Anaerobic Digestion from the Laboratory to the Field: An Experimental Study into the Scalability of Anaerobic Digestion

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Abstract

Bench-scale laboratory experimentation is an essential component of anaerobic digestion (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 optimization. To be of use there must be a strong correlation between results obtained in the lab, and the 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. Cumulative and daily biogas production, methane content, VS-destruction, and pH of the digestate were compared. A strong correlation was found between the three digester sizes, indicating the scalability of AD is tenable. However, some statistically significant differences in biogas production showed that there is a scaling effect that must be taken into account.

Keywords

Anaerobic digestion, biogas, scalability, bench-scale, horse manure.

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.

Anaerobic digestion (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. 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 technology is capable of handling many different forms and amounts of organic

waste. AD not only serves to improve sanitation, but also soils through the application of the treated liquid effluent (a viable replacement for chemically engineered fertilizers), and the production of renewable energy in the form of clean-burning biogas.

Laboratory experimentation is an essential component of AD 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. This study seeks to address this issue by examining the scalability of AD within three differently sized digesters on the bench-scale.

Technical Background

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. The four stages of anaerobic digestion are hydrolysis, acidogenesis, acetogenesis, and methanogenesis [1,2].

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 primarily on temperature, the type of feedstock, moisture content and any agitation/stirring of the digestate. In continuously-fed systems, waste is added to the digester at pre-designated times, again dependent on aforementioned 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[2].

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[1-4]. Biogas composition, especially the CH_4:CO_2 ratio, will vary greatly depending on the type of feedstock, or feedstocks (if co-digesting).

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[1,2,4]. The %TS represents the mass percent of dry solids in the wet material. The %VS is a percentage of the total solids, and represents the digestible material in the sample. 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. FS, due to their inorganic nature, are unable to be anaerobically digested and in some cases can even hinder or terminate the digestion process[2]. 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[1,3]. 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[5]. 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[1,2].

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.

The operating 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)[1,2]. Anaerobic digestion 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[1,2]. 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[2].

The organic loading rate (OLR) is a measure of the amount of digestible solids entering the bioreactor each day. This measurement 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/0.001m^3/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[3].

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%[2]. Dry fermentation systems can operate with a slurry solids content as high as 30%-40%[5]. House[3] and Leckie et al.[4] recommend that the slurry be kept between 7%-9% for most reactors, as this facilitates mixing and pumping of the digestate. 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[2].

Research into the use of horse manure as a feedstock for AD is surprisingly scant[6-9]. 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. 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.

Kalia and Singh[6] 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. While horse manure alone was found to be unsuitable as a sole feedstock for digestion, when used as a substitute (20%) for cattle manure, 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. Kusch et al.[7] 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. These researchers utilized horse dung mixed with straw bedding as their feedstock source. Mönch-Tegeder et al.[8] conducted an experiment to test the effects of different bedding materials on both the total biogas production and the methane content of the biogas. Wartell et al.[9] studied the effects of stall waste mixed with various ratios of softwood beddings on methane production. Table 1 shows the methane production of the various studies.

Table 1. *Cross-study comparison of methane production from horse manure*

Study	Sample	Size	(days)	(ml/g VS)
Wartell et al. [9]	Stable Manure	160ml	33	56 ±14
	"		40	122 ±78
	"		46	53 ±15
	"		59	231 ±18
	"		79	133 ±6
Kusch et al. [7]	Stable Manure	50L	40	170
Mönch-Tegeder [8]	Field Manure	100ml	35	171

Mönch-Tegeder et al.[8] 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. 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). Similar results were determined by Wartell et al.[9] who found that straw alone and horse manure alone were roughly equal in terms of methane yield. These findings led both sets of researchers 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" (p. 46)[9]. According to the Mönch-Tegeder et al.[8] 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.[9] came to similar conclusions as Mönch-Tegeder et al.[8], 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 on 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. Table 2 shows the feedstock characteristics of horse manure collected from various sources.

