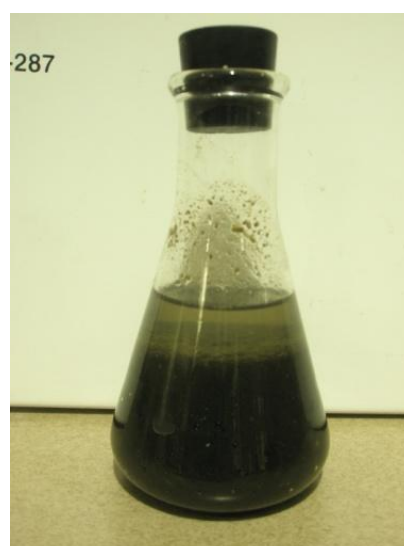


Figure 5. Diluted sludge inoculum.

### **Digesters and gas collector construction and set up**

Ten bench scale reactors were constructed, each having an approximate total volume of 6 L and a working volume of 4.8 L (Figs. 6 and 7). The digesters were built using clear PVC pipe and had a length of 30.5 cm (1 ft) and inside diameter (i.d.) of 15.2 cm (6 in.). In addition, one 15.2-cm PVC cap was used as the bottom cover. Similarly, the top cover was comprised of a 15.2-cm threaded PVC fitting with a 15.2-cm threaded PVC plug. The bottom and top covers were glued to the pipe with PVC cement. The

threaded plug connection was sealed with thread sealant to achieve an air-tight seal. Clear PVC pipe was chosen to allow viewing the material inside the digesters.

Each digester top cover had two holes drilled and tapped for outlets. One outlet was used for feeding and consisted of a mini valve (0.635 cm) and the other outlet consisted of a tubing connector (0.635 cm) and was used to transfer biogas to the gas collector. The bottom of the digester had one outlet, which was used to take liquid samples from the digester and consisted of a mini valve (0.635 cm). The connections of both valves and the fittings for the three outlets were sealed with a thread sealant or thread tape to prevent any gas or liquid leaks.

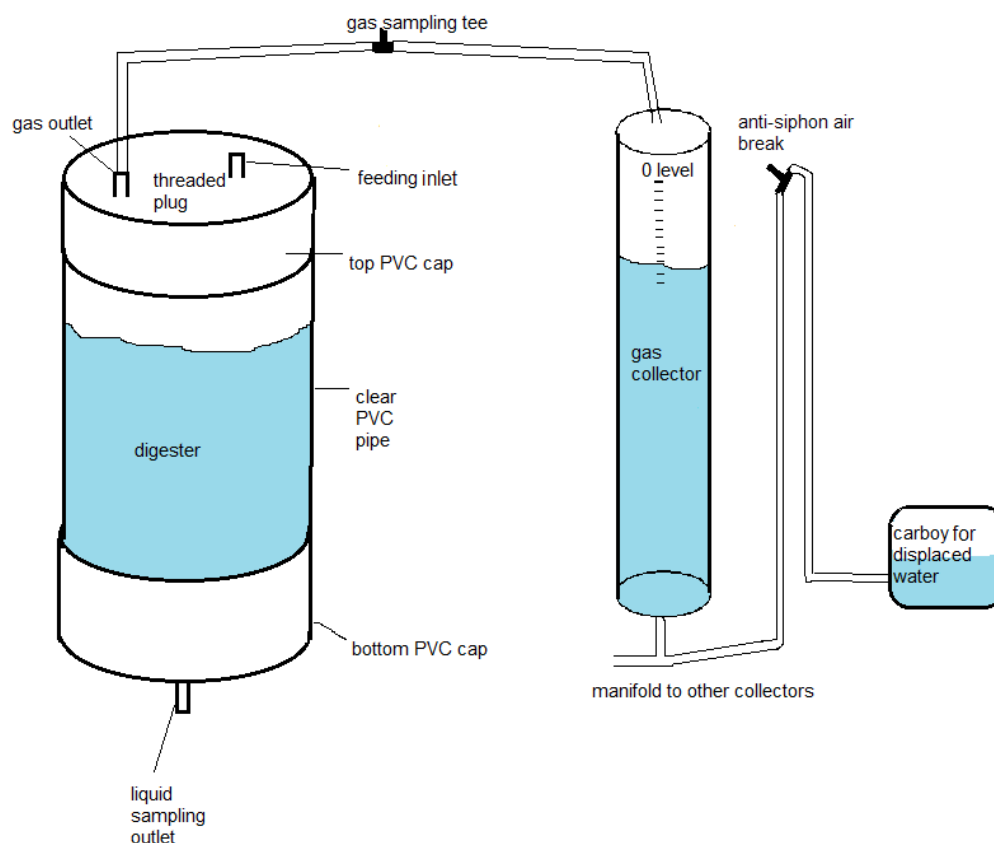


Figure 6. Digester and gas collector. Diagram includes the carboy for collecting displaced water located on the floor (not to scale).

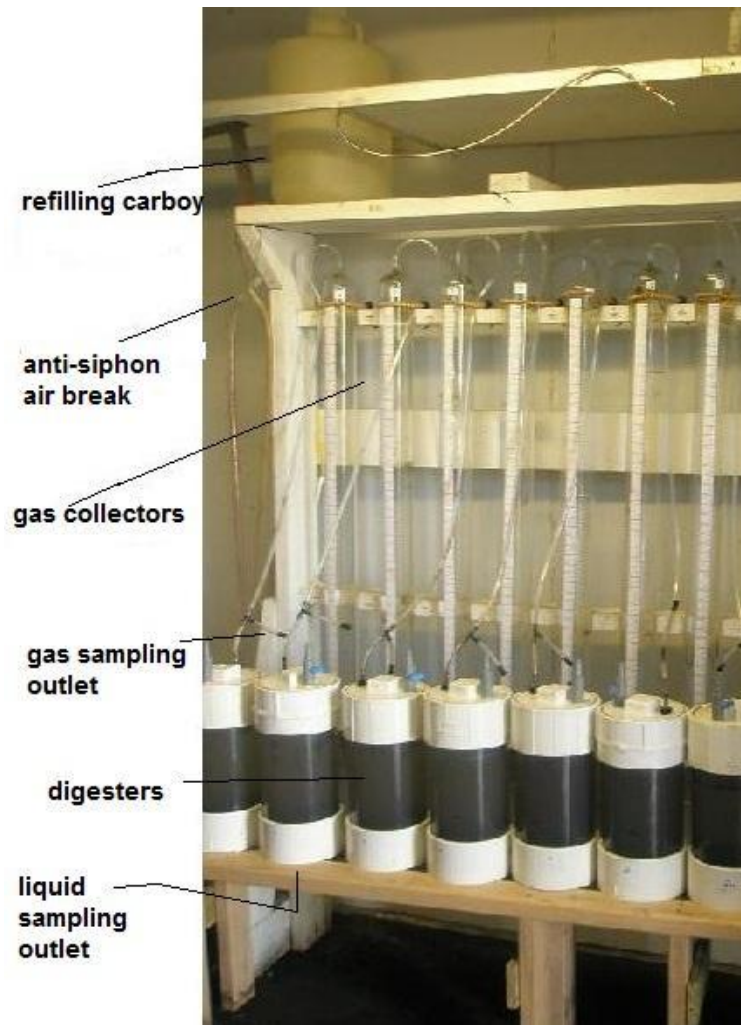


Figure 7. Reactors (7 of 10 shown) inside environmental chamber. Diagram includes refilling carboy on top of gas collectors (a second carboy located on the floor for collecting displaced water is not shown).

The gas collectors, which had been constructed for previous work with biogas, were glass tubes of 7.6 cm i.d. (3 in.), and approximately 122 cm (48 in.) long, with rounded domes at both ends. They had integral tubing connections at the top and bottom of 0.635 cm (0.25 in.) and 1.27 cm (0.5 in.), respectively. In addition, two new gas collectors were constructed from clear PVC tubing (7.6 cm i.d. and 122 cm long) for a total of ten gas collectors. The new gas collectors had PVC caps glued to each end which were drilled and tapped to accept a 0.365-cm tubing connector at the top and a 1.27-cm tubing

connector at the bottom. Each digester gas outlet was connected to the top of its gas collector using PVC tubing. The bottom of each gas collector was connected through a manifold to a liquid overflow located near the top of the collector and equipped with an air break to prevent siphoning. Gas collectors were filled with tap water. All connections were checked for gas leaks prior to starting the trials. No gas leaks were noticed during the digestion trials.

### **Digestion trials**

Three batch trials were performed to investigate combinations of wastewater and the two types of glycerol. For each batch trial, reactors were filled with various combinations of wastewater (WW), industrial glycerol (IG), and farm glycerol (FG). Control reactors containing only inoculum sludge and tap water were maintained during each batch trial. Feed compositions used for the batch trials are given in Table 1. Each reactor had a total working volume of approximately 4.8 L, and duplicate reactors were used for each feed composition. The trials were performed in an environmental chamber maintained at 35°C. The digesters were mixed daily by turning them upside-down and shaking for about 20 seconds.

Because of variability in the composition of the wastewater samples used, each batch trial included reactors containing wastewater only. The glycerol concentrations used in this study were 2.3, 3.4, and 4.6 g L<sup>-1</sup> for farm glycerol (density: 1.1 kg L<sup>-1</sup>) and 2.5, 5, and 10 g L<sup>-1</sup> for industrial glycerol (density: 1.2 kg L<sup>-1</sup>), the highest concentration producing inhibition. Holm-Nielsen et al. (2008) indicated that glycerol concentrations above 5 g L<sup>-1</sup> caused overload and inhibition of the digestion process.



Table 1. Batch trials components.

Trial ID	Waste water	Water	Industrial glycerol	Farm glycerol	Inoculum sludge	Glycerol concentration in reactor	Batch trial number
	L	L	mL	mL	L	g L <sup>-1</sup>	
1-WW-only	2.5				2.3		1st
1-WW+ FG	2.5			20	2.3	4.6	
1-WW+ IG	2.5		20		2.3	5.0	
1-WW+IGb	2.5		40		2.3	10.0	
1-Control		2.5			2.3		
2-WW-only	2.7				2.1		2nd
2-FG-only		2.7		10	2.1	2.3	
2-IG-only		2.7	10		2.1	2.5	
2-WW+FG	2.7			10	2.1	2.3	
2-Control		2.7			2.1		
3-WW-only	3.3				1.5		3rd
3-WW+FG I	3.3			10	1.5	2.3	
3-WW+FG II	3.3			15	1.5	3.4	
3-WW+FG III	3.3			20	1.5	4.6	
3-Control		3.3			1.5		

The wastewater used in the second batch trials contained more solid chunks, which were partially separated to prevent clogging. The wastewater used in the rest of the trials was fed as collected, without separation of solids. No other pretreatment of the wastewater was done. Both glycerol samples were settled overnight to remove any scum layers formed during storage. The industrial glycerol had a thin dark scum layer on top, which was separated before feeding, to prevent potential inhibition to the AD microbial populations. In contrast, the farm glycerol had no scum formation.

The wastewater samples were mixed and measured into a separate container for each reactor. For the co-digestion trials, glycerol was added to the feed container using a manual pipette. Similarly, glycerol was added to tap water for the glycerol-only trials. Once the volume of the diluted sludge inoculum was adjusted to 2.3 L for the first batch

trials, the feed volume was added. The reactors were filled through the feeding port using a funnel connected by a short piece of tubing.

The sludge used as inoculum was recycled after each batch. Once a batch trial finished, the digesters were emptied and the sludge was allowed to settle. Liquid was decanted from the settled sludge solids, and all the solids samples from the reactors were mixed together to provide uniform inoculum for the next batch. The amount of sludge inoculum returned to the reactors was decreased for each succeeding batch to allow processing more waste in each reactor.

A higher volume of feed and higher organic load were expected to require longer digestion times for the batch trials. The duration of the batch trials was 18 d for the first batch, 16 d for the second, and 66 days for the third. The digestion trials ended when biogas production ceased.

### **Sampling techniques**

Biogas volumes and pressures were recorded daily. The gas collectors were marked to provide a direct reading of volume. Pressure was measured as the distance in height between the water level in the gas collector and the water level in the overflow (near the anti-siphon air break) (Figs. 6 and 7). Pressure was measured in inches of water. Biogas volumes were converted to standard temperature and pressure (0°C and 1 atm) assuming ideal gas behavior. Biogas was discharged from the gas collectors by lifting the overflow carboy above the collectors to refill the collectors with water and opening the valves on top of the digesters to allow the gas to exit.

Gas sampling was done through use of a tee connection located between the gas collector and digester (see Figs. 6 and 7), which was sealed with a rubber stopper. The stopper was removed and a syringe was inserted into the tee to collect a gas sample.

While gas was flowing out of the gas collector, the syringe was gradually opened to withdraw a sample. Approximately 60 mL was collected for each biogas sample which was analyzed immediately using gas chromatography. Gas samples were collected periodically during the first batch trial. During the second batch trial, biogas samples were collected daily. During most of the third batch trial, the gas chromatograph was not functioning so the biogas samples could not be analyzed.

Liquid samples were taken only the first and last days of each batch trial to determine solids content, COD, and pH. The liquid samples were collected in duplicate, with approximately 75 mL of material collected for each sample. However, additional sampling was done to measure pH during digestion. The daily mixing of digesters was performed before taking liquid samples to obtain uniform sampling. After a sample was analyzed for pH, it was returned to the reactor through the feeding port to keep digester volume constant. The pH was monitored during each batch trial, but adjustments were made only if inhibition occurred. Once inhibition occurred, a 1.5M NaOH solution was used to adjust the pH to approximately pH 7.

## **Sample analyses**

### ***Total and volatile solids (TS and VS)***

Standard methods (APHA, 2005) were used to evaluate total and volatile solids. Each sample was mixed on a magnetic stirrer, and approximately 20 mL of sample was poured into a crucible (30 mL). The samples were first placed in drying oven at 105°C overnight to determine total solids. Then the dried samples were placed in a muffle furnace at 550°C for two hours to determine the volatile solids. A larger crucible was used for the glycerol samples to avoid loss of mass during ignition in the furnace. A balance with resolution of 0.1 mg was used to weigh samples. Triplicates were generally

done for solids analysis of wastewater, glycerol, and sludge samples, and duplicates were done for digester sampling.

### ***Chemical oxygen demand (COD)***

Chemical oxygen demand is another method of indirectly measuring the organic material in a sample; therefore, COD is a common parameter in AD. This test measures the amount of oxidant (dichromate ion,  $\text{Cr}_2\text{O}_7^{2-}$ ) that reacts in a sample after digestion under rigorous conditions, which is expressed in  $\text{mg O}_2$  equivalent per liter.

The COD test was performed using a colorimetric test (HACH®, method 8000, Loveland, Colo.). Reagent vials with a high COD concentration range (0–15,000 ppm) and the model 45600 COD reactor (both from HACH®, Loveland, Colo.) were used. Absorbance of the digested samples was measured at 620 nm using a Spectronic 20D<sup>+</sup> spectrophotometer (Milton Roy, Madison, Wisc.).

For the COD analysis, samples were mixed on a magnetic stirrer and allowed to settle. Because particulate matter interferes with absorbance readings, samples were centrifuged or allowed to settle and only the liquid fraction was used. A volume of 0.2 mL of the liquid sample was pipetted into a reagent vial; the vial was closed and then mixed by shaking. Then the vials were inserted into the COD reactor for heating at 150°C for two hours (rigorous conditions). After heating in the COD reactor, the vials were cooled to room temperature, mixed, and absorbance was measured with the spectrophotometer.

The COD calibration curve was linear, with 0 as intercept, and determined by using a prepared standard solution of potassium acid phthalate (KHP) having a concentration of 10,000 (mg COD)  $\text{L}^{-1}$  and diluted solutions of 6000, 5000, 2000, and 1000 (mg COD)  $\text{L}^{-1}$ . Deionized water was used for the blank and for diluting the solutions used for calibration. The glycerol samples were diluted prior to measuring the COD by adding

deionized water at a ratio 125 mL per mL of glycerol to bring the concentration into an acceptable range for the test.

### ***pH***

Prior to taking pH measurements, the pH meter (Hanna Checker, West Henrietta, N.Y.) was calibrated at pH 4 and pH 7 with fresh standard solutions. Each sample was mixed on a magnetic stirrer while measuring pH. The glycerol samples had to be diluted with deionized water at 125:1 ratio to measure pH.

### ***Alkalinity***

The alkalinity was measured using a 0.1N H<sub>2</sub>SO<sub>4</sub> solution to titrate to pH 4.5 according to standard methods (APHA, 2005). Correct alkalinity in a reactor helps prevent abrupt pH drops. However, alkalinity was only measured for the third batch trial to determine the causes of unexpected pH drops and inhibition observed during that trial. In addition, the alkalinity of the wastewater feed for the third batch was measured to compare to the alkalinity of the inhibited reactors.

### ***Biogas analysis***

Gas samples were analyzed with a model 8610C gas chromatograph (SRI, Torrance, Cal.) to determine O<sub>2</sub>, N<sub>2</sub>, CH<sub>4</sub>, and CO<sub>2</sub> concentrations. The columns used were a 1.8-m (6-ft) silica gel packed column and a 1.8-m molecular sieve 13X packed column, both supplied and installed by the GC manufacturer. The temperature of the GC oven was held at 37°C for the first 4 minutes after injecting the sample and then ramped up from 37°C to 220°C at a rate of 20°C min<sup>-1</sup>; the run was complete after 15 minutes. The carrier gas used was helium with a flow rate of 20 mL min<sup>-1</sup> at 27 psi. A thermal conductivity detector was used.

The GC was calibrated using various standard gas mixtures containing O<sub>2</sub>, N<sub>2</sub>, CH<sub>4</sub>, and CO<sub>2</sub> (Table 2). The standard mixtures had an accuracy of 2%. The calibration curve for each gas had three points except CO<sub>2</sub> which had only two points. The GC was calibrated approximately twice monthly using the GC manufacturer's software, and low variation was observed in peak areas for the calibrated gases. Data obtained from the GC were limited because of equipment malfunctions that occurred periodically.

Table 2. Gas composition of each standard mixture.

Standard	Volume in mixture (%)			
	CH <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub>	O <sub>2</sub>
Standard 1	10	10	10	5
Standard 2	4	15		1
Standard 3	20		40	
Air sample			79	21

## CHAPTER III

### RESULTS

#### Characterization of substrates and inoculum

Results for substrate and inoculum characterization are presented in Table 3. High variability was seen in the samples of wastewater feed. Specifically, wastewater from the third batch had much higher solids content than the wastewater used in the first two batches. In addition, sludge with low VS (d.b.) and COD content was used as inoculum. Finally, low ash (i.e., high VS) in both glycerol samples (Table 3) may imply few catalyst residues are present in each glycerol. Additionally, different pH values for each glycerol may imply different neutralization processes in the biodiesel plants.

Table 3. Characterization of substrates and inoculum.

