ANAEROBIC DIGESTION OF EQUINE WASTE

By

BRIAN A. WARTELL

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ABSTRACT OF THE THESIS

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By BRIAN A. WARTELL

Thesis Director: Professor Donna E. Fennell

The goals of this project were to determine the methane production potential of horse manure during anaerobic digestion; to examine the effect of softwood chip bedding, pelleted Woody Pet® softwood bedding, and straw on the methane production potential of equine stall waste; and to investigate the feasibility of co-digestion of waste food and equine waste under thermophilic conditions.

Initial results suggested that softwood bedding may have inhibited methane production in 15 L semi-continuous digesters. However, further extensive investigation in batch and continuous flow digesters determined that softwood bedding did not inhibit methane production and, on the contrary, contributed to methane production. The methane production potential for horse manure at 35°C averaged 139 ± 65 L/ kg VS (average ± standard deviation) and 29 ± 15 L/ kg wet weight, corresponding to 9.2 ± 4.8 x 10^5 kJ / metric ton wet weight. The energy production potential of stall waste with softwood chip bedding ranged from 4.0 ± 0.4 x 10^5 kJ / metric ton wet weight to 6.6 ± 0.8 x 10^5 kJ / metric ton wet weight, depending upon the relative amount of bedding present.

Co-digestion of equine waste and food waste under thermophilic conditions was performed at the 20 L and 6.3 m^3 scale. The 20 L thermophilic digesters were fed a variety of food wastes in addition to stall waste containing softwood bedding. The methane production from these digesters was 356 ± 61 L/kg VS-d. The large-scale (6.3

m^3) digester was operated in excess of one year primarily on waste food and horse manure (no bedding). The loading rate increased over time to 1.7 kg VS/m^3-d. The methane content of the biogas was 55.7 ± 5.2 %. Total ammonia nitrogen approached 5 g/L, suggesting a higher C:N ratio feed stock mixture than that afforded by the waste food and horse manure mixture might be necessary for future applications.

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Chapter I. Introduction

1.1. Rationale

The New Jersey equine industry has an economic worth of $1.1 billion (Gottlieb et al., 2007) and produces the largest quantity of livestock waste in New Jersey (Brennan et al., 2007; NJDA, 1996). Concentrated animal feeding operation (CAFO) and animal feeding operation (AFO) rules now require equine facilities to develop a manure management program (NJDA, 2006; AFBF, 2007). Many horse farms utilize or store manure on-site, and the application of manure and stall waste on fields and pastures is the primary means of disposal (Warren, 2003). Land application or nursery use of the manure often follows composting (Romano et al., 2006). Horse waste mixed with straw bedding is preferentially sought for use in mushroom production. However, not all owners wish to use straw bedding and not all equine facilities are within a geographic area that could serve mushroom facilities (Malinowski, 2007). Equine facilities are seeking economical and environmentally friendly options for manure disposal. As part of horse waste handling, anaerobic digestion could be employed to increase the value of horse manure and offset disposal costs through production of a biofuel (methane).

Most recoverable equine waste is obtained from stalls (Wheeler and Zajaczkowski, 2002; Westendorf and Krogmann, 2006). The characteristics of stall waste are dependent upon the type of stall bedding utilized (Chamberlain et al., 2004; Westendorf and Krogmann, 2006; Airaksinen, 2006). Softwood shavings are often used as bedding because of high absorbency, lack of palatability and low cost (Chamberlain et al., 2004; Airaksinen, 2006). One horse, defined as a 454 kg (1000 lb) animal, produces 17 kg (37 lb) feces and 9 L (2.4 gal) of urine per day, for a total of about 27 kg (60 lb) of

waste (Romano et al., 2006; Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002). Stalled horses require up to 9 kg (20 lb) of bedding per day (Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002). Combined, this accounts for up to 12,000 kg (13 tons) of waste per horse per year.

Anaerobic digestion takes advantage of the anaerobic microbial degradation process. This process occurs naturally in the gut of most animals, including humans. It is simply defined as the breakdown of large (carbon-based) molecules via several types of anaerobic microorganisms, ultimately yielding the production of methane and carbon dioxide (Rittmann and McCarty, 2001). More specifically, fermentative bacteria initially interact with large polymers and produce either acetate or short fatty acid chains, which are also converted to acetate by acetogenic bacteria. Other products produced by fermentative and acetogenic bacteria are hydrogen and carbon dioxide. Methanogens, anaerobic archaea, utilize acetate or carbon dioxide and hydrogen to form the final end product, methane. If anaerobic processes are implemented in engineered anaerobic digesters, methane, which can be used for heating or electricity production, may be recovered from a variety of feed stocks (Ahring, 2003).

If equine waste is to be anaerobically digested, it could be done on-farm as a single substrate, or at the regional scale with other feedstocks. A recent assessment of biomass energy potential in NJ found that as of 2007, about 286,000 dry tons of food waste was recoverable as a biomass source (Brennan et al., 2007). While there are many other waste biomass sources in NJ, the largest source of recoverable agricultural livestock waste in 2007 was equine waste at 102,400 dry tons, greater than the amount of all other agricultural livestock wastes combined (Brennan et al., 2007). There are a few published

studies regarding the potential for anaerobic digestion of horse manure (Kalia and Singh, 1998; Mandal and Mandal, 1998; Zuru et al., 2004; Kusch et al., 2008) but none of these studies addressed equine stall waste that contains softwood bedding. To date, there do not appear to be any published studies addressing the co-digestion of food and equine wastes.

1.2. Overall Goal and Objectives of this Study

Based on a lack of information about anaerobic digestion of horse waste, the overarching goals of this study were to examine the feasibility of anaerobic digestion of this material. The specific objectives of this project were to determine the methane production potential of horse manure, to investigate the effect of stall bedding on the methane production potential, and to examine the ability of horse waste to act as a co-substrate for food waste digestion.

1.3. Thesis Overview

This thesis is composed of four chapters. **Chapter 1** is the introduction and **Chapters 2**, **3** and **4** are designed as individual papers for submission to scholarly journals. This thesis is thereby classified as a "thesis of papers."

Chapter 2 comprises all of the mesophilic (35°C) batch tests conducted to determine the methane production potential from horse manure alone, horse manure plus fresh or used softwood shavings bedding, horse manure plus softwood bedding pellet product, Woody Pet® (Woody Pet, Surrey, BC), and horse manure plus straw bedding. This chapter also includes details of initial semi-continuous digesters that prompted further investigation of the effects of wood on anaerobic digestion of stall waste and

further experiments conducted to determine whether softwood bedding inhibits methane production.

Chapters 3 and **4** both describe thermophilic (55°C) digesters utilizing combined food and horse wastes. **Chapter 3** describes results from replicate semi-continuous-feed 20 L thermophilic digesters used to investigate the feasibility of the co-digestion of food and stall wastes. Digesters were fed both substrates at equivalent ratios on a volatile solids basis.

Finally, **Chapter 4** describes operation of a 6 m^3 large-scale adaptation of waste food and horse manure digestion under thermophilic conditions. This pilot experiment was run at the Rutgers University Eco-Complex in Burlington County, New Jersey. The ratio of food waste to horse waste varied over the course of operation as the digester was started up and eventually reach a loading of 204 kg (450 lb) wet solids per feeding every two to three days, maintained at a 3:1 ratio of food waste to horse manure on a volatile solids basis.

Chapter II. Methane Production Potential of Horse Manure and Stall Waste

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2.1. Introduction

The equine industry in the U.S. provides a valuable resource for racing and recreational riding. New Jersey has among the largest number of horses of any state. Consequently, the largest source of recoverable agricultural livestock waste in NJ Is equine waste at approximately 102,400 dry tons, greater than the amount of all other agricultural livestock wastes combined (Brennan et al., 2007). Horse owners are often located on small farms with encroaching development and have increasingly less available acreage for manure spreading.