Table 2. *Comparison of Horse Manure Feedstock Characteristics Across Studies*

Study	Feedstock	%TS	%VS	C/N
Wartell, et al. [9]	Horse manure from stables, no bedding	(20-42) *M* = 37	(76-92) *M* = 83.7	
Kalia & Singh [6]	Horse manure from unknown source	22.6	87	35
Mönch-Tegeder, et al. [8]	Horse manure from field	20-27	18-24	23-37
Kusch et al. [7]	Horse manure from stable, with bedding	32-58	85-89	
Leckie et al. [4]	Horse manure from unknown source	16	87	35

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 laboratory 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. Most of the research into scalability conducted to date has shown a strong correspondence between data gathered in a bench-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[10-14].

Gallert et al.[11] 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. A positive correlation was found between the lab and industrial-scale systems in regards to biogas production and COD removal. Researchers found a strong correlation between the simulations performed in the lab and the actual performance of the industrial-scale digester, supporting their claim that the "feasibility of laboratory simulation experiments for scale-up considerations" (p. 1440)[11].

Brunn et al.[10] experimented with the reproducibility and transferability of laboratory-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 lab-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 lN/kg VS in digester 1 and 754 lN/kg VS in digester 2, which the researchers attributed to changes in substrate composition[10]. The results from the two lab-scale digesters were averaged and then compared to the industrial-scale digester according to the same set of parameters previously mentioned. The industrial-scale digester produced, on average, 36% more gas than the lab-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 the 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[10].

Kowalczyk et al.[13] 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. 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 between 1.42 and 5.96%" (p. 54)[13]. Differences in substrate composition, as in the Brunn et al.[10] study, was 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 (p. 54)[13]. 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.

Methodology

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.

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 and the same analytic balance were used to determine the %VS of the 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 two 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. (Landtec GEM2000) was used for the analysis of biogas composition. Two gastight syringes were required for this study. Figure 1 shows the experimental set-up used to measure biogas volume and collect gas samples for each scale digester.

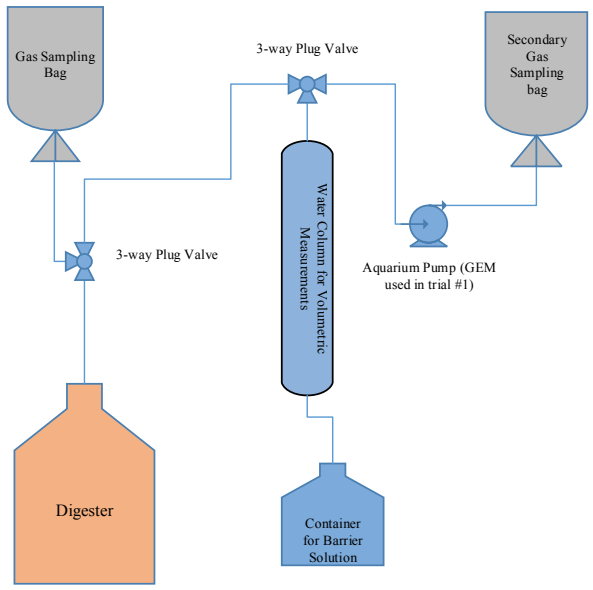

Figure 1. Schematic of water column for measuring volumetric biogas production.

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 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.

During gas measurements, one end of the valve would be connected to the gasbag, and the other end to an aquarium pump. 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.

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. 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 horse manure was chosen based on visual inspection for freshness and lack of contaminants such as soil, grasses, and rocks.