Sample	Density	Total solids (TS)	Volatile solids (VS)		COD	pH
	kg L <sup>-1</sup>	% (w.b.)	% (d.b.)	% (w.b.)	(mg COD) g <sup>-1</sup> (w.b.)	
Industrial glycerol	1.198 ± 0.031	77.1 ± 0.07	95.0 ± 0.05	73.1 ± 0.15	940 ± 52.0	4.7 ± 0.18 <sup>[a]</sup>
Farm glycerol	1.061 ± 0.044	78.2 ± 0.06 <sup>[b]</sup>	90.1 ± 0.21	70.5 ± 0.22	1535 ± 54.6	10 ± 0.08 <sup>[a]</sup>
Wastewater(1st batch)	1.005 ± 0.083 <sup>[c]</sup>	0.31 ± 0.07	65.7 ± 9.55	0.21 ± 0.08	3.93 ± 0.54	6.86 ± 0.03
Wastewater(2nd batch)	1.005 ± 0.083 <sup>[c]</sup>	0.57 ± 0.22	80.1 ± 6.89	0.46 ± 0.22	5.30 ± 0.05	7.81 ± 0.01
Wastewater(3rd batch)	1.005 ± 0.083 <sup>[c]</sup>	1.36 ± 0.57	89.4 ± 6.74	1.24 ± 0.57	7.27 ± 1.23	7.96 ± 0.05
Inoculum sludge	0.946 ± 0.085	4.81 ± 0.05	50.9 ± 0.22	2.45 ± 0.03	1.73 ± 0.20	7.32 ± 0.02

<sup>[a]</sup> diluted sample, <sup>[b]</sup> farm glycerol “moisture” was assumed to be methanol evaporated, <sup>[c]</sup> obtained from 9 samples from various WW feedstocks

The COD (d.b.) of the industrial glycerol was 1219 mg COD (g glycerol)<sup>-1</sup> (not shown in Table 3) and for farm glycerol was 1963 mg COD (g glycerol)<sup>-1</sup>. Pure glycerol has a

density of  $1.26 \text{ kg L}^{-1}$  and COD of  $1220 \text{ mg COD (g glycerol)}^{-1}$ , so the characteristics of the industrial glycerol were very similar to pure glycerol. However, characteristics of the farm glycerol were very different than pure glycerol, particularly the higher COD content. Approximate compositions of each glycerol source are presented in Table 4, along with crude glycerol composition data provided by the Houston biodiesel plant which supplied the industrial glycerol.

Table 4. Estimated compositions of the two types of glycerol along with data provided by the biodiesel plant.

Components in crude glycerol	Concentration (% wt)		
	Industrial glycerol	Farm glycerol	Biodiesel plant data
Glycerol	77	26.2	80–93
Lipid (e.g., $\text{C}_{57}\text{H}_{104}\text{O}_6$ )	0.13	52.0	0–20
Water	22.9	—	2–20
Catalyst	—	—	2–7
Methanol	—	21.8	0.1–0.5
pH	4.7	10.0	5.0–8.0

The estimated values in Table 4 were determined using the densities of each sample and solving for the fraction of pure glycerol ( $x$ ), methanol ( $m$ ), water ( $w$ ) and lipid ( $z$ ) assuming ideal mixing. Also, it was assumed that the lipid fraction included triglycerides material and had a density of  $0.915 \text{ kg L}^{-1}$ . Methanol was likely present in high amount in the farm glycerol, and had a density of  $0.791 \text{ kg L}^{-1}$ . However, any remaining methanol was assumed to be in negligible amounts for the industrial glycerol based on the plant data provided. The estimated values in Table 4 for farm glycerol were determined assuming the “moisture” fraction ( $m = 0.218$ ) was methanol, and making the sum of the other two components ( $x + z = 0.782$ ), and correcting the density of FG for the dissolved catalyst (ash):



$$x 1.26 + z 0.915 + m 0.791 = (1.06-0.082) \text{ kg L}^{-1} \quad (4)$$

For industrial glycerol, composition values were determined using  $w = 0.229$  and  $(x + z) = 0.771$ :

$$x 1.26 + z 0.915 + w 1.00 = (1.20) \text{ kg L}^{-1} \quad (5)$$

The results in Table 4 indicate that the lipid content was greater than the glycerol content in the farm glycerol. According to Thompson and He (2005), glycerol resulting from biodiesel produced from waste restaurant oil contained lipid concentrations (e.g., free fatty acids) up to 60%. Thompson and He (2005) also reported that glycerol from waste restaurant oil had a carbon content 50% higher than the average of other glycerol sources.

### **Biogas production**

Cumulative biogas volumes for the three batches are shown in Figures 8 through 10, with results for duplicate reactors designated by A and B. The first observation obtained from the three batch trials was that co-digestion trials produced more biogas than the WW-only trials. Secondly, biogas production from FG was higher than from IG as shown in the glycerol-only trials (Fig. 9). Furthermore, the co-digestion trial with farm glycerol (WW+FG) produced more biogas than the co-digestion trial with industrial glycerol (WW+IG) as shown in Figure 8.

Also, Figures 8 through 10 showed that daily biogas production variability was generally low for duplicate reactors as shown by very similar curves. Finally, biogas production from the control reactors was always low, implying that very little digestible organic matter remained in the sludge.

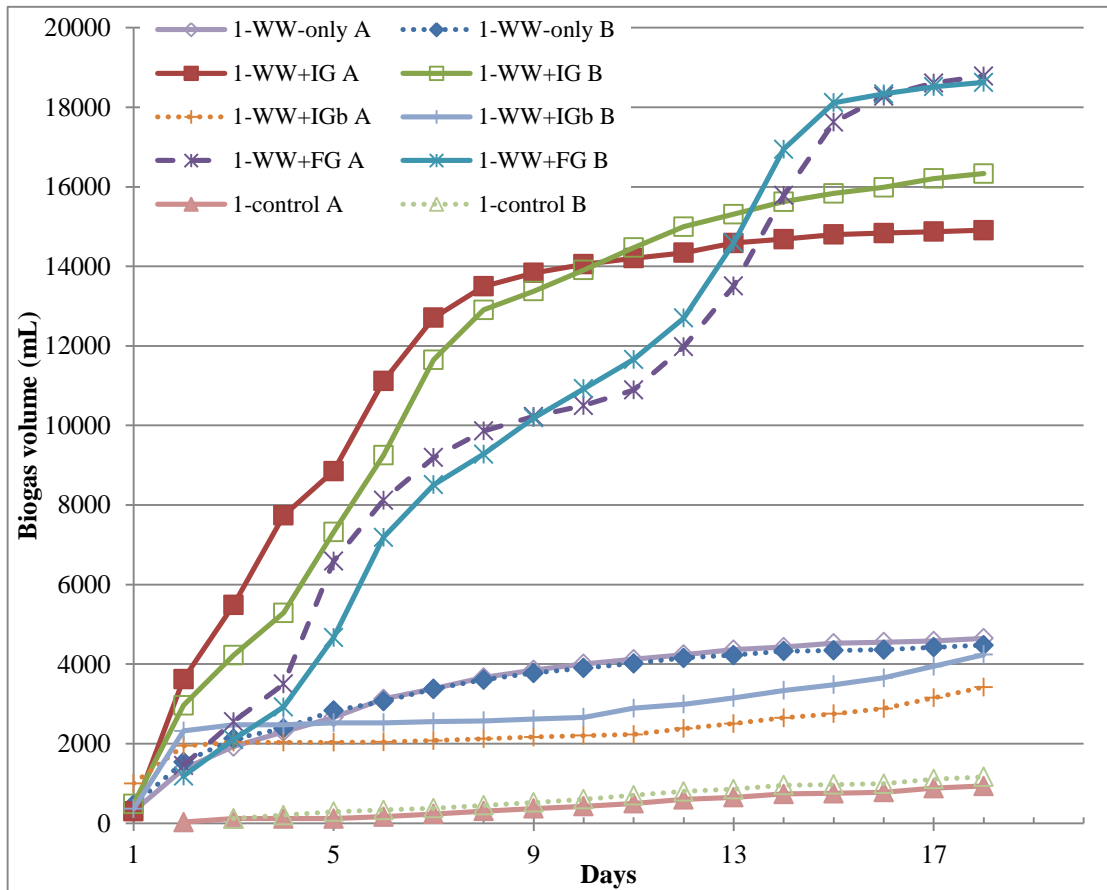


Figure 8. Cumulative biogas production for first batch trial.

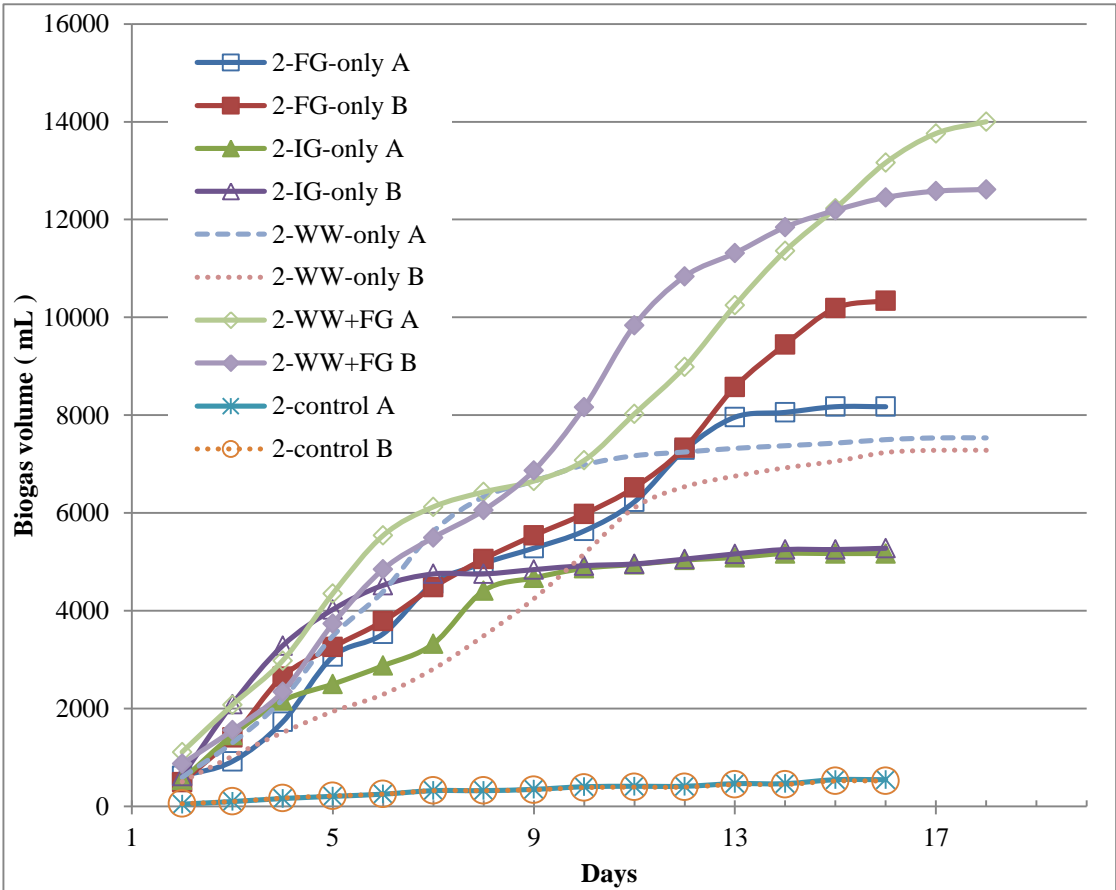


Figure 9. Cumulative biogas production for second batch trial.

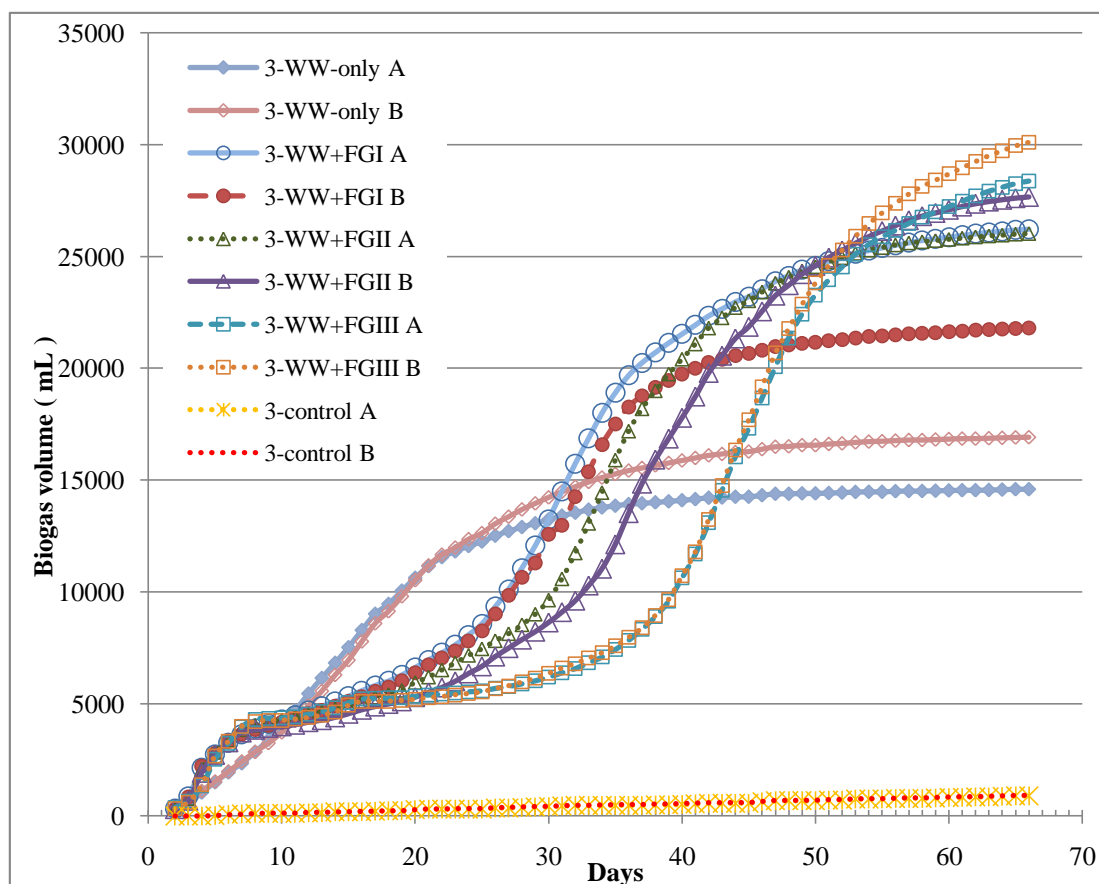
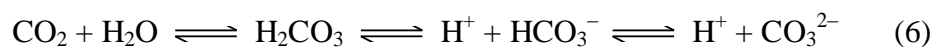


Figure 10. Cumulative biogas production for third batch trial.

Inhibition of gas production was observed in co-digestion Trial 1-WW+IGb (Fig. 8), which was likely caused by organic matter overload from the large amount of industrial glycerol added (40 mL). Similarly, the high organic load in the third batch co-digestion trials (3-WW+FGI, II, and III) (Fig. 10) led to inhibition of gas production. In both cases, a sudden drop in pH was observed in the reactors operated with high organic loadings. The pH was corrected in both trials, but the first trial was not operated long enough for recovery of gas production to occur. The third trial ran longer which allowed the reactors to recover normal gas production after several days.

## Biogas composition

Results from gas analysis showed that the CH<sub>4</sub> content in the biogas was generally much higher than the CO<sub>2</sub>. There were also varying amounts of N<sub>2</sub> and O<sub>2</sub> present (Table 5). The CO<sub>2</sub> content measured for the gas samples was likely lower than what was actually produced because of its solubility in the water used in the gas collectors. Not only is CO<sub>2</sub> soluble in water (1.135 g CO<sub>2</sub> per kg of water at 1 atm and 35°C), it reacts with water to form carbonic acid which can then dissociate to form bicarbonate (HCO<sub>3</sub><sup>-</sup>) and then carbonate (CO<sub>3</sub><sup>2-</sup>) according to Equation 6:



Because CH<sub>4</sub> is sparingly soluble in water (0.035 g CH<sub>4</sub> per kg of water at normal conditions), the biogas composition measured will be lower in CO<sub>2</sub> than what is being produced by the digestion process. Generally, CH<sub>4</sub>:CO<sub>2</sub> ratios that are produced in AD processes are closer to 1.5:1, the biogas composition being around 60% CH<sub>4</sub> and 40% CO<sub>2</sub> (Ward et al., 2008). Most of the gas samples gave initial CH<sub>4</sub>:CO<sub>2</sub> ratios greater than 2:1 at the beginning of a batch and by the last days of the batch, CH<sub>4</sub>:CO<sub>2</sub> ratios were generally greater than 5:1. The O<sub>2</sub> and N<sub>2</sub> present were assumed to be from air introduced during reactor start-up and while sampling, as any production of O<sub>2</sub> and N<sub>2</sub> by microbial populations should be negligible (Ward et al., 2008). The O<sub>2</sub> and N<sub>2</sub> contents gradually decreased during a reactor run indicating air initially in the reactor headspace and gas lines was being flushed out by the biogas production. Additional air could have entered the gas samples during the sampling process and when returning pH samples to the reactors. The N<sub>2</sub>:O<sub>2</sub> ratio in biogas samples was larger than the air ratio of 3.8:1 indicating some O<sub>2</sub> was consumed by facultative bacteria in the reactors.

Table 5. Biogas compositions for two reactors during the second batch trial.

Samples	Day	Concentration (% v v <sup>-1</sup> )				Sum
		O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub>	
2-WW-only B	2	2.37	42.7	36.6	14.1	95.8
	3	1.34	39.4	40.1 <sup>[a]</sup>	14.7	95.8 <sup>[b]</sup>
	4	0.98	34.4	44.7	14.9 <sup>[a]</sup>	95.8 <sup>[b]</sup>
	5	0.83	30.2	49.4	15.7	96.2
	6	0.68	24.2	54.1	17.2	96.2
	7	0.67	19.6	58.0	17.3	95.5
	8	0.40	14.2	62.2	18.7	95.5
	9	0.40	11.3	66.1	18.06	95.8
	10	0.60	9.2	68.6	17.9	96.3
	11	0.42	6.6	52.8 <sup>[a]</sup>	18.2	99.2 <sup>[c]</sup>
	13	0.56	5.5	77.0	15.8 <sup>[a]</sup>	98.9
17	0.63	6.4	79.9	12.6	99.5	
2-WW+FG A	2	2.81	49.3	29.2	error <sup>[a]</sup>	95.8 <sup>[b]</sup>
	3	0.980	31.5	43.3	19.8	95.6
	4	0.40	19.4	53.8	22.8	96.4
	5	0.35	11.0	61.4	23.6	96.4
	6	0.21	5.0	67.2	23.7	96.2
	7	0.33	4.2	71.0	error <sup>[a]</sup>	95.8 <sup>[b]</sup>
	8	0.45	4.1	72.0	19.25	95.8
	9	0.49	4.5	73.4	17.49	95.9
	10	0.70	4.5	72.6	17.58	95.3
	11	0.45	2.4	75.6	20.76	99.3
	13	0.29	1.5	76.2	20.95	99.0
17	0.52	2.1	79.0	17.76	99.4	

<sup>[a]</sup> GC equipment malfunctions caused concentration values to be incorrect or not detected, <sup>[b]</sup> average of sum of gases in all samples,

<sup>[c]</sup> average of sum of gases (second average after new calibration)

Because biogas produced by AD consists only of CH<sub>4</sub> and CO<sub>2</sub> along with trace amounts of H<sub>2</sub>S and other gases that were not measured, the composition of the biogas being produced by microbial activity was calculated assuming it consisted of only the CH<sub>4</sub> and CO<sub>2</sub> contents measured by GC. The fraction of CH<sub>4</sub> in the biogas for several reactors during the first batch trial is shown in Figure 11. Cumulative CH<sub>4</sub> production for each of the batch trials is shown in Figures 12 through 14. To calculate cumulative volumetric

methane production, it was assumed that the volume of biogas that accumulated in the gas collectors between readings was composed only of  $\text{CH}_4$  and  $\text{CO}_2$  as indicated above. The trends for cumulative  $\text{CH}_4$  production were similar to those for biogas production shown in Figures 8–10. The GC was not available for gas analysis during the third batch trial as it had been sent to the manufacturer for repair. Consequently, only biogas production was used for analysis of the digestion trials.