One horse (defined here as a 454 kg (1000 lb) animal) produces roughly 17 kg (37 lb) feces and 9 L (2.4 gal) of urine per day, for a total of about 27 kg (60 lb) of waste (Romano et al., 2006; Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002). Stalled horses require up to 9 kg (20 lb) of bedding per day (Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002). Combined, this accounts for up to 12,000 kg (13 tons) of waste per horse per year, with bedding constituting about 25% of the wet weight. Horse waste is often spread on land either before or after composting (Chamberlain et al., 2004; Krogmann et al., 2006; Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002).

Most recoverable equine waste is from stalls (Wheeler and Zajaczkowski, 2002; Westendorf and Krogmann, 2006). The characteristics of stall waste are highly dependent upon the type of stall bedding utilized and the nature of stall cleaning, e.g. spot

cleaning versus complete removal of bedding, that occurs at a particular facility (Chamberlain et al., 2004; Westendorf and Krogmann, 2006; Airaksinen, 2006). Softwood shavings are often used as bedding because of high absorbency, lack of palatability and low cost (Chamberlain et al., 2004; Airaksinen, 2006). Straw is often preferentially used as bedding for brooding mares because of its softness and low toxicity (Airaksinen, 2006) when compared with wood, which contains compounds with known toxic properties (Belmonte et al., 2006; Savluchinske-Feio et al., 2006) that could be harmful to foals (Malinowski, 2007). There is also a market for equine stall waste from horses bedded on straw from the mushroom industry (Poppe, 2000) and this may also affect the choice of bedding for a specific facility.

Horse manure and used bedding can attract insects and vermin in addition to producing unpleasant odors and potentially contaminating water sources (e.g. high nitrogen and phosphorus levels) via runoff from stored or land-applied waste (Airaksinen et al, 2006; Romano et al., 2006; McFarland, 2008). Roughly 75% of horse farms utilize or store manure on-site and the application of manure and stall waste on fields and pastures is the primary means of disposal (Warren, 2003). Land application or nursery use of the manure often follows composting (Romano et al., 2006).

Equine facilities are seeking new options for manure disposal. One of these options could be centralized processing that would remove manure from farms where there is inadequate land for spreading and treat it in locations that pose fewer water quality risks while producing valuable end products such as compost. Thus, in this study, the feasibility of applying anaerobic digestion as a step in centralized horse waste processing to increase the value of horse manure through production of a biofuel

(methane) was examined. Anaerobic digestion is widely applied for dairy, swine, and poultry wastes (Magbanua et al., 2001; Liu et al., 2009). The digestate from anaerobic digestion of animal waste still contains degradable organic material and nutrients and may be further stabilized by aerobic composting (Kusch et al., 2008; Adhikari, 2006), but is usually applied to crop or pasture land as the ultimate fate (Westendorf and Krogmann, 2004).

Research articles pertaining to anaerobic digestion of animal manures target primarily cattle and swine waste, and to a lesser degree, poultry waste. Very few published studies are available regarding the potential for anaerobic digestion of horse manure. This may be because it is a less abundant waste than cattle and swine manures in many parts of the US and the world, and because horse manure's higher solids content makes this material highly suitable for composting. However, several regions and states have robust equine industries with large numbers of animals producing substantial quantities of waste that need to be disposed of properly and cost-effectively. Anaerobic digestion of horse waste was investigated by researchers in India (Kalia and Singh, 1998; Mandal and Mandal, 1998), Nigeria (Zuru et al., 2004) and Germany (Kusch et al., 2008) and there were two press reports of digesters to be built at racetracks in the USA (Church, 2005; Stumbos, 2001), although no further publications or notices were found regarding actual contruction. Additionally, there is unpublished research on anaerobic digestion of horse waste in the US (Jewell, 2006). Kusch et al. (2008) have conducted the only extensive research of horse manure and have investigated the solid state anaerobic digestion of horse waste mixed with straw bedding, reporting successful digestion of this material. Because equine waste is collected in a solid state (25 to 40% TS), Kusch et al.

(2008) proposed that digestion of equine waste might be best accomplished in a batch wise manner using a static pile system. Their studies were conducted in 50 L laboratory-scale batch digesters and compared both percolation and flooding, and digestate recycling mechanisms as modes for increased methane production.

Much of the recoverable horse waste available for anaerobic digestion in New Jersey is intermingled with softwood bedding, and to date there is no information available on the methane production potential of this material. For on-farm applications, a system such as that proposed by Kusch et al. (2008) could be utilized where batchwise digestion of stored material is performed. Extended studies have not been conducted to test this idea, particularly with respect to stall waste and the biodegradability and effects of different types of stall bedding.

Based on lack of information about anaerobic digestion of equine waste, this study had as its overall goal to determine the methane production potential of horse waste. The specific objectives were to (1) determine the methane production from horse waste in semi-continuous flow (15 L) and simple high solids batch (125 L) reactors; (2) determine the effect of different types of beddings on the methane production from stall waste in 160 mL batch serum bottle studies; and (3) determine the methane production potential of different types of stall bedding alone in 160 mL batch serum bottle studies. Because wood contains resin-type compounds with known toxic and antimicrobial properties (Belmonte et al., 2006; Savluchinske-Feio et al., 2006) and because initial experiments performed as part of this study suggested that toxicity could be a problem, it was important to determine if equine stall waste from horses bedded on softwood chips is

amenable to anaerobic digestion and to determine if intermingled wood bedding has a negative effect on the conversion to methane.

2.2. Materials and Methods

2.2.1 Feed Stock and Inoculum

Horse manure without bedding was collected from loafing sheds and stall waste with softwood (pine) bedding was collected from stalls at the New Jersey Agricultural Experiment Station (NJAES) Animal Care Program on the Cook Campus of Rutgers University, New Brunswick, NJ. Fresh softwood chips and straw bedding were also provided by the Animal Care Program and a softwood pellet bedding, Woody Pet® (Woody Pet, Surrey, BC), was provided as a personal gift by Ms. Diana Orban of the Rutgers University Equine Science Center. Used softwood chips were obtained by removing them from stall waste manually. All wastes were stored at 4°C to minimize deterioration prior to use. Typical total solids (TS) and volatile solids (VS) content of the respective substrates are shown in Table 2.1.

Table 2.1. Average measured total and volatile solids content of the feedstocks obtained from the New Jersey Agricultural Experiment Station (NJAES) Animal Care Program (range values are shown in parentheses).

[a]Substrate	Total Solids (% Wet Weight)	Volatile Solids (% TS)
[b]Horse Manure	37.0 (20-42)	83.7 (76-92)
[c]Stall Waste (manure plus softwood bedding)	32.0 (22-40)	79.8 (79-91)
Softwood Bedding (fresh)	92.1 (91-93)	90.1 (89-99)
Softwood Bedding (manually separated)	31.2 (30-32)	92.8 (91-94)
Woody Pet®	93.8 (93-94)	90.8 (90-92)
Straw	93.3 (92-94)	97.9 (97-98)

[a] The number of samples analyzed (n) was: horse manure, 7; stall waste, 6; softwood bedding fresh, 2; softwood bedding manually separated, 2; Woody Pet®, 1; and straw, 1.
[b]collected from outdoor loafing sheds
[c]collected from stalls

For the 125 L solid state batch reactor study, stall waste was obtained from Oxbow Stables in Hamburg, NJ. The waste was generated from stalls bedded with Condensed Pine Wood Bedding Pellets (Guardian Horse Bedding Equistock, LLC, Rockford, IL). Stalls were spot cleaned twice per day. Based on the number of horses on site (58) and the amount of bedding purchased per year, it was estimated that the waste contained between a 1:1 and 2:1 wood to manure ratio on a VS basis. The waste had been stored on site in static piles for approximately two weeks prior to use, and had a total solids (TS) content of 41.3 ± 2.5 % and a volatile solids (VS) content of 82.0 ± 3.8 %.

