

ANAEROBIC DIGESTION FROM THE LABORATORY TO THE FIELD: AN EXPERIMENTAL
STUDY INTO THE SCALABILITY OF ANAEROBIC DIGESTION

A Thesis
by
KEVIN JAMES GAMBLE

Submitted to the Graduate School
Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2014
Department of Technology and Environmental Design

ANAEROBIC DIGESTION FROM THE LABORATORY TO THE FIELD: AN EXPERIMENTAL
STUDY INTO THE SCALABILITY OF ANAEROBIC DIGESTION

A Thesis
by
KEVIN JAMES GAMBLE
August 2014

APPROVED BY:

James B. Houser
Chairperson, Thesis Committee

Marie C. Hoepfl
Member, Thesis Committee

Michael S. Hambourger
Member, Thesis Committee

Jerianne S. Taylor
Interim Chairperson, Department of
Technology and Environmental Design

Max C. Poole
Dean, Cratis D. Williams Graduate School

Copyright © by Kevin James Gamble 2014
All Rights Reserved

Abstract

ANAEROBIC DIGESTION FROM THE LABORATORY TO THE FIELD: AN EXPERIMENTAL STUDY INTO THE SCALABILITY OF BENCH TO PILOT-SCALE ANAEROBIC DIGESTION

Kevin James Gamble
B.A., Appalachian State University
M.S., Appalachian State University

Chairperson: James B. Houser

With an ever-growing human population, there comes a need to deal with increasing amounts of organic wastes. Anaerobic digestion (AD) is a unique organic waste treatment method. This process improves sanitation and environmental quality through pollution control, while providing organic fertilizer in the form of a liquid digestate and renewable energy in the form of clean-burning biogas.

In designing AD systems efficiency is key, not just for pollutant removal, but also for biogas production. It can be costly in terms of time, money, and resources to dial-in systems once the full-scale has been constructed and is operational. Bench-scale laboratory experimentation is an essential component of AD research and development, as the ability to simultaneously test multiple variables on a small-scale to see their impact on AD efficiency helps in reducing the costs associated with system optimization.

In order for bench-scale research to be of use, there must be a strong correlation between results obtained in the lab and actual performance of large-scale anaerobic digesters. In this study, three differently sized bench-scale digesters (100 mL, 1 L, and 10 L) treating horse manure were tested side-by-side to determine the accuracy of scaling between digester sizes. Digesters were compared by the cumulative and daily biogas

production, methane content of the biogas, volatile solids-destruction (VS-destruction), and pH of the digestate.

Based on these results, a strong correlation was found between the yield of biogas and the digester sizes, showing that the scalability of AD is tenable; however, there were some statistically significant differences in biogas production between the digester sizes, showing that there is a scaling effect that must be taken into account. This study shows that while bench-scale studies are important, and can reflect (or predict) digester performance in larger-sized AD systems, some amount of caution must be taken in extrapolating results obtained in the lab directly to these larger scales.

Acknowledgments

There are a number of individuals who have helped me navigate the process of this thesis endeavor and who deserve my utmost gratitude. I would first like to thank my committee chairperson, Dr. James Houser for his support throughout the course of this study. Without his help and expertise, this project would have likely never happened. I would also like to thank Dr. Michael Hambourger for taking the time to introduce me to the world of gas analysis and for training me on the gas chromatograph, and Dr. Marie Hoepfl, who has been nothing but supportive throughout my graduate career, and who has helped me improve my writing and professional skills more than anyone.

I would also like to thank Keith Ward from Dutch Creek Trails for allowing me free access to his horse farm, and for providing the horse manure used in this study. I must also extend my thanks to the Office of Student Research and the Cratis Williams Graduate School. The GRAM, OSR and Cratis D. Williams grants I was awarded helped tremendously in funding this study, and in improving the biogas research laboratory for future students.

I would like to thank my friends and family for their continued support. I would especially like to thank my oldest friend, Cully Little, who has been a source of inspiration for much of my life, and who was the prime mover in getting me into the technical field. Finally, and most importantly, I would like to thank my incredible fiancée Renee Shulman. Without her sacrifices and ceaseless love and support, I would have never survived the past two years. Thank you for always having my back and for encouraging me to achieve my dreams.

Table of Contents

Abstract.....	iv
Acknowledgments	vi
Chapter 1: Introduction.....	1
Statement of the Problem.....	2
Research Questions.....	3
Purpose of the Study	3
Significance of the Study.....	4
Definition of Terms	4
Chapter 2: Review of Literature.....	5
Anaerobic Digestion.....	5
The Anaerobic Digestion Process.....	6
Feedstocks	8
Feedstock Characterization	8
Bioreactor Conditions	9
Horse Manure as AD Feedstock.....	12
Biogas Production Analysis	16
Biogas Volume.....	16
Biogas Composition.....	17
VS-destruction and COD.....	18

Scalability of Anaerobic Digestion	19
Chapter 3: Research Methods	22
General Overview	22
Equipment	22
Samples	24
Experimental Process	25
Feedstock Characterization and Preliminary Trial.....	25
New Components.....	29
Experimental Trials	32
Trial 1.....	33
Trial 2.....	39
Discussion of Methodology.....	42
Preliminary Trial.....	42
Experimental Trial 1	43
Experimental Trial 2.....	48
Chapter 4: Research Findings	51
Feedstock Characterization.....	51
Preliminary and Experimental Trials.....	52
Preliminary Trial.....	52
Experimental Trial 1	54
Experimental Trial 2.....	59
Chapter 5: Discussion and Conclusions.....	66
Scalability.....	66
Cumulative Biogas Production	66

Daily Biogas Yield.....	71
Biogas Composition.....	72
pH	75
Volatile Solids Destruction	76
Other Observations	78
Predictive Model	80
Simple Scale-up Predictive Model.....	81
Linear/Power Predictive Model	84
Conclusions	86
Limitations of Study.....	88
Suggestions for further research.....	89
References	91
Vita.....	94

Chapter 1: Introduction

As the world's population continues to grow, so grows the need to deal with increasing amounts of organic wastes. These organic wastes include, but are not limited to: human, animal/livestock, food waste, and wastes associated with some industries (such as dairies, breweries, paper mills, etc). If ignored, these organic wastes can quickly become environmental pollutants with the potential to negatively affect the quality of soils, groundwater, environmental habitats, and human and animal health; however, there are ways in which the problems associated with organic waste may be addressed, while simultaneously providing some added benefits.

AD is a method of organic waste treatment wherein wastes are decomposed in a controlled, oxygen-free environment for the purpose of pollution reduction and the generation of biogas, a renewable natural gas comprised primarily of methane and carbon dioxide. AD technology is quite versatile, in that it can be applied in many different situations and in many different scales. AD technology is capable of handling many different forms and amounts of organic waste, from relatively small, family-sized digesters in rural developing nations treating a few pounds of waste each day, to multi-million dollar projects treating hundreds of thousands of pounds of waste per day (such as wastewater treatment plants for major cities). AD not only serves to improve sanitation, but also soils through the application of the treated liquid effluent (a viable replacement for chemical fertilizers), and the production of renewable energy in the form of clean-burning biogas.

Laboratory experimentation is an essential component of anaerobic digestion research, as it allows for multiple experiments to be run simultaneously, granting the ability to

change numerous variables and to collect large amounts of data in relatively short periods of time. In the case of AD research, bench-scale experimentation also dramatically reduces the amount of raw materials required for experimentation, cutting down on waste, costs, and the need to dispose of (or use) large quantities of biogas. This type of bench-scale research is vital in helping to determine the proper application of AD, and in optimizing the AD process to improve efficiencies in both waste treatment and biogas production. Lessons learned on the bench-scale can have important implications regarding the design and operation of digesters at larger scales (such as industrial facilities.)

However, in order for bench-scale research to be of use, there must be a strong correlation between reactions in the laboratory and reactions in larger-scale digesters. The accuracy of this scalability is crucial to ensuring that work performed on the laboratory scale does not result in wasted time and resources or in findings that are not applicable to large-scale applications of AD. The present study seeks to address this issue by examining the scalability of AD within three differently sized digesters on the bench-scale.

Statement of the Problem

The Department of Technology and Environmental Design (TED) at Appalachian State University has a robust, and growing, biofuels program. In the autumn of 2013 construction began on a pilot-scale laboratory for AD research. Three pilot-scale digesters were constructed from plastic 275 gallon (~1,041L) Intermediate Bulk Container (IBC) totes that eventually will be housed in an insulated shed for pilot-scale experimentation and research into biogas production. The ultimate goal is to use this lab as both a research station and as a source of usable biogas for heating needs in the adjoining biodiesel research facility.

With the implementation of these new, larger digesters, comes the need to determine the accuracy of bench-scale digesters in predicting their performance. This

accuracy can prove highly beneficial in helping to determine the optimal feedstocks and reactor conditions to ensure the most efficient treatment of organic wastes and the highest outputs of methane. Performing these types of experiments in the final, full-scale digesters could not only result in a waste of time and resources, but could also lead to monetary losses as digesters are taken offline. For this reason, it is vital to know the predictive capacity of bench-scale digesters in modeling digestion in larger systems.

Research Questions

The work described herein was undertaken to address the following research questions:

1. Is there a significant correspondence in the daily and cumulative biogas yields from 100 mL, 1 L, and 10 L bench-scale digesters measured in milliliters per gram of volatile solid (mL/g VS)?
2. Are there significant differences among these digesters in regards to methane content of the biogas, VS destruction, and/or pH of the digestate?
3. Are there sufficient data to create a reliable model for predicting the performance of larger digesters based on results from bench-scale reactors?

Purpose of the Study

This experimental study seeks to begin the process of determining the scalability of AD. This will be accomplished by comparing three differently sized bench-scale digesters in terms of biogas production, biogas composition, VS-destruction and pH. The ultimate goal is to determine if biogas yield corresponds to the size of the digester. With these data, determinations can then be made as to whether data collected from one size digester is transferable to other sizes. By determining the scalability of the bench-scale protocol, specifically the reproducibility of results within digester sizes and the transferability of results across sizes, the hope is that future students will take these results and apply them to the forthcoming pilot-scale systems for final analysis. Another important aspect of this

research project is re-designing the bench-scale system set-up. As part of this process, efforts will be made in determining the ideal bench-scale system design, so that data collected from future students will be as accurate and consistent as possible.

Significance of the Study

By focusing on scalability and the reproducibility/transferability of results within and between digester sizes, the results obtained from this study should prove beneficial to other researchers working on AD. Specifically, the results will help in determining the predictive value of bench-scale studies, and will uncover potential issues associated with scaling. Closer to home, the work from this project will help shape the direction the TED department's biofuels lab will take in terms of future equipment purchases and bench-scale system designs by highlighting areas in which improvements can be made.

Definition of Terms

For the purposes of this study, "bench-scale" refers to experimentation carried out in small-scale laboratory bioreactors (in the case of this study, from 100 mL to 10 L). "Pilot-scale" refers to larger bioreactors (in the case of this study, ≥ 1000 L digesters).

"Barrier solution" refers to the liquid medium serving as the means of volumetric biogas measurement via liquid displacement in gasometers.

"Inoculum" refers to a healthy community of methanogenic bacteria, which can be acquired from the active sludge gathered from an operating anaerobic digester.

"Bioreactor," "biodigester," and "digester" are used synonymously and refer to the anaerobic digester, the physical vessel in which the anaerobic digestive process takes place.

"Slurry" refers to the final mix of distilled water and horse manure that will be the material undergoing anaerobic digestion.

Chapter 2: Review of Literature

Anaerobic Digestion

AD is an alternative form of waste treatment, whereby organic waste materials ranging from manures and food scraps to yard waste and industrial wastewaters may be decomposed in a controlled, oxygen-free environment in order to produce biogas, a renewable natural gas comprised primarily of methane (CH₄) and carbon dioxide (CO₂). Biogas may be used for heating, the production of electricity, or as a vehicle fuel in the form of compressed renewable natural gas (CRNG) (United States Environmental Protection Agency [USEPA], 2014a).

Methane itself is a highly potent greenhouse gas (GHG), up to 20 times more potent than CO₂ (USEPA, 2013). There are many sources of methane production in the environment. Wherever a pocket of organic matter has the ability to decompose free of oxygen, you are likely to find methane gas. Landfills are a major source of biogas (typically referred to as Landfill Gas due to some compositional differences), which is why modern landfills must include gas wells and flare stacks in order to mitigate the damage of freely releasing methane into the environment (or risking explosion hazard). Through flaring (combusting), methane is broken down into H₂O and CO₂, and the CO₂ is far less damaging than CH₄ (USEPA, 2014b).

The benefits of AD include reductions in GHG emissions and groundwater contamination due to the diversion of organic waste from landfills; the production of biogas, a clean-burning energy source that can be used to offset the use of fossil fuels; the production of a nutrient-rich liquid effluent and solid digestate, which can serve as

alternatives to chemical fertilizers and as valuable soil amendments, respectively; and finally, increased sanitation, especially on farms, as AD is a highly efficient method of waste disposal (Cheng, 2010; Drapcho, Nhuan & Walker, 2008; Liu, Ding & Wang, 2010).

The Anaerobic Digestion Process

AD occurs in a four-stage process whereby organic waste matter is broken down in a controlled environment in the absence of oxygen to produce biogas and a nutrient-rich effluent that can be utilized as a fertilizer (see Figure 1). The four stages of anaerobic digestion are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Cheng, 2010; Drapcho et al., 2008).

During hydrolysis long-chain organic polymers, including fats, proteins, and carbohydrates, are broken down into smaller components, such as amino acids and simple sugars, making them available for further degradation via acetogenic bacteria. In the acidogenic phase, bacteria break down the amino acids and sugars made available from hydrolysis into CO₂, hydrogen (H₂), ammonia (NH₃), volatile organic acids, and alcohols. In the next phase, acetogenesis, these byproducts are further converted by bacteria into H₂, CO₂, and acetic acid. In the fourth and final stage, methanogenesis, methanogenic archaea convert the byproducts created in the acetogenic phase into biogas, which is comprised primarily of CH₄ and CO₂, though other trace gases such as nitrogen gas (N₂) and hydrogen sulfide (H₂S) may also be present, depending on the feedstocks utilized and the reactor conditions in which the digestion took place (Cheng, 2010; Drapcho et al., 2008; Leckie, Masters, Whitehouse, & Young, 1981).

AD can take place in many different types of digesters. The majority of these digester types can be split into two groups: batch-fed and continuously-fed. The primary difference here is in the loading rate of the digester. In batch-fed systems, the digester is filled all at once. This waste will remain in the system until the end of the pre-specified

digestion time, upon which the waste will be removed altogether, and the reactor refilled. The reaction times are dependent on a number of factors that will be discussed later in more detail. In continuously-fed systems, waste is added to the digester at pre-designated times, again dependent on the process parameters. In continuously-fed systems, as new wastes are added, older pre-treated wastes are removed. The majority of large-scale industrial digesters operate in the continuously-fed mode as it allows the digester to continually produce biogas (Cheng, 2010).

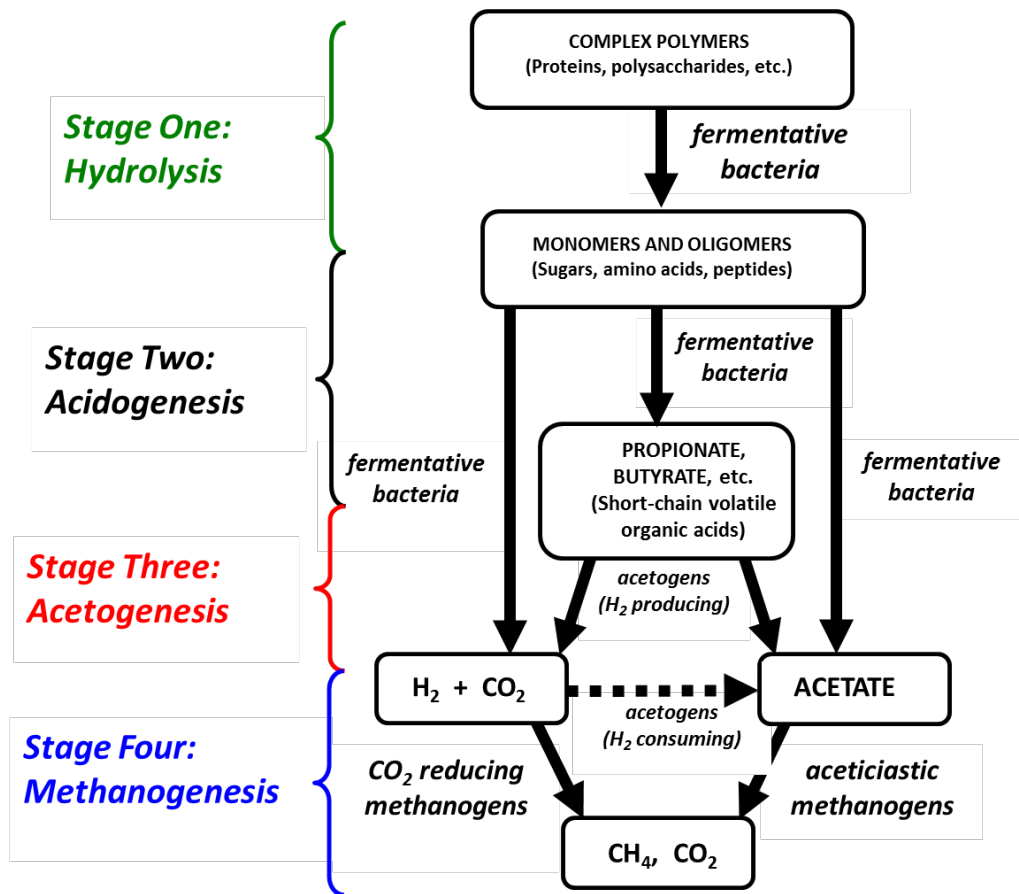


Figure 1. AD process (Hashsham, n.d.).

Feedstocks

There are numerous feedstocks (organic waste materials) that can be used in the AD process. Feedstocks can include animal and human manure, wastewater, food waste, garden/yard waste, greases, oils, fats, and some industrial waste/wastewaters, such as paper mill and brewery effluent (Cheng, 2010; Drapcho et al., 2008; House, 2010; Leckie et al., 1981). Biogas composition, especially the CH₄:CO₂ ratio, will vary depending on the type of feedstock, or feedstocks (if co-digesting). For example, biogas produced from feedstocks with greater carbohydrate content will tend to have a higher CO₂ concentration than feedstocks higher in lipids and proteins (Drapcho et al., 2008).

Feedstock Characterization

Feedstocks for AD are characterized by a particular set of parameters. Typically, the most important characteristics are: percent total solids (%TS), percent volatile solids (%VS), the carbon-to-nitrogen (C/N) ratio, and pH (Cheng, 2010; Drapcho et al., 2008; Leckie et al., 1981).

The %TS is the amount of dry, solid material present in a given feedstock sample. This measurement is determined by weighing the sample before and after drying the feedstock, evaporating off any water. The %TS represents the mass percent of dry solids in the wet material (see equation 1). The %VS is a percentage of the total solids, and represents the digestible material in the sample (see equation 2). Incineration of the feedstock at 550° C for at least 2.5 hours is used to determine the %VS, and the portion remaining after incineration is referred to as the fixed solids (FS), which are comprised of inorganic material. Fixed solids are unable to be anaerobically digested and in some cases can even hinder or terminate the digestion process.

$$\%TS = (W_d/W_w) \times 100 \quad \text{(Equation 1)}$$

$$\%VS = ((W_d - W_i)/W_d) \times 100 \quad \text{(Equation 2)}$$

Where:

W_w = Weight of wet feedstock

W_d = Weight of dried feedstock

W_i = Weight of incinerated feedstock (only fixed solids remain)

The C/N ratio refers to the ratio of carbon to nitrogen present in the feedstock. Ideally, a ratio of 30:1 is utilized for most anaerobic digestion reactions (Cheng, 2010; Leckie et al., 1981). A certain amount of nitrogen is essential for the growth of methanogenic bacteria, however, when the nitrogen content gets too high, the build-up of ammonia can lead to an increase in the pH, up to 8.5, which can harm the methanogens (Liu et al., 2010). The pH of the feedstock will have an effect on the stability of the reaction. Methanogens require a relatively neutral pH, so feedstocks outside of this range, either more acidic or more alkaline, may require the use of pH buffers in order to maintain reactor stability (Cheng, 2010; Drapcho et al., 2008).

Bioreactor Conditions

There are a number of reactor conditions that are important in ensuring a stable, productive anaerobic digestion process. These reactor conditions include (but are not limited to): temperature, pH, organic loading rate, moisture content, and retention time.

Temperature.

The temperature of the reactor is one of the more important decisions that must be made in designing an AD system. There are three primary temperature ranges in which AD can occur: psychrophilic (10-20° C), mesophilic (20-40° C), and thermophilic (40-60° C)

(Cheng, 2010; Drapcho et al., 2008). AD can occur in any of these ranges, although it is advisable to use an inoculum that has acclimated to the same temperature range to ensure a health community of microbes. The necessary retention time decreases with an increase in temperature (Cheng, 2010; Drapcho et al., 2008). This is true for both the difference in the three temperature ranges, as well as within those ranges. For example, a reactor running in the mesophilic temperature range will produce more biogas, and typically with a higher concentration of methane, if kept at 35-37° C, rather than at the lower end of the range (~20-30° C) (Drapcho et al., 2008).

pH.

The pH of the reactor should be maintained at a relatively neutral level. Methanogens are highly sensitive to changes in pH, and require a range of 6.7-7.4 in order to maintain reactor stability (Drapcho et al., 2008). As previously stated, the use of pH buffers may prove beneficial, or even necessary, in maintaining a neutral pH within the reactor. Common pH buffers include lime, sodium hydroxide, ammonia, ammonium bicarbonate, soda ash, and sodium bicarbonate (Liu et al., 2010).

Organic loading rate.

The organic loading rate (OLR) is a measure of the amount of digestible solids entering the bioreactor each day. Usually reactors are “fed” in one of two ways: batch, or continuously. Batch digestion involves taking the total volume of waste to be digested and adding it to the digester all at one time. Upon completion, the digestate is removed and the reactor is refilled. In continuously-fed systems, a fraction of waste is added regularly at pre-specified times, and an equal amount of effluent is removed. The amount fed to the digester each day is partially determined by the size of the digester and the desired retention time to ensure that all of the waste is fully treated; i.e., if the desired retention time of the digester is

30 days, and it is fed once per day, then 1/30th of the total volume of the digester worth of waste will be added each day.