Horse manure was combined with the proper amount of distilled water to make roughly an 8% slurry mix (Equation 1), and was thoroughly blended together using a paint-stirring attachment on a battery-powered drill. 12 gallons of distilled water were combined with 32 lbs. of fresh horse manure, with a %TS of 25%, for a final slurry concentration of 8% TS (Equations 2-3).

$$8\%\ \text{slurry} = 8\ \text{lb. TS}/\ 100\ \text{lb. } H_20 \quad (1)$$

$$8\ \text{lb. TS} \times (100\ \text{lb. fresh horse manure}/25\ \text{lbs TS}) = 32\ \text{lb. fresh horse manure} \quad (2)$$

$$100\ \text{lb. } H_2O \times \text{gallon}/8.34\ \text{lb.} = 11.99\ \text{gallons } H_2O \quad (3)$$

The experimental trial 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 preliminary trials. 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. Each of the digesters were sanitized with StarSan™ (Five Star Chemicals) to destroy any remaining, unwanted organisms. Even though this is a no-rinse sanitizer utilized in brewing, 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 pH was measured and the headspace and the slurry itself for each digester was flushed with pure N_2 for approximately one and a half minutes. 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. A 20% sample of inoculum from previous digestions was placed into each experimental 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.

Thirty days was chosen as the experimental trial length because it is a typical length for digesters operating in the mesophilic temperature range[2]. Volumetric measurements and gas composition measurements were taken on a daily basis, as close to every 24 hours as was possible. Each of the digesters fed into a gasbag: 12 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 (Figure 1).

Results

Before the start of the experimental trials, samples of horse manure were analyzed for %TS, %M, and %VS. Fourteen total samples were used for this characterization. %TS was 22.8 to 27.7 with a median of 25.5. %VS was 69.6 to 96.9 with a median of 79.5.

Table 3 provides the pre- and post-trial pH for the digesters in trial two.

Table 3. Comparison of Pre/Post-Trial pH's in All Digesters, Trial Two (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 2 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.

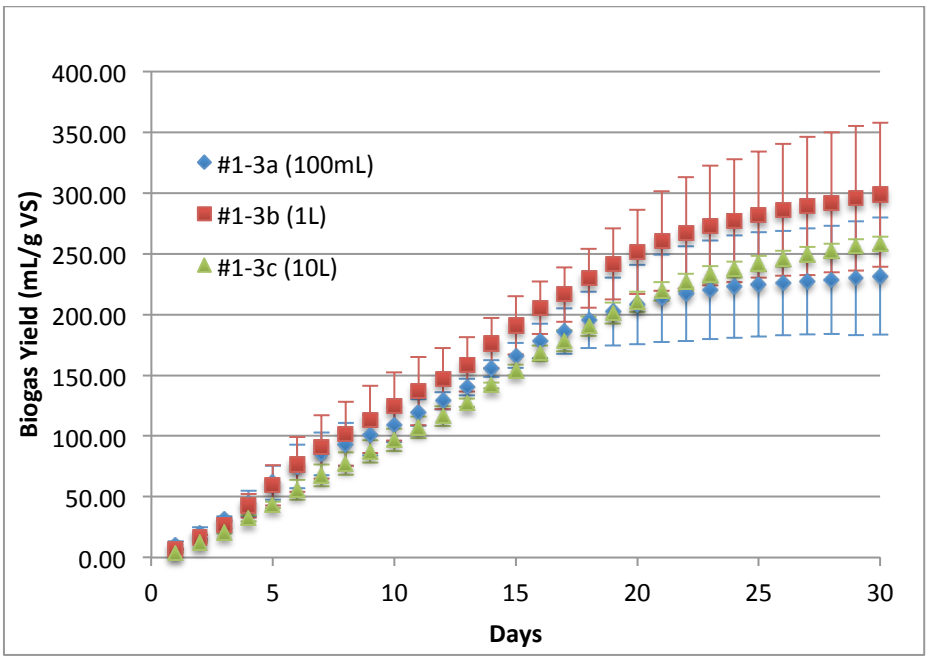

Figure 2. Comparison of average cumulative biogas yields, all non-control digesters.

Figure 3 shows a comparison of the average cumulative CH_4 production from the 100 mL, 1 L, & 10 L digesters. 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 (Table 4).