Municipal mesophilic (35°C) anaerobic digester sludge used as inoculum was obtained from the Joint Meeting of Essex and Union Counties wastewater treatment facility in Elizabeth, N.J.

2.2.2. Semi-Continuous-Flow Reactors: Setup and Operation

The methane production and percent conversion of VS to methane for horse waste was first investigated in semi-continuous flow reactors. Replicate (Reactors 1 and 2) semi-continuous-flow reactors (CFR) were developed in two 24.6 L (6.5 gal) polyethylene fermentation buckets (Beer and Wine Hobby, Woburn, MA) with gas-tight lids. Biogas was collected in 87 L Tedlar® gas bags (Cole-Parmer Instruments, Vernon Hills, IL). Reactors were filled with 14 L of anaerobic digester sludge and were purged with nitrogen gas prior to initiation of feeding. Feedings were performed by removing the lid, adding the substrate, replacing the lid and purging the headspace with nitrogen gas. After feeding had commenced, the CFRs were incubated at 25°C for the first 55 days of operation and at 35°C thereafter. Reactors were fed with bedding-free horse manure for 82 days and then were fed with stall waste (horse manure plus softwood bedding) until Day 126 when operation ceased.

During operation at 25°C (0 to 55 days) the reaction volume was maintained at 15 L and the total solids content of the reactor was maintained at a target of 12% TS, or a volatile solids concentration (X_v^o) of approximately 100 g VS/L. A volatile solids loading rate (VSLR) of 2.8 kg VS/m^3-d (42 g VS/d) was selected based on guidelines for municipal sewage sludge digesters (Rittmann and McCarty, 2001). The resulting solids retention time (θ_x) calculated from $\theta_x = X_v^o/VSLR$ was approximately 40 days. On an

organic loading rate (OLR) basis, this was approximately 4 kg COD/m^3-d, assuming 1.42 g COD/g biomass VS (Rittmann and McCarty, 2001).

During operation at 35°C (Day 55 onward) the operating volume maintained in the reactor was decreased to 10 L because of foaming problems. The target volatile solids feeding rate remained the same at 42 g VS/d, resulting in a corresponding increase of the VSLR to 4.2 kg VS/m^3-d (approximately 6 kg COD/m^3-d). The resulting solids retention time (θ_x) was approximately 24 days. On Day 82 the feed stock of the reactors was switched to stall waste consisting of approximately 25% softwood bedding on a wet weight basis, based on estimates of bedding used and waste produced per horse per day (Westendorf and Krogmann, 2004; Wheeler and Zajaczkowski, 2002) the VSLR, however, remained at 4.2 kg VS/m^3-d.

2.2.3. Methane production potential tests: Setup and Operation

Methane potential tests were performed in 160 mL batch serum bottles to examine the effect of bedding type on the methane production from anaerobic digestion of horse waste and to determine the methane production potential of the manure and bedding itself. The methane production potential tests described here were carried out using recommended procedures for such tests (for a review of recommended procedures see Rozzi and Remigi, 2004).

The experimental protocols are shown in Tables 2.2 through 2.6. Five batch reactor experiments (Exp.) were performed to examine the effect of stall bedding on methane production potential:

- Exp. 1, Effect of fresh softwood bedding on methane production (**Table 2.2**)
- Exp. 2, Effect of fresh softwood bedding on methane production (**Table 2.3**)

- Exp. 3, Effect of different bedding types—softwood bedding, Woody Pet® and straw—on methane production and methane production potential of bedding alone (**Table 2.4**)
- Exp. 4, Effect of used softwood bedding on methane production (**Table 2.5**)
- Exp. 5, Methane production potential of softwood bedding alone (**Table 2.6**).

For Exp. 1 and 2, each bottle (except those containing only inoculum or only softwood bedding) received 0.5 g VS of fresh horse manure. Fresh, unused softwood bedding was then added at various ratios of soft wood bedding VS to horse manure VS to determine whether the presence of the material (perhaps because of leaching of resin compounds from the material) might inhibit methane production (**Tables 2.2 and 2.3**). Reactors were inoculated with 10 mL of municipal anaerobic digester sludge, purged with oxygen-free nitrogen while anaerobic minimal salts medium (Fennell et al., 1997) was added to achieve an operating volume of 100 mL. Serum bottle reactors were operated as stirred (shaken) batch systems at 35°C for periods of approximately one to three months.

All treatments were performed in triplicate. Each experiment also included three types of control treatments receiving: only inoculum plus mineral medium to serve as controls for methane produced from the inoculum alone; inoculum plus manure alone to determine the methane production potential of the manure; and treatments containing only inoculum and bedding to examine the amount of methane produced from bedding alone.

Table 2.2. Experimental protocol for methane production potential batch test Exp. 1[a] to determine the effect of fresh softwood stall bedding on methane production from horse manure.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 1	Effect of fresh softwood bedding on methane production			
Bottle Set 1	Inoculum control	--	--	10
Bottle Set 2	Manure control	0.5	--	10
Bottle Set 3	Bedding: Manure ratio 0.01:1 bedding VS: manure VS	0.5	0.005	10
Bottle Set 4	Bedding: Manure ratio 0.05:1 bedding VS: manure VS	0.5	0.025	10
Bottle Set 5	Bedding: Manure ratio 0.1:1 bedding VS: manure VS	0.5	0.05	10
Bottle Set 6	Bedding: Manure ratio 0.25:1 bedding VS: manure VS	0.5	0.125	10
Bottle Set 7	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	0.5	0.25	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 59 days at 35°C*

Table 2.3. Experimental protocol for methane production potential batch test Exp. 2[a] to determine the effect of fresh softwood stall bedding on methane production from horse manure.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 2	Effect of fresh softwood bedding on methane production			
Bottle Set 1	Inoculum control	--	--	10
Bottle Set 2	Manure control	0.5	--	10
Bottle Set 3	Bedding: Manure ratio 0.05:1 bedding VS: manure VS	0.5	0.01	10
Bottle Set 4	Bedding: Manure ratio 0.1:1 bedding VS: manure VS	0.5	0.05	10
Bottle Set 5	Bedding: Manure ratio 0.25:1 bedding VS: manure VS	0.5	0.125	10
Bottle Set 6	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	0.5	0.25	10
Bottle Set 7	Bedding: Manure ratio 1:1 bedding VS: manure VS	0.5	0.5	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 40 days at 35°C*

Exp. 3 (**Table 2.4**) also tested the degradability and methane potential of Woody Pet®, a commonly used softwood pelleted bedding that disintegrates into small wood particles under the influence of moisture, and straw, which is known to degrade rapidly and produce high methane concentrations during anaerobic digestion (Møller, et al., 2003). Each substrate was also tested independently, to ascertain methane production potential from the bedding alone.

Table 2.4. Experimental protocol for methane production potential batch test Exp. 3[a] to determine the effect of fresh softwood bedding, Woody Pet®, and straw bedding on methane production from horse manure and to determine methane production potential from the bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 3	Effect of bedding on methane production and methane production potential of bedding alone			
	Softwood bedding			
Bottle Set 1	Inoculum control	--	--	10
Bottle Set 2	Manure control	0.5	--	10
Bottle Set 3	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	0.5	0.25	10
Bottle Set 4	Bedding: Manure ratio 1:1 bedding VS: manure VS	0.5	0.5	10
Bottle Set 5	Bedding: Manure ratio 2:1 bedding VS: manure VS	0.5	1	10
Bottle Set 6	Bedding: Manure ratio 4:1 bedding VS: manure VS	0.5	2	10
Bottle Set 7	Bedding Only	--	1	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 34 days at 35°C*