For continuously-fed systems, the OLR is typically expressed as the weight of VS or chemical oxygen demand (COD) per unit of volume of reactor per day (e.g., 1 gram VS/0.001m³/day). The OLR can have dramatic effects on the stability and the pH of the reactor. As new substrate enters the reactor the acid-forming bacteria quickly break down material into volatile acids, which the methanogens will further convert into biogas. If the OLR is too high, and the methanogenic community is not strong enough, the volatile acids can build up and lower the overall pH of the reactor, “souring” it and potentially killing off the methanogenic community and halting the reaction (House, 2010).

Moisture content.

The moisture content of the reactor is a measure of the solids content of the influent. In wet fermentation systems the total solids of the slurry (influent) is usually maintained at 2%-10% (Drapcho et al., 2008). Dry fermentation systems can operate with a slurry solids content as high as 30%-40% (Liu et al., 2010). House (2010) and Leckie et al. (1981) recommend that the slurry be kept between 7%-9% for most reactors, as this facilitates mixing and pumping of the digestate.

Retention time.

Retention time is the time required for the feedstock to remain in the bioreactor before exiting as effluent. The retention time in great part depends on the temperature of the bioreactor. Typically, 40-100 days retention is necessary for bioreactors running in the psychrophilic range, 25-40 days for mesophilic, and 15-25 days for thermophilic (Drapcho et al., 2008). For continuously-fed systems increased retention time necessitates larger systems, as the digestate must remain in the digester for longer periods of time. Therefore, it can sometimes be economically beneficial to run bioreactors at higher temperatures, thus

limiting the size of the digester to be constructed. However, if this method is employed, the energy input required to maintain these higher temperatures must be taken into consideration.

Horse Manure as AD Feedstock

Research into the use of horse manure as a feedstock for AD is surprisingly scant (Kalia & Singh, 1998; Kusch, Oechsner, & Jungbluth, 2008; Mönch-Tegeger, Lemmer, Oechsner, & Jungbluth, 2013; Wartell, Krumins, Alt, Kang, Schwab & Fennell, 2012). All of these studies have shown that horse manure is viable as a feedstock for AD, but that certain considerations specific to horse manure can have drastic effects on biogas production, the methane content of the biogas, and the long-term stability of the reaction (Kalia & Singh, 1998; Kusch et al., 2008; Mönch-Tegeger et al., 2013; Wartell et al., 2012).

According to the literature, the biggest issue surrounding the use of horse manure as an AD feedstock is the collection point for the manure (pasture vs. stable) and, if the manure is collected from a stable, the type of bedding that is used. For this reason, the majority of the available literature on horse manure centers on studies looking into the effects of different bedding materials on methane production.

Mönch-Tegeger et al. (2013) conducted an experiment to test the effects that these factors have on both the total biogas production and the methane content of the biogas. The researchers tested horse manure that was collected from a field ("horse dung"), manure that had been piled and stored, and manure collected from stables. Manure collected from the stables was further separated based on bedding type: straw, straw pellet, straw/flax, flax, wood-pellets, and sawdust. Bench-scale batch digesters were employed to compare the suitability of these manures for the production of biogas. The bedding materials themselves were digested independently of the manure/bedding mixes, to determine their contribution to biogas and methane production.

Wartell et al. (2012) studied the effects of stall waste mixed with various ratios of softwood beddings on methane production. These researchers compared the methane production potentials of stall waste mixed with dry softwood bedding; used softwood bedding (bedding that had been exposed to urine and/or feces); Woody Pet®, “a softwood pellet bedding that disintegrates into small wood particles under the influence of moisture;” and straw (Wartell et al., 2012, p. 43). Included in this study were control groups testing the methane production potential of the individual bedding materials on their own (both fresh and used), as well as horse manure by itself (meaning horse manure without bedding.)

Kusch et al. (2008) studied the effects of different ratios of inoculum to horse manure in a “solid phase digestion process,” looking primarily at the stability of the reaction and methane production (p. 1280). These researchers utilized horse dung mixed with straw bedding as their feedstock source. The goal of the study was to first determine the ideal ratio of inoculum to feedstock for biogas production and reactor stability by using pre-digested horse manure collected from a functioning digester treating only horse manure. The second experiment involved using this inoculum-feedstock ratio and then testing the effects of percolation (recirculation of digester leachate within the bioreactor) and flooding on the digestion process, specifically in terms of methane production potential. The average methane production for horse manure, collected from stable, was found to be 170 mL/g VS over the course of 40 days.

Kalia and Singh (1998) ran a study in which they considered the effect of co-digesting horse manure along with cow manure in family-sized bio-digesters operating in rural northern India. The researchers compared different ratios of horse to cattle manure, and the effect those ratios had on biogas production and the stability of the reaction. While horse manure alone was found to be unsuitable as a sole feedstock for digestion, when used

as a substitute (20%) for cattle manure biogas production was found to be about equal to that of cattle manure alone. One important effect the researchers noted in the digestion of horse manure was the tendency for the solid and liquid portions of the digestate to separate within the reactor, leading to less efficient digestion and mixing problems.

Mönch-Tegeder et al. (2013) concluded that of the types of stable manures they examined, manure collected from straw and straw-pellet bedding led to the greatest biogas production with the highest methane content, while the woody bedding materials (wood chips and sawdust) produced far less methane (see Table 1). Interestingly, however, straw alone was found to have a slightly higher methane yield than both fresh horse manure from a straw bedding mix, and horse dung (manure without bedding). Horse dung, that is manure collected from the field, was found to produce an average of 171 mL/g VS of methane over the course of the trial (35 days).

Similar results were determined by Wartell et al. (2012) who found that straw alone and horse manure alone were roughly equal in terms of methane yield. These findings led both sets of researchers (Mönch-Tegeder et al., 2013; Wartell, et al., 2012) to determine that the addition of straw to horse manure would only serve to increase the potential methane production. In fact, it was found that “[s]traw bedding contributed substantially to methane production...increasing methane production nearly linearly up to a 4:1 ratio of bedding to horse manure” (Wartell et al., 2012, p. 46). The methane production results from this study were much more varied, partly a factor of the duration of the reaction. In this study, horse manure (collected from stables) was found to produce an average of 56 ± 14 , 122 ± 78 , 53 ± 15 , 231 ± 18 , 133 ± 6 mL/g VS over the course of 33, 40, 46, 59, and 79 days, respectively.

According to the Mönch-Tegeder et al., (2013) study, woody material beddings led to the creation of “sinking layers” in the digester, resulting in higher failure rates. For this reason the researchers advised against using these types of bedding in AD systems, as they

are better suited for combustion or composting. Wartell et al. (2012) came to similar conclusions as Mönch-Tegeder et al. (2013), but did not find any inhibitory effects on the digestion process with the use of softwood bedding, although there was a positive correlation between an increasing dilution effect and methane production with an increase in the ratio of softwood bedding to manure. In other words, while digestion was not completely inhibited by the bedding, the concentration of methane in the resultant biogas was diminished (diluted).

Table 1. CH_4 Production from Stable Manure Samples (Mönch-Tegeder et al., 2013, p. 165)

Barn	Bedding	Specific methane yield /Nm ³ CH ₄ kg ⁻¹ VS	Variation coefficient /%	Methane energy /MJ kg ⁻¹ VS	Energy recovery /%	<i>P</i>	<i>R_m</i>	λ	<i>R</i> ²
A	Straw	0.180	6.17	7.18	38.96	0.175	0.0119	1.48	0.996
B	Straw	0.164	1.81	6.53	36.19	0.177	0.0076	2.02	0.858
C	Straw	0.212	1.39	8.44	45.14	0.207	0.0122	1.70	0.997
D	Straw	0.208	3.29	8.27	45.76	0.205	0.0120	2.72	0.998
E	Straw	0.191	2.46	7.59	40.50	0.190	0.0097	2.60	0.997
F	Straw-pellets	0.191	2.43	7.60	39.85	0.192	0.0102	2.03	0.998
G	Straw/Flax	0.215	0.69	8.55	46.19	0.214	0.0124	3.60	0.989
H	Flax	0.150	3.17	5.98	32.23	0.155	0.0072	3.01	0.998
I	Wood-pellets	0.104	2.73	4.14	21.88	0.100	0.0064	1.23	0.991
J	Sawdust	0.114	2.13	4.53	24.41	0.109	0.0078	1.73	0.989

Note: VS = volatile solids; *P* = methane production potential, Nm³ CH₄ kg⁻¹ VS; *R_m* = maximum daily methane yield, Nm³ CH₄ kg⁻¹ VS; λ = lag time, d.

An interesting aside from the Mönch-Tegeder study came about from the feedstock characterization measurements and analysis. Mönch-Tegeder et al. (2013) included a trace-element composition test of the collected manures and found that horse manure lacks many of the trace elements present in other agricultural animal manures. In fact, according to the study, “...horse manure cannot provide sufficient amounts of trace elements for a stable biogas process. In comparison with the silages, the concentrations of the micronutrients are equal to the ones for grass silage” (Mönch-Tegeder et al., 2013, p. 166). For this reason,

and coupled with the relatively high C/N ratios of horse manure, the researchers recommended that horse manure be co-digested with nitrogen-rich manures, such as swine and chicken, for the greatest biogas and methane production potential. This finding meshes nicely with the conclusions drawn by Kalia and Singh (1998), in which horse manure was best suited as a co-digestate feedstock, rather than as the sole feedstock for anaerobic digestion.

Biogas Production Analysis

Biogas Volume

Biogas volume can be measured by a number of methods. One of the most commonly employed and simplest methods is through liquid displacement (Veiga, Soto, Méndez & Lema, 1990; Walker, Zhang, Heaven & Banks, 2009). Of course, there are other, potentially more accurate ways of measuring biogas production, such as with the use of wet-gas meters and flow meters. In the absence of access to such equipment, however, the liquid displacement method is viable (Johnson, 2009; Trowbridge, 2013).

If using the liquid displacement method for measuring biogas production, there are some important parameters that must be addressed to ensure that the data are accurate. The two most important of these are the choice of a proper barrier solution to prevent the loss of gases through dissolution, and the normalization of volumetric measurements to standard temperature and pressure (STP) (Hawkes and Young, 1980; Walker, et al. 2009).

Research has shown that the ideal barrier solution for reducing gas solubility is an acidified, saturated NaCl solution (Walker et al., 2009). This is due to the propensity of CO₂ to dissolve in water over time. Walker et al. (2009) tested different barrier solutions, and how much CO₂ and/or CH₄ was lost through dissolution over a period of 17 days. A 100% saturated NaCl solution was found to be optimal for preventing the loss of CO₂ into solution, but that such a concentration can lead to the build-up of salts on the equipment and is

therefore not recommended. However, “a 75% saturated NaCl solution was comparable to that of the saturated solution, with 96% of the methane and 88% of the carbon dioxide remaining in the column headspace at the end of the 8-day test period” (Walker et al., 2009, p. 6343).

The primary reason for preventing the dissolution of gases into the barrier solution is to ensure that gas-composition measurements are not negatively affected. For example, the loss of CO₂ into solution will result in both skewed gas volume and composition readings, resulting in volumetric measurements less than the actual amounts, which in turn will show a higher concentration of methane than is actually present in the biogas.

Biogas Composition

Biogas is comprised primarily of CH₄ and CO₂. CH₄ typically makes up 60-70% of the total volume, and CO₂ 30-40% (Cheng, 2010; Drapcho et al., 2008). Depending on the reactor conditions and the wastes undergoing treatment, there are sometimes trace amounts of other gases, primarily H₂S and N₂. For the purposes of energy production CH₄ is the primary gas of interest. In many large-scale digester systems, CO₂ and other trace gases will be scrubbed out to improve the purity of the gas to be burned, increasing efficiency and energy output (Cheng, 2010).

Monitoring the biogas composition throughout the digestion process can provide valuable insights into the health/stability of the reaction. Early on in the process analyzing the biogas composition can also cue the operator into what stage of the AD process is currently underway (this is especially important in the start-up of new digesters and with the addition of new, untested feedstocks or co-digestion schemes). For example, in the early stages of the digestion process the wastes undergo three stages before the methanogenic bacteria take-hold and methane starts to be produced. During this time, acid-

forming bacteria are the prominent microbial communities. This can be seen directly in the production of primarily CO₂ in the early stages of the reaction.

Spikes in CO₂ content during the reaction can signal a potential souring effect, which can slow, and sometimes even halt the AD process. This can occur when too much waste is added at one time to the digester, or when a highly decomposable waste, such as food waste, is added. This can cause the acid-forming bacteria to dominate the process, thereby lowering the pH of the digestate, potentially killing off the methanogenic bacteria necessary for methane production (House, 2010).

VS-destruction and COD

VS-destruction and COD are two indicators used for determining both the feeding schedule for the digesters (the OLR) and also for the effectiveness of the digester in treating wastes (i.e. removing potential pollutants.) VS-destruction is a measurement of the amount of volatile solids destroyed (converted) during the digestion process. As mentioned previously, VS is determined through incineration of the feedstock/digestate sample. VS-destruction (sometimes called VS-removal) is used in many AD studies, and can be a useful indicator of the effectiveness/efficiency of the AD process for a given digester and/or feedstock (Brunn, Dornack, & Bilitewski, 2007; Bouallagui, Marouani, & Hamdi, 2010; Kowalczyk, Schwede, Gerber, & Span, 2011).

The other primary measurement for determining the efficiency of an AD reaction is COD. Some researchers have argued that VS-destruction/removal is not the most accurate method for measuring the removal of pollutants. More specifically, VS measurements run the risk of over-estimating the available material for degradation through anaerobic digestion. Leckie et al. (1981) argues that COD is a better indicator for pollution-removal and for determining the OLR for a particular digester. The reasoning given for this is that while VS represents the organic fraction of the total solids, it is measured as the fraction

that will burn off at 550° C. During a digestion process, the materials are not being incinerated, but rather biodegraded via chemical processes. Leckie, et al. (1981) claims that only 50% of the total VS is actually degradable through digestion. For that reason, COD is a better measurement, as it represents the amount of oxygen that is necessary to oxidize (degrade) the organic material through chemical means rather than through incineration.

Scalability of Anaerobic Digestion

Similar to investigations into horse manure as an AD feedstock, there has been relatively little research done into the scalability of AD research, and the transferability of results from bench-scale studies to industrial-scale biogas plants. Research into the effectiveness of different feedstocks and co-digestion schemes is most often performed at the bench-scale, and it is of utmost importance to know whether or not the results obtained in the laboratory are transferable to larger-scale systems (Kowalczyk et al., 2011). Most of the research into scalability has shown a strong correspondence between data gathered in a laboratory-scale reactor and its predictive value for determining performance on larger scales, provided that the reactor and process conditions are kept as similar as possible (Bouallagui, et al., 2010; Brunn, et al., 2007; Gallert, Henning, & Winter, 2003; Kowalczyk, et al., 2011; Massé, Croteau, Masse, & Danesh, 2004).

Gallert et al. (2003) researched how effective and accurate data gathered from laboratory-scale digesters are at predicting the performance of industrial-scale digesters operating under the same basic parameters. The intent of their research was to determine in the lab the effects of increasing the OLR on reactor performance, in order to determine a maximum, stable OLR that could be maintained, thus allowing for an increased use of organic waste and a higher efficiency in waste treatment. The goal was to extrapolate the results obtained in the lab to an operating, industrial-scale digester in Karlsruhe, Germany.

The bench-scale digesters were constructed to mirror the same system parameters as the industrial-scale reactor, with liquid volume being the primary variable. A positive correlation was found between the lab and industrial-scale systems in regards to biogas production and COD removal. At this point, the OLR was slowly ramped up in the lab-scale digesters to find the maximum OLR, while still maintaining a stable reaction. The researchers found data from the laboratory-scale digesters and the industrial digesters correlated in an increase of the OLR from $8.5 \text{ kg COD m}^{-3}\text{d}^{-1}$ to $19 \text{ kg COD m}^{-3}\text{d}^{-1}$ (Gallert et al., 2003). Based on this result, the city of Karlsruhe increased its OLR from $8.5 \text{ kg COD m}^{-3}\text{d}^{-1}$ to $15 \text{ kg COD m}^{-3}\text{d}^{-1}$. The large-scale reactor ran at this OLR for a year and a half (at the time the paper was published) and researchers found a strong correlation between the simulations performed in the lab and the actual performance of the industrial-scale digester, supporting the “feasibility of laboratory simulation experiments for scale-up considerations” (Gallert et al., 2003, p. 1440).

Brunn et al. (2007) experimented with the reproducibility and transferability of bench-scale digestion experiments to the industrial scale. Two identical 120 L reactors (80 L process volume) operating under the same conditions were run in parallel, and compared to an industrial-scale reactor with a process volume of 4.6 million liters (4600 m^3). The reactors were compared by the degree of VS degradation, total organic carbon (TOC), ammonium nitrogen content, organic acids, and specific biogas production.

Compared to each other, the two bench-scale bioreactors exhibited a high degree of agreement, given identical conditions. There was some variation, specifically in biogas production. The authors reported a total of $827 \text{ l}_N/\text{kg VS}_{\text{in}}$ in digester 1 and $754 \text{ l}_N/\text{kg VS}_{\text{in}}$ in digester 2 (Brunn et al., 2007), which the researchers attributed to changes in substrate composition. The results from the two bench-scale digesters were averaged and then compared to the industrial-scale digester according to the same set of parameters. The

industrial-scale digester produced 36% more gas on average than the bench-scale digesters, which the researchers could not explain except as a possible result of different feeding schedules and substrates: the lab-scale digesters were only fed three times a week, compared to the industrial-scale digester which was fed daily, and the substrate for the digesters was taken from a different plant. The researchers concluded that there is a high rate of reproducibility between digesters of the same scale, and that transferability to a larger scale is possible if the same process conditions are used (Brunn et al., 2007).

Kowalczyk et al. (2011) researched the scalability of AD by analyzing the performance of three identical 22 L digesters and a single 390 L digester, operating under identical process conditions. The goal of the work was to serve as a “pre study” to determine the transferability between digesters of two different scales, as well as reproducibility between digesters of the same size (Kowalczyk et al., 2011, p. 48). The results of the study show a high level of correspondence between the four digesters based on the measured parameters (biogas volume, biogas composition, % dry matter (DM), and %VS.) The three smaller (22 L) reactors showed a high rate of reproducibility, with a “daily relative standard deviation of...between 1.42 and 5.96%” (Kowalczyk et al., 2011, p. 54). Differences in substrate composition, as in the Brunn et al. (2007) study, were given as the reason for this variation. In comparing the 22 L digesters to the 390 L digester, a relative deviation between “-6.92 and 18.07% with an average of 6.33%” was found (Kowalczyk et al., 2011, p. 54). The researchers claimed that this was not due to the OLR, which was varied during the test, but was likely a result of differences in substrate composition, geometry, and construction materials of the digesters, as well as the mixing method.

Chapter 3: Research Methods

General Overview

In this experimental study, efforts were made to begin determining the transferability of findings from laboratory-scale AD trials to larger pilot-scale systems, as well as to examine the reproducibility of results within scales. The pilot-scale biodigesters that are currently under construction are each sized to hold roughly 1000 L, so for that reason this study tested three differently-sized bench-scale digesters: 100 mL, 1 L, and 10 L. By logarithmically increasing the size of the digesters, the goal was to facilitate development of a predictive model for biogas production, particularly for the forthcoming pilot-scale system. The next logical step in building this predictive model for scale-up would be 100 L digesters, but due to certain constraints this study was unable to test a bioreactor of that size.

Data collected from the three differently-sized digesters were compared with one another in order to determine the transferability of results between different digester sizes. Data within each of the size groups were also examined to determine the reproducibility of results in digesters of the same size. The parameters for determining the transferability and reproducibility of laboratory-scale AD experimentation included daily and cumulative biogas production, biogas composition, VS-destruction, and pH.

Equipment

A laboratory pH meter (OakTon Instruments pH/Ion 510 Bench pH/Ion/mV Meter) was used for determining the pH of the digestate before and after the digestion process. This meter was calibrated using commercial pH standards (pH 4.0, 7.0, and 10.0).

A drying oven and an analytic balance were used to determine the %TS and moisture content (%M) of the horse manure prior to the start of the two trials.

A muffle furnace (ThermoScientific Thermolyne™ #F6010) and the same analytic balance were used to determine the %VS of the horse manure prior to experimentation.

A CHN analyzer (Perkin Elmer CHN 2400), which measures the mass percent of carbon, hydrogen, and nitrogen present in a given sample, was used to determine the C/N ratio of horse manure prior to experimentation.

A hot water circulation bath was used to house the 100 mL and 1 L bioreactors and to maintain consistent temperatures in the mesophilic range.

An immersion water heater/circulator (Anova Suis Vide Immersion Circulator, 120 V) was used in the secondary water bath to heat and maintain proper temperatures for the 10 L bioreactors.

A bi-directional aquarium pump was used in Trial 2 for moving biogas, recharging the water columns, and assisting in taking volumetric measurements (Stock pump, purchased from HerbalAire Ltd.).

Multi-foil gas collection bags (Restek Multi-layer Foil Gas Sampling Bags) of various sizes (12 L, 10 L, 3 L, & 1 L) were used for the collection of biogas from the 10 L digesters in Trial 1, and for all of the digesters in Trial 2.

A landfill gas analyzer (Landtec GEM2000) was used for the analysis of biogas composition. In the first trial, this device also assisted in volumetric measurement, utilizing the on-board pump to move biogas, and to recharge the inverted cylinders. In the second trial the GEM was used solely as a gas analysis device.

Two gastight syringes were required for this study. A 10 mL syringe (Hamilton gastight® 1700 model #1010 LTN) was used for the removal of biogas samples from the gas collection bags for injection into 5 mL glass vials for transport. A 10 µL Hamilton

gastight® syringes (model #1701 N) was used for taking samples from the vials/gas collection bags and injecting them to the gas chromatograph.

A gas chromatograph (GC) (Shimadzu GC17A, molecular sieve column, helium carrier gas) was used to analyze biogas produced by the digesters, and was also used as a way to determine the performance and accuracy of the GEM landfill gas analyzer as well as to troubleshoot potential leaks and areas of contamination in biogas sampling.

Samples

The feedstock used in samples for all experimental trials of this study was horse manure gathered from a local horse farm. The manure was collected prior to the start of each trial. Horse manure was chosen due to the large number of horse farms in the area (Watauga County, NC), coupled with the fact that relatively little research has been done on this particular feedstock. The horse manure was collected from Dutch Creek Trails in Vilas, NC. The owner of the farm, Keith Ward, was kind enough to grant free access to the farm to collect feedstock samples as needed. This particular horse farm does not have a central stable, so all manure was collected directly from the field. Efforts were made to take samples from the most recent manure piles to ensure freshness of the feedstock.