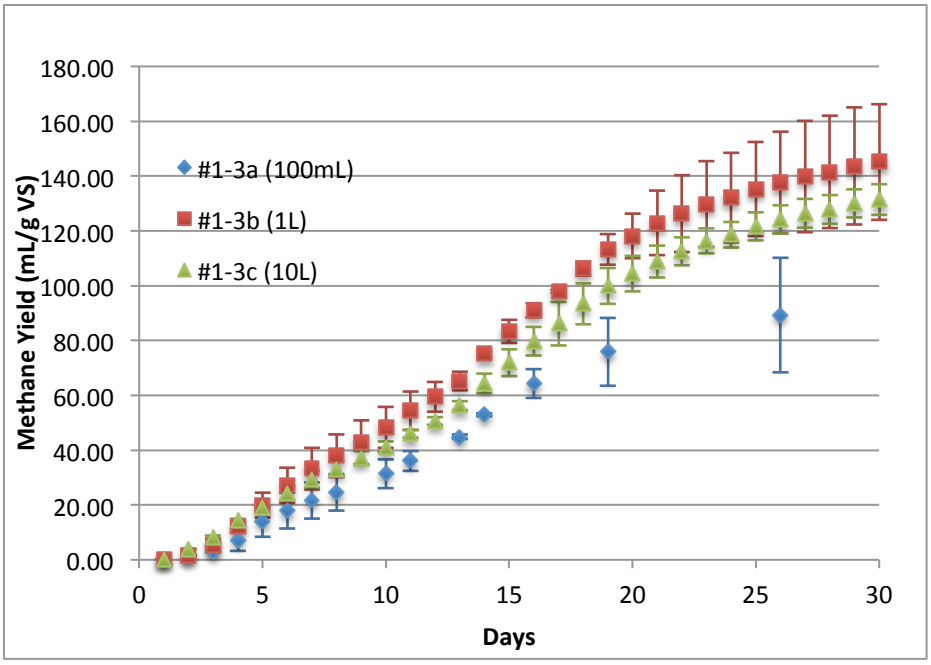

Figure 3. Comparison of average cumulative CH_4 production, all non-control digesters.

Table 4. Methane production from different scale digesters

Sample	Size	Days	ml/g VS
Horse manure	100ml	30	89 ±21
"	1L	30	145 ±21
"	10L	30	131 ±6

Figure 4 provides a comparison of the average daily biogas production for the 100 mL, 1 L, and 10 L digesters, not including the three controls. All three trend lines are six-factor polynomial, and R-squared values for each digester's trend line are included.