Table 2.4. Continued. Experimental protocol for methane production potential batch test Exp. 3[a] to determine the effect of fresh softwood bedding, Woody Pet®, and straw bedding on methane production from horse manure and to determine methane production potential from the bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 3	Effect of bedding on methane production and methane production potential of bedding alone			
	Woody Pet® Bedding			
Bottle Set 8	Inoculum control	--	--	10
Bottle Set 9	Manure control	0.5	--	10
Bottle Set 10	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	0.5	0.25	10
Bottle Set 11	Bedding: Manure ratio 1:1 bedding VS: manure VS	0.5	0.5	10
Bottle Set 12	Bedding: Manure ratio 2:1 bedding VS: manure VS	0.5	1	10
Bottle Set 13	Bedding: Manure ratio 4:1 bedding VS: manure VS	0.5	2	10
Bottle Set 14	Bedding Only	--	1	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 34 days at 35°C*

Table 2.4. Continued. Experimental protocol for methane production potential batch test Exp. 3[a] to determine the effect of fresh softwood bedding, Woody Pet®, and straw bedding on methane production from horse manure and to determine methane production potential from the bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (gVS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 3	Effect of bedding on methane production and methane production potential of bedding alone			
	Straw Bedding			
Bottle Set 15	Inoculum control	--	--	10
Bottle Set 16	Manure control	0.5	--	10
Bottle Set 17	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	0.5	0.25	10
Bottle Set 18	Bedding: Manure ratio 1:1 bedding VS: manure VS	0.5	0.5	10
Bottle Set 19	Bedding: Manure ratio 2:1 bedding VS: manure VS	0.5	1	10
Bottle Set 20	Bedding: Manure ratio 4:1 bedding VS: manure VS	0.5	2	10
Bottle Set 21	Bedding Only	--	1	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 34 days at 35°C*

Exp. 4 (**Table 2.5**) examined the effect of fresh and used softwood bedding on methane production potential from horse manure and determined the methane production potential of the bedding alone. Each bottle (except those containing only inoculum or only

softwood bedding) received 2.38 g VS of fresh horse manure. Used softwood bedding, previously manually removed from the stall waste mixture, was then added at ratios of 0, 0.25, 0.5, 1, 2, and 4 g bedding VS to g horse manure VS either in addition to the horse manure or alone, to test the methane production potential of the bedding alone. Controls included those with horse manure alone, unused softwood bedding alone and horse manure plus fresh (unused) softwood bedding at a 1:1 weight ratio of bedding VS to horse manure VS. All treatments during this experiment were performed in triplicate. Reactors were inoculated as described for Exp. 1 and operated as stirred (shaken) batch systems at 35°C for 79 days.

Table 2.5. Experimental protocol for methane production potential Exp. 4[a] to determine the effect of fresh and used softwood bedding on methane production from horse manure and to determine methane production potential from the bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 4	Effect of fresh and used softwood stall bedding on methane production			
	Fresh (never used) softwood bedding			
Bottle Set 1	Inoculum control	--	--	10
Bottle Set 2	Manure control	2.38	--	10
Bottle Set 3	Bedding: Manure ratio 1:1 bedding VS: manure VS	2.38	2.38	10
Bottle Set 4	Bedding Only	--	2.38	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 79 days at 35°C*

Table 2.5. Continued. Experimental protocol for methane production potential Exp. 4[a] to determine effect of fresh and used softwood bedding on methane production from manure and to determine methane production potential from bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	Stall Bedding (g VS)	Inoculum (mL)
Exp. 4	Effect of fresh and used softwood stall bedding on methane production			
	Used (manually separated from stall waste) softwood bedding			
Bottle Set 5	Inoculum control	--	--	10
Bottle Set 6	Manure control	2.38	--	10
Bottle Set 7	Bedding: Manure ratio 0.25:1 bedding VS: manure VS	2.38	0.595	10
Bottle Set 8	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	2.38	1.19	10
Bottle Set 9	Bedding: Manure ratio 1:1 bedding VS: manure VS	2.38	2.38	10
Bottle Set 10	Bedding: Manure ratio 2:1 bedding VS: manure VS	2.38	4.76	10
Bottle Set 11	Bedding: Manure ratio 4:1 bedding VS: manure VS	2.38	9.52	10
Bottle Set 12	Bedding Only	--	0.595	10
Bottle Set 13	Bedding Only	--	1.19	10
Bottle Set 14	Bedding Only	--	2.38	10
Bottle Set 15	Bedding Only	--	4.76	10
Bottle Set 16	Bedding Only	--	9.52	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 79 days at 35°C*

For Exp. 5 (**Table 2.6**), each bottle (except those containing only inoculum or only bedding) received 2.38 g VS of fresh horse manure. Fresh softwood bedding was added at ratios of 0, 0.5, 1, and 2 g bedding VS to g horse manure VS. Further, a series of bottles were prepared with different amounts of bedding alone to assess the potential for methane production from its degradation. Reactors were inoculated as described for Exp. 1 and operated as shaken batch systems at 35°C for 33 days. All treatments were performed in triplicate, including bottles receiving only inoculum plus mineral medium and bottles containing only inoculum and softwood bedding, with the wood being equivalent on a volatile solids basis to the horse manure added to other bottles.

2.2.4. Solid State Batch Reactors: Setup and Operation

Anaerobic digestion of stall waste from Oxbow Stables, Hamburg, NJ (section 2.2.1) was performed in high solids, batch stainless-steel water-jacketed reactors covered with foam-insulation as described previously in detail (Hull, et al., 2002; Krogmann, et al., 2003; Hull, et al., 2005). Each reactor had a total capacity of 125 L, with a height of 100 cm and a diameter of 40 cm. The reactors were equipped with stainless-steel screens near the bottom so that a waste pile could be held in place while free liquid could drain and be collected in the bottom of the reactor. Prior to use, reactors were tested for ability to hold pressure at approximately 14 kPa (2 PSI). At start-up, each reactor was filled to approximately 100 L with 29 kg wet weight (9.8 kg VS) stall waste plus 2 L of inoculum (2% volume:volume amendment). Reactors were separately initiated seven days apart. On Days 50 and 43, respectively, each reactor was opened and 10 L additional inoculum (10% volume:volume amendment) was added.

Table 2.6. Experimental protocol for methane production potential Exp. 5[a] to determine effect of fresh softwood bedding on methane production from manure and to determine methane production potential from bedding alone.

Exp.	Description	Substrates and Inoculum		
		Manure (g VS)	**Stall Bedding (g VS)**	**Inoculum (mL)**
Exp. 5	Effect of fresh softwood stall bedding on methane production from manure and methane production from bedding			
	Fresh softwood bedding			
Bottle Set 1	Inoculum control	--	--	10
Bottle Set 2	Manure control	2.38	--	10
Bottle Set 3	Bedding: Manure ratio 0.25:1 bedding VS: manure VS	2.38	0.595	10
Bottle Set 4	Bedding: Manure ratio 0.5:1 bedding VS: manure VS	2.38	1.19	10
Bottle Set 5	Bedding: Manure ratio 1:1 bedding VS: manure VS	2.38	2.38	10
Bottle Set 6	Bedding: Manure ratio 2:1 bedding VS: manure VS	2.38	4.76	10
Bottle Set 7	Bedding Only	--	0.595	10
Bottle Set 8	Bedding Only	--	1.19	10
Bottle Set 9	Bedding Only	--	2.38	10
Bottle Set 10	Bedding Only	--	4.76	10

[a] *Experimental bottles were filled with anaerobic mineral medium to 100 mL and operated for 33 days at 35°C*

The top of each reactor was equipped with four ports. One port was connected by 1.3 cm. diameter braided Tygon® tubing to a wet test meter (Precision Scientific, Chicago, IL) through which the biogas flow from the reactor was continually measured. Measured biogas was discharged to a chemical fume hood. The second port accommodated a temperature probe that was extended to just above the bottom of the reactor. The third port was connected to a liquid distribution manifold on the inside of the lid of the reactor and was connected by Tygon® tubing and a pump to a port at the bottom of the reactor. This system was used for re-circulating leachate that drained from the waste pile to the bottom of the reactor, back to the top of the reactor every 2 to 5 days to maintain moisture in the pile. During each leachate re-cycling event, a 50 to 200 mL sample was collected for pH and ammonia-N determination. The fourth port was connected to a pressure gauge, which was used initially to assure proper sealing conditions and later to ensure no pressure buildup occurred (e.g., from clogging of lines).