The original plan was to collect horse manure all at once, obtaining enough to perform the feedstock characterization analysis and to conduct a preliminary trial and two experimental trials, as this would ensure better consistency in feedstock samples across trials. However, due to a lack of space, specifically a refrigerator large enough to store this much manure, the manure had to be collected three separate times: first for the feedstock characterization/analysis and a preliminary trial run, then before the start of each of the experimental trials.

At each of the three site visits, the collected horse manure was chosen based on visual inspection for freshness and lack of contaminants such as soil, grasses, and rocks.

Efforts were made to collect samples from as many different piles as possible, in as many locations as were accessible. Coupled with this, twice the amount of manure as was needed was collected during each trip. The purposes of these actions were to ensure that the samples taken were as representative as possible of the horses at this horse farm.

Experimental Process

Feedstock Characterization and Preliminary Trial

This study began with both a feedstock characterization and a preliminary trial that was run using solely horse manure as the feedstock. This trial run was performed with the help of students enrolled in an undergraduate Biofuels course in the TED department, instructed by James Houser, Ph.D.. The goal of this preliminary trial was to gain an understanding of the effectiveness of horse manure as a feedstock prior to the experimental trials, and to determine if there would be any obstacles or problems to overcome using this feedstock.

Feedstock characterization.

On February 5, 2014 the first collection trip was made to Dutch Creek Trails. The weather was overcast and cold, barely above freezing. This proved useful because it helped in distinguishing the freshest manure piles from the older ones (though usually this could be done by sight alone). Three five-gallon buckets were filled with horse manure, totaling roughly 20 kg of fresh manure. The manure was returned to the laboratory where it was thoroughly homogenized by mixing the contents of each of the buckets using a hand trowel. Samples were placed into gallon-sized bags and vacuum-sealed. These samples were then placed into a small refrigerator (4° C) for storage for two days until they would be prepped for characterization by the undergraduate students. The horse manure was tested for %TS, %VS, %FS, and the C/N ratio.

Fourteen total samples were taken for the purposes of characterization. Seven pairs of undergraduate students each filled two ceramic crucibles, and myself four, with the homogenized manure. One of the crucibles in each pair was smaller, and one larger, for sample sizes of between 32g-36g and 65g-76g, respectively (wet weight manure). The samples were then placed in a drying oven for 48 hours at 100°-105° C in order to drive off moisture. Once the samples had dried, they were each weighed again to determine their dry weight. Upon determining the dry weight, fourteen of the samples were placed in a muffle furnace at 550° C and held there for 2.5 hours to incinerate them. After this time, the samples were removed from the furnace, allowed to cool, and weighed again. These values were used to determine the %TS and %VS (see Equations 1 and 2).

The other two dried samples, which were not incinerated, were used to determine the C/N ratio. The two samples were combined, thoroughly ground with a mortar and pestle, and then run through a food processor for approximately one minute, until the sample was reduced to a powder-like consistency. This homogenized sample was then combusted in the CHN analyzer in order to determine the C/N ratio of the manure. Samples (~2 mg) of this dried manure were measured out and run through the CHN for analysis. Unfortunately, there were a number of problems with the data collected from the CHN analysis.

Numerous trials were run through the CHN analyzer (Perkin Elmer CHN 2400), using 1-3 mg-sized samples of dried, powderized horse manure. With each trial the results were wildly varying, the only common factors being their inconsistency with the literature, and their abnormally high C/N ratios (between 90-750). One hypothesis to explain these findings may be the seemingly high volume of hay present in the manure samples, based upon visual inspection. Because such small samples had to be taken for use in the CHN analyzer it was difficult to get a representative measure for the manure alone. Another,

more likely explanation was that the reduction tube of the CHN analyzer was packed with an old reagent. The oxidation of the copper reagent led to skewed N measurements, which could explain the abnormally high C/N ratio.

The unused portions of horse manure from this characterization process were resealed and placed back into the small refrigerator in the lab. This sample served as the feedstock for the undergraduate students' next class, which involved making small digesters out of 150 mL Erlenmeyer flasks, the same type of digester design that has been used in past AD experimentation in the TED department.

Preliminary trial.

On February 14th, 2014 students in the undergraduate biofuels course split into the same seven pairs used the previous week for the characterization lab. Each pair took a sample from the same homogenized horse manure as from the previous week (which had been stored in a refrigerator at 4° C) and prepared an 8% slurry (based on dry mass of manure) using distilled water, with a final slurry volume of 100 mL. This slurry was placed into a 150 mL Erlenmeyer flask, sealed, and transported to the biofuels laboratory in Edwin Duncan Hall.

There the digesters were uncapped and the pH was measured in each using a laboratory pH meter. These numbers were recorded and the headspace of each digester was then flushed with pure N₂ for approximately one minute. The reason for the flushing with nitrogen is to try and maintain as anaerobic of an environment as possible before the start of the reaction. This is standard practice in many anaerobic digestion bench-scale studies (Møller, Sommer & Ahring, 2004; Wartell et al., 2102). In past TED department biogas experiments this step has been ignored. Not flushing with N₂ resulted in very slow lag times before gas production began, and also the formation of white mold on the top slurry. For these reasons this study incorporated an N₂ purge. After this purge, each of the

digesters was re-capped and placed into the hot water bath where they were connected to the bench-scale set-up that had been used in previous undergraduate, and graduate experiments (see Figure 2 & Figure 3).

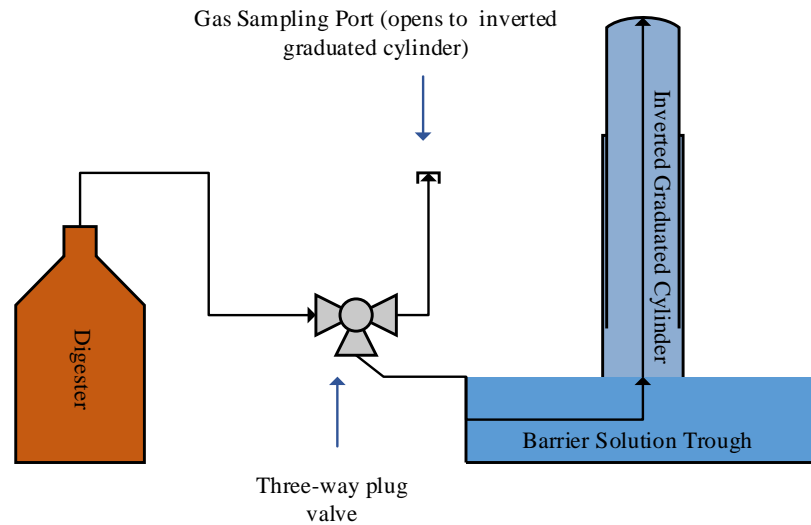


Figure 2. Schematic of bench-scale system for 100 mL & 1 L digesters (preliminary and Trial 1).

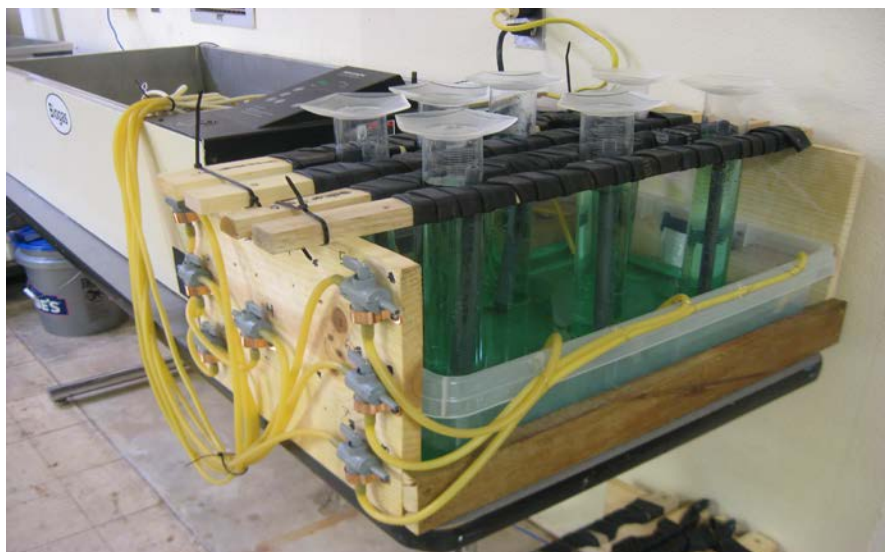


Figure 3. Bench-scale set-up for preliminary trial and previous TED department biogas experiments.

The preliminary trial lasted for 30 days. Every day, each digester was agitated by hand for at least 30 seconds to ensure a proper mixing of the substrate. Gas volume was recorded by determining the difference of the meniscus height of the barrier solution in the inverted graduated cylinders between days of observation. For example, if the meniscus was at the 50 mL mark one day, and was at the 75 mL mark 24 hours later, 25 mL of biogas would be recorded as having been produced that day. The barrier solution in the trough and inverted graduated cylinders consisted of purely tap water from the lab, with a green food coloring to make reading the level of the meniscus easier.

After the 30-day trial had been completed, the digesters were pulled from the system set-up and the system itself was taken down. As will be discussed in the Discussion and Conclusions section, the preliminary trials shed some light on a number of problems with the way the bench-scale system was designed. These issues were addressed in the experimental trials by implementing a number of design changes.

Prior to the start of each of the experimental trials some changes were made to both the bench-scale system set-up as a whole as well as to the volumetric measuring devices. These changes were made in response to issues encountered in the preliminary trial and in prior student lab and research projects (Trowbridge, 2013). Although the ultimate goal of this project was to determine the accuracy of scalability, calibrating and troubleshooting the bench-scale AD set-up became a major focal point of the project, and an activity of great importance for future AD experiments in the TED department. The reasons for these changes will be discussed in further detail in the Discussion and Conclusions section.

New Components

In order to operate the 10 L digesters used in the two experimental trials a new hot water bath had to be created, because the three-gallon carboys used for the 10 L digesters were too large to fit inside the laboratory hot water bath. A simple 2' x 2' x 2' box was

constructed out of a sheet of 1/4" plywood, with 1" x 4" braces. The inside, including the bottom, was lined with a double-layer of 1" blue board to help provide insulation to the box and to better maintain heat. Over the insulation, three layers of 6mm black vinyl plastic were glued down to waterproof the container. The bath can accommodate up to four three-gallon carboys with enough space in the center to mount a heater.

An immersion heater/hot water circulator was chosen as the heating element for this water bath. The reasons for this included the integration of a circulator and a heater on the unit, as well as the easy-to-read LCD display that allowed for changes to the temperature and the setting of timers. The circulator was mounted above the water line on the bath, in between the four digesters, allowing for maximum circulation of hot water throughout the bath. Pieces of blue board were cut to size in order to fit tightly over the top of the carboys, trapping as much heat and condensation as possible within the bath.

Multi-layer foil gas collection bags were utilized for the 10 L digesters in Trial 1, and eventually for all of the digesters in Trial 2. This is the first time these bags have been used in the TED department's AD research. On each bag there is polypropylene combo valve with a 3/16" OD connection for the gas line and a syringe port with replaceable septum. The 10 L digesters were equipped with 12 L gasbags for Trial 1.

Due to the size of the 10 L digesters, and the volume of gas they were likely to produce once they were fully operational, the use of inverted graduated cylinders was not possible for gas volume measurements, since 2 L is the largest graduated cylinder readily available. To solve this problem I developed a simple technique using a water column to pull a vacuum on the gasbags to measure the volumes of biogas produced each day (see Figure 4).

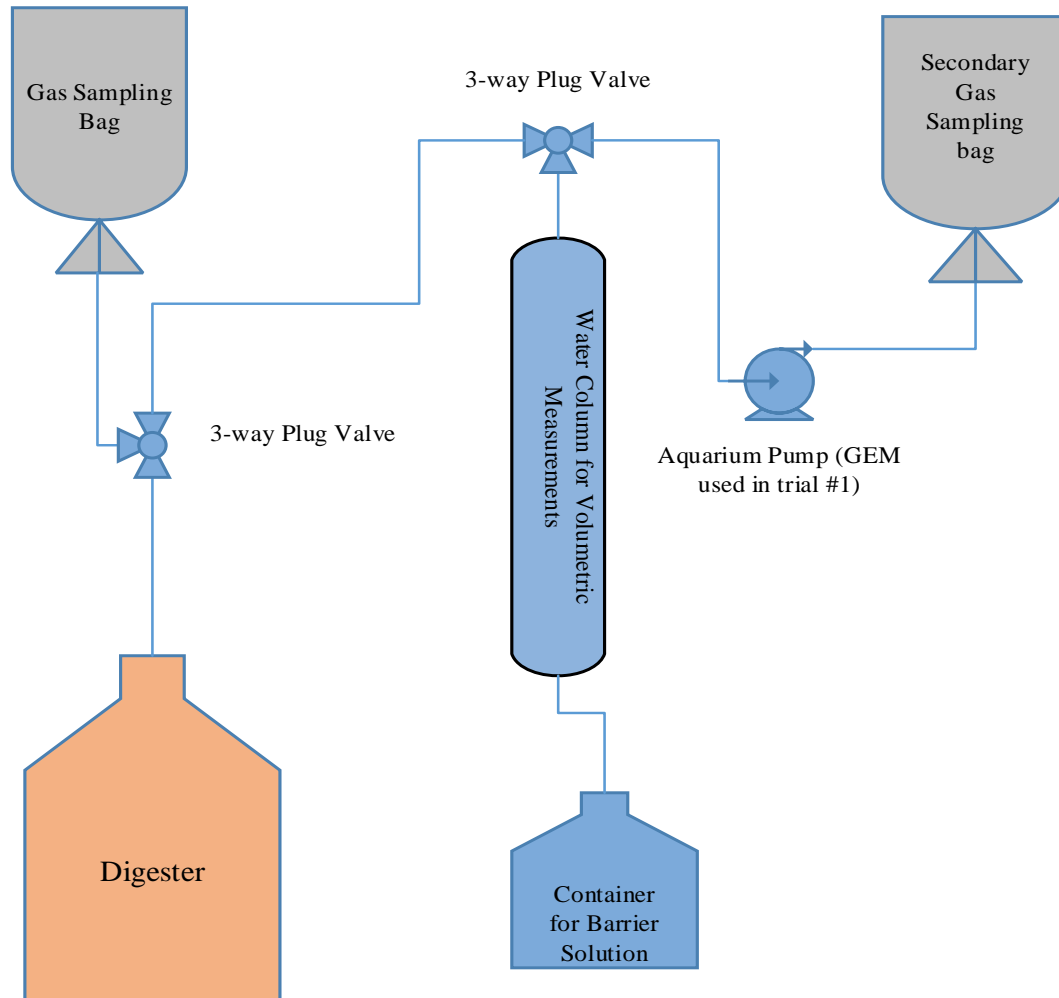


Figure 4. Schematic of water column for measuring volumetric biogas production.

The water column was constructed out of an 18" length of clear acrylic 2 1/4" ID pipe. Connected to both ends of this pipe was a PVC slip coupling, a female threaded coupling, and a plastic 1/4" OD hose barb connector. Connections and seals were made using a plastic-bonding adhesive, and silicone caulk where needed to ensure that the device would be water-tight. The top barb on the column served as a connection point between the water column and a three-way plug valve.

During gas measurements, one end of this valve would be connected to the gasbag, and the other end to the GEM (in Trial 2 an aquarium pump was used instead). Attached to the bottom barb connector on the water column was a length of natural rubber tubing which was submerged in a three-gallon carboy containing a barrier solution. This container served as both dump site and source for barrier solution during volumetric measurements, as well as a means to keep out air infiltration and maintain the vacuum. A piece of measuring tape was placed along the side of the column to serve as a sight gauge for taking volume measurements. More detail into the operation of this water column is included in the following sections.

Experimental Trials

Following the preliminary trial, two experimental trials were performed in this study. Each trial consisted of 12 digesters, four each for the three sizes: 100 mL, 1 L, & 10 L. Each trial took place over a 30-day period, with the bioreactors operating at a temperature of 37.8° C. The temperature was maintained in each of the hot water baths utilized in this study with the use of digital temperature controllers. The 100 mL digesters were constructed out of 250 mL Erlenmeyer flasks. The 1 L digesters, similarly, were constructed from 2 L Erlenmeyer flasks. The 10 L digesters were constructed out of three-gallon plastic (PET) fermentation carboys. The difference in digester geometry between the 10 L digesters and the two smaller sizes was, unfortunately, an uncontrolled variable that was monitored throughout the experiment, and is addressed in the discussion and conclusions section (see Figure 5).



Figure 5. Comparison of the three digester scales, from left to right: 10 L, 1 L and 100 mL.

Trial 1

Trial 1 began on March 12, 2014 and was a 30-day trial. Thirty days was chosen because it is the traditional trial length for studies in the TED department Biofuels Technology course and because it is a typical length for digesters operating in the mesophilic temperature range (Drapcho et al., 2008). The horse manure was collected from Dutch Creek Trails on the morning of March 12th and stored in four sanitized five-gallon buckets. The manure was then delivered to the biofuels lab in Edwin Duncan Hall on the Appalachian State University campus. Outside of the lab, the manure was combined with the proper amount of distilled water to make roughly an 8% slurry mix (see Equation 3), and was thoroughly blended together using a paint-stirring attachment on a battery-

powered drill. 12 gallons of distilled H₂O were combined with 32 lbs. of fresh horse manure, with a %TS of 25%, for a final slurry concentration of 8% TS.

$$8\% \text{ slurry} = 8 \text{ lb. TS}/100 \text{ lb. H}_2\text{O} \quad (\text{Equation 3})$$

$$8 \text{ lb. TS (100 lb. fresh horse manure}/25 \text{ lbs. TS)} = 32 \text{ lb. fresh horse manure}$$

$$100 \text{ lb. H}_2\text{O} \times \text{gallon}/8.34 \text{ lb.} = 11.99 \text{ gallons H}_2\text{O}$$

Homogenization of feedstock and preparation of digesters.

Following this homogenization process, the slurry mix was distributed into each of the 12 sanitized and covered digesters. The digesters had previously been washed with Powdered Brewery Wash™ (PBW) (Five Star Chemicals) to ensure that no residues remained in the vessels from previous reactions. Each of the digesters was then sanitized with StarSan™ (Five Star Chemicals) to destroy any remaining, unwanted organisms. Even though this is a no-rinse sanitizer utilized in brewing processes, the vessels were briefly rinsed out with distilled water as an added precaution against potentially killing-off desirable microorganisms in the manure sample.

The Erlenmeyer flasks and the carboys were marked to show the fill lines for the 100 mL, 1 L, and 10 L samples. The digesters were filled, and quickly capped with a rubber stopper. The digesters were then brought into the lab where the pH was measured and each digester was flushed with pure N₂ for approximately one and a half minutes. Both the headspace and the slurry itself were flushed with N₂. Following this, each of the digesters was placed into its respective hot-water bath, and the connections were made from each digester to the gas collection systems.

Volumetric biogas measurements (100 mL and 1L).

For Trial 1, biogas production was measured using two forms of liquid displacement. The smaller digesters (100 mL and 1 L) used inverted graduated cylinders to measure biogas production via the displacement of a barrier solution. The 10 L digesters used the water column device (Figure 4) to handle the larger volumes of gas being produced. The first method involved using inverted graduated cylinders in a trough of barrier solution (see Figure 6).

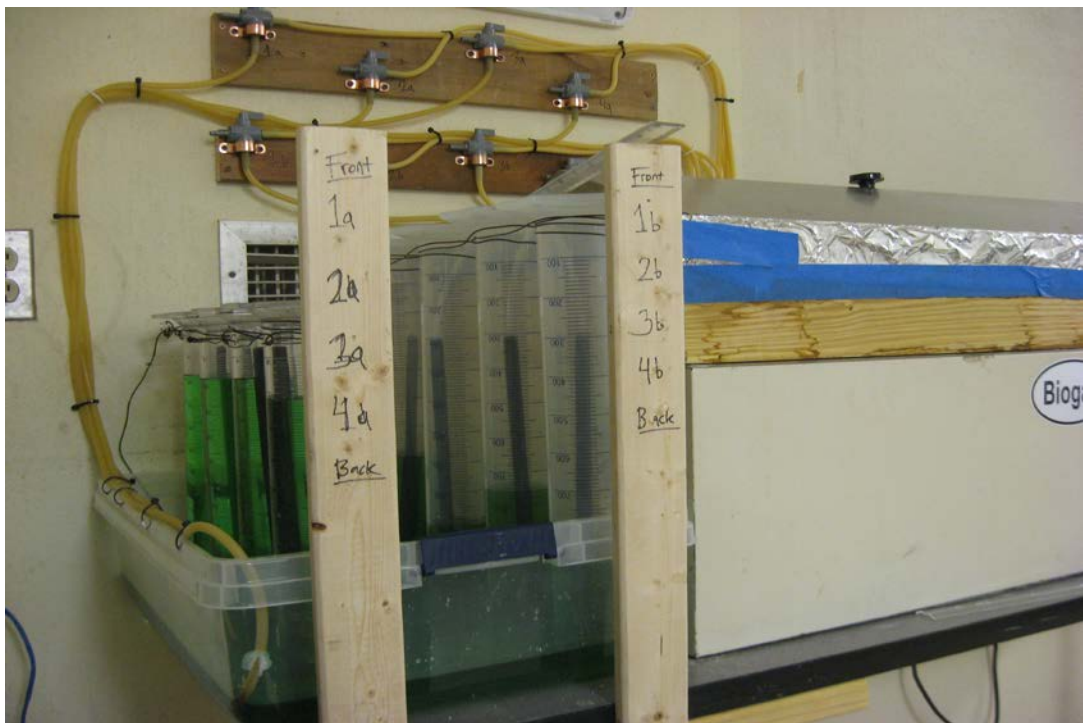


Figure 6. Bench-scale set-up for 100 mL and 1 L digesters in Trial 1.

Biogas produced in the 100 mL and 1 L digesters traveled through a length of 3/16" ID latex natural rubber tubing to a three-way valve. This three-way valve served as a sample port to allow gas samples to be drawn off from the inverted cylinders. From there the gas traveled via the same size natural rubber tubing to a length of 1/4" PVC piping, to which the tubing had been connected via a brass barbed fitting. This piece of PVC extended

to the top of the graduated cylinder, just above the top of the barrier solution line. As gas entered the cylinder, the barrier solution was displaced and emptied into the trough; this displacement was measured via the hash marks on the graduated cylinder (see Figure 6).