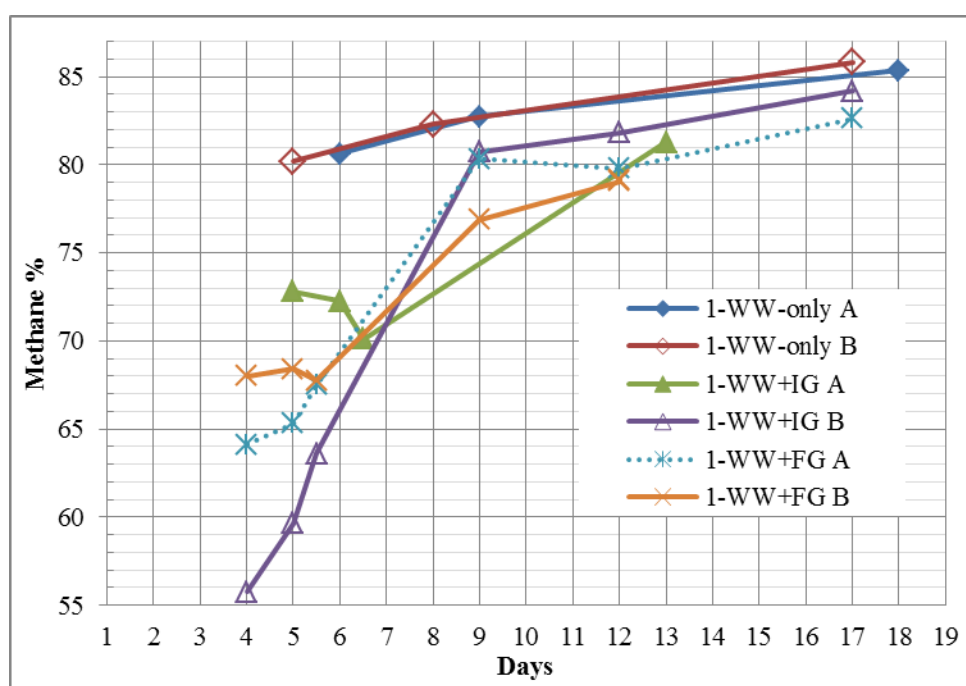


Figure 11.  $\text{CH}_4$  content of biogas produced during the first batch trials.

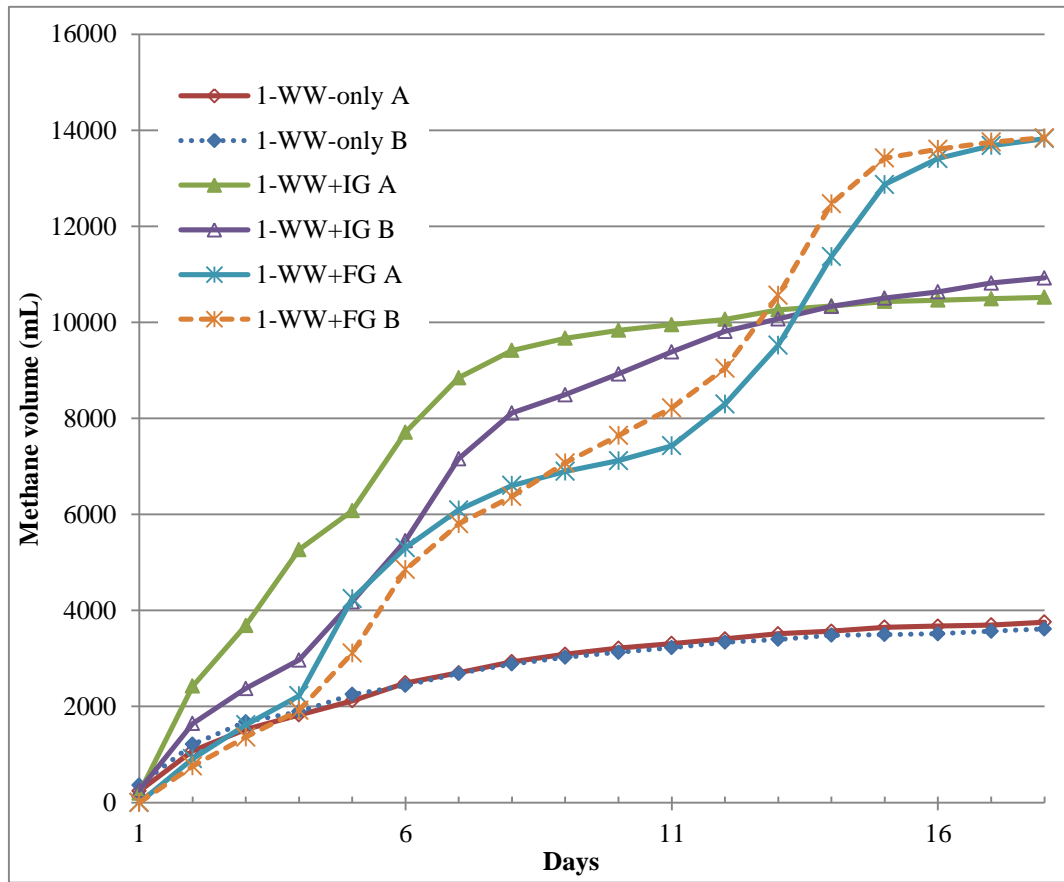


Figure 12. Cumulative CH<sub>4</sub> production for first batch.



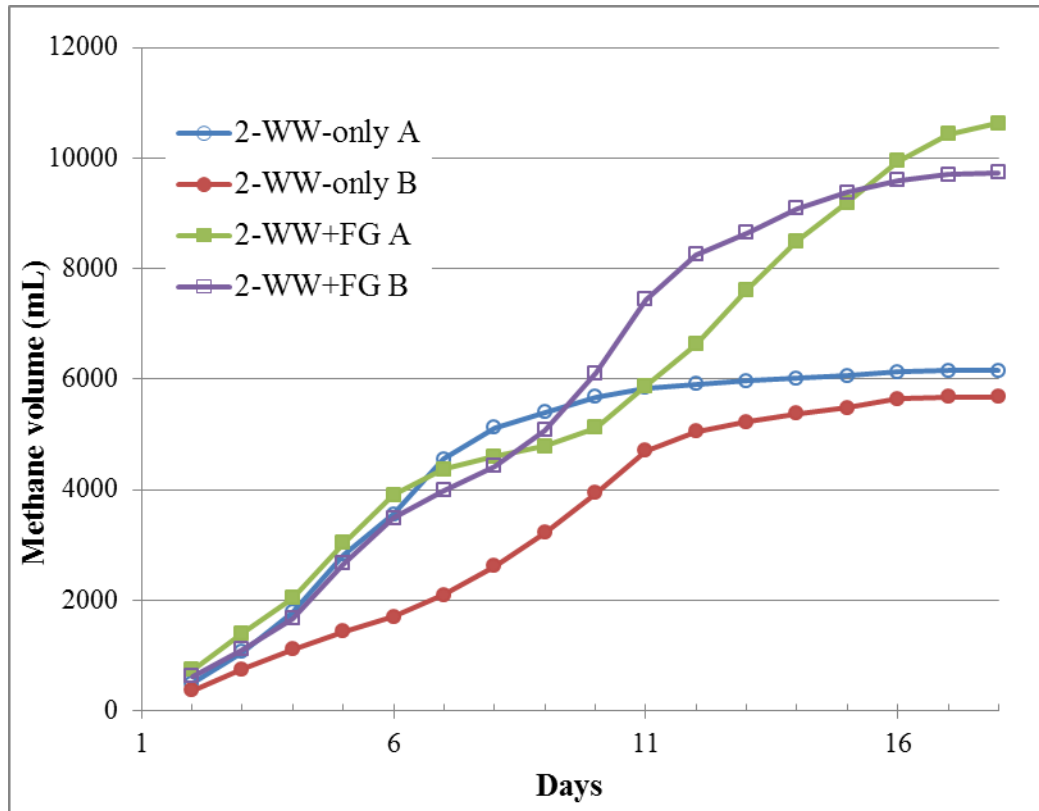


Figure 13. Cumulative CH<sub>4</sub> production for second batch.

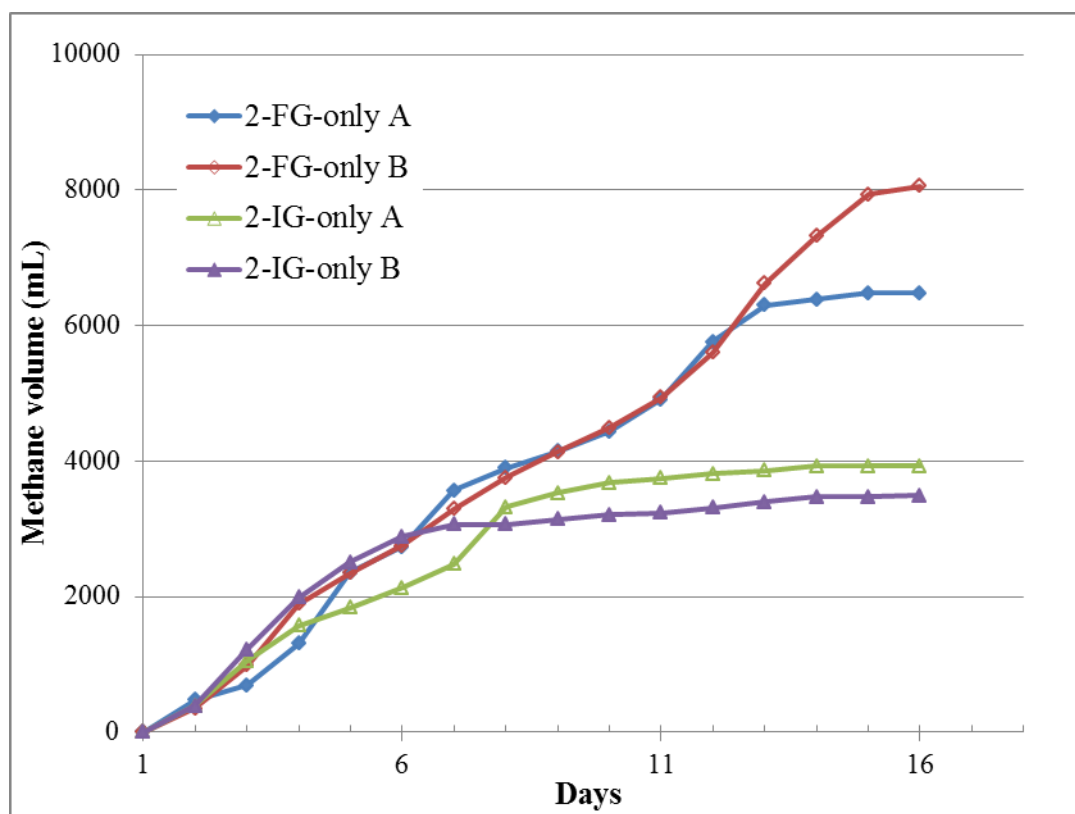


Figure 14. Cumulative CH<sub>4</sub> production for second batch (glycerol-only trials).

### Analysis of wastewater-only trials

Amounts of feed added and feed concentrations for all wastewater-only trials are shown in Table 6. The wastewater feedstock had high variability in VS and COD concentrations; consequently, the amount of organic matter varied considerably among the batch trials.

Table 6. Feed characteristics of wastewater-only trials.

Trial ID	Wastewater			Feed concentration in reactor	
	Volume	VS	COD	(g VS) L <sup>-1</sup>	(g COD) L <sup>-1</sup>
	L	g	mg COD		
1-WW-only	2.50	5.10	9,882	1.1	2.1
2-WW-only	2.70	12.54	14,395	2.6	3.0
3-WW-only	3.25	40.41	24,111	8.5	5.1

Biogas production for all wastewater-only trials is shown in Figure 15. Biogas production increased as the concentration of VS in the reactor increased. The greatest biogas production was seen in Trial 3-WW-only which had the highest VS and COD loadings in the reactors. Finally, biogas production was seen to be successful for all wastewater-only trials, with all trials having normal cumulative curves.

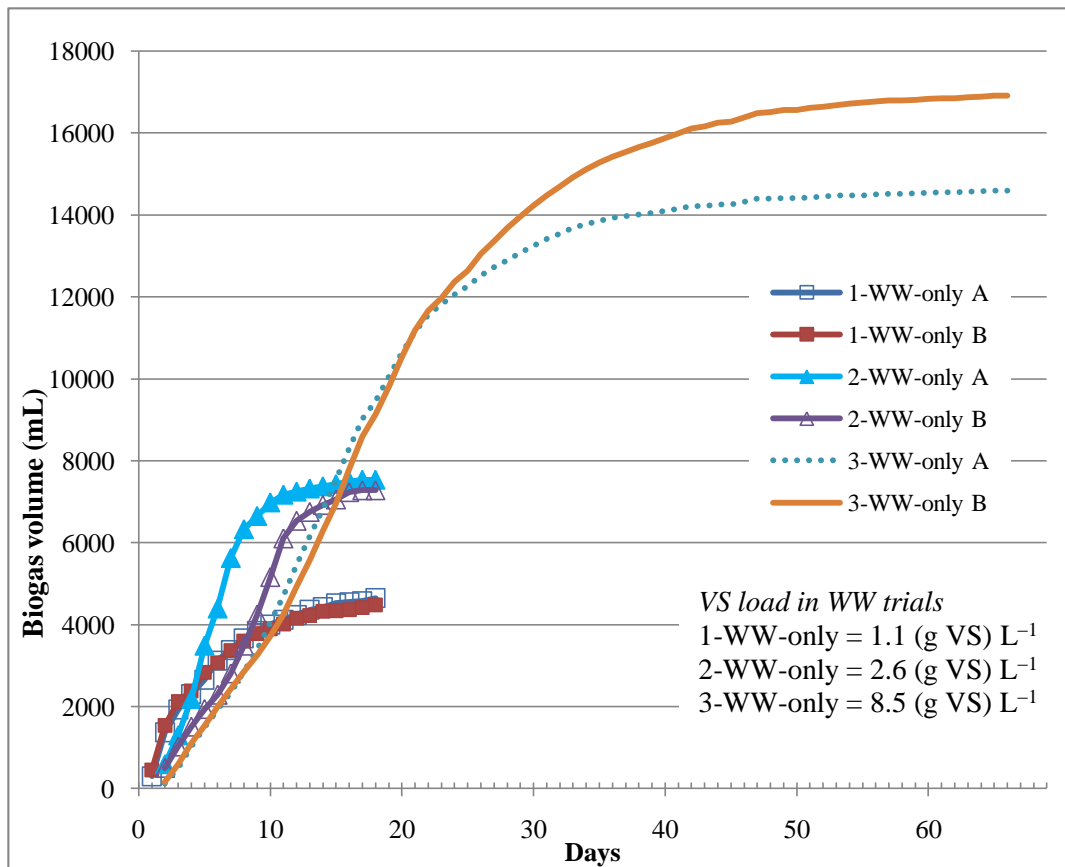


Figure 15. Cumulative biogas volumes of wastewater-only trials.

Biogas yields on VS basis for the wastewater-only trials are shown in Figure 16. The trend for biogas yield was opposite to that for biogas production (Fig. 15) with the highest biogas yield obtained in the first batch although it had the lowest biogas production. In conclusion, a high VS concentration in the wastewater increased biogas production but resulted in lower biogas yield. This could be explained by the higher VS wastewater containing more hard-to-digest solids (e.g., feathers) thereby decreasing biogas yield. However, it is possible that an improvement in mixing of the reactor or pretreatment of the wastewater feed (e.g., blending, crushing feathers) could improve digestibility and increase the biogas yield.

Biogas yields on COD basis (Fig. 17) were opposite to the biogas yields determined on VS basis (Fig. 16). This resulted from differences in the COD:VS ratio for the wastewater samples. In theory, the COD:VS ratio for samples from the same source should be approximately constant. However, the COD:VS ratios for the wastewater samples in this study were 2:1, 1:1, and 0.6:1 for batches 1, 2 and 3, respectively. This likely resulted from removing suspended solids from the sample prior to analyzing for COD. Since these suspended solids contained additional COD that could have contributed to biogas production, further analysis of biogas yield was done only on VS basis ( $\text{L (g VS added)}^{-1}$ ).

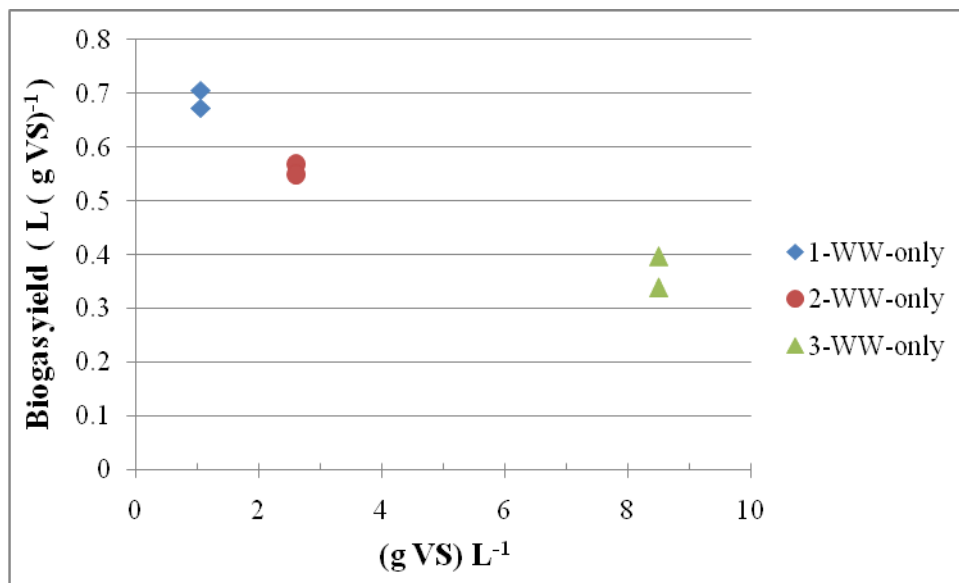


Figure 16. Biogas yield ( $\text{L (g VS)}^{-1}$ ) vs. volatile solids concentration in wastewater-only trials. The two data points for each VS concentration are for the duplicate reactors used.