The reactor temperature was maintained by heated water supplied by a 75.3 L (19.9 gal.) electric water heater (Reliance, Ashland City, TN) and recirculated with a UP15-42 F pump (Grundfos, Olathe, KS) through 1.3 cm. (0.5 in.) diameter PVC tubing. The pump operation was controlled by a TA-3 controller (SUPCO, Allenwood, NJ) that monitored the temperature probe inside the reactor and a temperature probe inside the water jacket. The internal reactor temperature was maintained between 34.0°C and 36.0°C for both reactors during the course of the experiment.

2.2.5. Analyses

Solids analysis.

Total and volatile solids analyses for all materials was performed according to Standard Methods (Clesceri et al, 1998).

Biogas and methane measurements.

The volume of biogas collected in the gas bags attached to semi-continuous flow reactors (section 2.2.2) was measured twice weekly using a wet test meter (Precision Scientific).

Gas was wasted from the 160 mL batch serum bottle reactors (section 2.2.3) every three to four days and the volume was measured at atmospheric pressure using a gas-tight plastic syringe or a water displacement system constructed from a 100 or 500 mL burette.

For the solid state 125 L batch reactor tests (section 2.2.4), biogas production was determined by noting the reading on the wet test meter every one to four days and a daily average biogas flow (L/ day) was calculated. Results are reported as aligned with Day 1 of Digester #1. Every two to three days, a 3 L Tedlar® gas bag (CEL Scientific, Santa Fe Springs, CA) was connected to the outlet of the wet test meter to obtain a biogas sample to determine the methane content.

The methane concentration in the biogas was analyzed via a 0.5 mL gas sample collected at atmospheric pressure using a glass-Teflon®-stainless-steel gas-tight syringe equipped with a side port needle (Valco® Precision Sampling, Baton Rouge, LA) and injected into an Agilent® 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a GS-GasPro capillary column (30 m x 0.32 mm I.D.; J&W

Scientific, Folsom, CA) and a flame ionization detector. Helium was the carrier gas at a constant pressure of 131 kPa (19 PSI). The oven temperature was held at 150°C. The resulting chromatographic peak area was compared to a five-point calibration curve prepared using mixtures of 0 to 100% methane created by mixing volumes of methane (99% purity; Matheson Tri-Gas, Inc., Montgomeryville, PA) and air in a 0.5 mL gas-tight syringe (Valco® Precision Sampling, Baton Rouge, LA). Volumes of biogas and methane produced were corrected and reported at standard temperature (25°C) and pressure (1 atm) using the ideal gas law. Other components of the biogas were not analyzed but were assumed to be primarily CO_2 as the other main digestion end product and N_2 (from purge gas), along with trace amounts of NH_3 and H_2S.

For batch serum bottle studies, the average methane production relative to the control bottle receiving horse manure but no bedding plus or minus one standard deviation was reported for each bottle set. Efficiencies of methane production based on the input of feed stock biomass VS was estimated by assuming 1 g COD stabilized = 0.35 liters of methane at STP and that 1 g COD = 1.42 g VS (Rittmann and McCarty, 2001).

The potential energy production from the waste in kJ per metric ton was determined by dividing the total cumulative volume of methane produced by the total wet weight of waste (manure and/or bedding) added to each bottle (converted to metric tons). This amount was then converted to mol of methane per metric ton and multiplied by the energy potential of the methane (802 kJ / mol methane (Schwarzenbach, et al., 2003)).

pH

The pH was measured using an Accumet® 900 pH meter (Fisher Scientific), according to Standard Methods *(*Clesceri et al, 1998*)*.

Total Ammonia Nitrogen (TAN) and Ammonia

For TAN determination, 1 mL samples were first centrifuged at 10,000 *g* and then the supernatant was removed and filtered through a 25 mm nylon membrane syringe filter (PALL, East Hills, NY). The filtrate was diluted 1000:1 using milliQ water and analyzed using a Dionex® ICS-1000 Ion Chromatograph (Sunnyvale, CA) with a Dionex® CSRS Ultra II 4-mm cation column. The resulting chromatographic peak areas were compared to a five point curve generated from analysis of standards prepared over a concentration range from 0.0625 to 1.0 mM NH_4^+-N/L, according to standard methods (Clesceri et al, 1998).

Data Analyses

Analysis of variance (ANOVA) was conducted using Microsoft Excel® to determine the statistical significance of differences between methane production from manure and softwood bedding mixtures, relative to controls receiving horse manure only. A significance level of 0.05 was used.

2.3. Results and Discussion

2.3.1. Semi-Continuous-Flow, Complete-Mix Reactor (CFR) Results

During start-up, duplicate CFRs, 1 and 2, were operated with a feedstock of horse manure alone (no softwood bedding). The solids content of the digesters was allowed to

increase from 2.9% (initial TS of the inoculum) to approximately 12% TS over the first 30 days of operation (**Figure 2.1a**). Thereafter, the average content was 12.8 ± 1.7 % TS and the corresponding VS concentration (X_v^o) based on an average digestate VS of 74% (**Figure 2.1b**) was 96 g VS/L. The pH was 7.3 ± 0.2 throughout the entire period of operation of both reactors (**Figure 2.2a**). Alkalinity ranged from 3 to 7 g as $CaCO_3$/L (**Figure 2.2b**).

Biogas and methane production from the CFRs is shown in **Figure 2.3.** Biogas production was somewhat variable between the duplicate reactors, as was the corresponding methane production. Methane production at 25°C was 1.2 ± 1.1 L/d and the percent methane was 30.8 ± 17 %. Methane production increased approximately 5-fold when the temperature was increased from 25°C to 35°C after Day 55. At 35°C with a substrate of horse manure alone the methane production rate averaged for the two CFRs was 7.7 ± 2.8 L/d and the percent methane was 57.9 ± 6.6 %.

The highest estimated yield of methane from the volatile solids loaded during operation at 35°C with horse manure only (VS estimated to be converted to methane) was approximately 35% for reactor 1 and 38% for reactor 2. The methane production potential of the horse manure, based on a VS loading of 42 g VS/d, was thus 183 ± 67 mL methane/g VS.