Each day, approximately every 24 hours, the volume of biogas produced in each digester was recorded. Before these measurements were taken, each digester was agitated by shaking for approximately 30 seconds. Because these digesters were running in batch mode and there was no means of mechanical stirring, they had to be agitated each day to ensure a complete digestion, and to prevent the forming of scum layers, or solid/liquid separations within the digester, which has been demonstrated to be a problem with horse manure (Kalia and Singh, 1998).

Once the digesters had been agitated, the biogas volume was determined by subtracting the initial height of the barrier solution from the current level, the same method employed in the volumetric measurements in the preliminary trial. These measurements were recorded each day in both a laboratory logbook and an Excel spreadsheet.

With this particular volume-measuring scheme, the graduated cylinder served as both the gas storage device and the volume-measuring device for the 100 mL and 1 L digesters. In past experiments, the GEM landfill gas analyzer has been hooked up to the sample port on the 3-way valve and used to take biogas samples. The GEM is equipped with an on-board pump that draws gas samples in from one port, runs them through the internal cells for analysis, and exhausts them through another port. Thus, the GEM served not only to take gas samples for composition analysis, but it also simultaneously “recharged” the graduated cylinders, bringing the barrier solution level back to zero.

Biogas composition measurements (100 mL and 1 L).

When possible, gas composition measurements were taken daily. Recording the volume of the gas produced was possible at any time, as it could be ascertained by looking

at the level of the barrier solution in the graduated cylinder. However, taking gas composition measurements with this particular set-up was dependent on the volume of gas produced, as the GEM requires a certain amount of gas for accurate readings.

Due to the fact that the graduated cylinders served dual functions, as both a measuring device and storage vessel for the biogas, considerations had to be made as to the solubility of the biogas into the barrier solution. As previously discussed in the literature review, the use of a NaCl saturated solution helps to mitigate the dissolution of carbon dioxide into water. This was especially important for the smaller digesters where the biogas had to remain in the cylinder until enough gas was produced to run it through the GEM gas analyzer. For this reason, a 20% NaCl solution was chosen, based on evidence gleaned from the literature about salt build-up at solutions more concentrated than this (Walker et al., 2009).

Volumetric biogas measurement (10 L).

For taking volumetric measurements from the 10 L digesters, one central water column was used to take gas samples from each of the four 10 L digesters. Rather than using this column for both measurement and gas storage, as with the 100 mL and 1 L digesters, 12 L multi-foil gasbags were used for storage, and the water column was used only intermittently to measure biogas volume. Each of the 10 L digesters had its own 12 L gas bag, which was connected to the digester via the same type of 3/16" ID natural rubber tubing as the smaller digesters. Halfway up each gas line was a 1/4" OD plastic barbed tee coupling. One side of this coupling served as a sample port where the intake line for the water column connected. When disconnected from the water column, this sample port was kept closed and sealed with a hose clamp to prevent an influx of ambient air. On the other end of the port, 3/16" ID natural rubber tubing was used to connect into the 12 L gas bags.

In order to take volumetric measurements a clamp was placed on the gas line between the digester and the tee coupling, cutting off the flow of gas from the digester to the gas bag. The connection was then made between the water column and the sample port on the gas line, allowing access to the biogas collected in the gas bag. Once the lines were securely fastened, the three-way valve on top of the water column was opened to the gas bag. Once this valve was opened the barrier solution began to drop in the water column, creating a vacuum, and thus removing the biogas from the gasbag into the water column. A piece of measuring tape was placed on the water column to act as a sight gauge. Once the water level dropped to a certain point, the three-way valve on top of the water column was closed. From here the amount of biogas produced could be ascertained in the same way as with the smaller digesters, by determining the difference between the initial and final water levels in the column.

Biogas composition measurement (10 L).

Once the gas volume had been determined and recorded, the GEM gas analyzer was connected to the other end of the three-way valve on the water column. Once the lines were secure, the valve was switched to the GEM, and the GEM was activated, allowing the biogas to be pumped out of the water column and into the GEM. This simultaneously allowed for biogas composition measurements to be taken, while also “recharging” the water column for the next round of volumetric measurements. Gas from the exhaust on the GEM was directed to a large gas collection bag constructed for this purpose. This was to avoid venting biogas back into the laboratory. At the end of each day, the gas collection bag was taken outdoors and vented. Unfortunately, a safe flaring method could not be built in time for the experiment.

Once biogas production began to ramp up it was necessary to do multiple runs from the same gas bag, emptying the bag and recharging the water column until the bag had

completely emptied. The gas itself was removed from the bag through a vacuum created by the barrier solution (distilled water, no NaCl) falling within the water column (see Figure 4). It was easy to tell when the bag had emptied because the barrier solution would stop falling inside the water column, held aloft by the empty bag. The difference(s) in height between the final and initial barrier solution levels were determined, and combined across multiple runs, if required. These measurements were then recorded in both the laboratory logbook and on an Excel spreadsheet.

Trial 2

Trial 2, like Trial 1, was a 30-day trial, beginning on April 14, 2014. The manure was collected on April 13, 2014. The operating temperature of the digesters was 37.8° C. The primary feedstock was also horse manure, and was collected from Dutch Creek Trails horse farm. However, unlike in Trial 1, an inoculum was used for this trial to help ensure a healthy digester start-up, and to serve as a comparison to Trial 1 for the effects of inoculum on the digestion process. Also, unlike Trial 1, the 100 mL and 1 L digesters utilized the same type of gas measuring device as the 10 L digester from Trial 1. Gas bags were used for all digesters, and the inverted graduated cylinders were done away with entirely. Finally, a two-way aquarium pump was utilized to vent gas and “recharge” the water column, as opposed to using the GEM device as described in Trial 1..

Inoculum preparation.

The digestate from Trial 1 served as the inoculum for Trial 2. Inoculum refers to a sample of manure, or sludge, which has already been acclimated to an anaerobic environment, and which contains a healthy community of methanogens (Drapcho et al., 2008). Horse manure previously has been shown to be a good host for methanogenic bacteria (Kusch et al., 2008). For Trial 2, 20% of the total volume of the final horse manure slurry was comprised of this inoculum.

The inoculum was created by taking the remaining digestate from the digesters in Trial 1 and combining it into a single large vessel. This slurry was then homogenized with the use of a battery-powered drill with a paint-stirring attachment. Once the slurry was thoroughly homogenized, samples were taken and placed into each of the now empty and cleaned/sanitized digesters. A 20% sample of the inoculum was placed into each digester according to its final volume. For example, 2 L of pre-digested horse manure was added to each of the 10 L digesters. One of the reactors for each size was filled with just the inoculum (for instance, 10 L of inoculum was used as the control for the 10 L scale bioreactor), which served as a control for the determination of the inoculum's contribution to biogas and methane production.

Homogenization of horse manure sample.

Following this, as in Trial 1, the horse manure samples were combined, mixed with the proper amount of distilled water to make an 8% slurry, and thoroughly blended together with the use of a drill and paint-stirring attachment. From this homogenized slurry, samples were taken and placed into each of the digesters, bringing the total volumes for the three sizes to 100 mL, 1 L, and 10 L respectively. Each of the digesters was capped with a rubber top as soon as it was filled and then brought into the lab. Samples for pH determination were quickly taken from each of the digesters, and following that, they were each flushed with pure nitrogen, as in Trial 1. Both the headspace and the slurry were flushed with nitrogen. After this the digesters were placed into the hot water baths and connections were made to the gas bags. The digesters were each agitated for roughly 30 seconds after the connections had been made.

Biogas volumetric and composition measurements.

As with Trial 1, the volumetric measurements and gas composition measurements were taken on a daily basis, as close to every 24 hours as was possible. Unlike Trial 1,

however, all digesters utilized the same basic set-up (see Figure 7). Each of the digesters fed into a gasbag: 10 L bags for the 10 L digesters, 3 L for the 1 L digesters, and 1 L for the 100 mL digesters. Halfway up the line to each gas bag was a 1/4" OD tee coupling, one side of which served as the sampling port. In order to take volumetric measurements, the same method was followed as with the 10 L digesters from Trial 1, utilizing the water column.



Figure 7. Bench-scale set-up for 100 mL and 1 L digesters in experimental Trial 2.

One change, however, was made to the system design. After Trial 1 it was determined that it was likely detrimental to the lifetime of the GEM to use it for lifting water within the graduated cylinders and water column. As a solution to this problem, a bi-directional aquarium pump was used in its place. Volumetric measurements were taken in the same basic way, but instead of pumping the biogas directly into the GEM, it first passed

through the aquarium pump and into an extra 12 L multi-layer foil gas bag. Once the digester gas bag had been emptied, and all of the gas moved to another sampling bag via the aquarium pump, the GEM was hooked up to the bag and gas analysis was performed. As in Trial 1, the exhausted gas from the GEM was directed to a large gas collection bag. This bag was taken outdoors each day and vented to the atmosphere. Each of the digesters was agitated each day before measurements were taken, and those measurements were recorded in both the laboratory logbook and on an Excel spreadsheet.

Discussion of Methodology

One important aspect of this project was the redesign of the bench-scale AD system. Throughout the course of this study changes were made to the design of the bench-scale system, specifically the methods for measuring the volume of biogas production. The following sections will highlight some of the changes made to the system over the course of the study.

Preliminary Trial

Observations from the preliminary trial led to three important changes to the system design. The first change involved a switch from 150 mL Erlenmeyer flasks to 250 mL Erlenmeyer flasks for use as the 100 mL digesters. The primary reason for this switch was due to the small amount of headspace, and the conical shape of the flask. This led to a number of clogged digesters during the preliminary trial, wherein the solid portions of the digestate would separate from the liquid and get forced up the digester, creating a “plug” of solid digestate and limiting the release of biogas from within the digester (as could be determined via visual inspection with the presence of biogas bubbles trapped in the liquid portion). This likely had an effect on the measured data. By switching to 250 mL flasks the headspace was expanded, preventing the clogging of digesters, as well as better matching the headspace volume ratio as compared to the other two digester sizes (1 L and 10 L).

Beyond the switch from 150 mL to 250 mL Erlenmeyer flasks for the 100 mL digesters, the gas lines themselves were reorganized. Rather than orienting the gas lines so that they ran below the level of the digesters and the barrier solution trough, they were oriented upwards, above the system components and water/digestate levels (see Figure 6). The reason for this was to prevent clogging of the gas lines with digestate, which occurred during the preliminary trial as well as in previous experiments. By orienting the gas lines above the system, any digestate that might make it to the gas line would fall back via gravity into the digester, preventing clogs.

The preliminary trial also showed that CO₂ will solubilize into water, given time. There were periods of time in the preliminary trial where gas remained in the inverted graduated cylinders for 7-10 days. Once biogas was analyzed for composition, the CO₂ levels were lower than expected. This only reinforced the need for the use of a 20% NaCl barrier solution, which was used in experimental Trial 1, as opposed to purely tap water.

Experimental Trial 1

The results of experimental Trial 1 led to some further conclusions regarding the bench-scale set-up, leading to some changes that have been briefly described. Three important points can be drawn from experimental Trial 1. First, 100 mL may be too small of a size for bench-scale research, given the lab's current capabilities. Secondly, the use of graduated cylinders for biogas collection/measurement may not be the most effective method. And finally, it was determined that using the on-board pump on the GEM could be detrimental to the life of the equipment, so the volume-measuring method was slightly reconfigured.

Issues associated with 100 mL digesters.

In the comparison of average cumulative CH₄ production for Trial 1 (see Figure 14), there was a fair amount of discrepancy between the results across digester sizes,

specifically between the 100 mL digesters and the 1 L and 10 L digesters. One reason for this disparity was that the 100 mL digesters did not produce enough biogas each day for gas composition measurements to be taken, at least given the current laboratory capabilities (specifically, because the GEM requires a certain amount of gas to be available for accurate readings). The inability to take daily composition measurements limited the number of data points available for comparison. Without access to a GC, it was impossible to take daily biogas composition measurements from 100 mL digesters.

Another issue associated with using only 100 mL of digestate was that in all three trials, and in previous experiments performed in the lab, digesters of this size tended to cease biogas production earlier than expected, while also producing less gas than would be expected, especially when compared to the results from larger digesters. One reason for this could simply be that heterogeneity in the substrate resulted in non-uniform substrate composition when working on a scale this small. Such inhomogeneity could have a much more dramatic effect on biogas production in a small-scale reactor than on a larger scale. Likewise, without the use of an inoculum, as in the preliminary trial and experimental Trial 1, there is a chance that sample sizes of only 100 mL could greatly vary in terms of the diversity and amount of healthy microorganisms. One final possibility could be simply related to the loss of gas through leaks in the system. Since all digesters were plumbed using the same materials, with the same basic gas line lengths, leaks in the 100 mL digesters would be much more significant than in the larger digesters as per a percentage of biogas produced.

Issues associated with inverted graduated cylinders.

The 100 mL and 1 L digesters in Trial 1 utilized inverted graduated cylinders for volumetric measurements, and even though a 20% NaCl barrier solution was used, there was likely still some CO₂ lost to solution as the gas remained in the headspace. This would

be especially true towards the end of the trial as less biogas was being produced each day, resulting in longer retention times within the inverted cylinder.

Experimentation with the GEM in past digestion experiments has shown that it is important to have a volume of at least 50-100 mL of gas for the GEM to be able to take an accurate reading. With volumes below this amount, the gas composition analysis is skewed by what we hypothesize to be a mixing of the biogas being sampled and atmospheric air in the intake line and on the internal cells. Finally, error/noise in the gas composition measurements from the GEM have been noted in numerous bench-scale trials using the inverted cylinders. No determination has been made as to the root cause, be it leaks in the lines, loss of gas to solution, collection of water vapor from the cylinders, or other factors. These errors, specifically an over-estimation in the measurement of balance gases (those gases not CO₂, CH₄, or O₂) were not detected when composition measurements were taken from the gas bags with the 10 L digesters.

After Trial 1 it was determined that it was likely detrimental to the lifetime of the GEM to use it for lifting water within the graduated cylinders and water column. While this was convenient, allowing for analysis to take place at the same time as recharging the cylinders, it was putting unnecessary stress on the GEM's internal pump. As a solution to this problem, a bi-directional aquarium pump was used in its place. Volumetric measurements were taken the same basic way, but instead of pumping the biogas directly into the GEM, it first passed through the aquarium pump, and then into a larger, empty gas bag.

This method was chosen for two reasons. First, it relieved the stress on the GEM's internal pump. Since the GEM is primarily an analysis device, not a pump, this is both practical and suggested. Secondly, it allowed for the biogas produced to be mixed and measured all at once. In Trial 1, at least with the 10 L digesters, biogas composition analysis

was somewhat skewed due to having to recharge the water column multiple times. This meant that the GEM was not taking gas samples and running analysis all at once, but in bits and pieces. Using the aquarium pump and an extra gas bag ensured that the GEM could then sample the entire volume of gas at once, with no interruptions. As in Trial 1, the exhausted gas from the GEM was directed to a large gas collection bag. This bag was taken outdoors each day and vented to the atmosphere.

Exploded digesters and solution to problem.

During the third week of Trial 1, two of the 10 L digesters burst (see Figure 8). As biogas built up within the digester, and the solid/liquid separation occurred, some digestate was forced up through the gas lines and into the gas collection bags. This prevented the gas collection bag from relieving pressure in the digester, and as a result the rubber stopper blew off. Following this event, steps were taken to prevent this in the remaining 10 L digesters, and for future experiments (including Trial 2 of this study).



Figure 8. 10 L digesters #1c and #4c burst during the third week of Trial 1.

150 mL Erlenmeyer flasks were plumbed in series into the gas line (see Figure 9). This allowed the flask to serve as a “pressure relief/condenser,” ensuring that any digestate that made it into the gas lines would fall to the bottom of the flask, thus relieving pressure and preventing digestate from reaching the gas collection bags. This solution proved valuable for the remainder of the trial, as some digestate did overflow from the remaining digesters into the flasks, but with no loss of digestate or gas.



Figure 9. 150 mL Erlenmeyer flasks used as pressure breaks to prevent clogged lines.

Experimental Trial 2

No major changes were made to the bench-scale set-up as a result of Trial 2, though 100 mL still appears to be too small of a sample size for bench-scale studies, as shown in the data from Trial 2 (next chapter). However, one important note should be made regarding biogas analysis and the use of a gas chromatograph (GC).

Originally, it was hoped that samples could be taken and run through a GC over the course of each of the trials. However, there was trouble with the GC from the start. During Trial 1 schedules were in conflict too often, and there were some problems with the GC, particularly involving the random nature of the output analyses. It seemed that every

fourth or fifth run of the same gas of the same amount would produce drastically different results. Due to this, it was decided that samples would instead be taken during Trial 2, while the problem were resolved during the remainder of the first trial.

Issues with the gas chromatograph.

During Trial 2 it was soon discovered that there were yet more complications with the GC, as well as with the gas sampling method. Originally, the intent was to use a 10 mL gas-tight syringe to take biogas samples from the gas collection bags and transfer them to 5 mL serum vials with rubber septum tops. This involved taking four to five 10 mL samples and flushing out each of the vials with biogas to ensure that the ambient air within the vial was removed. The reason for this was due to the lack of a GC on site, and the need to transport samples safely across campus without contaminating the biogas. Unfortunately, this method was rather unsuccessful, even though different numbers of “flushes” were tried, up to six 10 mL flushes per 5 mL vial. Regardless of the number of flushes, the data from the GC showed contamination of both O₂ and N₂. At this point, efforts to take samples of the biogas from Trial 2 were scrapped.

Issues with GEM landfill gas analyzer.

However, there was still question as to the results of the composition analyses from the GEM. It was noted that there had been “noise” in the data in the past, and that part of this was suspected to be due to the system set-up and the use of graduated cylinders. Data collected from the gas bags helped strengthen this hypothesis, given the cleaner data (little to no detection of O₂ or N₂ in samples). With this in mind, a five-point calibration curve was finally made for the GC, specifically for the detection of CH₄, as at this point efforts to find the CO₂ peak were dropped. Gas collection bags were taken to the chemistry department and the samples of the same biogas were analyzed by the GEM and GC side-by-side. The results of the GC confirmed those of the GEM, and although CO₂ data could not be compared

the CH₄ from both devices measured within $\pm 3-5\%$ of each other. The GEM itself measures to an accuracy of $\pm 3\%$. These results further confirmed the notion that the system set-up was a major part of the problem involving skewed data from the GEM. Results from the GC/GEM comparison are included in the research findings section.

Chapter 4: Research Findings

Feedstock Characterization

Before the start of the experimental trials, samples of horse manure were analyzed for %TS, %M, %VS, and the C/N ratio. Fourteen total samples were used for this characterization. The results obtained from the feedstock characterization analysis are presented in Table 2 and compared with values from the literature. As can be seen, these results are quite consistent with the literature. For example, all of the studies, including this one, found the %VS to be in the 80-80% range.

Table 2. Comparison of Horse Manure Feedstock Characteristics Across Studies

Source	Feedstock	%TS	%VS	C/N
This study n=14	Horse manure from field	(22.8-27.7) <i>M</i> = 25.5	(69.6-96.9) <i>M</i> = 79.5	Unknown
Wartell, et al. (2012)	Horse manure from stables, no bedding	(20-42) <i>M</i> = 37	(76-92) <i>M</i> = 83.7	
Kalia & Singh (1998)	Unknown	22.6	87	35
Mönch- Tegeder, et al. (2013)	Horse manure from field	20-27	18-24 ^a	23-37
Kusch et al. (2008)	Horse manure from stable, with bedding	32-58	85-89	
Leckie et al. (1981)	Unknown	16	87	35

a. Mönch-Tegeder, et al. (2013) based %VS on wet mass of feedstock, rather than the more conventional calculation based on dry mass (as noted in equation 2).

Preliminary and Experimental Trials

Provided data include the value \pm the 95% confidence interval (CI.) Differences between trials were analyzed using ANOVA in order to determine if the means between treatments (reactor sizes) were significantly different. Microsoft Excel was used for most data analysis. The ANOVA analyses were performed in the R software package.

Preliminary Trial

Table 3 highlights the pre- and post-trial pH of the seven 100 mL digesters for the preliminary trial (G1-G7). The pH remained relatively constant for the preliminary trial, lowering only slightly over the course of the reaction. The gas production results obtained from the preliminary trial are presented in Figure 10. As can be seen in the graph, there is a fair amount of inconsistency between digesters, although each used the same volume and type of feedstock. Possible reasons for this will be addressed in the Discussion and Conclusions section.

Table 3. *Pre- and Post-Trial pH for the Digesters in the Preliminary Trial (100 mL)*

	100 mL						
Preliminary Trial	G1	G2	G3	G4	G5	G6	G7
Pre-trial pH	7.36	7.36	7.4	7.37	7.64	7.69	7.64
Post-trial pH	7.13	7.18	7.27	7.21	7.19	7.27	7.12
Difference	0.23	0.18	0.13	0.16	0.45	0.42	0.52

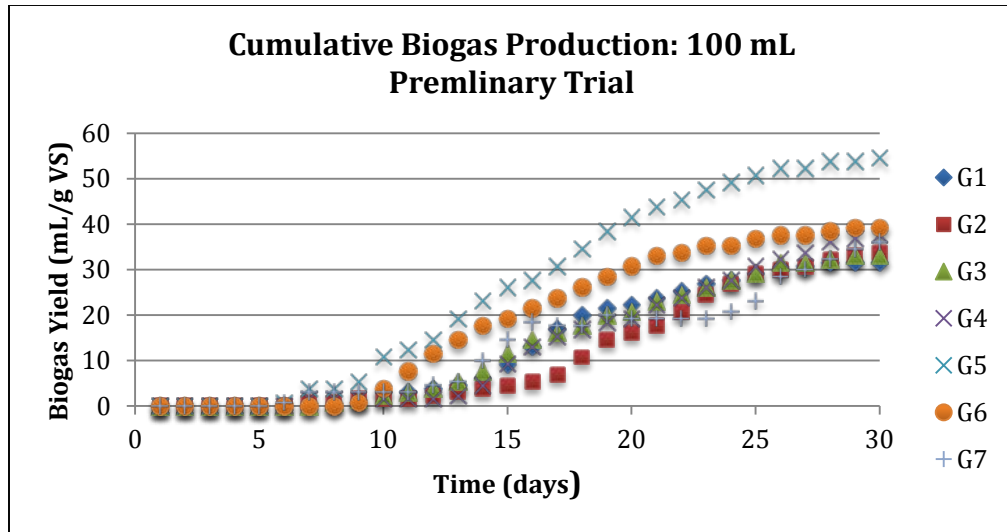


Figure 10. Comparison of cumulative biogas yields for 100mL digesters (preliminary trial).