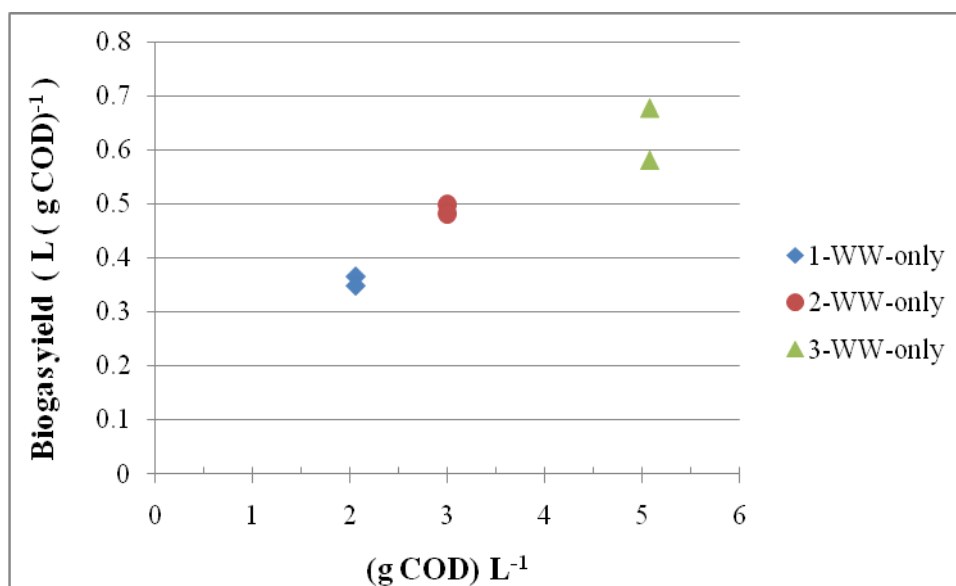


Figure 17. Biogas yield ( $\text{L (g COD)}^{-1}$ ) vs. chemical-oxygen-demand concentration in wastewater-only trials. The two data points for each COD concentration are for the duplicate reactors used.

### Analysis of glycerol-only trials

The amount of feed added and the feed concentrations of glycerol-only trials are shown in Table 7. In addition, the biogas cumulative production of glycerol-only trials is shown in Figure 18.

Table 7. Feed characteristics of glycerol-only trials.

Trial ID	Glycerol				Feed concentration in reactor	
	Volume mL	Conc. (w.b.) $\text{g L}^{-1}_{\text{reactor}}$	VS g	COD mg COD	$(\text{g VS}) \text{L}^{-1}$	$(\text{g COD}) \text{L}^{-1}$
2-FG-only	10	2.3	9.75	16,885	2.0	3.5
2-IG-only	10	2.5	8.77	11,280	1.8	2.4

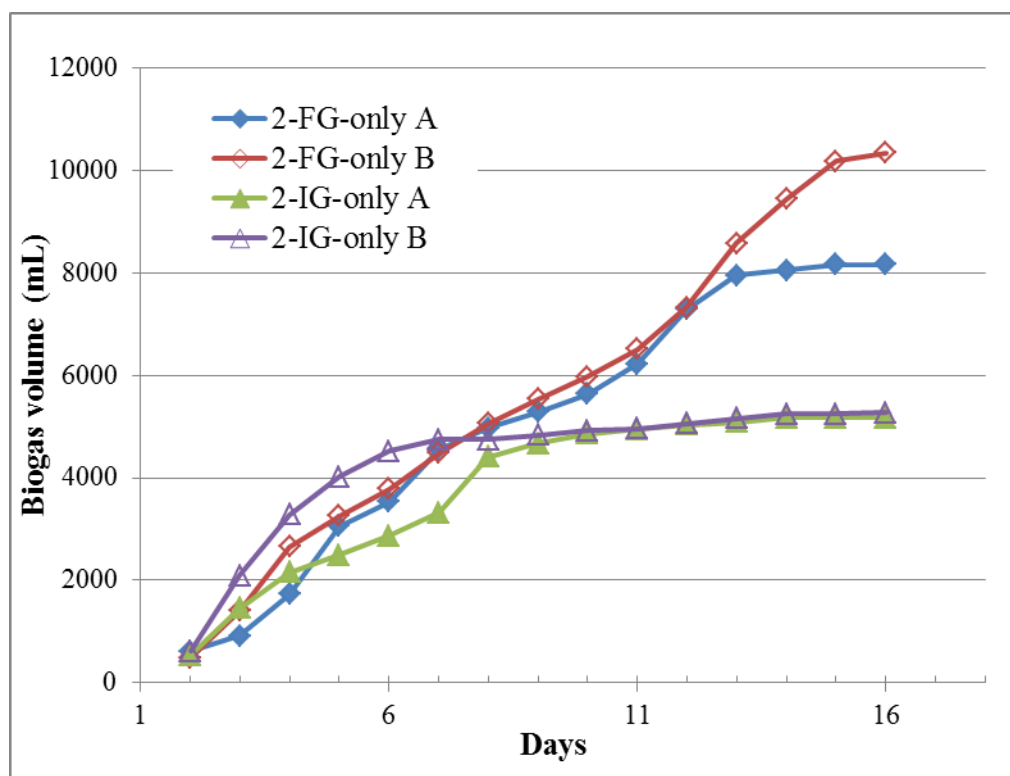


Figure 18. Cumulative biogas volumes of glycerol-only trials.

Farm glycerol produced more biogas than the industrial glycerol when comparing trials with similar glycerol concentrations. The cumulative curves are similar for both types of glycerol during the first seven days, but then the FG curves continued to increase while the IG curves became constant implying the FG had more degradable matter than IG. The variability in daily biogas production between duplicate reactors was probably caused by differences in methanogenic populations within the digesters.

Figure 19 shows the  $\text{CH}_4$  yields for crude glycerol obtained from glycerol-only trials along with the theoretical  $\text{CH}_4$  yield for pure glycerol. The average  $\text{CH}_4$  yields measured were  $0.375$  and  $0.702 \text{ L (g VS)}^{-1}$  for industrial and farm glycerol, respectively. On COD basis, the average  $\text{CH}_4$  yields were  $0.292$  and  $0.406 \text{ L (g COD)}^{-1}$  for industrial and farm glycerol, respectively. The  $\text{CH}_4$  yield for lipids ( $\text{C}_{57}\text{H}_{104}\text{O}_6$ ) is found from Buswell

equation as  $1.014 \text{ L (g VS)}^{-1}$ , similarly, the  $\text{CH}_4$  yield for pure glycerol is  $0.426 \text{ L (g VS)}^{-1}$ . The “moisture” content of the farm glycerol (21.8%) was assumed to be unreacted methanol, which has a  $\text{CH}_4$  yield of  $0.525 \text{ L (g VS)}^{-1}$ , giving a theoretical yield for FG of approximately  $0.773 \text{ L (g VS)}^{-1}$ . In addition, the theoretical  $\text{CH}_4$  yield for IG was determined to be  $0.427 \text{ L (g VS)}^{-1}$ . The considerably higher  $\text{CH}_4$  yields for farm glycerol suggest that this source contained significant amounts of other organic matter, such as lipids, that give substantially higher methane yields than glycerol.

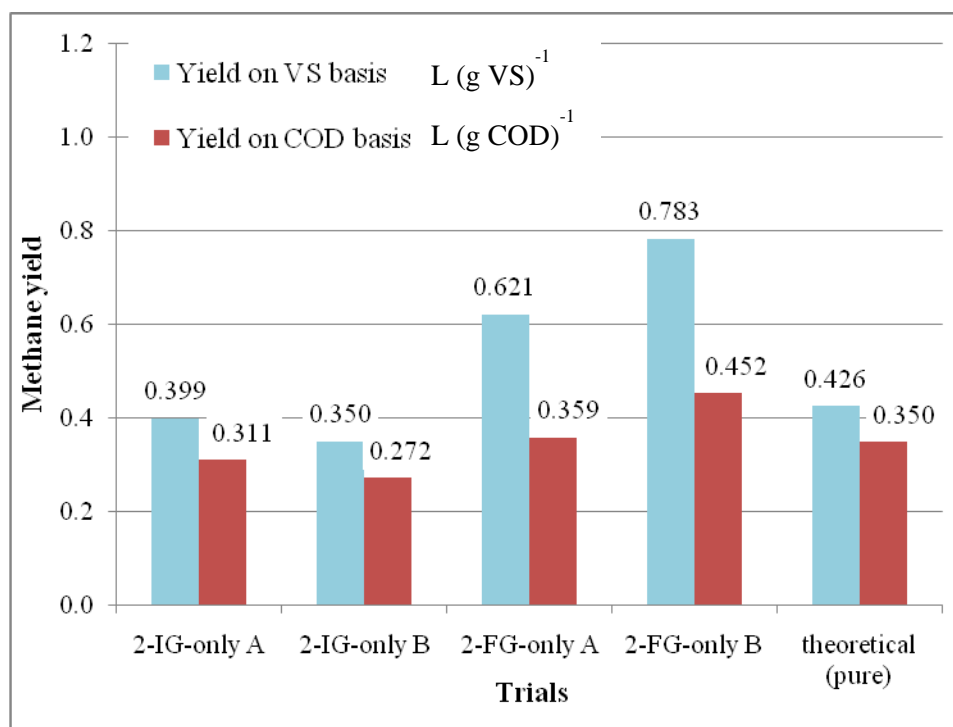


Figure 19.  $\text{CH}_4$  yields on VS and COD basis for glycerol-only trials and theoretical yield for pure glycerol.

Furthermore, the  $\text{CH}_4$  yields obtained were relatively similar to the values of the literature. For example, Chen et al. (2008a) obtained a  $\text{CH}_4$  yield from crude glycerol of  $0.360 \text{ L (g VS)}^{-1}$ . Furthermore, Siles Lopez et al. (2009) obtained a  $\text{CH}_4$  yield of  $0.302 \text{ L (g COD)}^{-1}$  for glycerol (acidified). Similarly, Ma et al. (2008) obtained a  $\text{CH}_4$  yield from



crude glycerol of  $0.393 \text{ L (g COD)}^{-1}$ . Finally, Amon et al. (2006) reported a  $\text{CH}_4$  yield from crude glycerol of  $0.750 \text{ L (g VS)}^{-1}$ . The variability on  $\text{CH}_4$  production from glycerol found in the literature is considered to agree with our results for industrial glycerol. Also, crude glycerol composition was not reported in any of these studies, including Amon et al. (2006), but their compositions are believed to include lipid residues.

### **Analysis of co-digestion trials**

Feed compositions for the co-digestion trials are shown in Table 8. High variability in VS content resulted not only from addition of different volumes of glycerol but also from the variability of the WW samples. Significantly higher VS concentrations were observed in Trial 1-WW+IGb (due to VS from glycerol) and in Trials 3-WW+FG I, II, and III (mainly due to VS from WW). As mentioned earlier, biogas production in these four trials became inhibited within the first few days of digestion by rapid production of acids which lowered the pH. Moreover, this rapid production of acids occurred only when the increased level of VS was supplied by glycerol rather than wastewater since the organic load for Trial 3-WW-only shown earlier was as high as for the inhibited trials but it was not observed to be inhibited.

Table 8. Feed characteristics of co-digestion trials.

Trial ID	Glycerol				Chicken process wastewater			Feed concentration in reactor	
	Volume	Conc. (w.b)	VS	COD	Volume	VS	COD	(g VS) L <sup>-1</sup>	(g COD) L <sup>-1</sup>
	mL	g L <sup>-1</sup>	g	mg COD	L	g	mg COD		
1-WW+IGb	40	10.0	35.10	45,120	2.50	5.10	9,882	8.4	11.5
1-WW+IG	20	5.0	17.50	22,560	2.50	5.10	9,882	4.7	6.8
1-WW+FG	20	4.4	19.50	33,770	2.50	5.10	9,882	5.1	9.1
2-WW+FG	10	2.2	9.75	16,885	2.70	12.50	14,395	4.6	6.5
3-WW+FG I	10	2.2	9.75	16,885	3.30	41.00	24,111	10.6	8.5
3-WW+FG II	15	3.3	14.60	25,328	3.30	41.00	24,111	11.6	10.3
3-WW+FG III	20	4.4	19.50	33,770	3.30	41.00	24,111	12.6	12.1

The COD:N ratios of the co-digestion feeds were approximated assuming N concentrations for WW of 135 mg L<sup>-1</sup> (according to data from the chicken processing wastewater treatment plant) and for glycerol of 1.2 mg L<sup>-1</sup> (according to Thompson and He, 2005). The co-digestion feeds had COD:N ratios ranging from approximately 90:1 to 130:1 g COD per g N. These ratios indicate that supplemental N would have to be added in continuous AD processes treating this combination of substrates to maintain digester operation.

Cumulative biogas production for the co-digestion trials is shown in Figure 20. Except for Trial 1-WW+IGb, which was not operated long enough to recover, all inhibited reactors were able to recover from inhibition (3-WW+FG I, II, III) after adjusting the pH. It can be observed in Figure 20 that the time required for recovery from inhibition increased with increasing amounts of glycerol added (3-WW+FG I, II, III). Also, the more glycerol added, the greater the amount of biogas produced. In addition, co-digestion with farm glycerol (1-WW+FG) produced more biogas than with industrial glycerol (1-WW+IG).

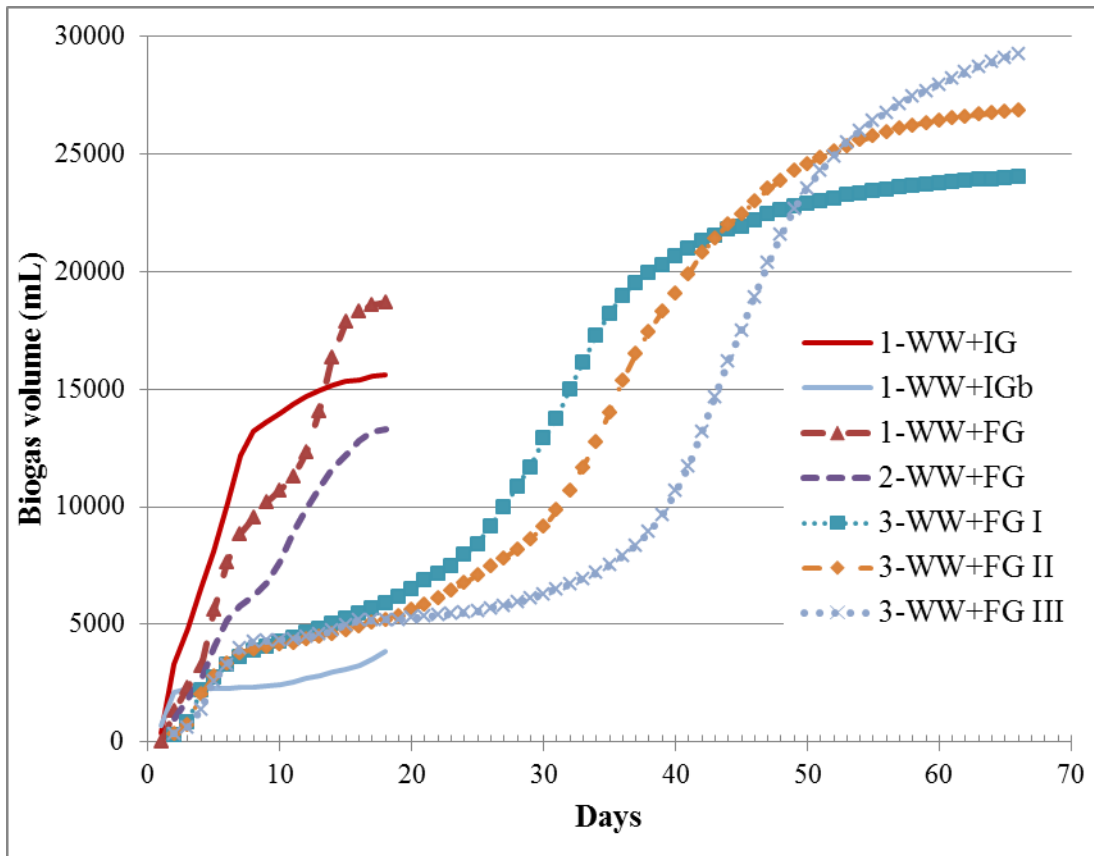


Figure 20. Biogas cumulative volumes of all co-digestion trial (average of duplicates).

Figure 21 shows the biogas production with respect to the VS concentration in the co-digestion trials (excluding Trial 1-WW+IGb since digestion was not complete).

Generally, higher biogas production was observed for higher VS loads in co-digestion trials.

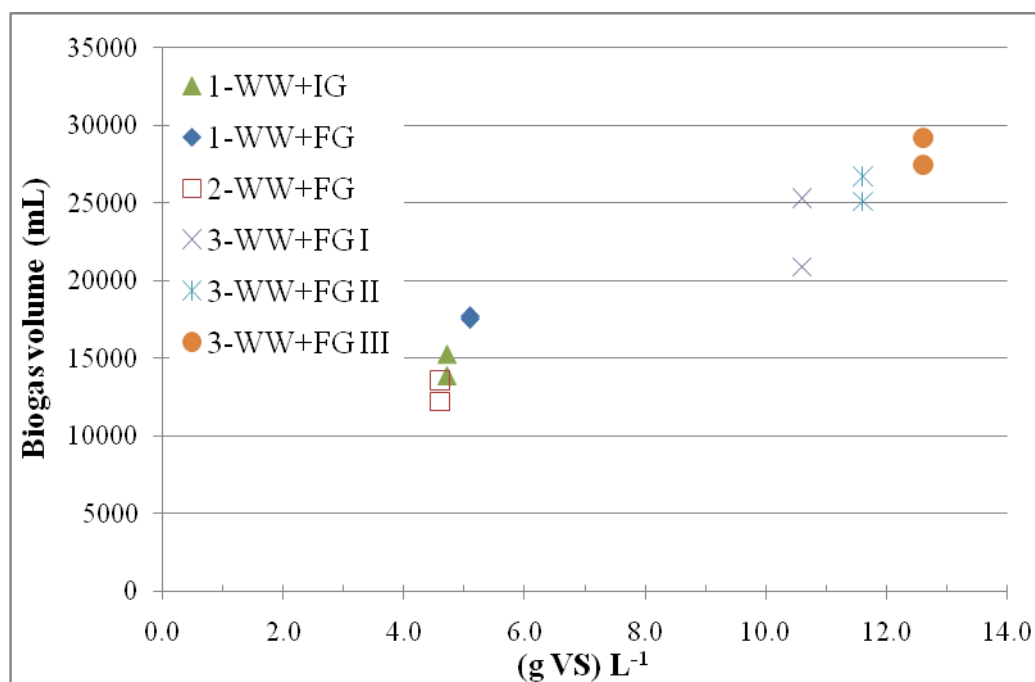


Figure 21. Biogas production vs. concentration of VS in co-digestion trials. The two data points for each VS concentration are for the duplicate reactors used.

The biogas yields of both co-digestion and wastewater-only trials are shown in Figure 22 for comparison. These data show that biogas yield generally decreases with increasing VS load. The primary factor driving this trend appears to be increasing VS concentration in the wastewater substrate, most likely because of hard-to-digest solids (e.g., feathers) present in the WW feedstock.