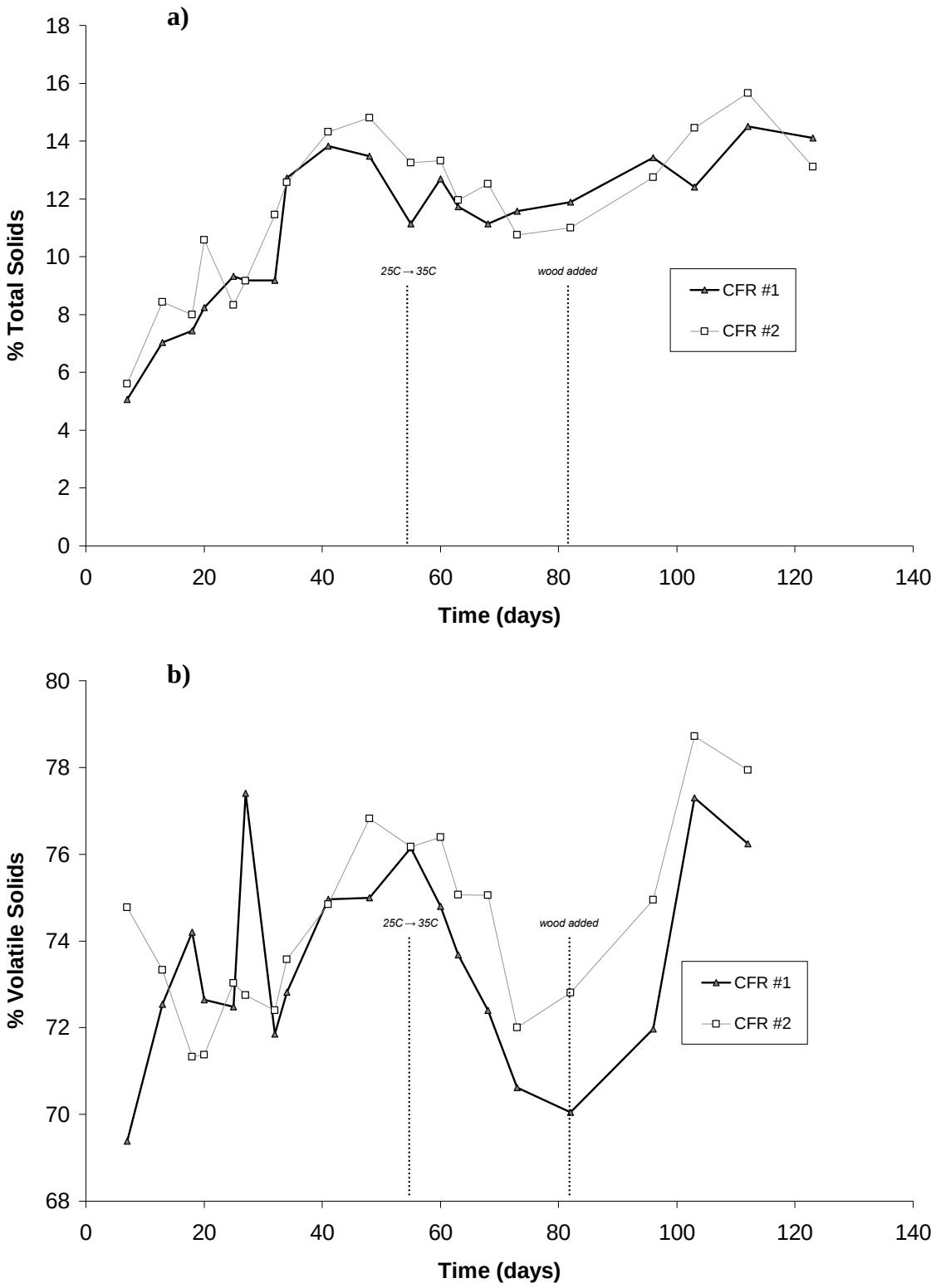


Figure 2.1. Solids content in semi-continuous flow reactors (CFRs). a) change in % total solids over time ; b) change in % volatile solids

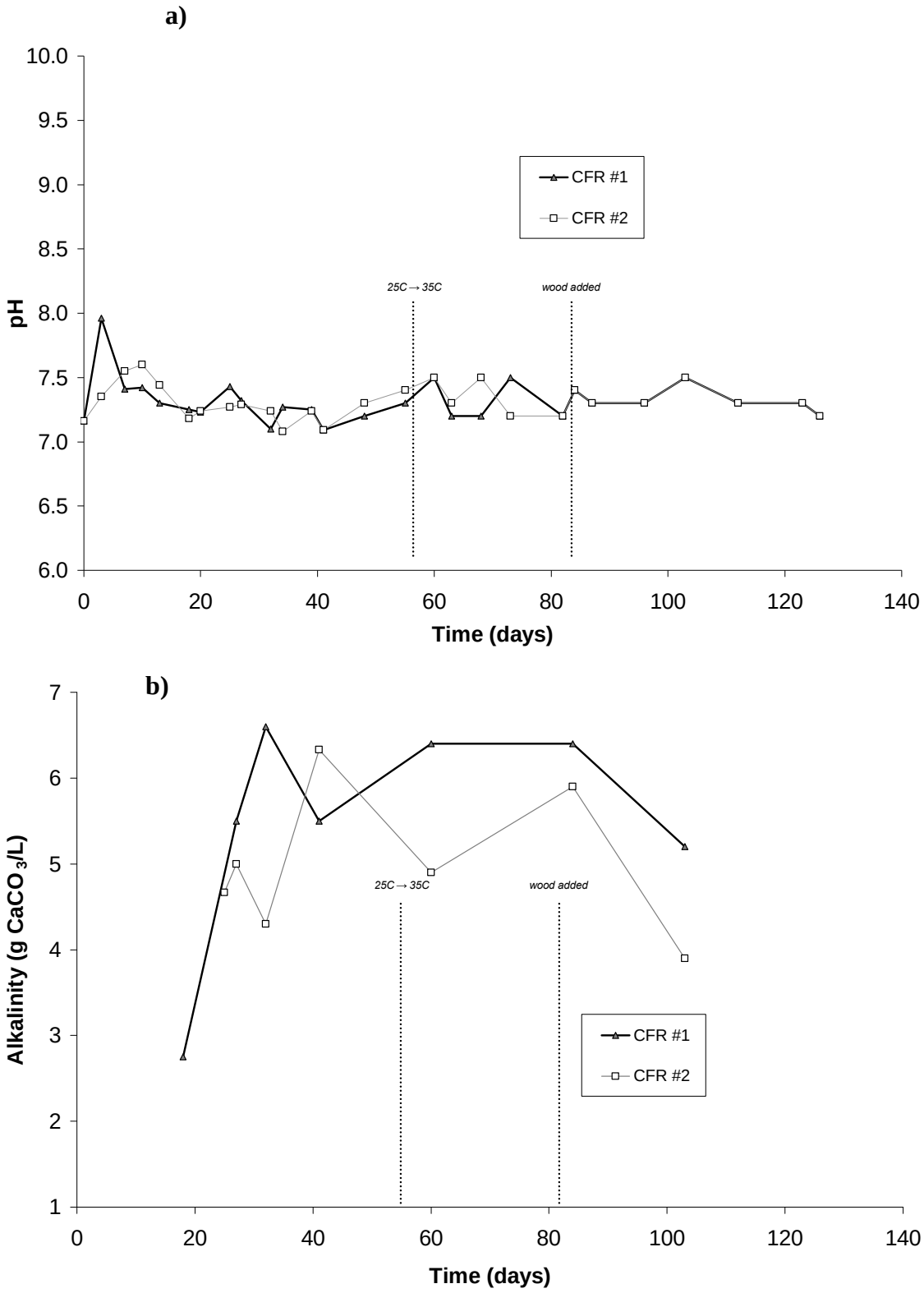


Figure 2.2. pH and alkalinity in semi-continuous flow reactors (CFRs). a) pH values; b) alkalinity.