Figure 11 shows the average cumulative biogas production for the digesters in the preliminary trial. The average cumulative biogas production was 39.12 ± 7.02 mL/g VS.

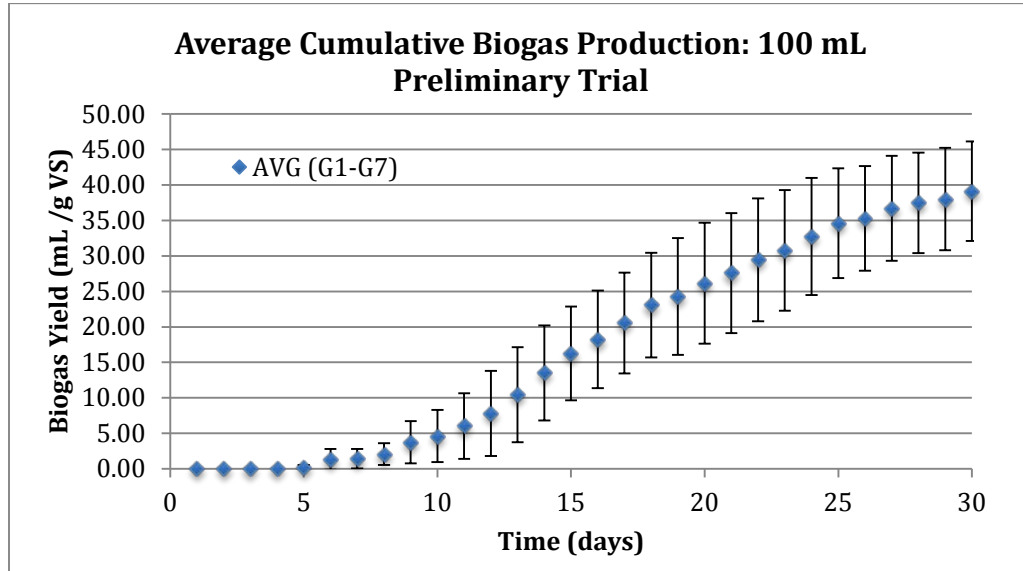


Figure 11. Average cumulative biogas production for 100mL digesters (preliminary trial).

Experimental Trial 1

Experimental Trial 1 consisted of 12 digesters, four each of the three sizes, performing in batch-mode for 30 days, with an operating temperature of 37.8° C. Each of the digesters was filled with fresh horse manure. On day 25 of the trial, two of the 10 L digesters burst, the pressure inside the digesters having become too great and liquid digestate having made its way up through the gas lines and into the gas collection bags. This resulted in the loss of data for digesters #1c & #4c, for days 25-30 of the trial. Table 4 presents the pre and post-trial pH for each of the digesters. The pH dropped in all digesters, with a tendency towards becoming more neutral.

Table 4. Comparison of Pre- and Post-Trial pH for all Digesters in Trial 1.

	100 mL				1 L				10 L			
TRIAL #1	#1a	#2a	#3a	#4a	#1b	#2b	#3b	#4b	#1c	#2c	#3c	#4c
Pre-Trial pH	7.25	7.26	7.25	7.27	7.23	7.28	7.24	7.3	7.23	7.26	7.31	7.24
Post-Trial pH	7.19	7.2	7.16	7.16	7.18	7.2	7.21	7.16	7.14	7.2	7.23	7.15
Difference	0.06	0.06	0.09	0.11	0.05	0.08	0.03	0.14	0.09	0.06	0.08	0.09

Figure 12 provides a comparison of the average cumulative biogas production from the 100 mL digesters from the preliminary trial and from experimental Trial 1. The four digesters in experimental Trial 1 averaged a cumulative biogas yield of 52.01 ± 9.06 mL/g VS, while the seven digesters from the preliminary trial averaged 39.12 ± 7.02 mL/g VS.

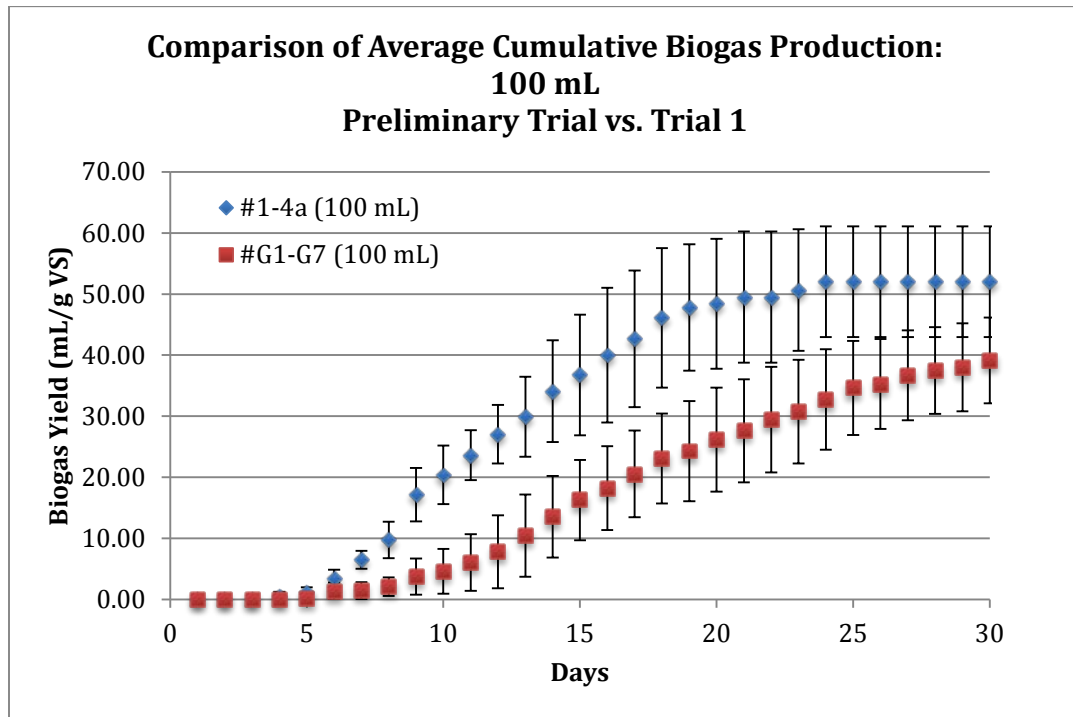


Figure 12. Average cumulative biogas production, Trial 1 and preliminary trial.

Figure 13 highlights the average cumulative biogas production for the three different digester sizes in experimental Trial 1. The average cumulative biogas yields for the digesters were: 52.01 ± 9.06 (100 mL), 103.39 ± 9.99 (1 L), and 119.79 ± 2.38 (10 L) mL/g VS.

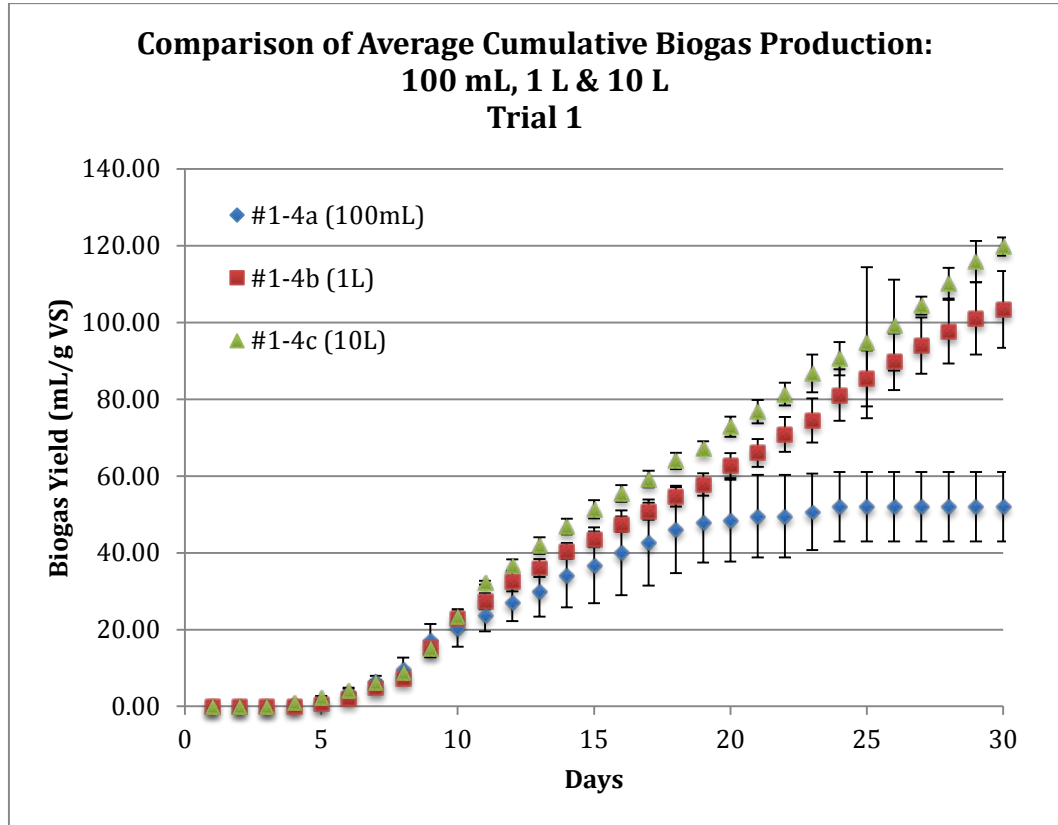


Figure 13. Comparison of average cumulative biogas yield from all digesters in Trial 1.

Figure 14 highlights the average cumulative CH₄ production for the digesters from Trial 1. The 100 mL, 1 L, and 10 L digesters produced a cumulative average of 4.59 ± 1.48, 33.56 ± 5.51, and 54.50 ± 5.30 mL/g VS of CH₄, respectively.

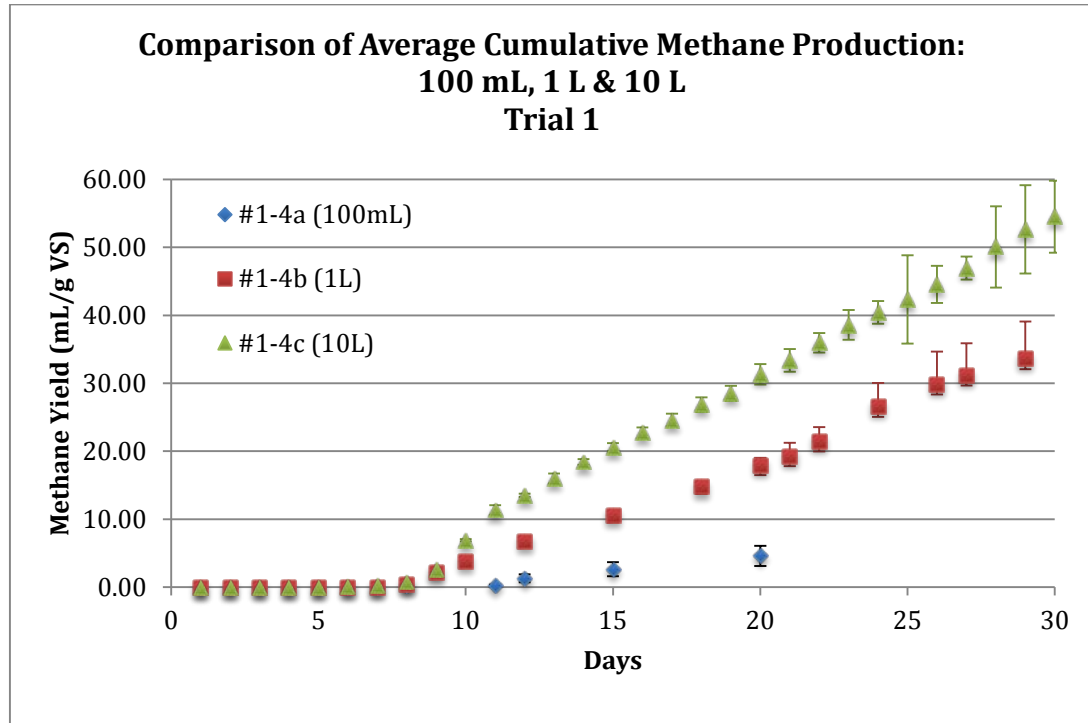


Figure 14. Comparison of average cumulative biogas yield from all digesters in Trial 1.

Figure 15 highlights the average daily biogas production for the 100 mL, 1 L, and 10 L digesters from experimental Trial 1. Included are trend lines fit with six-factor polynomials.

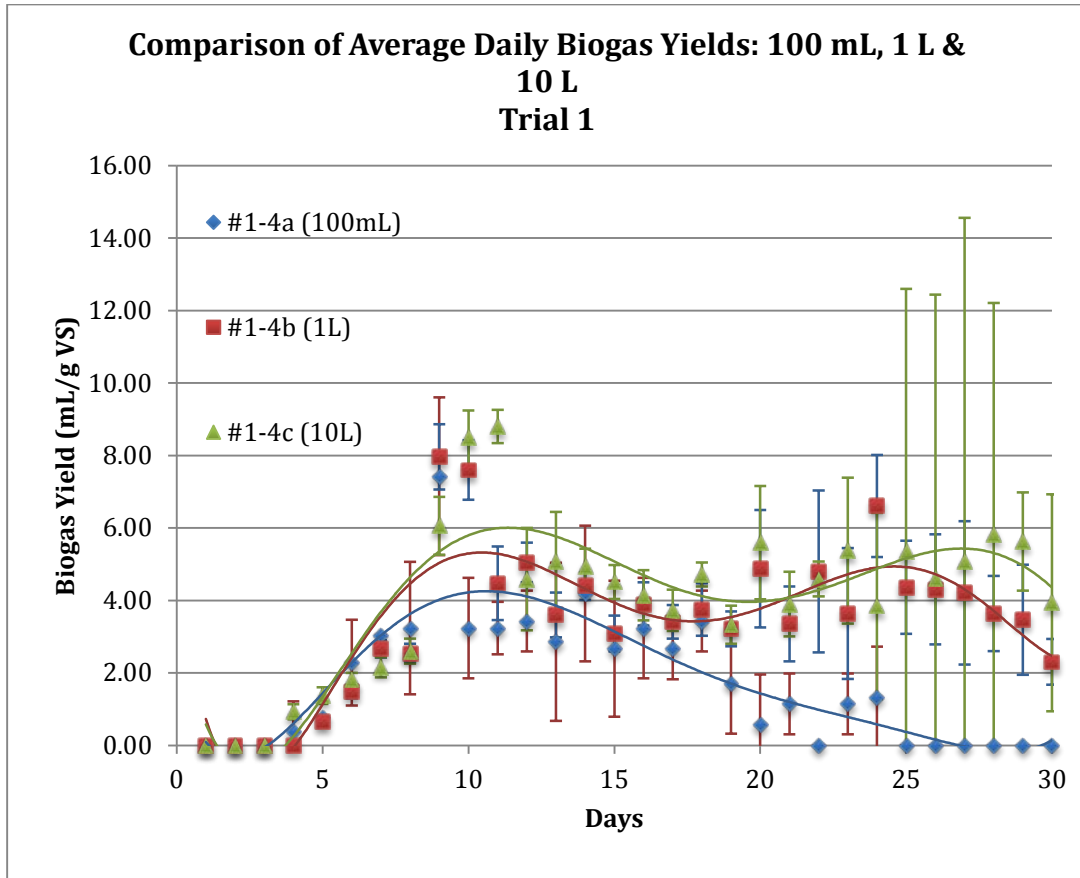


Figure 15. Comparison of average daily biogas yields, all digesters, Trial 1.

Table 5 highlights the pre- and post-trial volatile solids, specifically the %VS of the digestate before and after the trial.

Table 5. Comparison of %VS and Volatile Solids Destruction for Experimental Trial 1.

	100 mL				1 L				10 L			
TRIAL #1	#1a	#2a	#3a	#4a	#1b	#2b	#3b	#4b	#1c	#2c	#3c	#4c
%VS (pre)	79.5	79.5	79.5	79.5	79.5	79.5	79.5	79.5	79.5	79.5	79.5	79.5
%VS (post)	53.2	51.7	52.4	51.5	51.9	52.2	53.7	51.6	51.9	56.3	53.5	51.1
%VS destroyed	33	34.9	34	35.2	34.7	33.9	32.4	35	34.7	29.1	32.7	35.7

Experimental Trial 2

Experimental Trial 2 consisted of 12 total digesters, four each of the three different scales. Three of the digesters were filled with fresh horse manure, homogenized, and mixed to an 8% slurry. 20% of the total volume of these nine digesters consisted of inoculum, a mix of homogenized, pre-digested horse manure from the first trial. One digester in each of the three scales was filled with the inoculum only (no fresh manure), to the same volumes as the other digesters (i.e., 10 L of inoculum was used in the 10 L control). They served as controls in order to determine the contribution of the inoculum to the total biogas production.

Table 6 provides the pre and post-trial pH for the digesters in Trial 2. The pH was slightly more acidic in this trial than in the preliminary trial and in experimental Trial 1.

Table 6. Comparison of Pre/Post-Trial pH's in All Digesters, Trial 2 (a=100 mL, b=1 L, c=10 L).

	100 mL				1 L				10 L			
TRIAL #2	#1a	#2a	#3a	control	#1b	#2b	#3b	control	#1c	#2c	#3c	control
Pre-Trial pH	6.68	6.71	6.65	7.16	6.57	6.72	6.63	7.15	6.59	6.71	6.66	7.16
Post-Trial pH	6.93	6.98	6.94	7.13	6.93	6.97	6.93	7.14	6.99	6.91	7.02	7.16
Difference	-0.25	-0.27	-0.29	0.03	-0.36	-0.25	-0.3	0.01	-0.4	-0.2	-0.36	0

Figure 16 provides a comparison of the average cumulative biogas production for the three digester scales. Only the three inoculated digesters from each group were averaged, and the control digesters were examined separately. On average, the 100 mL digesters produced a total of 231.6 ± 43.48 , the 1 L digesters 298.64 ± 59.40 , and the 10 L digesters 258.49 ± 5.54 mL/g VS of biogas.

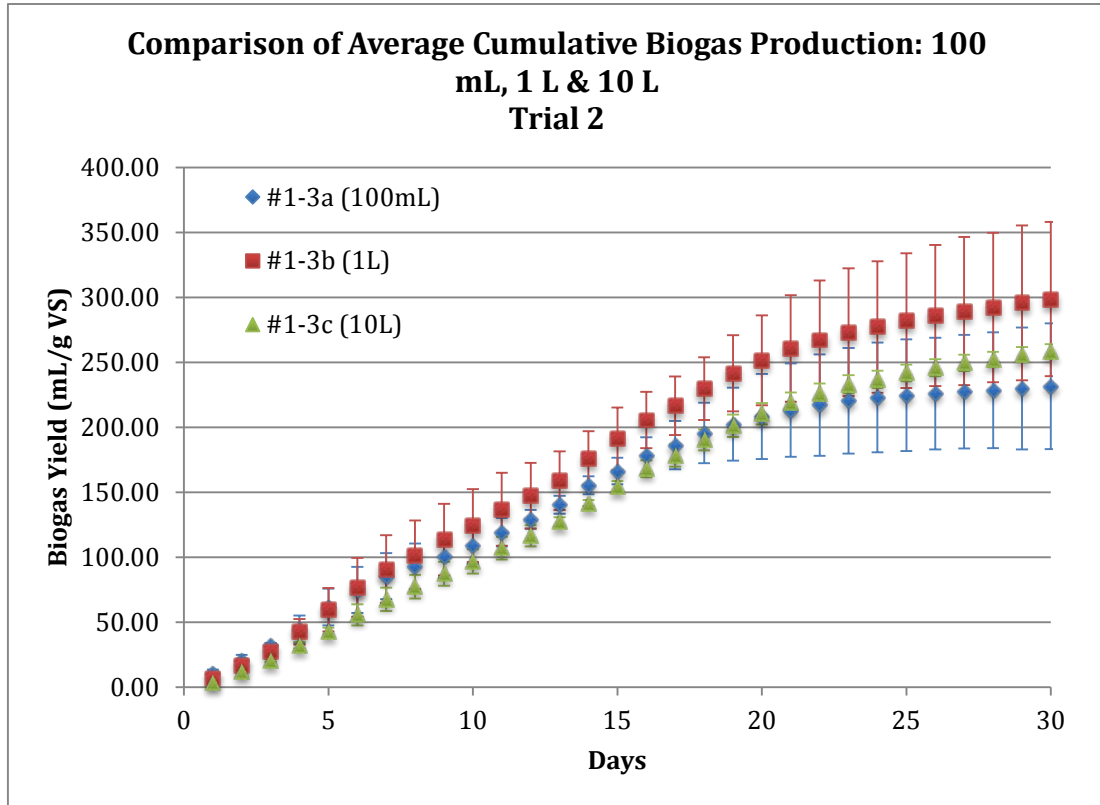


Figure 16. Comparison of average cumulative biogas yields, all non-control digesters.

Figure 17 shows the comparison in biogas production between the three control digesters, one each of the three different sizes. The 100 mL digester produced a total of 105.99 mL/g VS, the 1L 137.68 mL/g VS, and the 10 L 148.62 mL/g VS. The 10 L digester produced 7.53% more biogas than the 1 L and 41.2% more biogas than the 100 mL. The 1 L digester produced 31.3% more biogas than the 100 mL digester.