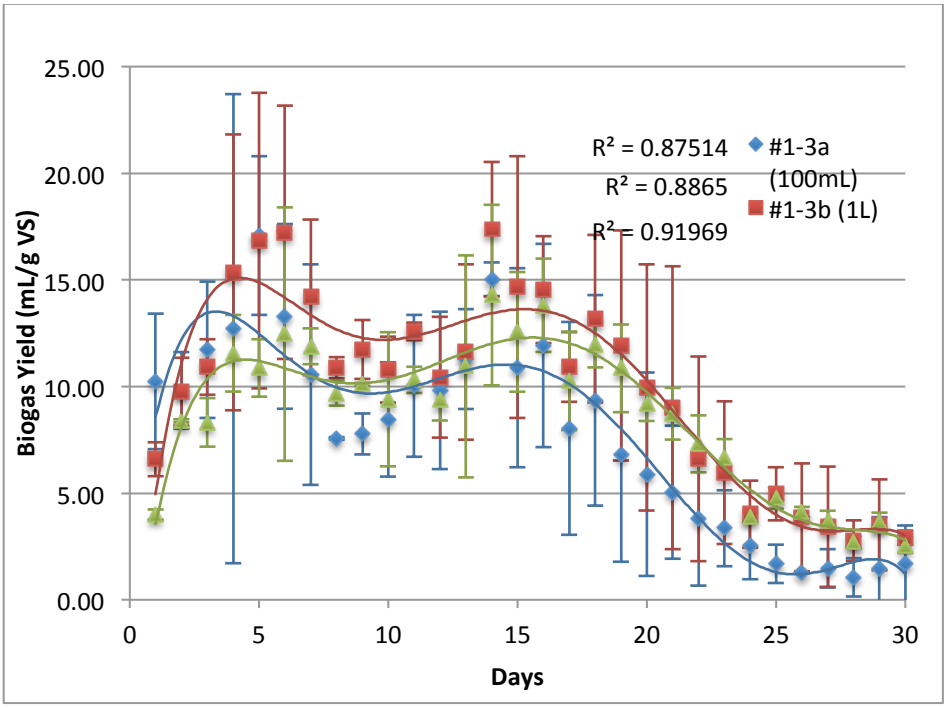

Figure 4. Comparison of average daily biogas yields, all non-control digesters

Table 5 shows the pre and post %VS for the digesters.

Table 5. Comparison of Pre/Post %VS for all Digesters in Trial Two

	100 mL				1 L				10 L			
TRIAL #2	#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**

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), efforts were made to answer this question. This research 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?

All of the digesters showed a high rate of correspondence in terms of total biogas production and their respective production curves (see Figure 2). 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 among each of them. The difference was smallest (though still significant) between the 10 L digesters and the 100 mL digesters ($P=0.012$.)

Although biogas composition measurements could not be performed every day on the 100 mL digesters due to their low gas output, the biogas composition measurements were in agreement with one another and with expectations for biogas (see Figure 3). In future studies, 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 a GC. Compared to the literature, the methane production (see Table 4) is slightly less than that reported by other researchers[7-8], but is similar to the results of Wartell et al.[9] (see Table 1). It should be noted that the research we are comparing our results to all had longer periods of data collection (33-79 days) that would raise the total cumulative amount of biogas and methane produced compared to our shorter 30-day period. If the reactions were allowed to continue our 1 L and 10 L digesters may have reflected the numbers presented by other researchers[7-9]. Our 1 L and 10 L digesters show a higher rate of correspondence with the literature; though our 100 mL digesters produced more methane than the Wartell et al.[9] 160 mL digesters at day 33 (see Tables 1 and 4). The source of the manure samples is a potential reason for the difference in reported values. This trial utilized horse manure gathered directly from the field, as did Mönch-Tegeder et al.[8]. Wartell et al.[9] and Kusch et al.[7], on the other hand, took horse manure gathered from stables, which was likely contaminated with bedding materials.

Interestingly, the digesters across scales tended towards a bi-phasic production curves with two peaks (see Figure 4). 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. In the case of the digesters for this experiment there were two peaks in production. All digesters experienced two production peaks, first around day four and again around day 15 (Figure 4). 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.

The pH of the feedstock was more acidic than was expected (based on preliminary trials), measuring as low as 6.66 (see Table 3). Reasons for this are unknown, but a visual inspection of the feedstock may yield some potential causes. Compared to the horse manure in preliminary trials, the manure in the experimental trial 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 preliminary trials was collected during two of the colder months in an already exceptionally cold winter for Watauga County, NC (February/March.) Mönch-Tegeder et al.[8] 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 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 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, which could also have had an effect on the nitrogen content and thus the pH of the feedstock (making it more acidic).

Within each of the experimental trials there was a high rate of correspondence between the pre and post %VS. A 20% inoculum made up of pre-digested horse manure 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 (see Table 5). 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. 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. 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.[4] mention that only about 50% of the reported available VS is actually digestible via anaerobic digestion. This trial supports that argument, the %VS destroyed was only between 50-60% (see Table 5). 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[10,11].

Based on the data, 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 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 is presented in Table 6.

Table 6. 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	15,940.35	18,809.73	14,693.33	**N/A**

For the simple model, 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. 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 two (see Table 6), 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 shows 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 . Further study into this scaling effect, especially utilizing these predictive models, is recommended.

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