The average biogas yield for co-digestion trials (FG and IG) was  $0.555 \text{ L (g VS)}^{-1}$ , whereas for wastewater-only trials it was  $0.540 \text{ L (g VS)}^{-1}$ . Furthermore, the biogas yields for co-digestion were slightly greater than wastewater-only trials when comparing them batch by batch. One exception was the co-digestion trial with industrial glycerol (1-WW+IG) which had a slightly lower biogas yield than Trial 1-WW-only.

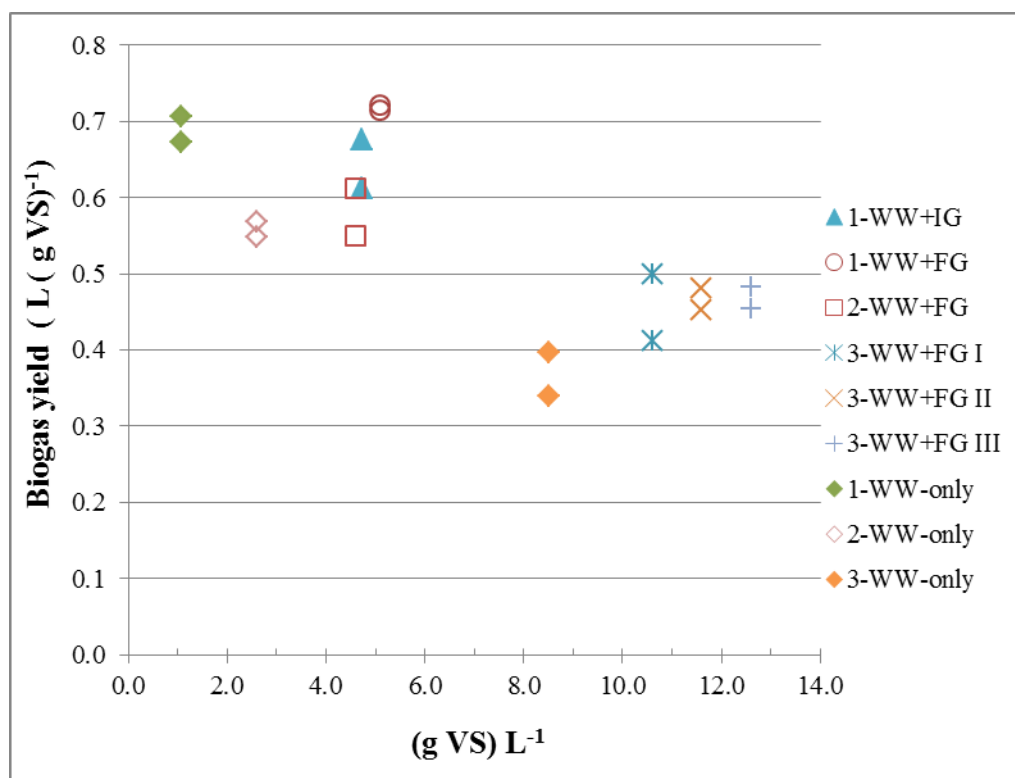


Figure 22. Biogas yields for co-digestion and wastewater-only trials. The two data points for each VS concentration are for the duplicate reactors used.

Finally, the biogas yields from glycerol are shown in Figure 23 with respect to volumes of glycerol added. The biogas production from glycerol was obtained by subtracting the biogas production from wastewater (WW-only) and the control (inoculum) for each batch from the biogas production of the co-digestion or glycerol-only trials. It is shown in Figure 23 that the biogas yields from each glycerol in co-digestion are very similar to biogas yields from glycerol-only trials. Also, the yields for FG in co-digestion are relatively constant among different glycerol volumes added. The average biogas yields from FG and IG (Fig. 23) were  $0.753$  and  $0.585$  L (g VS)<sup>-1</sup>, respectively. The higher biogas yield for FG is explained by the higher organic matter present in the crude glycerol, such as lipids and methanol.

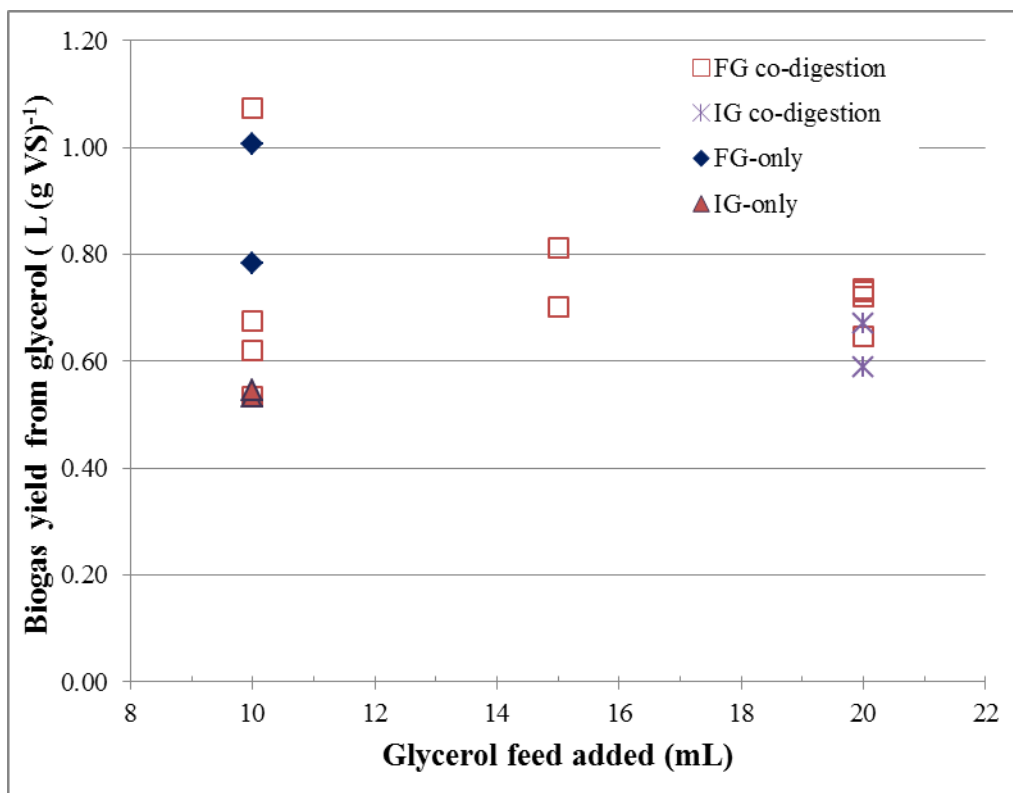


Figure 23. Biogas yields from each glycerol. All duplicates are shown (some data points are crowded).

### Degradability results

The degradability of organic matter measured as COD and VS removed is shown in Table 9. Several outliers in the VS and COD data were omitted from the analysis, as it was believed that those digester and liquid samples were not mixed well prior to solids content or COD analysis. Table 9 also shows mean and standard deviation values for similar trials. High values for standard deviations in % VS removed were observed due to sampling. Moreover, inoculum sludge was removed by centrifugation of samples from the second batch trials, but this was not done for the other batch trials which could have caused additional variability in COD and VS removal efficiencies.

Table 9 shows that the substrates wastewater and glycerol had no effect on degradability of organic matter. All the data are within one standard deviation, so no significant differences were observed among trials. Some difference was seen in % COD removed between wastewater and glycerol, but in practice this difference was smaller because COD of suspended solids in the wastewater was not measured.

### **pH analysis**

The pH profiles for the three batches are shown in Figures 24 to 26. Initially, the pH decreased slowly, but at the end of digestion the pH increased, as remaining acids were converted to  $\text{CH}_4$ . In general, the pH for all the non-inhibited trials, including wastewater-only trials and the control trials, had approximately pH 6.5 to pH 7.5 for the duration of the batch. However, the pH values for glycerol-only and co-digestion trials were slightly lower than for wastewater-only and control trials during the initial portion of the batch trials (Figs. 24 to 26), implying more acids were produced lowering the pH.

The pH decreased sharply, however, in the inhibited trials during the first two or three days of digestion (about the same time that the inhibition occurred) and remained low until pH was adjusted (Figs. 24 and 26). For example, the pH of Trial 1-WW+IGb-B dropped to pH 4.6 (Fig. 24) while the rest of the inhibited trials had a less severe pH drop: Trial 3-WW+FG III dropped only to pH 6 (Fig. 26). After adjusting the pH with 1.5M NaOH, the pH increased to approximately pH 6.4 for 1-WW+IGb and to approximately pH 7.3 for 3-WW+FG III by the end of the batch.

Table 9. VS and COD removal efficiencies for all trials.

Trials	% VS removed	% COD removed
1-WW-only A	27.4	24.6 <sup>[a]</sup>
1-WW-only B	71.3 <sup>[a]</sup>	56.0
2-WW-only A	40.5	71.1
2-WW-only B	66.7	65.8
3-WW-only A	21.5	56.9
3-WW-only B	35.7	65.4
<b>AVG±ST.DEV.</b>	<b>38.4 ± 15.6</b>	<b>63.0 ± 5.8</b>
2-FG-only A	17.7 <sup>[a]</sup>	76.8
2-FG-only B	61.4	77.0
<b>AVG±ST.DEV.</b>	<b>61.4</b>	<b>76.9 ± 0.1</b>
2-IG-only A	59.6	75.8
2-IG-only B	49.2	76.9
<b>AVG±ST.DEV.</b>	<b>54.4 ± 5.2</b>	<b>76.4 ± 0.6</b>
1-WW+FG A	-4.9 <sup>[a]</sup>	60.7
1-WW+FG B	49.0	59.0
2-WW+FG A	64.5	84.7
2-WW+FG B	67.6	87.0
3-WW+FGI A	31.5	74.0
3-WW+FGI B	40.7	73.5
3-WW+FGII A	24.2	74.6
3-WW+FGII B	13.9	73.8
3-WW+FGIII A	33.3	74.0
3-WW+FGIII B	26.5	77.7
<b>AVG±ST.DEV.</b>	<b>39.0 ± 17.2</b>	<b>73.9 ± 8.4</b>
1-WW+IGb A	27.1 <sup>[a]</sup>	-60.8 <sup>[a]</sup>
1-WW+IGb B	47.7	-67.6 <sup>[a]</sup>
1-WW+IG A	-1.1 <sup>[a]</sup>	65.6
1-WW+IG B	47.0	67.7
<b>AVG±ST.DEV.</b>	<b>47.4 ± 0.4</b>	<b>66.7 ± 1.0</b>

<sup>[a]</sup> outlier due to inconsistencies because of unmixed samples



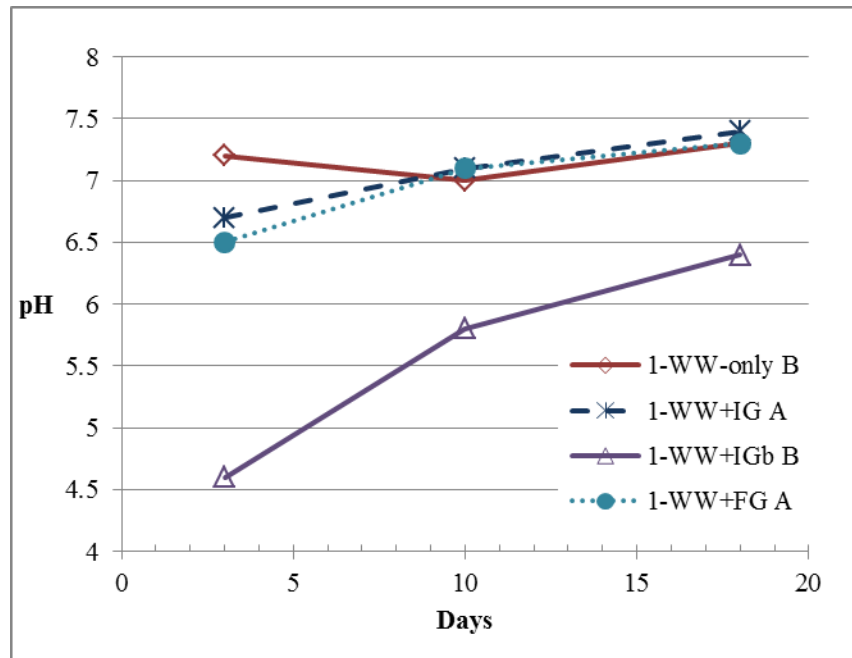


Figure 24. Profiles of reactor pH during first batch (one reactor pH plotted).

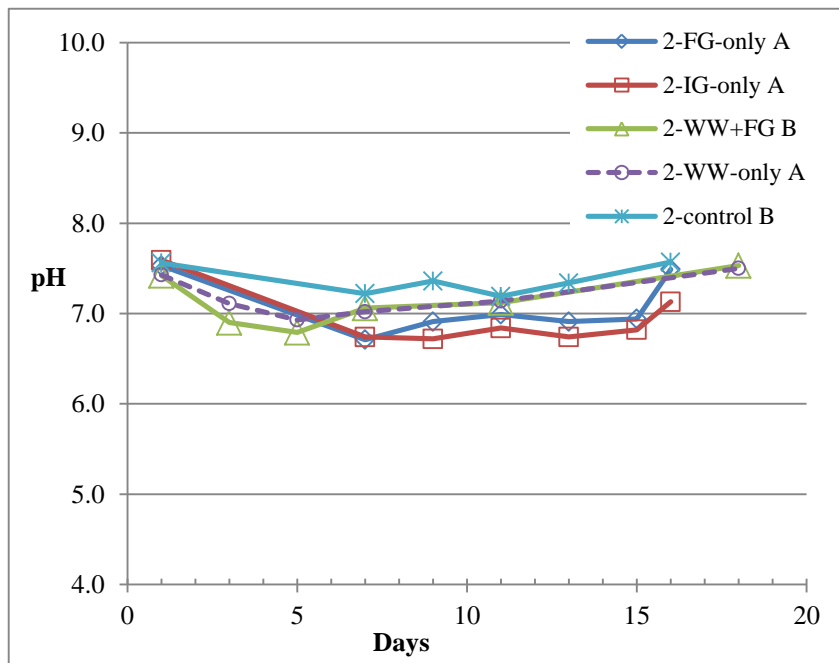


Figure 25. Profiles of reactor pH during second batch (one reactor pH plotted).

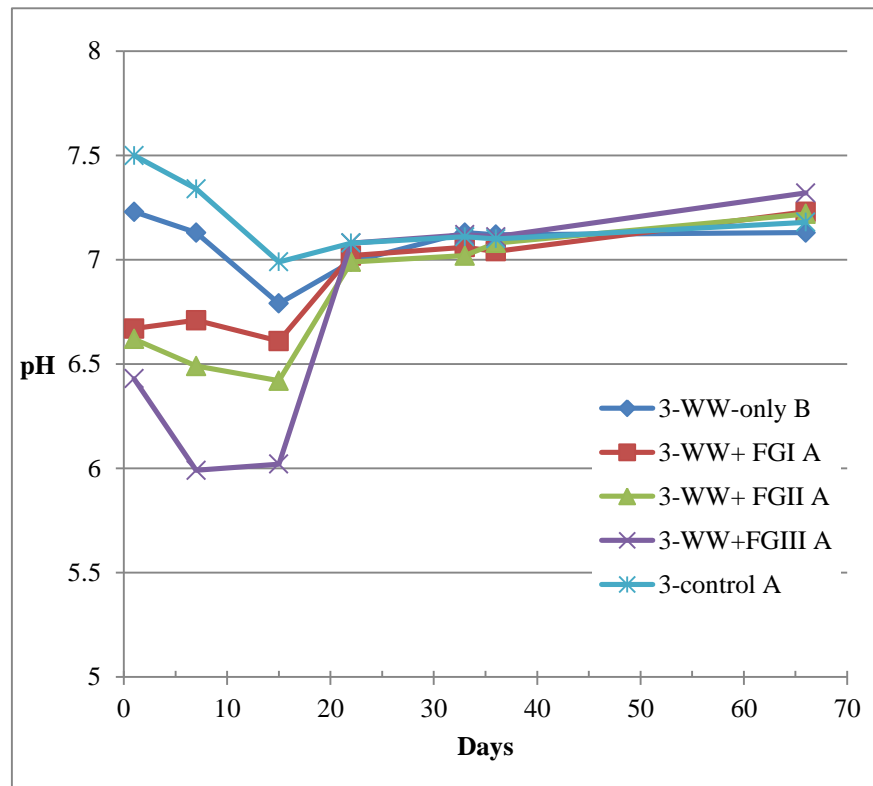


Figure 26. Profiles of reactor pH during third batch (one reactor pH plotted).

For these experiments, the pH was adjusted only after a drop in pH was observed. The large changes in pH observed in some trials might have been prevented or reduced with greater alkalinity in the reactors. For instance, less inoculum was used for the third batch trials, which likely reduced the amount of alkalinity available. The alkalinity was not determined in all batch trials, so it could not be confirmed if alkalinity present in the reactors could have prevented inhibition from high VFA accumulation.

## CHAPTER IV

### CONCLUSIONS

In agreement with prior studies on co-digestion with glycerol, this study showed that glycerol could be a co-substrate of interest to biogas producers using chicken processing wastewater. Results confirmed that co-digestion of chicken processing wastewater and glycerol improved biogas yields over the wastewater alone, even though slightly. The biogas yield for co-digestion with glycerol was  $0.555 \text{ L (g VS)}^{-1}$  and for wastewater-only trials was  $0.540 \text{ L (g VS)}^{-1}$ . Therefore, glycerol addition increased the biogas yield by 3% on average, but biogas yields up to 26% greater were obtained in one batch (3<sup>rd</sup> batch).

Glycerol feed provided more organic matter to the microorganisms, however, the degradability of the co-digestion trials was not significantly different from the degradability of wastewater-only trials, 38.4% and 39.0% VS removed, respectively. Therefore, glycerol addition did not affect effluent quality adversely, as the degradability of wastewater co-digested with glycerol was similar to that for wastewater alone.

The different processing methods for glycerol had an effect on glycerol composition and on biogas and  $\text{CH}_4$  production from glycerol. A considerably higher  $\text{CH}_4$  yield was obtained for the glycerol from an on-farm biodiesel plant compared to one from an industrial/commercial biodiesel operation. The farm glycerol organic composition was estimated at approximately 52.0% lipids, 26.2% glycerol and 21.8% methanol, but the industrial glycerol composition was estimated to contain only glycerol and negligible lipids. The  $\text{CH}_4$  yield for farm glycerol was  $0.702 \text{ L (g VS)}^{-1}$  and for industrial glycerol it was  $0.375 \text{ L (g VS)}^{-1}$ . Therefore, crude glycerol containing substantial amounts of lipid (e.g., free fatty acids) and methanol, such as the farm glycerol, can be expected to give higher  $\text{CH}_4$  yields than more highly purified glycerol.

Severe inhibition was observed in co-digestion trials with high organic loadings, implying acidification occurred during these trials. The inhibition was observed only in co-digestion trials, implying that glycerol was rapidly degraded and produced high amounts of intermediate acids which caused the inhibition of biogas production. However, the inhibition of the microbial populations was reversible, even though it took several days until inhibited trials were able to recover biogas production. However, co-digestion trials produced more biogas than wastewater-only trials after recovering from inhibition.