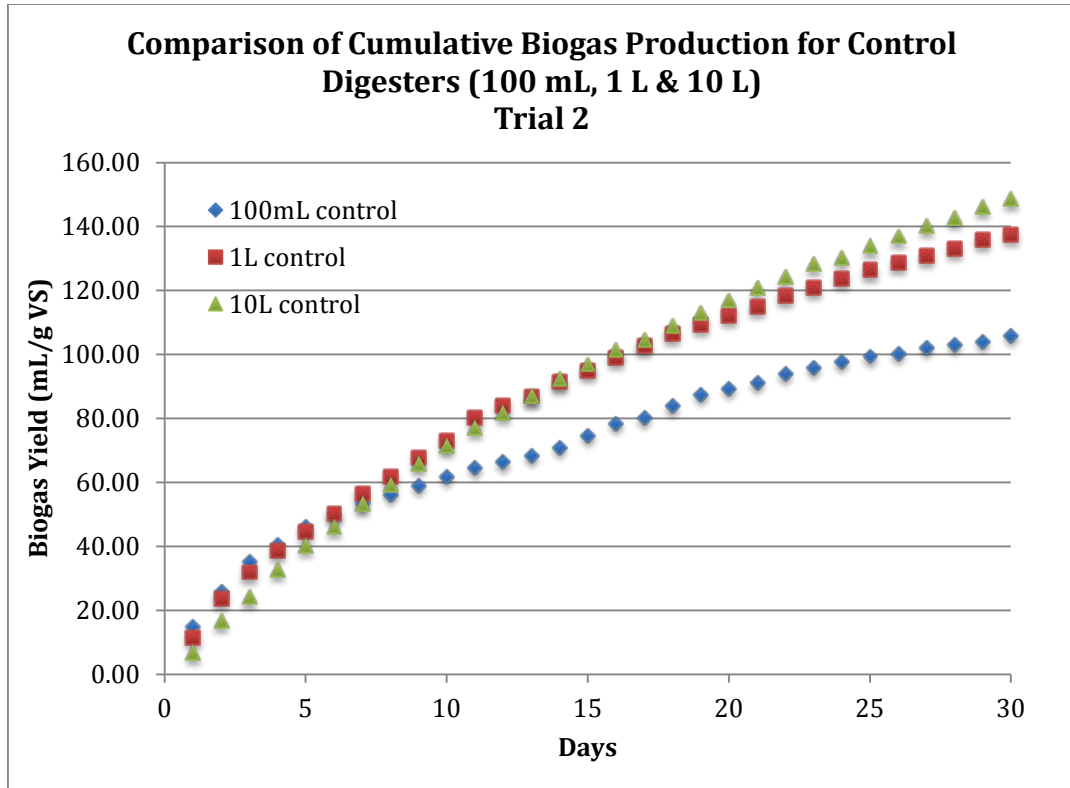


Figure 17. Comparison of average cumulative biogas yields, control digesters.

Figure 18 shows a comparison of the average cumulative CH₄ production from the 100 mL, 1 L, & 10 L digesters from experimental Trial 2. The 100 mL digesters produced, on average, 89.26 ± 20.91, the 1 L digesters produced 145.18 ± 21.14, and the 10 L digesters produced 131.41 ± 5.61 mL/g VS.

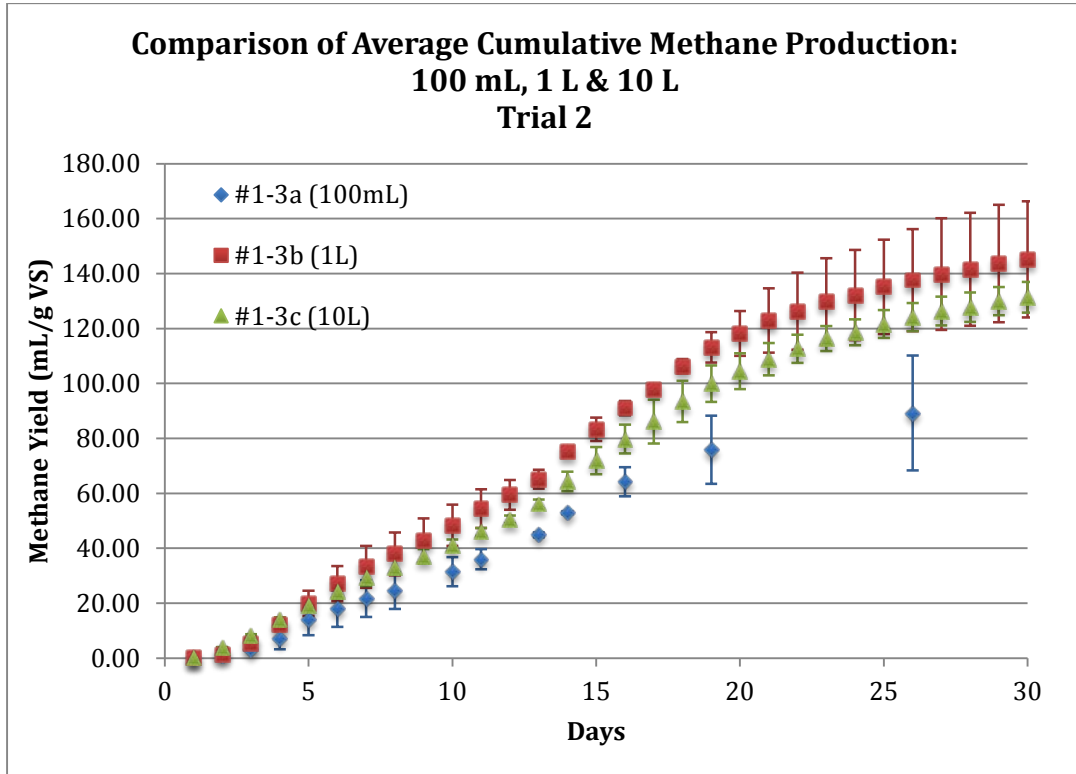


Figure 18. Comparison of average cumulative CH₄ production, all non-control digesters.

Figure 19 highlights the comparison of cumulative CH₄ production in the three control digesters. The 100 mL, 1 L, and 10 L digesters produced a total of 30.43, 53.36, and 66.71 mL/g VS of CH₄, respectively.

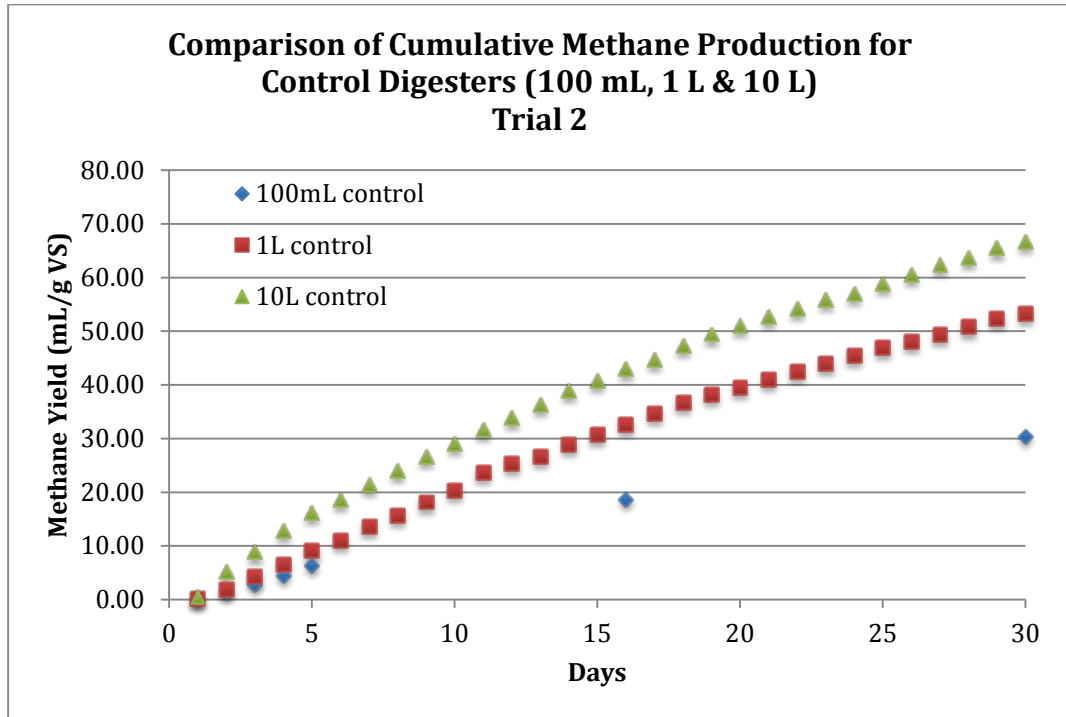


Figure 19. Comparison of average cumulative CH₄ production, control digesters (Trial 2).

Figure 20 provides a comparison of the average daily biogas production for the 100 mL, 1 L, and 10 L digesters in Trial 2, not including the three controls. All three trend lines are six-factor polynomial, and R-squared values for each are included.

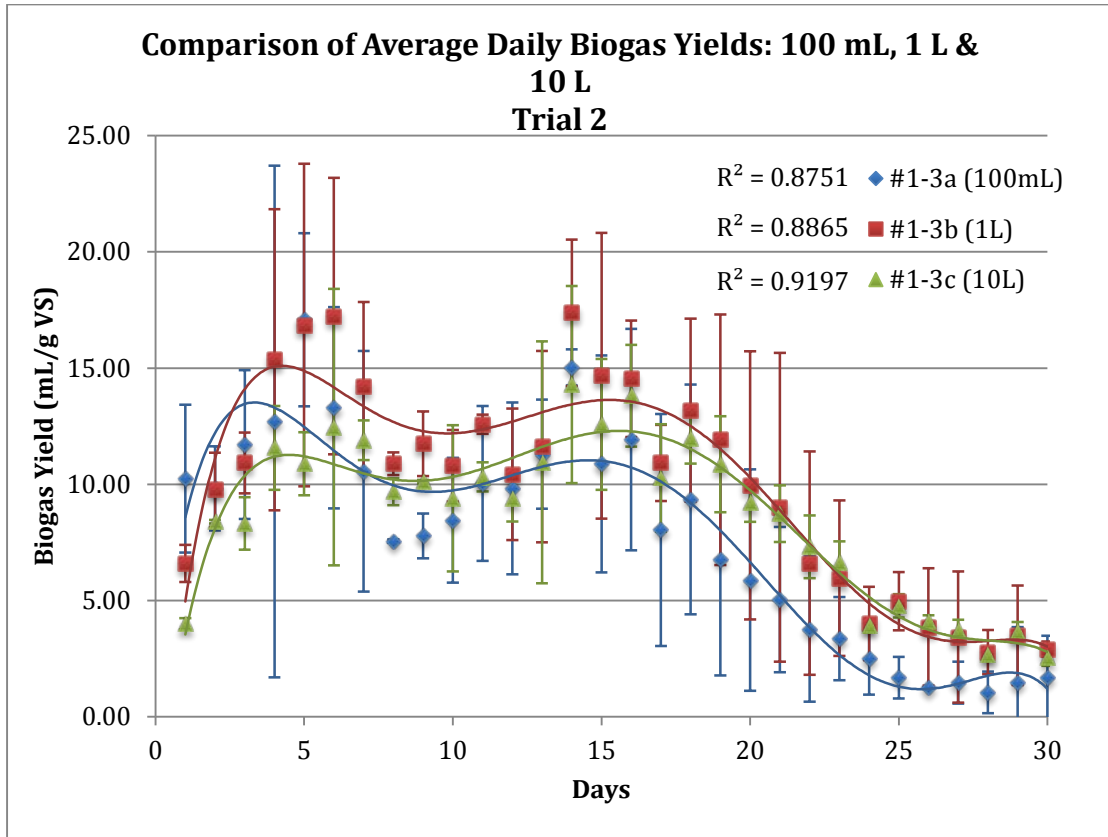


Figure 20. Comparison of average daily biogas yields, all non-control digesters (Trial 2).

Figure 21 highlights the daily biogas production for the three control digesters (one of each size). A trend line was fit using six-factor polynomial equation, and R-squared numbers for each are given.

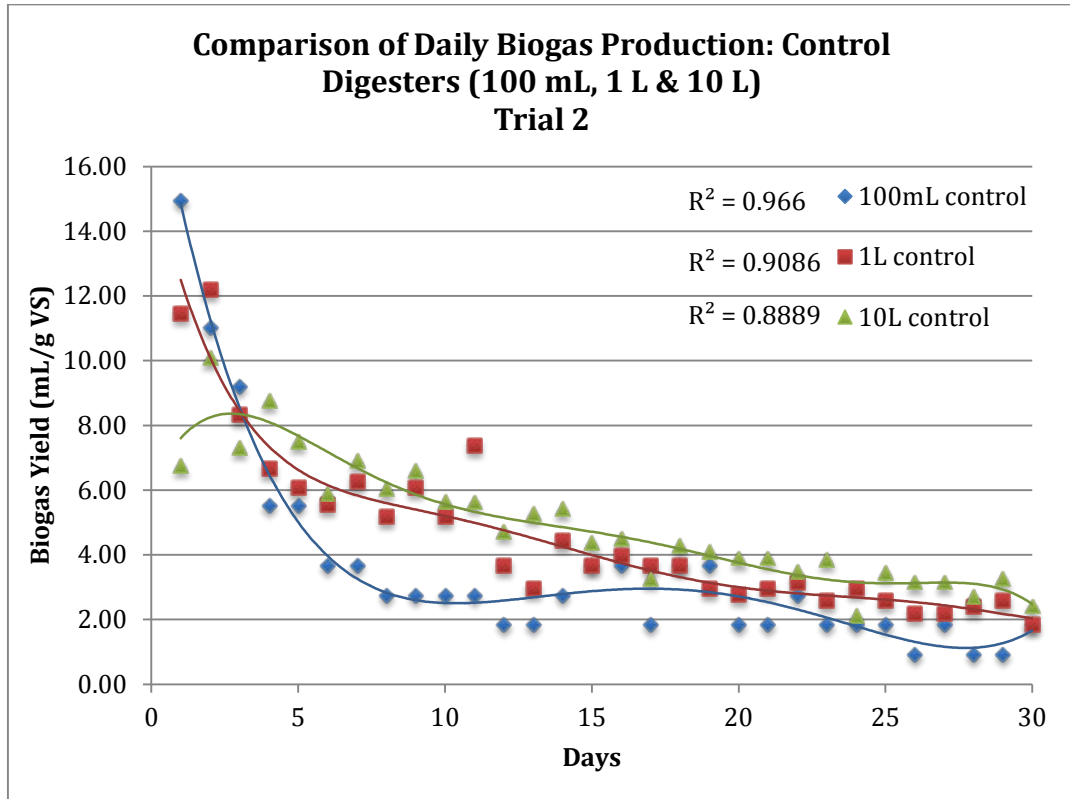


Figure 21. Comparison of average daily biogas yield for three control digesters (Trial 2).

Table 7 shows the pre and post %VS for the digesters in Trial 2. All of the #4 digesters (a, b & c) were control digesters, which is why their pre-digestion %VS figures are lower.

Table 7. Comparison of Pre/Post %VS for all Digesters in Trial 2.

TRIAL #2	100 mL				1 L				10 L			
	#1a	#2a	#3a	control	#1b	#2b	#3b	control	#1c	#2c	#3c	control
%VS (pre)	81.6	82.3	81.9	52.7	79.4	82.8	83.3	52.3	80.4	80.2	79.3	53.1
%VS (post)	30.8	28.6	29.4	26.3	30.8	30.8	35.7	23.5	32	34.5	31.4	23.3
%VS Destroyed	62.3	65.2	64.1	50.1	61.2	62.8	57.1	55.1	60.2	57	60.4	56.1

Chapter 5: Discussion and Conclusions

The ultimate goal of this research endeavor was to begin the process of determining how accurately the results of anaerobic digestion can be scaled from bench-scale digesters to large-scale biogas plants. Specifically, are the results from bench-scale anaerobic digestion research transferable to larger scales? Through comparing three differently sized digesters (100 mL, 1 L, and 10 L) over the course of two experimental trials, efforts were made to answer this question. Specifically, this project set out to understand if there is a strong correspondence between cumulative and daily biogas production across digester sizes, and whether there are any significant differences among digester sizes in regards to the methane content of the biogas, the destruction of volatile solids, or the pH of the digestate. With this information, is it possible to predict biogas production on a larger scale through the use of bench-scale biodigesters?

Scalability

Cumulative Biogas Production

There was a relatively high level of correspondence in the cumulative biogas production both within and between digester sizes within each trial performed for this study. However, there remains considerable run to run variability between trials. Because Trial 2 utilized a 20% inoculum, and a slightly different feedstock, comparing the two trials directly is somewhat problematic. As shown in Figure 21, Trial 1 experienced a week-long induction period prior to the onset of biogas production. In contrast, Trial 2 began producing biogas shortly after beginning the trial. This difference is attributable to the need to establish a robust microbial community in Trial 1, whereas these microbes were

provided by the inoculum in Trial 2. Consequently, direct comparison of the cumulative biogas yield in these two trials is problematic, since Trial 2 essentially operated for an extra week in the presence of an established microbial community. However, the trials can still be compared in terms of maximum cumulative and daily biogas/methane production (see Figure 22 and 23). It should be noted that the inoculum contribution was removed for the production values for Trial 2 for the purposes of comparison with Trial 1. As seen in Figure 22, the maximum daily rate of biogas production (approximately 16 mL/gVS/d) is considerably greater than that in Trial 1 (approximately 9 mL/gVS/d). This suggests that digesters in Trial 2 worked better than those in Trial 1. The cause for this discrepancy is not fully clear, but could be due to differences in feedstock composition or the improvements made to the experimental design following Trial 1. This result hints at considerable run to run variability that should be further explored before extrapolating laboratory-scale results to larger digesters. The preliminary trial can be compared to experimental Trial 1 because the feedstocks were the same, but only with the 100 mL digesters, as this was the only digester size used in the preliminary trial. Since neither of these trials utilized an inoculum, comparisons can be made in the lag times in digester start-up.

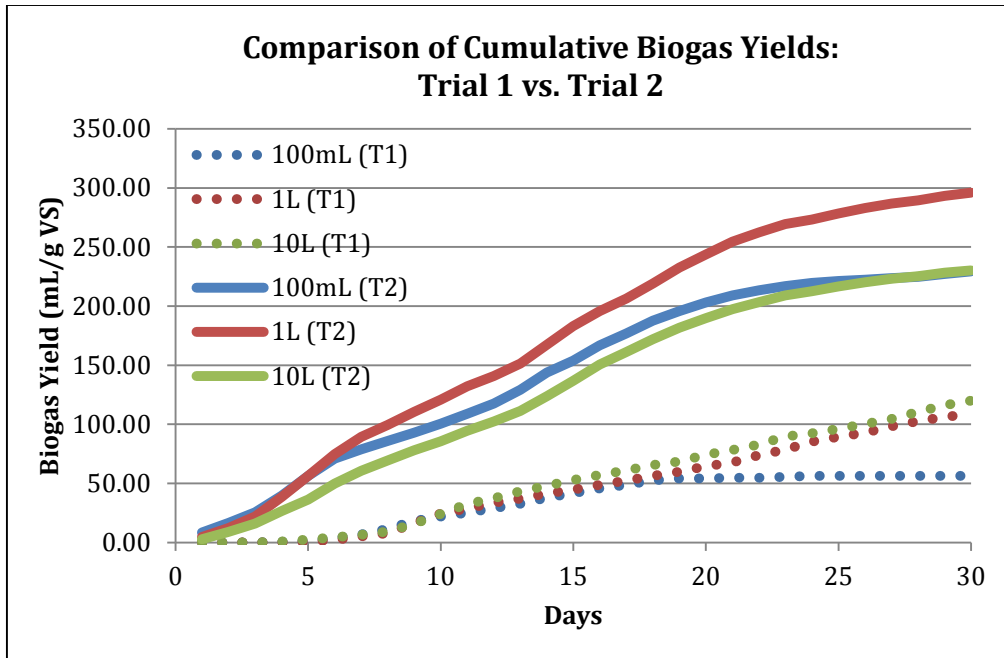


Figure 22. Comparison of cumulative biogas yields, Trial 1 and Trial 2.

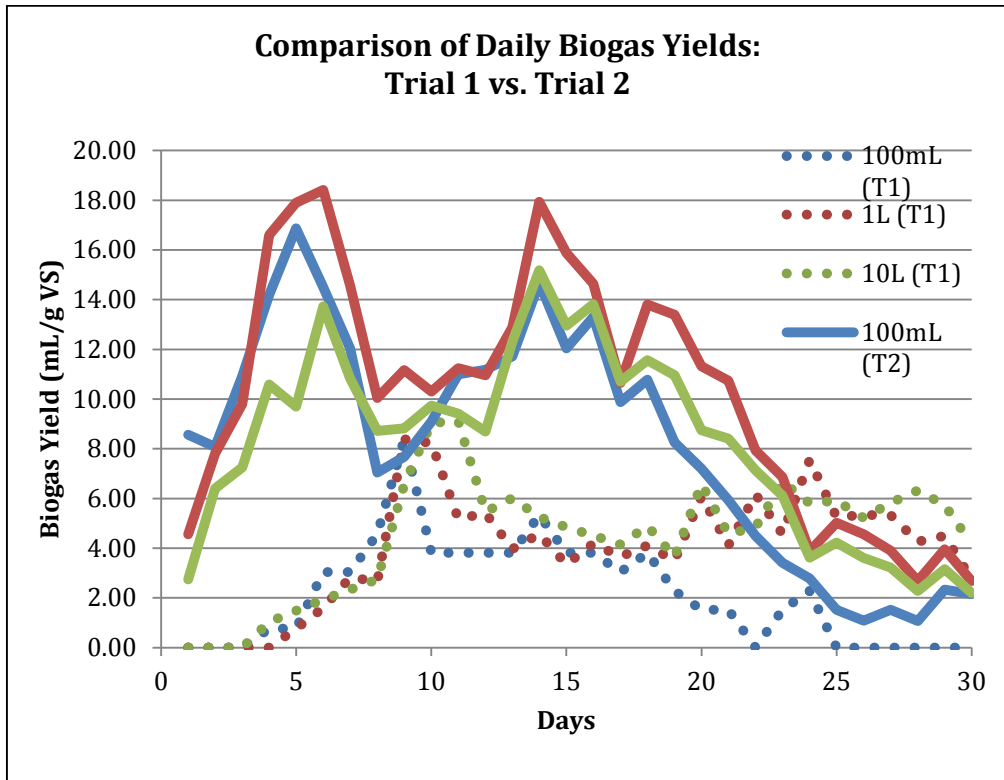


Figure 23. Comparison of maximum daily biogas yields, Trial 1 and Trial 2.

Preliminary trial.

Comparing the 100 mL digesters in the preliminary trial to those in Trial 1, which used the same feedstock and no inoculum, one can still find some variability (see Figure 12). There are a number of reasons that might account for these inconsistencies. To begin with, undergraduate students working in pairs made the slurry preparations rather than myself. There was also no large, primary slurry used for filling individual digesters (as was done in the experimental trials). Rather, each group took a sample of manure and created their own slurry sized for the digester. This could have resulted in differences between substrate compositions, which in turn could have an impact on biogas production. Furthermore, the digesters were built from 150 mL Erlenmeyer flasks, which may be too small of a container for this volume of digestate. This is especially true for horse manure, which unlike many other feedstocks that have been examined in the lab tends to separate out into solid and liquid levels, an effect also reported by Kalia & Singh (1998).