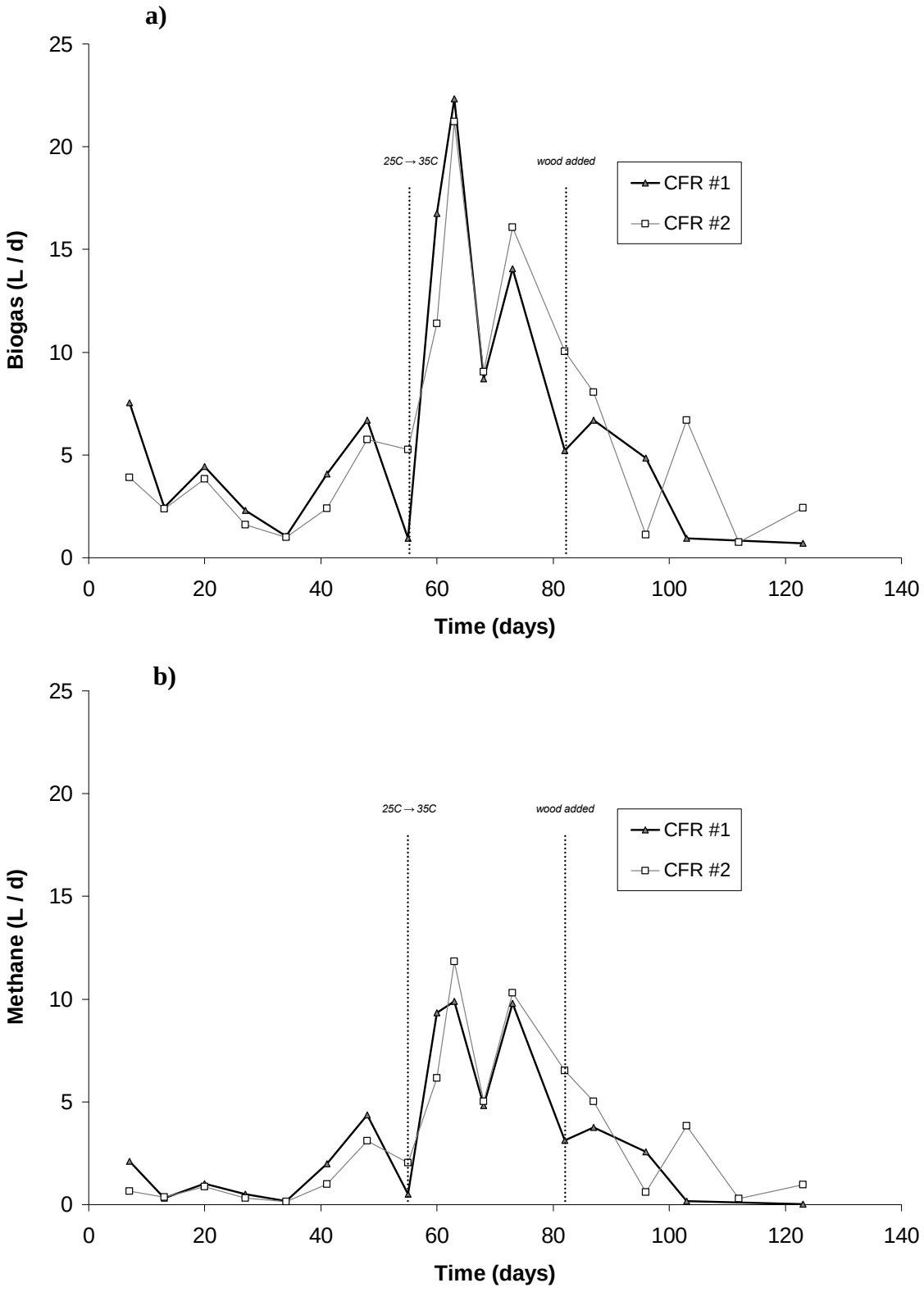


Figure 2.3. Biogas and methane production by semi-continuous flow reactors (CFRs). a) biogas production L/d; b) methane production (L/d).

On Day 82, both digesters were switched from horse waste without bedding to stall waste (horse manure intermingled with softwood bedding). The horse stall waste with bedding contained about 25% wood chips by wet weight or approximately 0.4 g bedding VS per g manure VS. Although the overall mass loading of VS remained the same, the wood was not expected to be degraded or converted to methane under these conditions (Gunaseelan, 1997). Thus, the readily available VS and the effective VSLR were expected to decrease by up to 30%, accordingly, when the feedstock was switched from manure to stall waste. Methane production indeed declined upon switching to stall waste, and reached levels that were lower than those observed during operation at 25°C. The overall biogas production declined to approximately 14% of that produced by manure alone after Day 82 (**Figure 2.3**), much greater than the expected decrease that could be caused by the lower degradability of the wood bedding. The methane content of the biogas also decreased from 57 ± 13% from Day 60 to 96 to 9 ± 9% from Day 103 to 123 for reactor 1 and 59 ± 6% from Day 60 to 96 to 39 ± 1% from Day 112 to 123 for reactor 2. On some days following addition of stall waste in reactor A, the percent methane in the biogas was <1%. It was hypothesized based on these results that addition of stall waste (including the softwood chips) may have inhibited the microbial community and methane production, through the presence of anti-microbial compounds (Belmonte et al., 2006; Savluchinske-Feio et al., 2006).

2.3.2. Methane production potential of horse waste

To further investigate the effects of bedding type and the general digestibility of horse manure, five individual batch experiments (Exp. 1, 2, 3, 4 and 5)

were conducted as described in section 2.2.4. and Tables 2.2 through 2.6. In each of these experiments, the cumulative methane production from horse manure was determined over incubation times ranging from 33 to 79 days. The methane produced ranged from 70 to 120 mL over 40 to 60 days in the batch tests with 0.5 g horse manure VS added (Exp. 1, 2, and 3) and from 135 to 620 mL over 33 to 79 days in the batch tests with 2.38 g horse manure VS added (Exp. 4 and 5). The methane production potential for horse manure at 35°C ranged from 45 ± 13 L/ kg VS to 114 ± 73 L/ kg VS over approximately 40 days of incubation to 134 ± 7 L/ kg VS over 79 days of incubation. (Note: one additional experiment produced 215 ± 17 L/ kg VS over 59 days.) The methane production potential of horse manure averaged over all batch experiments was 139 ± 65 mL methane per g horse manure VS, similar to that observed during CFR operation. Note that the inoculum alone produced an average of 0.4 ± 0.35 mL methane per 10 mL or 0.01 ± .01 mL methane per g VS over periods of 49 to 79 days of incubation. These levels were considered negligible and were not subtracted from the methane production values reported for other treatments.

2.3.3. Toxicity of softwood bedding and methane production potential of different bedding types

Exp. 1, 2 and 3 were performed to determine the effect of potentially toxic softwood bedding on methane production from horse manure. Methane produced in bottles with different bedding VS to horse manure VS ratios was expressed relative to the horse manure only control for each batch test. Since each treatment received the same amount of horse manure VS (0.5 g), the ratio of methane produced by each treatment

relative to the control treatment that received no bedding, was expected to be 1:1, since the wood was not expected to be highly biodegradable. If production was less than 1:1, then this would have suggested inhibition of methanogenesis by the presence of the wood.

As seen in **Figure 2.4**, contrary to the hypothesis, fresh softwood bedding did not appear to substantially inhibit methane production relative to controls over a wide range of loadings from 0.01:1 to 4:1 softwood bedding VS to horse manure VS. The average of the ratios of methane production in treatments receiving softwood bedding relative to the controls with horse manure only was 0.84 ± 0.24 (average ± one standard deviation), i.e. less than 1 However, there was no indication of a dose response wherein higher ratios of softwood bedding resulted in successively less methane production relative to controls. Further, analysis of variance of data from all treatments in Exp. 1, 2 and 3, indicated that there was no statistically significant difference between the methane production relative to the control (p = 0.36), nor was there a statistically significant difference between groups of treatments receiving softwood bedding (p = 0.19). Taken together, these results indicated that regardless of the amount of fresh softwood bedding present in the stall waste mixture, the full amount of potential methane production would be realized from the degradable horse manure fraction contained in the waste mixture. No apparent toxicity or inhibition was observed.