Finally, biodiesel producers may profit by selling the crude glycerol to biogas producers as a co-substrate for anaerobic co-digestion. In practice, biodiesel producers could obtain energy from glycerol using their own biogas reactor, but they need a primary substrate to supply nutrients for the AD process. Biodiesel producers would not only generate revenue from the low-value glycerol waste but also improve their environmentally friendly image if glycerol is used in biogas reactors.

### **Recommendations for future studies**

Co-digestion of these two substrates should be examined in continuous AD reactors to make sure the nutrient balance, including nitrogen and micronutrients, is sufficient to maintain biogas production. Future continuous AD studies should determine the optimum organic loading rate and the maximum glycerol concentration for efficient biogas production, and avoid accumulation of intermediate acids and inhibition of methanogenic bacteria. Furthermore, supplementation of alkalinity should be done if necessary to avoid inhibition of methanogenic bacteria due to low pH.

It is recommended that future AD studies should examine the crude glycerol quality in order to increase CH<sub>4</sub> production. The crude glycerol should be characterized for lipids, glycerol, methanol and other components to determine the theoretical CH<sub>4</sub> yield from the

crude glycerol. The Buswell equation may be used to determine the theoretical CH<sub>4</sub> (and biogas) yield of crude glycerol containing organic impurities.

A pilot-scale AD study should examine the effluent quality resulting from co-digestion treatment of chicken processing wastewater and crude glycerol. Our study determined the degradability in terms of VS and COD reduction, but degradability of other parameters such as total and soluble COD and suspended solids should be evaluated to determine if the AD effluents will meet the environmental norms for discharge. Also, pretreatment of the chicken processing wastewater, such as crushing or removing feathers, and efficient mixing in the reactor may increase biogas production and degradability.

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**APPENDIX A**  
**ADDITIONAL DATA**

Table A-1. Substrate and inoculum characterization (raw data).

Samples	% TS	% VS	Density
	(w.b.)	(d.b.)	kg L <sup>-1</sup>
<b>Industrial glycerol</b>	77.1	no data <sup>[a]</sup>	1.169
	77.2	no data <sup>[a]</sup>	1.210
	77.1	94.3 <sup>[b]</sup>	1.240
	74.9	95.0	1.159
	77.1	95.0	1.217
	77.2	95.1	1.193
<b>Farm glycerol</b>	88.4	no data	1.129
	88.5	no data	1.058
	88.4	59.1 <sup>[b]</sup>	1.070
	78.2	90.0	1.040
	78.3	90.3	1.009
<b>Wastewater (1st)</b>	0.36	72.4	no data
	0.26	58.9	no data
<b>Wastewater (2nd)</b>	0.46	78.5	no data
	0.82	87.7	no data
	0.42	74.2	no data
<b>Wastewater (3rd)</b>	1.83	93.8	1.216
	0.57	79.4	no data
	1.70	93.2	0.976
	1.34	91.3	0.986
<b>Wastewater (samples not used for trials)</b>	0.48	no data	0.980
	0.47	no data	0.999
	0.51	no data	1.012
	0.29	51	0.917
	0.29	57.3	0.976
	0.29	54.8	0.984
<b>Dewatered sludge as collected in plant</b>	16.7	58.9	no data
	16.8	59.0	no data
	16.8	59.0	no data
<b>Sludge inoculum (used in 3rd batch)</b>	4.84	51.1	0.970
	4.75	50.8	0.892
	4.84	50.6	1.056
	4.84	51.1	0.868
<sup>[a]</sup> faulty data, sample spill in ash oven			
<sup>[b]</sup> ash oven at 350°C			
ashes were brownish color for industrial glycerol			
and black for farm glycerol: different inorganic matter			

Table A-2. Chicken processing wastewater data from Sanderson Farms.

Components measured	units	June 2010		July 2010		2-month	
		average	std dev	average	std dev	average	std dev
Biological Oxygen Demand (5 day)	mg/L	2966	603	3578	647	3238	665
Chemical oxygen demand	mg/L	6300	2009	7218	924	6708	1604
Ammonia as N	mg/L	7.4	2.3	10	3.7	8.4	3.1
Total Kjeldahl Nitrogen as N	mg/L	143	15	125	12	135	16

Table A-3. Sample of wastewater data provided by Sanderson Farms.

**DRAFT: Sanderson WWTP - Process****DRAFT: Inorganic Parameters (Bryan Facility)**

	<b>Analysis</b>	<b>SQL</b>	<b>Result</b>	<b>Units</b>
NEL	BOD (5 day)	780	3290	mg/L
NEL	Chemical Oxygen Demand	240	7500	mg/L
NEL	Ammonia as N	0.15	13.2	mg/L
NEL	Total Kjeldahl Nitrogen as N	8.33	126	mg/L

The above sample was received in acceptable condition according to Aqua-Tech Laboratories, Inc. Subchapter D, Part 136.3, TABLE II.-*Required containers, preservation techniques, and holding*

## APPENDIX B

### BIOGAS AND METHANE RAW DATA

Table B-1. Biogas production (mL) for first batch trials (raw data).

Day	1-WW-only				1-WW + IGb				1-WW + IG				1-WW + FG				1-Control				
	A		B		A		B		A		B		A		B		A		B		
	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	Volume mL	Pressure in. H <sub>2</sub> O	
1	340	4	510	6	1100	10.5	400	4.5	330	4	540	5	?	1	?	0.5	?	?	?	?	?
2	1160	13.5	1170	15.5	1020	18.5	2100	22	3480	32	2620	27	1580	14	1300	11.5	30	4.5	?	?	
3	620	6	650	6.5	100	?	170	3	2010	18	1380	13	1210	11	1010	9.5	100	2.5	130	2	
4	420	3.5	290	9	0	?	0	0	2420	21	1140	22	1020	20	890	17	?	?	100	3	
5	400	7	490	12.5	0	?	60	3.5	1220	11	2180	21	3260	28	1880	18	0	0	90	4.5	
6	510	9	260	4.5	10	0	0	0	2420	23	2080	18.5	1660	17	2680	25	50	6.5	50	5	
7	290	4.5	340	7.5	40	0	30	1	1730	16	2570	22.5	1180	11	1450	13	80	7	50	5.5	
8	310	7	260	9.5	50	0.5	20	1	870	8.5	1370	13	740	7	850	8	80	8	80	6	
9	210	9	190	2.5	50	1	50	1.5	380	4	510	17	400	10.5	990	16	70	8.5	80	7	
10	170	2.5	140	4	40	1	50	2	240	6	590	6	310	3	810	8	60	9	90	7.5	
11	130	3.5	130	5	30	1.5	260	2.5	170	7.5	620	11	430	7	800	14.5	80	10	110	8.5	
12	130	5	150	5.5	170	2.5	110	3.5	150	8.5	570	15.5	1180	16.5	1120	20.5	110	10	110	9.5	
13	140	5.5	80	6.5	140	2	180	2.5	270	10	350	4	1660	15	2040	18.5	60	11	60	10	
14	70	6	110	7	160	3.5	210	4.5	110	2	350	7	2440	21.5	2520	22	90	11.5	110	11	
15	110	7	20	7.5	110	4.5	160	5.5	130	3	230	9	1990	18	1280	12	20	11.5	20	11	
16	30	7	20	7.5	150	5.5	190	7	40	3.5	170	10	730	7	250	3	30	12	20	11	
17	30	7	70	1.5	300	8	320	10	40	3.5	240	12	360	10	200	5	110	12.5	130	12	
18	80	1.5	60	2	300	3.5	330	3.5	40	4	140	2	200	2.5	120	6	60	13	60	12.5	

The calculations for the conversion of biogas volumes to standard temperature and pressure are included in a separate file.

Table B-2. Biogas daily production (mL) for first batch trials (standard temperature and pressure).

day	1-WW- only		1-WW+ IG		1-WW + FG		1-WW + IGb		1-control	
	A	B	A	B	A	B	A	B	A	B
1	304	459	295	485	?	?	1000	358	?	?
2	1062	1077	3327	2477	1449	1185	945	1962	27	?
3	558	585	1861	1262	1102	916	89	152	89	116
4	375	263	2256	1065	949	822	0	0	0	89
5	361	448	1111	2032	3089	1740	0	54	0	81
6	462	233	2266	1928	1533	2522	9	0	45	45
7	260	307	1594	2404	1074	1326	35	27	72	45
8	280	236	787	1253	667	768	44	18	72	72
9	190	169	340	471	364	912	44	44	63	72
10	152	125	216	531	277	732	36	45	54	81
11	116	117	153	564	388	734	27	232	73	100
12	117	135	136	525	1088	1043	152	98	100	100
13	126	72	245	313	1526	1891	125	161	55	54
14	63	99	98	316	2277	2355	143	188	82	100
15	99	18	116	208	1842	1168	99	144	18	18
16	27	18	36	154	658	223	135	171	27	18
17	70	62	36	219	327	179	271	291	101	119
18	110	53	36	125	178	108	268	295	55	55

Table B-3. Biogas production (mL) for second batch trials (raw data).

day	2-FG-only				2-IG-only				2-WW-only				2-WW+FG				2-Control			
	A		B		A		B		A		B		A		B		A		B	
	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O
2	690	6	550	5	600	4.5	690	5	660	6.5	560	5.5	1220	11	960	9	50	2	60	2.5
3	330	8.5	1000	13.5	1010	10	1600	15	780	7.5	580	6	1060	10	760	7.5	60	1.5	50	2
4	890	9	1360	13	780	7.5	1310	12	980	9	540	5.5	1000	9	870	8	70	2	80	2.5
5	1460	13	670	6.5	380	4.5	820	8	1420	13	480	5	1500	13.5	1530	11.5	50	2	50	2.5
6	520	5.5	590	5	420	4.5	550	5.5	1000	9.5	390	4	1300	12	1220	11	50	2.5	40	2.5
7	1130	10.5	770	7.5	500	5	260	3	1350	12	580	5	650	6.5	720	7	80	3.5	80	3.5
8	460	5	640	6.5	1210	4.5	0	0	780	7.5	750	7.5	340	4	620	6.5	0	0	0	0
9	350	2.5	540	3	290	7	100	2	360	4	840	8	250	3	900	8.5	30	3.5	20	3.5
10	390	6	490	5	220	3	90	2	370	4.5	1010	9.5	470	5	1410	13	60	4	50	4
11	660	6.5	600	6	90	2	40	2	210	3.5	1040	9.5	1050	9	1820	16	10	4	10	4
12	1170	11	900	8.5	100	2.5	110	3	85	1	480	5	1060	9.5	1100	10.5	0	0	0	0
13	740	7	1360	12.5	60	1.5	120	2	85	2.5	240	7	1350	21	520	14.5	60	5	60	4.5
14	110	2	960	9	90	2.5	100	3	60	1	190	2.5	1220	11	590	6	0	5	0	4.5
15	130	3	820	8	0	2.5	0	3	60	1	150	4	940	19	380	9	90	5	80	5
16	0	3	170	9.5	0	2.5	30	1	80	2	200	5.5	1030	9.5	290	10.5	0	5	0	5
17									40	2.5	50	6	650	15	140	13	10	5	10	5
18									0	0	0	0	270	3.5	40	1.5	0	0	20	0

Table B-4. Biogas daily production (mL) for second batch trials (standard temperature and pressure).

	2-FG-only		2-IG-only		2-WW- only		2-WW+ FG		2-control	
Day	A	B	A	B	A	B	A	B	A	B
2	621	494	538	619	594	503	1111	870	45	54
3	299	916	917	1471	704	522	963	686	53	45
4	806	1244	704	1195	888	485	906	786	62	71
5	1336	603	341	741	1299	431	1374	1395	45	45
6	467	529	376	494	907	349	1186	1111	45	36
7	1028	695	449	232	1232	520	585	649	72	72
8	413	576	1084	0	704	677	304	558	0	0
9	312	482	261	89	322	759	223	814	27	18
10	351	440	196	80	332	916	422	1290	54	45
11	594	540	80	36	188	943	951	1677	9	9
12	1065	814	89	98	76	431	962	1000	0	0
13	667	1243	53	107	76	216	1258	477	54	54
14	98	870	80	89	53	169	1111	531	0	0
15	116	741	0	0	53	134	872	344	81	72
16	0	154	0	27	71	180	934	264	0	0
					36	45	597	128		
					0	0	241	36		

Table B-5. Biogas production (mL) for third batch trials (raw data).

Day	3-WW+FGI				3-WW+FGII				3-WW+FGIII				3-WW-only				3-Control				
	A		B		A		B		A		B		A		B		A		B		
	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	
2	360	4	330	4	370	4	340	4	420	4.5	350	4	140	2.5	180	2.5	0	0	0	0	0
3	600	6	620	6	500	5	420	4.5	300	2.5	330	4	410	4.5	480	5	0	0	0	0	0
4	1400	13	1480	14	1430	13.5	1360	13	760	7.5	850	8.5	600	6	580	6	0	0	0	0	0
5	650	6.5	650	6.5	840	8.5	890	9	1300	12.5	1420	13.5	510	5.5	470	5	20	0	20	0	0
6	580	6	560	5.5	590	6	620	6	800	7.5	720	7	490	5	500	5	40	0	40	0	0
7	420	9.5	380	9	520	10.5	480	10.5	800	14.5	730	13	450	9	490	9.5	40	0.5	20	0.5	0.5
8	300	3.5	240	3	220	3	140	2	380	4	250	3	570	6	480	5	20	0.5	40	0.5	0.5
9	230	3	200	2.5	140	2	90	1.5	50	1.5	40	1.5	610	6	440	4.5	0	1	20	0	0
10	220	3	180	2.5	130	2	80	1.5	20	1	10	1	730	7	530	5.5	0	1	0	0	0
11	210	4	180	4	120	3	80	2	60	1.5	60	1.5	700	13	570	10.5	0	1	0	0	0
12	250	6.5	220	5.5	160	4.5	130	3	130	2.5	80	2	820	18	740	16.5	40	1.5	40	0.5	0.5
13	200	8.5	180	7	120	5.5	100	4	120	3.5	120	3	740	26	690	22	0	1.5	0	0	0
14	240	3	210	2.5	160	2.5	140	2	200	2.5	160	2.5	750	7.5	800	7.5	30	1.5	20	0.5	0.5
15	260	5	230	4.5	180	4	180	3.5	250	4.5	330	4.5	760	13.5	720	13.5	20	2	10	1	1
16	280	3.5	250	3	210	3	200	2.5	180	2.5	200	2.5	850	8.5	920	9	0	1	20	1	1
17	260	3.5	260	3.5	180	3	160	3	30	0	0	0	800	10.5	890	11	20	1	10	1	1
18	220	5.5	210	5	140	4	130	4	30	0	10	0	480	14.5	590	16	20	1	20	1	1
19	300	8	300	8	210	6	130	5	10	0	30	0	640	18.5	710	22	0	1	0	1	1
20	370	4	410	4.5	390	4	210	3	70	0	60	0	640	6.5	800	7.5	50	1.5	50	1.5	1.5
21	380	7	400	7.5	270	6.5	240	4.5	70	0.5	90	1	570	11	720	13.5	30	1.5	40	2	2
22	350	10	340	10.5	350	9.5	240	6.5	40	1	50	1.5	440	14.5	520	18	0	1.5	0	2	2
23	390	4.5	340	4	350	4	310	3.5	60	0	90	1.5	290	3.5	350	4	10	1	20	1	1
24	500	6	490	6	380	5	390	5	60	1	60	2	270	4	420	5.5	0	0.5	0	1	1
25	540	10.5	500	10	320	7.5	350	8	50	1.5	90	2.5	220	6	300	8	0	0.5	0	1	1
26	830	17.5	830	17	400	11	480	12	120	2.5	160	4	290	8.5	460	11.5	40	1	40	1	1
27	860	8	900	8.5	350	4	420	4.5	100	1.5	140	2	230	2.5	350	3.5	20	1	20	1	1
28	1030	16.5	890	16	440	7.5	420	8	130	3	190	3.5	200	4.5	360	6.5	20	1	20	1	1
29	1060	25.5	690	21.5	520	12	390	11	150	4	200	5.5	190	6	300	9	20	1.5	20	1.5	1.5
30	1280	11.5	1380	12.5	700	6.5	460	4.5	180	2.5	230	3	190	2.5	300	3.5	20	0.5	10	1.5	1.5
31	1360	23	440	16	1030	15.5	510	9	220	4	270	5	190	4	280	5.5	40	1	30	2	2
32	1350	12.5	1410	12.5	1270	11.5	570	5.5	210	3	220	3	150	2	250	3	10	1	20	2	2
33	1200	22.5	1200	22.5	1410	23.5	740	12	290	5	270	5	150	3.5	240	5	0	1.5	0	2	2
34	1240	12	1320	12.5	1500	14	830	8.5	280	1.5	270	1.5	110	1.5	220	1.5	10	0.5	20	1.5	1.5



Table B-5. Continued.

Day	3-WW + FGI				3-WW + FGI II				3-WW + FGI III				3-WW - only				3-Control			
	A		B		A		B		A		B		A		B		A		B	
	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O	Volume mL	Pressure in. H2O
35	990	12.5	1010	13	1570	18.5	1190	13.5	380	5	340	4.5	90	2	180	3	10	0.5	10	1.5
36	860	8.5	840	8.5	1410	13	1520	13.5	460	5	430	4.5	80	1.5	170	2.5	0	0.5	10	1.5
37	620	12.5	550	12.5	1070	21	1410	22	540	9	470	7.5	40	1.5	130	3.5	0	0	0	1
38	510	5.5	420	4.5	890	8.5	1180	11	630	6.5	620	6	50	1.5	130	2	20	0.5	20	1.5
39	470	9.5	330	7.5	810	15.5	1010	19.5	760	12.5	780	13	50	2	120	3	0	0.5	10	1.5
40	445	4.5	310	3	740	6.5	1025	9	1140	10	1155	10	55	2.5	125	4	20	0.5	20	1.5
41	445	8.5	310	6	740	13.5	1025	18	1140	20.5	1155	21	55	3	125	5	20	1	20	2
42	460	5	280	3.5	790	8	1160	11	1530	14	1580	14.5	70	1.5	130	2	30	1	20	2
43	340	8	170	4.5	500	12	830	17.5	1530	27	1610	28	10	1.5	70	2.5	0	1	0	2
44	350	4	180	2.5	510	5.5	850	8.5	1640	15.5	1720	16	40	1.5	90	1.5	20	1	10	2
45	260	3	100	1.5	340	4	580	6	1380	13	1460	13	0	1.5	30	2	0	1	0	2
46	385	4.5	175	3.5	435	5	765	7.5	1480	14	1615	15	75	1.5	120	2	55	1.5	50	2.5
47	385	8	175	6	435	9	765	14	1480	27	1615	20	75	2	120	3	55	2	50	3
48	270	3.5	80	2	300	3.5	520	5.5	1410	13.5	1210	12	0	1	20	1	0	2	0	2.5
49	300	3.5	90	1.5	310	4	550	6	1170	11.5	1180	11.5	20	1	60	2	0	2	10	2.5
50	190	5	30	2	210	5.5	410	9.5	920	19	1030	20.5	0	1	0	1.5	0	1.5	0	2
51	230	3	90	1.5	250	3.5	410	4.5	740	7.5	870	9	20	1	60	2	30	1.5	30	2.5
52	190	4.5	60	2	200	5	350	7.5	640	12.5	740	15	20	1	30	2	10	1.5	10	2.5
53	200	3	80	2	220	3	360	4.5	630	7	690	7	30	1	50	2.5	40	2	30	3
54	160	4.5	60	2.5	160	4.5	330	7	470	10.5	610	12.5	10	1.5	40	3	10	2	10	3
55	130	2	40	1.5	130	2	280	3.5	390	4.5	540	6	0	1	20	1	0	2	0	3
56	130	2	60	1.5	130	2	270	3.5	320	3	480	5.5	30	1	30	1.5	30	1.5	20	3
57	120	3	50	1.5	100	3	270	5.5	350	3	460	5	10	1	30	1.5	10	1	20	1
58	120	4	20	2	60	3.5	200	7.5	300	5.5	380	8.5	0	1	0	1.5	0	1	0	0.5
59	83	4.5	37	2	60	4	157	8.5	263	7.5	303	11	13	1	23	1.5	17	1	20	0.5
60	83	5	37	2.5	60	4.5	157	9.5	263	10	303	13	13	1	23	1.5	17	1.5	20	1
61	83	6	37	3	60	5	157	11	263	12.5	303	15.5	13	1	23	2	17	2	20	1
62	80	1.5	40	1	60	1.5	140	2	270	3.5	310	4	0	1	0	1	10	1	0	1
63	70	2	30	1.5	50	2	110	3	230	5.5	290	6.5	10	1	20	1	10	1	20	1
64	60	1.5	30	1.5	40	2	90	4	200	3	250	3.5	20	1	20	1	20	1	20	1
65	60	2	40	2	50	2.5	80	4.5	180	4.5	240	5.5	20	1	20	1	20	1	20	1
66	40	2	10	2	30	3	60	5	130	5.5	180	7	0	1	10	1	10	1	0	1

Table B-6. Biogas production (mL) for third batch trials (standard temperature and pressure).