For a period of time during the preliminary trial there was concern that the digestion process would not begin, because there was no biogas production for the first 7-9 days. However, this was somewhat in agreement with the literature (and the results from Trial 1), where digestion of horse manure alone did not typically start until at least day four or five (Wartell et al., 2012). Possible reasons for this extended lag time in the preliminary trial could be due to not creating a fully homogenized slurry to begin with. Another factor could be that only the headspace of the digesters was flushed with N₂, not the slurries themselves, meaning that there could have been higher levels of O₂ within the digester than is desirable.

Trial 1.

Within Trial 1, there appeared to be a high level of correspondence between the 1 L and 10 L digesters (see Figure 13), while the 100 mL digesters clearly produced

significantly less biogas. Up until days 15-17 the three scales corresponded in terms of biogas production; however, after this point, the 100 mL digesters started to slow and eventually ceased production entirely, while the 1 L and 10 L digesters remained relatively consistent. Utilizing a one-way Analysis of Variance (ANOVA) with blocking for time, significant difference among all of the digesters was determined. While not as great as the other size comparisons, the 1 L and 10 L digesters were still significantly different in terms of their biogas production ($P=0.010$).

Reasons for the difference in the early plateauing behavior of the 100 mL digesters in Trial 1 could be the use of inverted graduated cylinders. One concern regarding the use of the inverted cylinders was the negative pressure placed on the inside of the digester as a result of having to support a column of barrier solution. This pressure may have had an inhibitory effect on the kinetics of the digesters within both the preliminary trial and experimental Trial 1. It was observed during Trial 1 that when the inverted cylinders were purged of gas and returned to the zero-mark the meniscus would drop down between 5-10 mL on the cylinders for the 100 mL digesters, and 10-30 mL on the cylinders for the 1 L digesters. This drop of barrier solution level indicated a clear pressure effect on the inside of the digester. An experiment comparing a 100 mL digester using an inverted cylinder to one using a gas bag for collection at ambient pressure would help shed light on this.

Trial 2.

All of the digesters within Trial 2 showed a high rate of correspondence in terms of total biogas production and their respective production curves (see Figure 16). Though the error bars representing the 95% confidence interval for each scale seem to overlap at every point over the 30-day trial, showing no significant differences in cumulative biogas production for the three scales, this is in fact not the case. Using a one-way ANOVA with blocking (to remove time as a factor) an analysis of the three control digesters yields

statistically significant differences amongst each of them. The difference was smallest (though still significant) between the 10 L digesters and the 100 mL digesters ($P=0.012$).

Given the TED department's current capabilities and equipment, the use of 100 mL digesters may be too small to provide data accurate enough for the prediction of biogas production at larger scales, even though the 100 mL digesters in Trial 2 did show a stronger correspondence with the larger scales in terms of specifically biogas production (unlike the 100 mL digesters in Trial 1.) While this proves their effectiveness in predicting biogas production, this process volume (100 mL) is still too small to take daily biogas composition measurements. For this reason, a minimum digester size of 200-250 mL is suggested for future biogas experiments, at least until a GC is installed on site, or a bench-scale biogas production unit is purchased with the capabilities of measuring low gas flow rates and methane production.

Daily Biogas Yield

Interestingly, the digesters across scales, in both experimental trials, tended towards a bi-phasic production curves with two peaks (see Figure 15 for Trial 1, and Figure 20 for Trial 2). This is somewhat unusual given that most daily production curves involve a steady ramp-up in production, followed by a peak and then a steady decline before production ceases entirely, essentially a bell curve. In the case of the digesters for this experiment there were two peaks in production. During Trial 1, there was a production peak around day 10 and again around day 25, though only for the 1 L and 10 L digesters, the 100 mL digesters from Trial 1 followed the typical production curve for biogas experiments. In Trial 2, all digesters experienced two production peaks, first around day four and again around day 15.

A review of the literature has thus far yielded no comparative production curves where there are two distinct peaks. The first peak in each of the trials does follow the

general trend for biogas production, highlighting the change in microbial communities from the acid-formers to the methane-formers. It is the second peak in each of the trials that is apart from the norm. Further research is recommended to see if this bi-phasic curve was just a fluke, or if there are other explanations.

Biogas Composition

Comparing the digesters based on biogas composition proves more troublesome and prone to error than comparing them on biogas production alone. This is especially true for experimental Trial 1, wherein inverted graduated cylinders were used for storing and measuring gas volume in the 100 mL and 1 L digesters, as opposed to gas collection bags.

Trial 1.

Trial 1 shows considerable variability in the rate of production of CH₄ between the different digester sizes (see Figure 14). In terms of total production, the 10 L digesters appeared to have produced more methane than the 1 L and far more than the 100 mL digesters. Two primary reasons may account for this significant difference in biogas composition, and both are related to the use of inverted graduated cylinders. As mentioned in the literature review and methodology discussion in Chapter Three, the use of inverted graduated cylinders and a barrier solution can lead to the loss of CO₂ to the barrier solution.

Even though a 25% NaCl solution was used, there were still periods of time where gas remained in the headspace of the inverted cylinders for the 100 mL digesters for five days or more, and during this time up to 20% or more of the CO₂ could have been lost to solution. This issue is primarily a result of the combined use of 100 mL digesters and the GEM gas analyzer, wherein the 100 mL digesters did not produced enough gas on any given day to allow for gas analysis via the GEM, thus necessitating the storage of biogas over a period of time.

The results from the 100 mL digesters are typical of the results that have been obtained in the laboratory in past biogas experiments. As mentioned previously, the GEM unit has consistently given suspect gas composition measurements. Knowing that anaerobic digestion occurs in the absence of oxygen, and that biogas is typically 60-65% CH₄ and about 30-35% CO₂, one would expect the composition measurements to reflect this. However, more often than not the GEM has reported high percentages of O₂ and “balance” gases. The balance gases would sometimes read as high as 40-50%. In support of the argument that this skewed data is a result of the use of inverted graduated cylinders (combined with the use of the GEM), the biogas composition data from the 10 L digesters in Trial 1 (which utilized gas collection bags) reflected what one would expect from biogas, with little to no detection of O₂ or “balance” gases. One possible reason for these faulty readings could be due to using the on-board pump on the Gem for lifting water. The GEM is not designed for this application, and the added pressure on the system through lifting the columns of water could have exposed leaks within the lines on the GEM itself, thus contaminating the biogas sample.

Trial 2.

Trial 2, compared to Trial 1, shows a higher level of correspondence between the three scales in terms of biogas production and methane content. Unlike Trial 1, in Trial 2 all digesters utilized gas collection bags, preventing the loss of CO₂ into the barrier solution, and also preventing the effects of negative pressure on the 100 mL and 1 L digesters as a result of supporting the columns of barrier solution in the inverted cylinders. Although biogas composition measurements still could not be performed every day on the 100 mL digesters due to their low gas output, the composition of the measured gas was seemingly unaffected by storage in the gas bags, as opposed to the use of inverted cylinders in Trial 1. As with the 10 L digesters from Trial 1, the biogas composition measurements for Trial 2

were in agreement with one another and with expectations for biogas. Unlike the digesters from Trial 1 that used inverted cylinders, none of the digesters from Trial 2 had any excess O₂ or balance gases, typically reading only CO₂ and CH₄.

In future studies, having access to a fully functional GC would make the comparison of biogas composition across samples far more accurate. The ability to take gas samples directly from the headspace of the digesters via a gas-tight syringe would help prevent the contamination of the sample with ambient air. It would also enable gas composition measurements to be taken daily from each of the digesters, since as little as 500 µL of biogas is required for analysis in the GC.

Comparison with other studies.

Compared to the literature, the methane production is slightly less than that reported by other researchers (see Table 8). The digesters in Trial 1 produced significantly less methane than did the digesters in Trial 2. Only one of the digesters from Trial 1 (10 L) comes close to producing the amount of methane reported by other researchers digesting horse manure, and even then it is on the low end. The 1 L and 10 L digesters in Trial 2 show a higher rate of correspondence with the literature, but still do not produce as much.

There are a few reasons that may account for these discrepancies. The first is the use of inverted cylinders in Trial 1, and the issues associated with flawed biogas analysis measurements when using the GEM gas analyzer. Time, too, could play a role, as the studies presented in Table 8 run for longer than 30 days. It is possible, especially with the digesters in Trial 2 that if the reaction were allowed to continue the 1 L and 10 L digesters may have more closely matched the numbers presented by other researchers.

The source of the manure samples is another potential reason for the difference in reported values. This trial utilized horse manure gathered directly from the field, as did Mönch-Tegedder et al. (2013). Wartell et al. (2012) and Kusch et al. (2008), on the other

hand, took horse manure gathered from stables, which likely included bedding materials. Moreover the disparate results found between trials one and two in the present study suggest there may be considerable variability in the composition of the manure collected, even from the same field.

Table 8. *Cross-study comparison of methane production from horse manure.*

Cross-study Methane Production Comparison				
<u>Study:</u>	<u>Sample</u>	<u>Size</u>	<u>(days)</u>	<u>(mL/g VS)</u>
Wartell et al. (2012)	Stable Manure	160ml	33	56 ±14
	"		40	122 ±78
	"		46	53 ±15
	"		59	231 ±18
	"		79	133 ±6
Kusch et al. (2007)	Stable Manure	50L	40	170
Mönch-Tegedder (2013)	Field Manure	100ml	35	171
Gamble (this study)	Field Manure	100ml (T1)	30	5 ±2
	"	1L (T1)	30	34 ±6
	"	10L (T1)	30	55 ±5
	"	100ml (T2)	30	89 ±21
	"	1L (T2)	30	145 ±21
	"	10L (T2)	30	131 ±6

pH

The pH was quite consistent both within and across trials. The pH seemed to hover around 7.3-7.5 for the pre-digested horse manure in the preliminary trial and in Trial 1. Over the course of the experiment the pH dropped slightly (to around 7.1-7.2), which is still within acceptable ranges for the growth of methanogenic microorganisms. It would be interesting to carry out the trial further to see if the pH continues to drop, or if it stabilizes at some point in the reaction.

The pH in Trial 2 (see Table 6) was slightly different from Trial 1 (see Table 4) and the preliminary trial. The pH of the feedstock was more acidic than was expected, measuring as low as 6.66. Reasons for this are unknown, but a visual inspection of the feedstock may yield some potential causes. Compared to the horse manure in the first two trials, the manure in experimental Trial 2 was noticeably greener and fouler smelling than previous samples. This may be in part due to a change in seasons resulting in a change in the horses' diet.

The manure for the preliminary trial and experimental Trial 1 was collected during two of the colder months in an already exceptionally cold winter for Watauga County, NC (February/March 2014). Mönch-Tegeger et al. (2013) mentioned in their study that during winter months the nutritional needs of the horses was less, resulting in smaller amounts of feed, and a change in the composition of the manure. This is primarily due to the fact that the horses are not working as much during the winter and therefore have much more "down time."

However, due to a lack of stables at Dutch Creek Trails, where the manure was collected for this study, it is more likely that the change in diet is the operative variable, specifically the lack of access to green pastures during the winter. The manure for Trial 2 was collected in early April when the weather had warmed significantly, and the grasses began to return in force. This could have resulted in a change in feeding habits, and thus manure composition. The greener appearance of the manure could have been related to an increase in the intake of fresh grasses, potentially altering the pH of the feedstock. Ideally, a CHN analysis would have been helpful in this determination.

Volatile Solids Destruction

VS destruction was measured in the experimental trials, but not in the preliminary trial. Within each of the experimental trials there was a high rate of correspondence

between the pre and post %VS. In Trial 2, a 20% inoculum made up of pre-digested horse manure from experimental Trial 1 was utilized in nine of the twelve digesters. Three of the digesters served as controls, which is why their pre-trial %VS is much lower than the other digesters. Compared to Trial 1, the digesters in Trial 2 destroyed a higher percentage of volatile solids (see Tables 5 and 7). The pre-trial %VS was also slightly lower in Trial 1 than in Trial 2. Again, one potential reason for this could be related to the differences in the manures between the two trials.

Unfortunately, only one sample from each of the digesters could be analyzed post-trial due to the limited number of crucibles and space within the muffle furnace, limiting the sample size to one per digester. Ideally, triplicates should be run for each of the digesters so that more robust data may be acquired. Due to the use of single samples, the measurements taken may not be entirely representative of the actual VS-destruction. A larger refrigerator for the laboratory could assist in future experimentation, allowing samples to be stored between analyses.

As mentioned in the literature review, some authors have expressed concerns over the use of VS/VS-destruction as a measure of both available organic materials and the measure of their degradation (Leckie et al., 1981). Leckie et al. mention that only about 50% of the reported available VS is actually digestible via anaerobic digestion. This trial supports that argument. In Trial 1 only about a third of the given VS was digested after 30 days. In Trial 2, even with nearly a doubled increase in biogas production, the %VS destroyed was still only between 50-60% (see Table 7). Given more time, a greater percentage may have been destroyed, but these numbers are actually quite consistent with the literature, wherein measured VS-destruction remains in the 55-75% range (Brunn et al., 2007; Bouallagui et al., 2010).

Another piece of evidence for the potential error in utilizing %VS destruction as a measure of pollution reduction stems from this study. In Trial 1, the 100 mL digesters plateaued and ceased producing biogas long before the 1 L or 10 L digesters. One reason for this could have been that the 100 mL digesters degraded all of the available organics. However, this does not appear to be the case; comparing the %VS destruction figures across the digester sizes, the %VS-destroyed for each size are roughly the same (see Table 5). Normalizing the biogas data to mL/ g VS-destroyed as opposed to mL/g VS, yields no changes in the production curve. One would expect that had the 100 mL digesters in Trial 1 digested all of the available organic material (as seems to be the case given the plateau effect), then the curve would have matched those of the 1 L and 10 L digesters more closely.

Part of the reason for this apparent error could be related to the use of VS- destruction as a measurement of pollution reduction, for the reasons mentioned previously. Another source of error could be related to the fact that only single samples from each of the digesters were tested. Ideally, triplicates of each would have been run to help account for error in sampling, but due to limited equipment this was not possible at the time of the study.

Other Observations

There were some other factors that may be examined in order to determine any other differences between the digesters across the three sizes. While these factors are not readily quantifiable, they could nonetheless highlight operational differences between the digesters. These factors include appearance of the digestate, growth/formation of molds in the digestate, viscosity, and proneness to clogging.

Efforts were made at the start of each trial to create a fully homogenized mixture of feedstock. The purpose was to limit the variability in substrate so that all digesters would be digesting nearly identical samples. Within both trials, the substrates appeared the same

across all digester sizes and they all had similar viscosities, as based on observations made during the agitation of the digesters each day.

Over the course of the preliminary trial and experimental Trial 1, there was some visible mold growth on the digestates, limited to the 100 mL digesters and 1 L digesters. This growth primarily took place in the first week of the preliminary trial and experimental Trial 1. Since this growth only occurred in the digesters using the inverted cylinders for volumetric measurements, it is possible likely that this played some role in the growth of mold. The exact reasons are unknown, but one possible cause could be the transfer of oxygen from the barrier solution to the headspace of the digester via the gas lines.

In the 10 L digesters in Trial 1, and all the digesters in Trial 2, the digesters and gas collection bags were completely cut off from ambient air. The digesters using the inverted cylinders in the preliminary trial and experimental Trial 1, however, were indirectly exposed to ambient air via the barrier solution. Coupled with this, the gas-lines and headspace of the inverted cylinders were not flushed with N₂ before being connected to the digesters (which were flushed). Past AD experimentation in the TED department has also observed the growth of mold, in all of those experiments no N₂ flushes were performed, in the digesters nor the gas lines, and the same problematic system design was used at that from the preliminary trial.

Another possible reason for the mold growth observed in Trial 1 was the use of an inoculum in Trial 2. By providing a healthy community of microbes at the start of the reaction in Trial 2, the likelihood of a healthy reaction was increased. In Trial 1, the microbes necessary for anaerobic digestion could have been in greater competition with other microbial communities, including those involved in aerobic degradation. These aerobic microbes would have also had an increased chance of survival given the potential for oxygen contamination in Trial 1.

Another observable differences involved the interaction between the digestate and the digesters themselves, specifically the geometry of the digesters. Once the switch was made to 250 mL Erlenmeyer flasks for the 100 mL digesters, clogging ceased to be a problem. In fact, there were no clogging problems at all with the 100 mL or 1 L digesters throughout either of the two experimental trials (only in the preliminary trial). This was not the case, however, for the 10 L digesters. Unfortunately, 3.5 gallon plastic carboys could not be found, so 3-gallon carboys were used instead. This led to a reduction in headspace within the 10 L digesters, as compared with the 100 mL and 1 L digesters using Erlenmeyer flasks (see Figure 5). The limited headspace led to two burst digesters during Trial 1, resulting in the loss of data for the last five days of the trial (digesters #1c and #4c), and one partially-burst digester in Trial 2, which resulted in a minimal loss of digestate, but some loss of biogas.

The reactions within the 10 L digesters appeared to be far more vigorous than in the smaller scales, though this could have been a result of the more compact digesters size, and the pressure associated with it. In future studies, if large bench-scale digesters are utilized, either a larger carboy should be used, or the volume of the digestate should be reduced to prevent clogging and potential explosion.

Predictive Model

Based on the data from Trial 1, total biogas production seems to be a better marker for prediction than CH_4 in determining the volume of gas created at different sizes and scales. Three different prediction curves/methods were applied to the actual biogas production data sets from experimental Trial 2 for purpose of comparison. The three models are: a simple scale-up predictive model, a model based on a power trend line fit, and a model based on a linear trend line fit. A comparison of these three predictive models with the actual data from Trial 2 (in L) are presented in Table 9.

Simple Scale-up Predictive Model

Using this assumption, prediction curves were generated for the 1 L and 10 L digesters by multiplying the average cumulative biogas production (in mL) of the smaller scales by ten, or one hundred, depending on the change in scales. For example, using the results from the 100 mL digesters, for predictive purposes they would be multiplied by ten to predict the 1 L values, and 100 to predict the 10 L values. Figure 24 shows the predicted curve for the 1 L digesters based on the average performance of the 100 mL digesters in Trial 1. Figure 25 highlights the predicted production curves for the 10 L digesters, based on both the 100 mL and the 1 L digesters in Trial 1. As can be seen in the graphs, the 100 mL digesters in this particular trial are not representative of production on larger scales. However, the 1 L and 10 L digesters are very close, with the 10 L digesters producing, on average, 15.9% more biogas than the prediction.

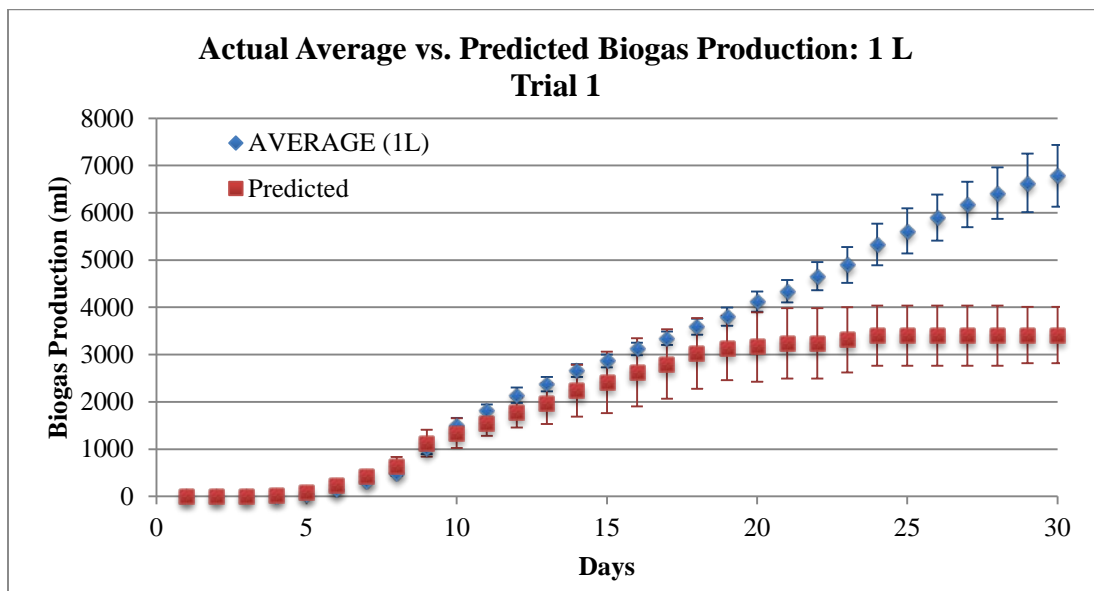


Figure 24. Comparison of actual average biogas production (1 L) and simple prediction (10x 100 mL).

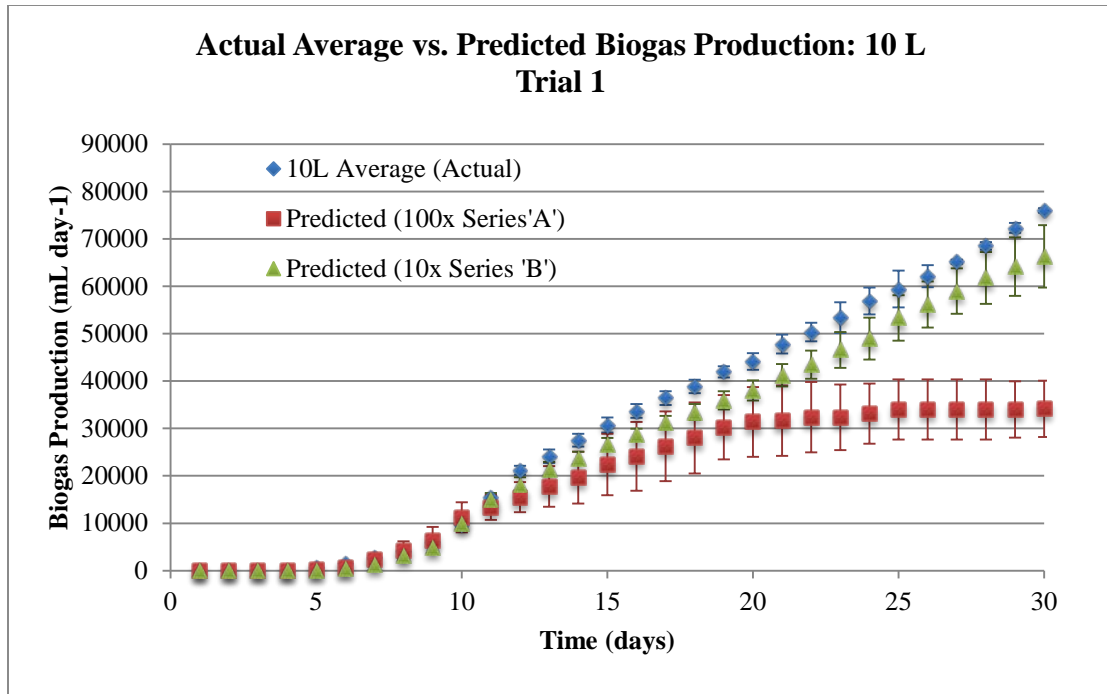


Figure 25. Comparison of actual average biogas production (10 L) with simple prediction (100x 100 mL and 10x 1 L).