Day	3-WW+FG I		3-WW+FG II		3-WW+FG III		3-WW-only		3-control	
	A	B	A	B	A	B	A	B	A	B
2	322	295	331	304	376	313	125	161	0	0
3	540	558	449	376	268	295	367	431	0	0
4	1281	1357	1310	1244	686	769	540	522	0	0
5	585	585	760	806	1188	1300	458	422	18	18
6	522	503	531	558	722	649	440	449	35	35
7	381	344	473	436	734	668	408	444	36	18
8	268	214	196	125	340	223	513	431	18	36
9	205	178	125	80	44	36	549	394	0	18
10	196	161	116	71	18	9	658	476	0	0
11	188	161	107	71	53	53	640	518	0	0
12	225	198	143	116	116	71	759	683	36	36
13	181	162	108	90	107	107	698	645	0	0
14	214	187	143	125	178	143	677	722	27	18
15	233	206	161	161	224	296	696	659	18	9
16	250	223	188	178	161	178	769	834	0	18
17	232	232	161	143	27	0	727	810	18	9
18	198	188	125	116	27	9	441	544	18	18
19	271	271	189	117	9	27	593	663	0	0
20	331	367	349	188	62	53	576	722	44	44
21	343	361	243	215	62	80	519	659	27	36
22	318	309	317	216	36	44	404	481	0	0
23	350	304	313	277	53	80	259	313	9	18
24	450	441	341	350	53	53	242	377	0	0
25	491	454	289	316	44	80	198	271	0	0
26	767	766	364	438	107	143	262	419	36	36
27	777	814	313	376	89	125	205	313	18	18
28	950	820	397	380	116	170	179	324	18	18
29	999	644	475	355	134	180	171	272	18	18
30	1167	1261	630	412	161	205	169	268	18	9
31	1274	405	948	462	197	242	170	252	36	27
32	1233	1288	1158	512	188	196	134	223	9	18
33	1123	1123	1322	675	260	242	134	215	0	0
34	1132	1206	1375	751	249	240	98	196	9	18

Table B-6. Continued.

Day	3-WW+FG I		3-WW+FG II		3-WW+FG III		3-WW-only		3-control	
	A	B	A	B	A	B	A	B	A	B
35	905	924	1455	1090	341	305	80	161	9	9
36	778	760	1290	1392	413	385	71	152	0	9
37	566	503	997	1317	489	424	36	116	0	0
38	458	376	805	1074	567	558	44	116	18	18
39	426	298	745	938	694	714	45	107	0	9
40	399	277	666	929	1035	1049	49	112	18	18
41	403	279	678	949	1061	1077	49	112	18	18
42	413	250	714	1056	1403	1450	62	116	27	18
43	307	152	456	767	1446	1525	9	62	0	0
44	313	161	458	769	1509	1585	36	80	18	9
45	232	89	304	522	1262	1336	0	27	0	0
46	345	156	390	691	1357	1484	67	107	49	45
47	348	157	394	701	1399	1502	67	107	49	45
48	241	71	268	467	1291	1104	0	18	0	0
49	268	80	277	495	1066	1076	18	53	0	9
50	170	27	189	372	854	959	0	0	0	0
51	205	80	224	367	668	788	18	53	27	27
52	170	53	179	316	585	680	18	27	9	9
53	179	71	196	323	568	622	27	45	36	27
54	143	54	143	298	427	557	9	36	9	9
55	116	36	116	250	350	486	0	18	0	0
56	116	53	116	241	286	431	27	27	27	18
57	107	44	89	243	313	413	9	27	9	18
58	107	18	54	181	270	344	0	0	0	0
59	74	33	54	142	238	276	12	21	15	18
60	74	33	54	142	239	277	12	20	15	18
61	75	33	54	143	240	279	12	20	15	18
62	71	36	53	125	241	277	0	0	9	0
63	62	27	45	98	207	261	9	18	9	18
64	53	27	36	81	179	224	18	18	18	18
65	53	36	45	72	161	216	18	18	18	18
66	36	9	27	54	117	162	0	9	9	0

Table B-7. Gas chromatography raw data for first batch trials.

Reactors	Day	Date	Volume concentration (%)				Sum of gases
			O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub>	
1-WW-only A	5	17-Jul	0.769	11.3	69.1	16.6	97.77
	8	20-Jul	0.812	8.40	73.0	15.2	97.41
	17	29-Jul	0.413	7.96	75.7	13.0	97.07
1-WW-only B	4	17-Jul	0.683	10.0	69.2	17.1	96.98
	7	20-Jul	0.769	8.28	72.9	error <sup>[a]</sup>	97.6 <sup>[b]</sup>
	16	29-Jul	0.502	8.44	75.6	12.5	97.04
1-WW+IGb A	1	13-Jul	2.88	15.5	58.0	21.5	97.85
	16	29-Jul	0.376	10.0	66.7	20.8	97.88
1-WW+IGb B	16	29-Jul	0.380	12.5	53.5	31.2	97.58
1-WW+IG A	4	16-Jul	0.247	1.73	69.6	19.6 <sup>[a]</sup>	97.6 <sup>[b]</sup>
	5	17-Jul	0.167	1.34	69.5	26.7	97.71
	5.5	17-Jul	0.183	1.06	67.3	28.7	97.24
	12	24-Jul	0.466	3.32	76.4	17.6	97.79
1-WW+IG B	3 <sup>[a]</sup>	16-Jul	0.3	4	52	42	97.6 <sup>[b]</sup>
	4	17-Jul	0.333	2.88	55.3	37.4	95.91
	4.5	17-Jul	0.212	1.96	60.3	34.5	96.97
	8	21-Jul	0.247	1.63	77.6	18.5	98.05
	11	24-Jul	0.281	1.89	78.8	17.5	98.42
	16	29-Jul	0.532	error <sup>[a]</sup>	78.3	14.7	97.6 <sup>[b]</sup>
1-WW+FG A	3	16-Jul	0.463	11.2	55.4	31.0	98.06
	4	17-Jul	0.350	6.25	59.7	31.7	98.00
	4.5	17-Jul	0.270	3.76	63.1	30.4	97.53
	8	21-Jul	0.353	2.62	76.2	18.6	97.78
	11	24-Jul	0.422	2.53	76.0	19.2	98.13
	16	29-Jul	0.398	2.08	78.0	16.4	96.88
1-WW+FG B	3	16-Jul	0.560	8.9	60.1	28.3	97.86
	4	17-Jul	0.292	6.25	62.3	28.8	97.64
	4.5	17-Jul	0.312	4.85	63.0	30.0	98.16
	8	21-Jul	0.372	2.68	73.1	22.0	98.15
	11	24-Jul	0.2 <sup>[a]</sup>	1.98	76.3	20.2	98.48
	11	24-Jul	0.257	1.73	76.3	20.1	98.39
1-control A	18	2-Aug	4.32	26.4	52.4	13.9	97.02
1-control B	18	2-Aug	0.4	7.2	72.5	17	97.10
<sup>[a]</sup> GC equipment malfunctions caused no detection or inaccurate values							
<sup>[b]</sup> average sum							

Table B-8. Gas chromatography raw data for second batch trials.

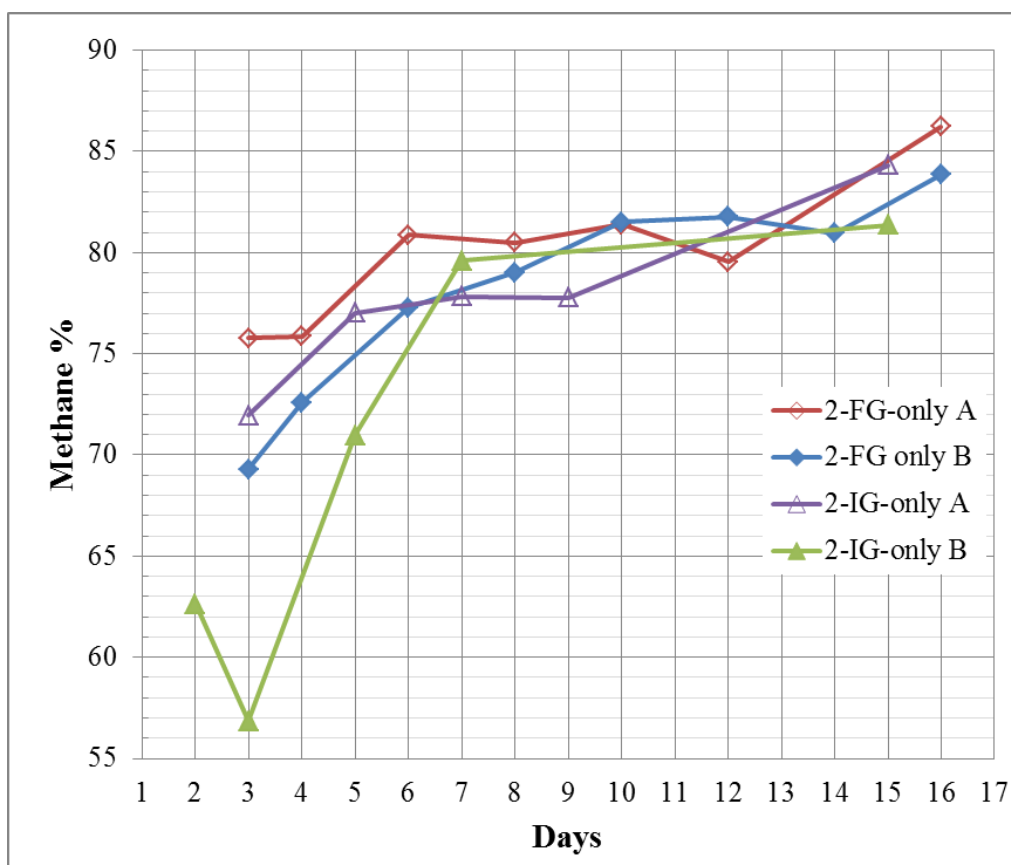
Reactors	Day	Date	Volume concentration (%)				Sum of gases
			O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub>	
2-FG-only A	3	11-Aug	4.04	39.9	39.1	12.5	95.47
	4	12-Aug	1.01	27.1	51.2	16.3	95.58
	6	14-Aug	0.612	14.2	66.4	15.7	97.00
	8	16-Aug	0.486	8.6	69.4	16.8	95.29
	10	18-Aug	0.502	error <sup>[a]</sup>	72.3	16.5	95.78 <sup>[b]</sup>
	12	20-Aug	0.38	3.9	72.8	18.7	95.78
	16	24-Aug	0.38	5.6	77.0	12.3	95.33
2-FG-only B	3	11-Aug	2.86	32.6	41.2	18.3	94.88
	4	12-Aug	1.02	20.2	54.2	20.5	95.77
	6	14-Aug	0.364	11.7	64.6	19.0	95.63
	8	16-Aug	0.372	7.2	69.5	18.5	95.51
	10	18-Aug	0.327	4.98	73.6	16.7	95.58
	12	20-Aug	0.341	3.54	74.8	16.7	95.37
	14	22-Aug	0.324	2.42	75.3	17.1 <sup>[a]</sup>	95.78 <sup>[b]</sup>
16	24-Aug	0.430	2.55	77.7	14.9	95.53	
2-IG-only A	3	12-Aug	0.64	27.1	48.2 <sup>[a]</sup>	19.1	95.78 <sup>[b]</sup>
	5	14-Aug	0.515	18.4	58.8	17.6	95.34
	7	16-Aug	0.413	12.4	64.2	18.3	95.24
	9	18-Aug	0.322	7.51	68.5	19.6	95.92
	15	24-Aug	2.76	16.3	64.2	11.9	95.19
2-IG-only B	2	11-Aug	0.881	51.5	25.4 <sup>[a]</sup>	16.2	95.78 <sup>[b]</sup>
	3	12-Aug	0.725	33.0	35.3	26.8	95.76
	5	14-Aug	error <sup>[a]</sup>	14.1	57.5	23.2 <sup>[a]</sup>	95.78 <sup>[b]</sup>
	7	16-Aug	0.626	11.5	66.3	17.0	95.43
	15	24-Aug	0.75	16.0	64.3	14.7	95.76
2-WW-only A	2	18-Aug	3.29	34.7	48.1	10.3	96.46
	3	19-Aug	1.25	28.5	54.2	12.7	96.62
	4	20-Aug	0.55	20.3	59.7 <sup>[a]</sup>	14.5	95.78 <sup>[b]</sup>
	5	21-Aug	error <sup>[a]</sup>	12.4	66.8	15.9	95.78
	6	22-Aug	0.22	8.2	70.8	16.9	96.12
	7	23-Aug	0.31	5.73	71.7	17.6	95.31
	8	24-Aug	0.35	error <sup>[a]</sup>	error <sup>[a]</sup>	16.8	95.78 <sup>[b]</sup>
	9	25-Aug	0.45	4.9	76.6	13.8	95.69
	10	26-Aug	0.44	4.7	77.4	13.9	96.49
	11	27-Aug	0.48	4.9	81.0	12.8	99.14
	13	29-Aug	0.54	6.0	81.3	11.6	99.44
17	2-Sep	0.76	error <sup>[a]</sup>	80.5	10.4	99.19 <sup>[c]</sup>	

Table B-8. Continued.

Reactors	Day	Date	Volume concentration (%)				Sum of gases
			O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub>	
2-WW-only B	2	18-Aug	2.37	42.7	36.6	14.1	95.80
	3	19-Aug	1.34	39.4	40.1 <sup>[a]</sup>	14.7	95.78 <sup>[b]</sup>
	4	20-Aug	0.98	34.4	44.7	14.9 <sup>[a]</sup>	95.78 <sup>[b]</sup>
	5	21-Aug	0.83	30.2	49.4	15.7	96.22
	6	22-Aug	0.68	24.2	54.1	17.2	96.15
	7	23-Aug	0.67	19.6	58.0	17.3	95.50
	8	24-Aug	0.40	14.2	62.2	18.7	95.52
	9	25-Aug	0.40	11.3	66.1	18.1	95.80
	10	26-Aug	0.60	9.2	68.6	17.9	96.25
	11	27-Aug	0.42	6.6	52.8 <sup>[a]</sup>	18.2	99.19 <sup>[c]</sup>
	13	29-Aug	0.56	5.5	77.0	15.8 <sup>[a]</sup>	83.08
17	2-Sep	0.63	6.4	79.9	12.6	99.54	
2-WW+FG A	2	18-Aug	2.81	49.3	29.2	error <sup>[a]</sup>	95.78 <sup>[b]</sup>
	3	19-Aug	0.980	31.5	43.3	19.8	95.58
	4	20-Aug	0.40	19.4	53.8	22.8	96.37
	5	21-Aug	0.35	11.0	61.4	23.6	96.35
	6	22-Aug	0.21	5.0	67.2	23.7	96.17
	7	23-Aug	0.33	4.2	71.0	error <sup>[a]</sup>	95.78 <sup>[b]</sup>
	8	24-Aug	0.45	4.1	72.0	19.3	95.81
	9	25-Aug	0.49	4.5	73.4	17.5	95.89
	10	26-Aug	0.70	4.5	72.6	17.6	95.34
	11	27-Aug	0.45	2.4	75.6	20.8	99.26
	13	29-Aug	0.29	1.5	76.2	21.0	98.96
17	2-Sep	0.52	2.1	79.0	17.8	99.36	
2-WW+FG B	2	18-Aug	2.93	46.0	33.7	13.3	95.96
	3	19-Aug	1.35	36.7	40.7	16.2 <sup>[a]</sup>	95.78 <sup>[b]</sup>
	4	20-Aug	0.73	26.8	47.8	20.3	95.67
	5	21-Aug	error <sup>[a]</sup>	15.9	57.7	22.8	96.38
	6	22-Aug	0.34	10.8	63.1	22.0	96.19
	7	23-Aug	0.57	9.2	66.3	error <sup>[a]</sup>	95.78 <sup>[b]</sup>
	8	24-Aug	0.31 <sup>[a]</sup>	6.6	69.5	19.1	95.23
	9	25-Aug	9.31	35.7	41.6	10.7	97.32
	10	26-Aug	3.00	13.0	63.9	16.3	96.30
	11	27-Aug	error <sup>[a]</sup>	error <sup>[a]</sup>	69.6	28.1	99.19 <sup>[c]</sup>
	13	29-Aug	0.32	1.9	78.2 <sup>[a]</sup>	17.5	99.19 <sup>[c]</sup>
17	2-Sep	0.69	3.1	81.5	13.7	98.88	
2-control A	16	25-Aug	0.85	49.2	35.4	10.3	95.80
	18	3-Sep	0.90	47.9	41.0	8.8	98.52
2-control B	16	25-Aug	0.95	41.4	42.7	11.4	96.53
	18	3-Sep	0.85	40.5	47.6	10	98.85
<sup>[a]</sup> GC equipment malfunctions caused no detection and inaccurate values							
<sup>[b]</sup> Average sum							
<sup>[c]</sup> Average sum after new calibration of GC							

Table B-9. Gas chromatography raw data for third batch trials.