The data gathered from experimental Trial 2 show a greater correspondence between scales than in Trial 1. This led to an increase in the accuracy of the simple predictive model in determining the biogas production in larger digesters (see Figures 26 and 27). However, using this particular predictive model is not ideal. This prediction is based off of an ideal notion that the amount of biogas produced is directly correlated with the amount of waste being treated. AD, however, is a biological process, and does not necessarily scale perfectly, as might a controlled chemical reaction. Based on the data from Trial 1, the issues associated with this predictive model become apparent. The 100 mL digesters clearly did not perform at all like the 1 L or 10 L digesters, meaning that had only 100 mL digesters been used for predicting biogas production in larger sizes (or scales), the results would have been quite flawed.

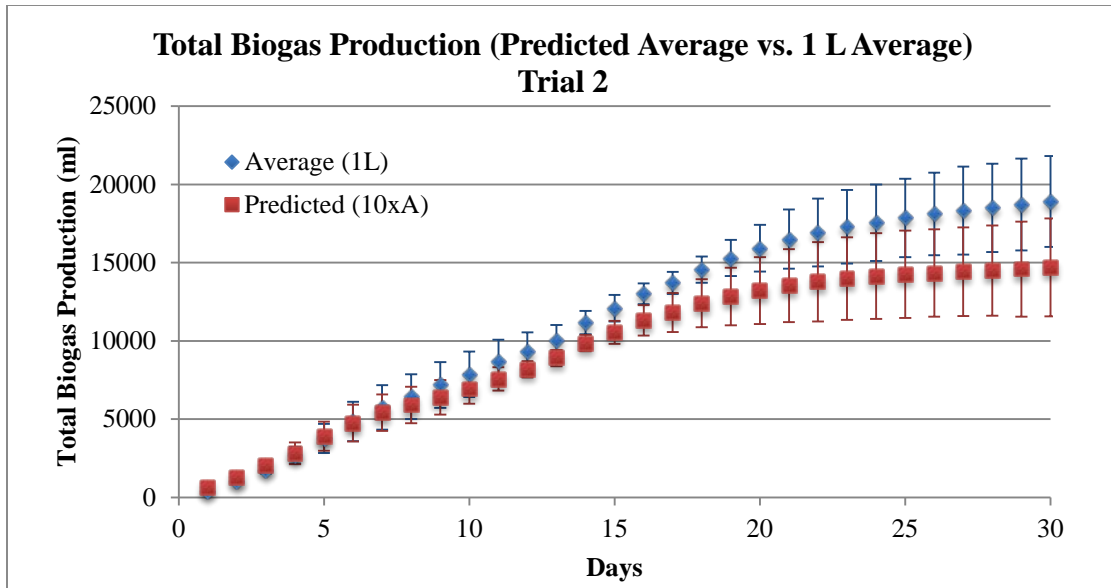


Figure 26. Comparison of actual average biogas production (1 L) with simple prediction (10 x 100 mL).

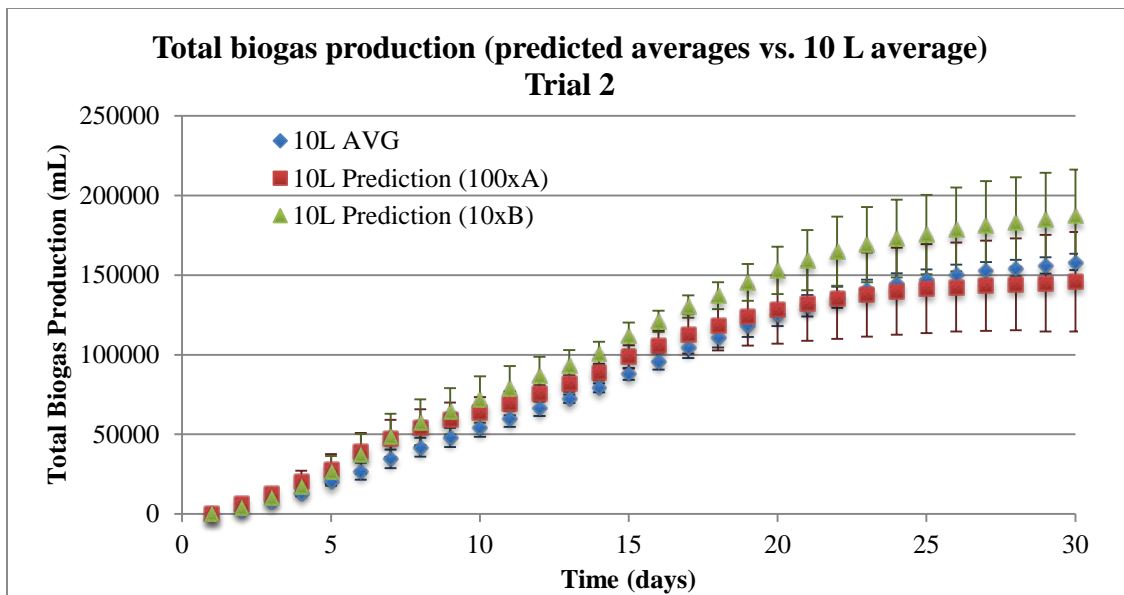


Figure 27. Comparison of actual average biogas production (10 L) with simple prediction (100x 100 mL and 10x 1 L).

Linear/Power Predictive Model

Using the data compiled from Trial 2, which showed a greater correspondence among digester sizes than Trial 1, the average cumulative biogas production (in L) for each of the digester sizes was plotted on a scatter chart with two trend lines fit to the data, one using a linear trend line, and the other a power trend line. Figure 28 provides an example of the linear predictive model and Figure 29 provides an example of the power-based predictive model, R^2 values are presented in the graph, along with the accompanying function.

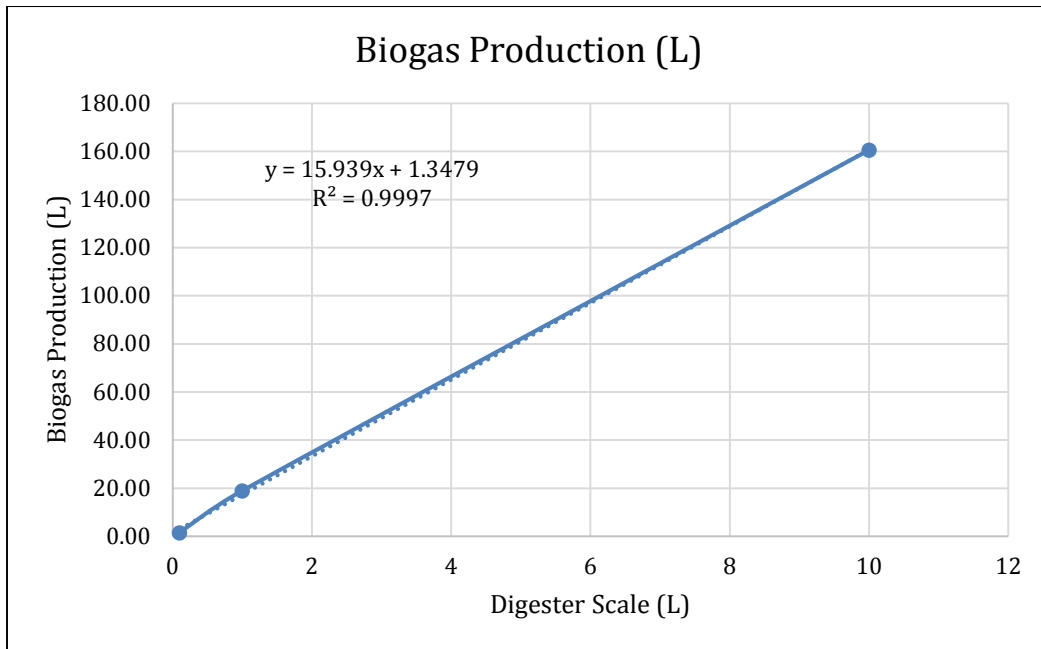


Figure 28. Biogas production linear predictive model.

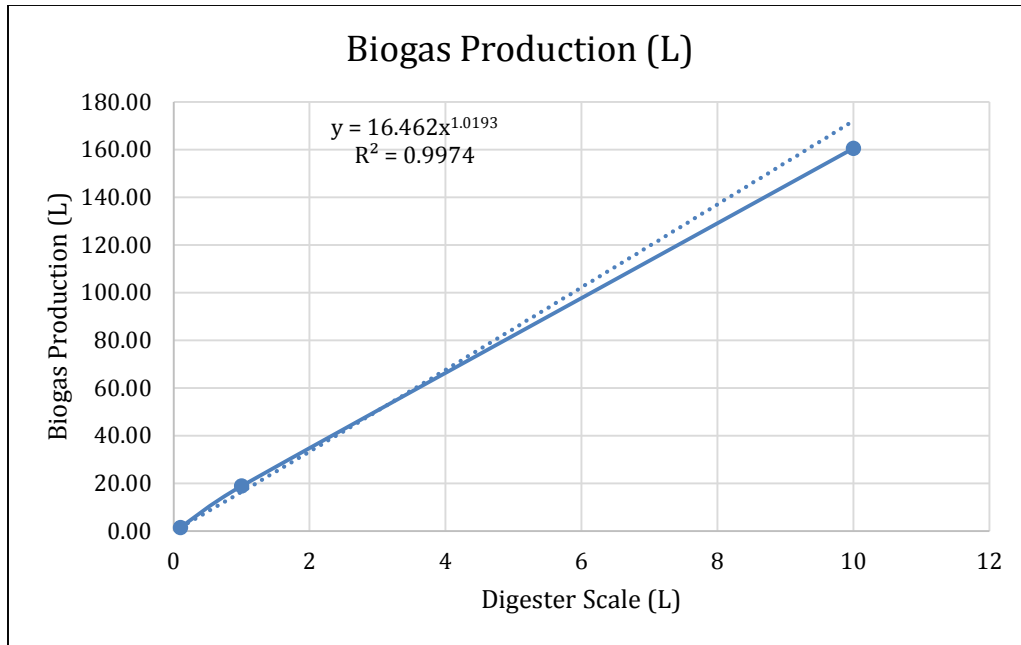


Figure 29. Biogas production, power predictive model.

Table 9. Comparison of actual biogas production (L) with predicted values.

Scale (L)	Linear (L)	Power (L)	Simple (L)	Actual (L)
0.1	2.94	1.57	N/A	1.47
1	17.29	16.46	14.69	18.91
10	160.74	172.10	146.93	160.59
250	3986.10	4578.29	3673.33	N/A
1000	15940.35	18809.73	14693.33	N/A

Using the power and linear predictive model, the idea for both is essentially the same. Once the actual data has been plotted, and the trend line fit, predictions can be made by substituting the “x” variable with the desired digester volume (L). Based on that one can get an idea of roughly how much biogas production can be expected from a given volume.

Based on these two predictive models, the linear model is slightly more accurate than the power model when compared to the actual biogas production values from experimental Trial 2 (see Table 9), especially in predicting the production of the 1 L and 10

L digesters. Given this, the scalability of AD seems to be viable, however, the data show that there is clearly some scaling effect which takes place, making exact extrapolations of results on the bench-scale to larger scales somewhat error prone if a model does not reflect these scaling effects. Further study into this scaling effect, especially utilizing these predictive models, is recommended.

Conclusions

In conclusion, this study proved to be a valuable one, both for the future of AD research in the TED department at Appalachian State University and also for other researchers working in AD studies. A significant correspondence was found between digester sizes and biogas production. Statistical analyses revealed, however, that while the correspondence is strong, there is still a slight scaling effect which must be taken into account. The addition of the use of an inoculum in Trial 2 proved useful, and the results obtained from that trial were much closer to those reported in the literature for horse manure.

The major differences in the methane content between digester sizes is likely the result of flawed readings on the GEM due to the system set-up in Trial 1, and the lack of available biogas in the 100 mL digesters for both trials. The addition of a GC to the laboratory will help reduce these discrepancies in the future, as much smaller biogas samples will be able to be drawn for the purposes of analysis.

VS-destruction and pH were relatively consistent between digester sizes within trials. However, there were some discrepancies in the %VS-destroyed within Trial 1, where the 100 mL digesters were shown to have destroyed an equal percentage of VS as the 1 L and 10 L digesters, but not an equivalent amount of biogas (normalized to mL/g VS). While the pH of the substrate in Trial 2 was more acidic than in Trial 1, both trials tended to become more neutral throughout the reaction.

Based on the collected data, there is sufficient agreement between digester sizes to allow for the creation of a predictive model. Two predictive models were developed out of this study, and each provide a formula for the prediction of biogas at different scales (based on data collected from the bench-scale.) While there is not perfect, linear scaling from 100 mL to 10 L, it is close, showing that there is a scaling effect which must be accounted for in predicting biogas production.

Finally, the work involved in troubleshooting the bench-scale system and the gas-collection/measurement systems will prove extremely useful to future students and faculty in their research into AD in the TED department. For other researchers, this work will prove useful as it confirms the scalability of AD, and highlights a scaling effect associated with increasing digester sizes/scales. This adds to the very limited research currently available on the scalability of AD. Hopefully, future students and studies can build on this work and compare data collected from the bench-scale with that collected from the forthcoming pilot-scale digesters.

Limitations of Study

This study was limited by a number of factors, first and foremost being a lack of equipment. Some pieces of equipment that would have been very helpful for this study are a properly functioning GC, a refrigerator for storing feedstocks between trials, and wet-gasometers(s) for measuring gas flow more accurately.

Ideally, having a GC on site in the laboratory would be most helpful because it would mitigate the need for flushing vials and transporting gas across campus. Likewise, it would enable the GEM to be retired from laboratory use and instead be used specifically for landfill gas composition measurements, which is what its intended function. This would save both time and effort, and would help to limit the contamination of the gas samples during the filling, transporting, and emptying of the vials.

Having wet-gasometers/flow meters would also be extremely beneficial to future bench-scale studies. Gasometers are available that can measure as little as 0.5 mL of gas/day, and that can upload this data in real time to a data-logging program. This would enable the use of smaller-scale digesters, but more importantly, would allow for real-time gas flow measurement, giving a more detailed look into the digestion process. This, coupled with a GC, could potentially enable the use of 100 mL digesters for bench-scale studies.

Suggestions for further research

There are a number of areas related to this project that need to be expanded upon. There is ample room for further student research. The following areas highlight the major points that ought to be addressed in future research:

1. The construction of a 100 L laboratory biodigester to complete the scale from 100 mL to 1000 L. Then, a side-by-side comparison of the 100 L and 1000 L digesters, this data should then be compared with the data generated from this study. Ideally using the same feedstock and inoculum ratios.
2. Experiments on the effect of agitation on the digestion process.
3. Experimentation on the effect of time in the digestion process on the bench-scale. Specifically, the ideal retention time for batch-mode digesters operating in the mesophilic temperature range.
4. Experiments on the effects of different ratios of inoculum on the digestion process.
5. A comparison of using VS and COD as a means for predicting the available organic material for degradation through digestion, and for the measurement of the efficiency of the AD reaction in removing pollutants.
6. Effects of digester geometry on digestion performance. Specifically a study into the differences headspace volume makes in digestion performance.
7. Determination of the smallest lab-scale digester size that provides accurate, consistent data, and produces enough gas to permit daily gas composition measurements via the GEM.
8. Further study into the accuracy of the water column device used for taking volumetric measurements in Trial 2. Determination into any variables that must be accounted for, and any normalizing formulas that must be applied to ensure accurate data.

9. Experimental trials utilizing the predictive models from this study. Ideally a study in which predictions will be made for biogas production using the power and linear-based predictive models, followed by experimental trials in which these predictive models are tested against. This would be useful not only for testing the utility of these predictive models, but also in the addition of more data which could be used to strengthen the models if they prove feasible.
10. Construction of a flare device for laboratory use so that biogas produced in the lab can be disposed of in a more environmentally conscious way.

References

- Bouallagui, H., Marouani, L., & Hamdi, M. (2010). Performances comparison between laboratory and full-scale anaerobic digesters treating a mixture of primary and waste activated sludge. *Resources, Conservation and Recycling*, 55(1), 29–33.
doi:10.1016/j.resconrec.2010.06.012
- Brunn, L., Dornack, C., & Bilitewski, B. (2007, October 1-5). *Application of laboratory scale experiments to industrial scale in case of anaerobic waste treatment*. Paper presented at the Eleventh International Waste Management and Landfill Symposium, Cagliari, Sardinia, Italy. Retrieved from <http://warr.org/223/>
- Cheng, J. (2010). Anaerobic digestion for biogas production. In J. Cheng (Ed.), *Biomass to renewable energy processes* (pp. 151-208). Boca Raton, FL: CRC Press.
- Drapcho, C. M., Nhuan, N. P., & Walker, T. H. (2008). *Biofuels engineering process technology*. New York, New York: McGraw-Hill Companies Inc.
- Gallert, C., Henning, A., & Winter, J. (2003). Scale-up of anaerobic digestion of the biowastes fraction from domestic wastes. *Water Research*, 37(6), 1433–1441.
doi:10.1016/S0043-1354(02)00537-7
- Hashsham, S. (n.d.). *Anaerobic digestion process* [diagram]. Retrieved from <http://fyi.uwex.edu/wimanuremgt/basics-of-anerobic-digestion/>
- Hawkes, F. R., & Young, B. V. (1980). Design and operation of laboratory-scale anaerobic digesters: operating experience with poultry litter. *Agricultural Wastes*, 2(2), 119–133.
- House, D. (2010). *The Complete Biogas Handbook*. USA: Alternative House Press.
- Johnson, B. (2009). *Characterization of institutional food waste: Anaerobic digestion of appalachian state university's food waste for biogas production* (Unpublished research paper). Appalachian State University, Boone, NC.
- Kalia, A. K., & Singh, S. P. (1998). Horse dung as a partial substitute for cattle dung for operating family-size biogas plants in a hilly region. *Bioresource Technology*, 64(1), 63–66.
- Kowalczyk, A., Schwede, S., Gerber, M., & Span, R. (2011, May 8-13). *Scale up of laboratory scale to industrial scale biogas plants*. Paper presented at the World Renewable Energy Congress 2011, Linköping, Sweden. Retrieved from http://www.ep.liu.se/ecp/057/vol1/007/ecp57vol1_007.pdf

- Kusch, S., Oechsner, H., & Jungbluth, T. (2008). Biogas production with horse dung in solid phase digestion systems. *Bioresource Technology*, 99(5), 1280–1292. doi:10.1016/j.biortech.2007.02.008
- Leckie, J., Masters, G., Whitehouse, H., & Young, L. (1981). *More other homes and garbage: Designs for self-sufficient living*. San Francisco, CA. Sierra Club Books.
- Liu, X. Y., Ding H. B., & Wang, J. Y. (2010). Food waste to bioenergy. In S. K. Khanal, R. Y. Surampalli, T. C. Zhang, B. P. Lamsal, R. D. Tyagi & C. M. Kao (Eds.), *Bioenergy and biofuel from biowastes and biomass* (pp. 43-70). Reston, Virginia: American Society of Civil Engineers.
- Massé, D. I., Croteau, F., Masse, L., & Danesh, S. (2004). The effect of scale-up on the digestion of swine manure slurry in psychrophilic anaerobic sequencing batch reactors. *Transactions-american society of agricultural engineers*, 47(4), 1367–1374.
- Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fraction of manure. *Biomass and Bioenergy*, 26, 485-495. doi:10.1016/j.biombioe.2003.08.008
- Mönch-Tegeder, M., Lemmer, A., Oechsner, H., & Jungbluth, T. (2013). Investigation of the methane potential of horse manure. *Agricultural Engineering International: CIGR Journal*, 15(2), 161–172.
- Trowbridge, J. (2013). *Thermophilic anaerobic digestion of brewery wastewater and food waste* (Unpublished research paper). Appalachian State University, Boone, NC.
- United States Environmental Protection Agency. (2013). *Overview of greenhouse gases: Methane emissions*. Retrieved from <http://epa.gov/climatechange/ghgemissions/gases/ch4.html>.
- United State Environmental Protection Agency. (2014a). *Anaerobic digestion 101*. Retrieved from <http://www.epa.gov/agstar/anaerobic/ad101/index.html>.
- United States Environmental Protection Agency. (2014b). *LFG energy projects*. Retrieved from <http://www.epa.gov/lmop/faq/lfg.html#11>.
- Veiga, M. C., Soto, M., Méndez, R., & Lema, J. M. (1990). A new device for measurement and control of gas production by bench scale anaerobic digesters. *Water research*, 24(12), 1551–1554.
- Walker, M., Zhang, Y., Heaven, S., & Banks, C. (2009). Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. *Bioresource Technology*, 100(24), 6339–6346. doi:10.1016/j.biortech.2009.07.018
- Wartell, B. A., Kruminis, V., Alt, J., Kang, K., Schwab, B. J., & Fennell, D. E. (2012). Methane production from horse manure and stall waste with softwood bedding. *Bioresource Technology*, 112, 42–50. doi:10.1016/j.biortech.2012.02.012

Zhu, B., Gikas, P., Zhang, R., Lord, J., Jenkins, B., & Li, X. (2009). Characteristics and biogas production potential of municipal solid wastes pretreated with a rotary drum reactor. *Bioresource Technology*, *100*(3), 1122–1129. doi:10.1016/j.biortech.2008.08.024

Vita

Kevin Gamble was born in Chapel Hill, North Carolina to Peter and Billie Gamble. He graduated from East Chapel Hill High School in 2003. After taking six months off, Kevin was accepted to Appalachian State University. There he studied Religious Studies, and was awarded a Bachelor of Arts degree in May 2009. Following graduation, Kevin took three years off to work and travel through Southeast Asia. It was on these travels that Kevin was motivated to return to school. In August of 2012, Kevin began study toward a Master of Science degree in Technology, with a concentration in Appropriate Technology, specifically organic waste treatment methods. He received the degree in August of 2014.

Kevin lives in Vilas, North Carolina with his fiancée, their two dogs, and an ever-increasing population of chickens.