Reactors	Day	Date	Volume concentration (%)				Sum of gases
			O <sub>2</sub>	N <sub>2</sub>	CH <sub>4</sub>	CO <sub>2</sub>	
3-WW+FG III A	55	2-Nov	0.58	2.94	82.4	12.9	98.82
	56	3-Nov	0.55	3.96	80.5	12.0	97.01
3-WW+FG III B	55	2-Nov	0.48	2.65	81.1	13.6	97.83
	56	3-Nov	0.41	2.49	81.0	13.8	97.70
3-control B	56	3-Nov	1.1	64.2	25.7	6.08	97.08

Figure B-1. CH<sub>4</sub> content of biogas produced during the second batch trials (glycerol-only trials).

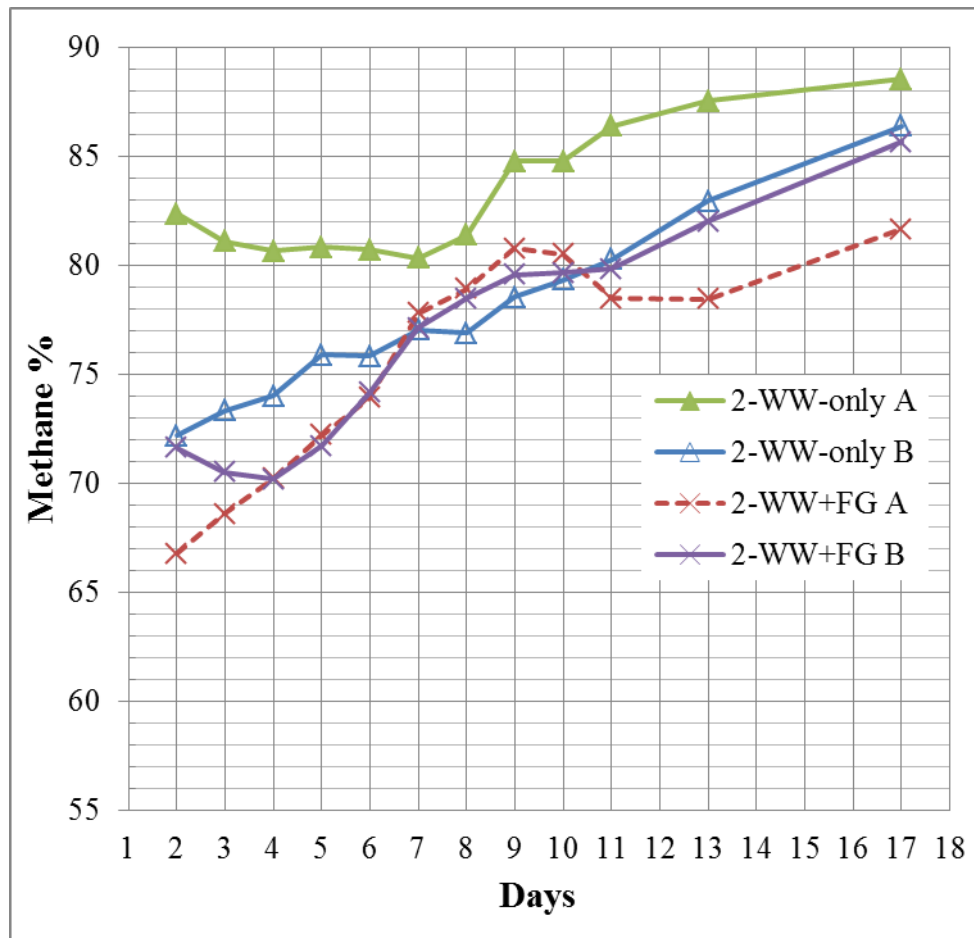


Figure B-2. CH<sub>4</sub> content of biogas produced during the second batch trials.



**APPENDIX C**  
**DEGRADABILITY RAW DATA**

Table C-1. Solid analysis for first batch trials.

Trials <sup>[a]</sup>	Initial Solids			Trials	Final Solids		
	% TS	%VS (d.b.)	% VS (w.b.)		% TS	%VS (d.b.)	% VS (w.b.)
WW-only A	2.74	54.58	1.50	WW- only A	2.34	54.58	1.28
WW-only B	2.70	55.40	1.50	WW- only B	1.72	54.70	0.94
WW+IGb A	2.63	55.10	1.45	WW- only B	0.76	53.63	0.41
WW+IGb B	3.16	58.50	1.85	WW+IGb A	0.85	53.69	0.46
WW+IG A	1.91	65.00	1.24	WW+IGb A	2.74	57.18	1.57
WW+IG B	2.02	66.00	1.33	WW+IGb A	1.97	57.26	1.13
WW+IG A	2.24	54.47	1.22	WW+IGb B	1.05	56.40	0.59
WW+IG B	2.13	59.40	1.27	WW+IG B	1.30	58.34	0.76
WW+FG A	2.33	59.50	1.39	WW+IG A	2.22	53.87	1.20
WW+FG B	4.44	55.50	2.46	WW+IG A	2.46	53.76	1.32
	2.17	59.80	1.30	WW+IG B	1.31	53.50	0.70
				WW+IG B	1.32	53.70	0.71
				WW+FG A	5.11	52.50	2.68
				WW+FG A	5.30	51.15	2.71
				WW+FG B	1.30	53.52	0.69
				WW+FG B	1.30	52.82	0.69
				control A	3.07	53.08	1.63
				control A	0.77	51.07	0.39
				control B	2.97	52.41	1.56
				control B	2.34	53.39	1.25

<sup>[a]</sup> control not measured, some trials had no duplicate sampled

Table C-2. Solid analysis for second batch trials.

Trials <sup>[a]</sup>	Initial Solids			Trials <sup>[a]</sup>	Final Solids		
	% TS	%VS (d.b.)	% VS (w.b.)		% TS	%VS (d.b.)	% VS (w.b.)
FG-only A	0.155	45.94	0.071	FG-only A	0.167	39.23	0.065
	0.181	53.44	0.097		0.172	42.21	0.073
FG-only B	0.217	49.33	0.107	FG-only B	0.161	26.73	0.043
	0.239	53.29	0.127		0.159	29.72	0.047
IG-only A	0.140	40.39	0.057	IG-only A	0.129	32.81	0.042
	0.230	67.71	0.156		0.124	34.85	0.043
IG-only B	0.185	53.71	0.099	IG-only B	0.141	37.50	0.053
	0.181	48.09	0.087		0.138	30.23	0.042
WW-only A	0.220	34.40	0.076	WW-only A	0.196	22.29	0.044
	0.214	35.54	0.076		0.191	24.37	0.047
WW-only B	0.271	51.90	0.141	WW-only B	0.197	31.40	0.062
	0.454	55.54	0.252		0.204	33.75	0.069
WW+FG A	0.257	60.25	0.155	WW+FG A	0.172	28.31	0.049
	0.254	55.90	0.142		0.184	30.95	0.057
WW+FG B	0.242	57.53	0.139	WW+FG B	0.173	27.05	0.047
	0.282	60.52	0.171		0.181	29.79	0.054
control A	0.151	30.36	0.046	control A	0.149	31.91	0.047
	0.152	34.88	0.053		0.150	36.10	0.054
control B	0.142	30.47	0.043	control B	0.152	35.13	0.053
	0.145	32.46	0.047		0.150	35.58	0.053

<sup>[a]</sup> solids content measured in liquid fraction of digesters only (no sludge sampled)

Table C-3. Solid analysis for third batch trials.

Trials	Initial Solids			Trials	Final Solids		
	% TS	%VS (d.b.)	% VS (w.b.)		% TS	%VS (d.b.)	% VS (w.b.)
WW+FG I A	3.45	53.58	1.85	WW+FG I A	2.47	48.88	1.21
	3.13	54.23	1.70		2.44	50.14	1.22
WW+FG I B	3.74	53.32	1.99	WW+FG I B	2.74	48.01	1.32
	4.25	52.63	2.24		2.51	47.38	1.19
WW+FG II A	3.86	53.9	2.08	WW+FG II A	3.29	49.46	1.63
	3.94	53.47	2.11		3.21	48.31	1.55
WW+FG II B	4.21	52.92	2.23	WW+FG II B	3.99	46.89	1.87
	3.99	53.36	2.13		3.88	48.55	1.88
WW+FG III A	4.09	53.33	2.18	WW+FG III A	3.30	46.89	1.55
	4.28	53.38	2.29		3.00	47.64	1.43
WW+FG III B	3.67	53.63	1.97	WW+FG III B	2.90	47.57	1.38
	3.40	54.03	1.84		2.99	47.40	1.42
WW-only A	4.08	52.11	2.13	WW-only A	3.08	47.56	1.47
	4.37	51.78	2.26		3.23	47.61	1.54
WW-only B	4.25	50.97	2.17	WW-only B	3.69	48.64	1.79
	4.21	51.67	2.17		3.42	48.22	1.65
control A	3.72	50.89	1.90	control A	3.01	49.08	1.48
	4.50	48.83	2.20		2.65	49.58	1.31
control B	3.77	50.51	1.91	control B	2.96	48.82	1.45
	3.92	51.05	2.00		2.90	49.66	1.44

Table C-4. COD analysis for all trials and substrates.

Sample vial	Absorbance	COD (mg L <sup>-1</sup> )	Date	Notes
Standard	0.057	1000	29-Jun	1000 mg L <sup>-1</sup> standard
Standard	0.225	5000	29-Jul	5000 mg standard
Standard	0.456	10000	29-Jul	10,000 mg L <sup>-1</sup> standard
blank	-0.004	0	13-Jul	zeroed
wastewater(1st)	0.154	3378	13-Jul	<b>new calibration</b>
wastewater(1st)	0.203	4452	15-Jul	<b>COD=Absorbance*21932</b>
wastewater(1st)	0.181	3970	15-Jul	
1-WW-only A	0.048	1053	15-Jul	initial COD - 1st batch
1-WW-only B	0.035	768	15-Jul	initial COD
1-WW+IGb A	0.373	8181	15-Jul	initial COD
1-WW+IGb B	0.364	7983	15-Jul	initial COD
1-WW+IG A	0.144	3158	15-Jul	initial COD
1-WW+IG B	0.125	2742	15-Jul	initial COD
1-WW+FG A	0.237	5198	15-Jul	initial COD
1-WW+FG B	0.262	5746	15-Jul	initial COD
1-WW-only A	0.035	768	29-Jul	final COD
1-WW-only A	0.039	855	29-Jul	final COD
1-WW+IG A	0.048	1053	29-Jul	final COD
1-WW+IG A	0.047	1031	29-Jul	final COD
1-WW-only B	0.041	899	30-Jul	final COD
1-WW-only B	0.040	877	30-Jul	final COD
1-WW+IGb A	0.600	13159	30-Jul	final COD
1-WW+IGb B	0.610	13379	30-Jul	final COD
1-WW+IG A	0.043	943	30-Jul	final COD
1-WW+FG A	0.097	2127	30-Jul	final COD
1-WW+FG B	0.112	2456	30-Jul	final COD
1-control A	0.061	1338	30-Jul	final COD
1-control B	0.075	1645	30-Jul	final COD
Standard	0.436	10000	10-Aug	10,000 mg L <sup>-1</sup> standard
Standard	0.267	6000	10-Aug	6000 mg L <sup>-1</sup> standard
Standard	0.089	2000	10-Aug	2000 mg L <sup>-1</sup> standard
blank	-0.023	0	17-Aug	zeroed
wastewater(2nd)	0.231	5267	17-Aug	<b>new calibration</b>
wastewater(2nd)	0.232	5289	17-Aug	<b>COD=ABSORBANCE*22799</b>
wastewater(2nd)	0.235	5358	17-Aug	
2-IG-only A	0.093	2120	10-Aug	initial COD - 2nd batch
2-IG-only B	0.103	2348	10-Aug	initial COD
2-IG-only B	0.101	2303	10-Aug	initial COD
2-control A	0.021	479	10-Aug	initial COD
2-control B	0.025	570	10-Aug	initial COD
2-FG-only A	0.108	2462	10-Aug	initial COD
2-FG-only B	0.128	2918	10-Aug	initial COD
2-WW-only A	0.076	1733	17-Aug	initial COD
2-WW-only A	0.090	2052	17-Aug	initial COD
2-WW-only B	0.098	2234	17-Aug	initial COD
2-WW+FG A	0.246	5609	17-Aug	initial COD
2-WW+FG A	0.207	4719	17-Aug	initial COD
2-WW+FG B	0.203	4628	17-Aug	initial COD
2-FG-only A	0.025	570	24-Aug	final COD
2-FG-only A	0.025	570	24-Aug	final COD
2-FG-only B	0.029	661	24-Aug	final COD

Table C-4. Continued.

Sample vial	Absorbance	COD (mg L <sup>-1</sup> )	Date	Notes
2-FG-only B	0.030	684	24-Aug	final COD
2-IG-only A	0.024	547	25-Aug	final COD
2-IG-only A	0.021	479	25-Aug	final COD
2-IG-only B	0.021	479	25-Aug	final COD
2-IG-only B	0.026	593	25-Aug	final COD
2-WW-only A	0.023	524	3-Sep	final COD
2-WW-only A	0.025	570	3-Sep	final COD
2-WW-only B	0.034	775	3-Sep	final COD
2-WW-only B	0.033	752	3-Sep	final COD
2-WW+FG A	0.034	775	3-Sep	final COD
2-WW+FG A	0.035	798	3-Sep	final COD
2-WW+FG B	0.026	593	3-Sep	final COD
2-WW+FG B	0.026	593	3-Sep	final COD
2-control A	0.027	616	3-Sep	final COD
2-control B	0.029	661	3-Sep	final COD
farm glycerol	0.726	16552	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
farm glycerol	0.614	13999	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
farm glycerol	0.606	13816	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
farm glycerol	0.569	12973	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
industrial glycerol	0.392	8937	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
industrial glycerol	0.428	9758	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
industrial glycerol	0.380	8664	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
industrial glycerol	0.384	8755	31-Aug	diluted sample (1:125 dH <sub>2</sub> O)
blank	-0.071	0	3-Sep	zeroed
wastewater (3rd)	0.365	8139	8-Sep	
wastewater (3rd)	0.289	6407	8-Sep	
sludge inoc	0.093	1938	8-Sep	
3-WW+FGI A	0.248	5517	10-Sep	initial COD - 3rd batch
3-WW+FGI B	0.240	5335	10-Sep	initial COD
3-WW+FGII A	0.292	6521	10-Sep	initial COD
3-WW+FGII A	0.310	6931	10-Sep	initial COD
3-WW+FGII B	0.315	7045	10-Sep	initial COD
3-WW+FGIII A	0.329	7364	10-Sep	initial COD
3-WW+FGIII B	0.343	7683	10-Sep	initial COD
3-WW-only A	0.136	2964	10-Sep	initial COD
3-WW-only B	0.145	3169	10-Sep	initial COD
3-control A	0.067	1391	10-Sep	initial COD
3-control B	0.065	1345	10-Sep	initial COD
3-WW+FGI A	0.062	1414	16-Nov	final COD
3-WW+FGI B	0.062	1414	16-Nov	final COD
3-WW+FGII A	0.075	1710	16-Nov	final COD
3-WW+FGII B	0.081	1847	16-Nov	final COD
3-WW+FGIII A	0.084	1915	16-Nov	final COD
3-WW+FGIII B	0.069	1573	16-Nov	final COD
3-WW+FGIII B	0.081	1847	16-Nov	final COD
3-WW-only A	0.047	1072	16-Nov	final COD
3-WW-only A	0.065	1482	16-Nov	final COD
3-WW-only B	0.048	1094	16-Nov	final COD
3-control A	0.058	1322	16-Nov	final COD
3-control B	0.043	980	16-Nov	final COD
Blank	0.004	0	16-Nov	zeroed

## APPENDIX D

### pH AND ALKALINITY

Table D-1. pH and alkalinity data.

1st batch		pH		
batch day		3	10	18
1-WW-only A		6.9	7.0	7.5
1-WW-only B		7.2	7.0	7.3
1-WW+IGb A <sup>[a]</sup>		5.0	6.5	6.9
1-WW+IGb B <sup>[a]</sup>		4.6	5.8	6.4
1-WW+IG A		6.7	7.1	7.4
1-WW+ IG B		6.7	7.1	7.2
1-WW+ FG A		6.5	7.1	7.3
1-WW+ FG B		6.7	7.2	7.4
1-control A		---	7.1	7.3
1-control B		---	---	7.3

<sup>[a]</sup> pH fixed after the 3rd day of batch

2nd batch		pH									
batch day		1	3	5	7	9	11	13	15	16	18
2-FG-only A		7.53	no data	no data	6.71	6.91	6.99	6.91	6.94	7.49	
2-FG-only B		7.31	no data	no data	6.73	6.99	7.01	6.86	7.01	7.35	
2-IG-only A		7.59	no data	no data	6.74	6.72	6.84	6.74	6.82	7.13	
2-IG-only B		7.64	no data	no data	6.82	6.93	7.00	6.91	6.99	7.56	
2-WW-only A		7.43	7.11	6.93	7.02	no data	7.14	no data	no data	no data	7.5
2-WW-only B		7.23	7.13	7.01	7.13	no data	7.15	no data	no data	no data	7.37
2-WW+FG A		7.36	6.79	6.80	6.89	no data	6.99	no data	no data	no data	7.53
2-WW+FG B		7.42	6.90	6.79	7.06	no data	7.12	no data	no data	no data	7.53
2-control A		7.54	no data	no data	7.25	7.39	7.27	7.26	no data	7.64	7.61
2-control B		7.56	no data	no data	7.22	7.36	7.19	7.34	no data	7.57	7.77

Table D-1. Continued.

<b>3rd batch</b>	<b>pH</b>						
batch day	2	8	16	23	34	37	66
3-WW-only A	7.15	7.11	no data	no data	7.17	7.14	7.2
3-WW-only B	7.23	7.13	6.79	6.99	7.13	7.12	7.13
3-WW+FG I A <sup>[b]</sup>	6.67	6.71	6.61	7.02	7.06	7.04	7.23
3-WW+FG I B <sup>[b]</sup>	6.72	6.69	no data	no data	7.07	7.14	7.19
3-WW+FG II A <sup>[b]</sup>	6.62	6.49	6.42	6.99	7.02	7.08	7.22
3-WW+FG II B <sup>[b]</sup>	6.54	6.36	no data	no data	7.1	7.06	7.22
3-WW+FG III A <sup>[b]</sup>	6.43	5.99	6.02	7.08	7.12	7.11	7.32
3-WW+FG III B <sup>[b]</sup>	6.38	6.03	no data	7.00	7.11	7.16	7.31
control A	7.50	7.34	6.99	7.08	7.11	7.10	7.18
control B	7.61	7.40	no data	no data	7.13	7.18	7.17

<sup>[b]</sup> pH fixed after the 16th day of batch

<b>Sample</b>	<b>pH</b>		
WW (1st batch)	6.90	6.82	
WW (2nd batch)	7.80	7.82	
WW (3rd batch)	7.91	8.01	
Farm glycerol <sup>[c]</sup>	10.1	10.0	
Industrial glycerol <sup>[c]</sup>	4.90	4.61	4.57
Inoculum sludge	7.31	7.34	

<sup>[c]</sup> diluted samples (1:125 deionized H<sub>2</sub>O)

<b>Alkalinity</b>	<b>(mg CaCO<sub>3</sub>)/L</b>	
WW (3rd batch)	938	878
3-WW+FG III A	1215	
3-control A	750	
3-WW-only B	1406	

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