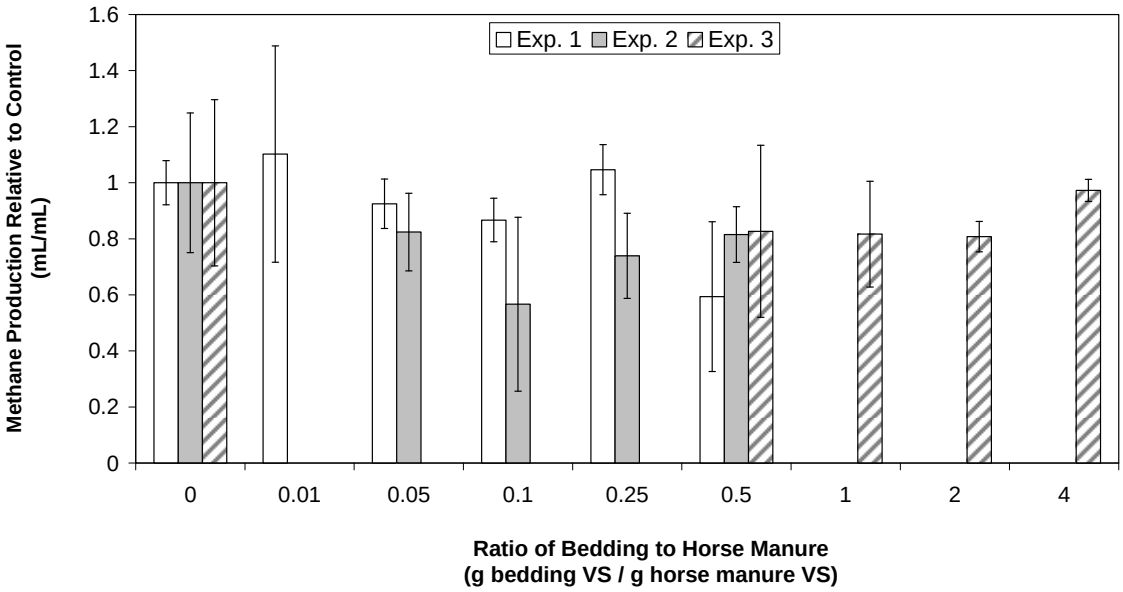


Figure 2.4. Methane production relative to manure only controls in batch anaerobic reactors amended with differing ratios of fresh softwood bedding VS to horse manure VS from Exp. 1, 2 and 3. Values are averages of triplicate bottles and error bars are one standard deviation.

In Exp. 3 the effect of Woody Pet® and straw on methane production was additionally examined (**Table 2.4**). Bottles containing manure and Woody Pet® produced 32 ± 8 mL methane over 46 days of incubation (**Figure 2.5**) or 72 ± 51 mL methane per g VS. Methane production from Woody Pet® alone (0.75 ± 0.19 mL) was nearly identical to that observed from fresh softwood bedding alone (0.85 ± 0.22 mL), yet all bottles containing a mixture of Woody Pet® and manure produced approximately 40% more methane than did bottles with manure alone, with similar methane concentrations (<1% different). Bottles containing manure and straw bedding produced 75 ± 33 mL methane over 46 days of incubation (**Figure 2.6)** or 111 ± 58 mL methane per g VS. Straw alone produced nearly identical methane volumes (27 ± 6 mL) as manure alone (26 ± 8 mL) and bottles containing manure and straw produced two to almost five times as much methane as mixtures of manure with softwood bedding depending upon the manure to

bedding ratio**.** Clearly, use of straw as bedding would result in higher production of methane than use of softwood bedding.

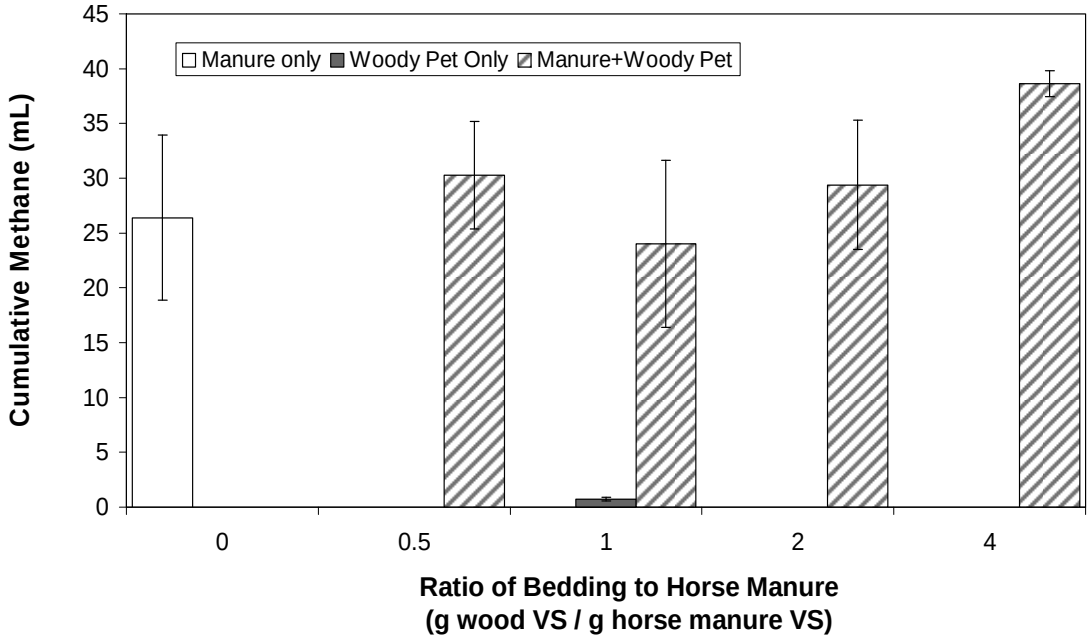


Figure 2.5. Methane production in batch anaerobic reactors amended with differing ratios of Woody Pet® to horse manure VS. Values are averages of triplicate bottles and error bars are one standard deviation.

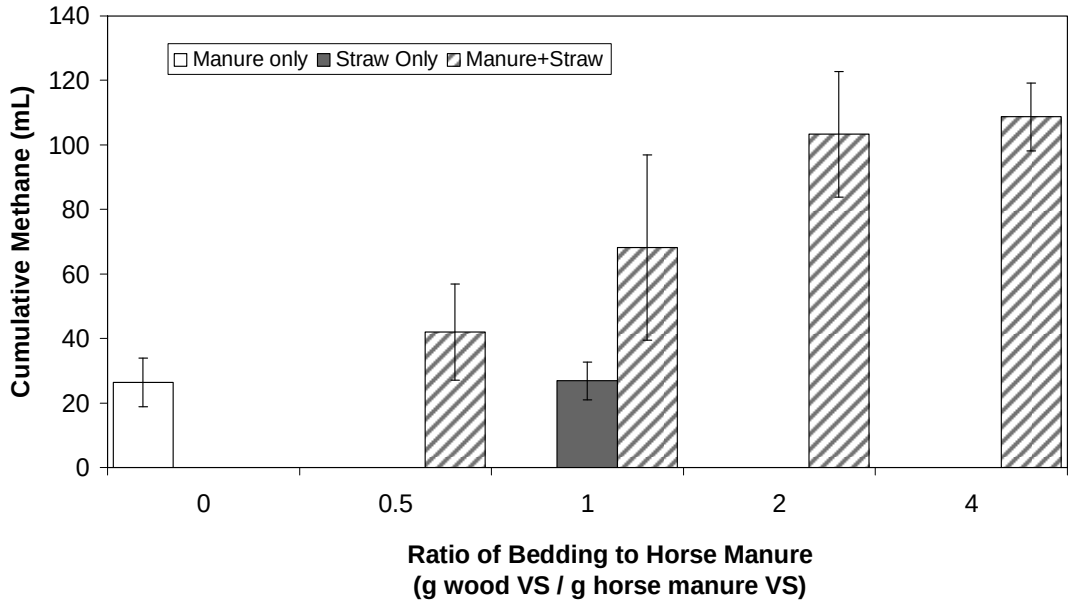


Figure 2.6. Methane production in batch anaerobic reactors amended with differing ratios of straw to horse manure VS. Values are averages of triplicate bottles and error bars are one standard deviation.

Exp. 4 utilized used softwood bedding that had been manually separated from stall waste, in addition to fresh softwood bedding, for testing for inhibition of methanogenesis. It was hypothesized that bedding that had been used and aged in stalls while exposed to urine, moisture and biological activity, may have been different than fresh, unused bedding with respect to the presence or availability of resin components that could be toxic to microbes. Used softwood bedding was added at ratios of 0, 0.25, 0.5, 1, 2, and 4 g bedding VS to g horse manure VS. However, results showed no inhibition caused by the presence of used softwood bedding, regardless of the amount added (**Figure 2.7**). Moreover, the presence of the bedding contributed positively to methane production with the manually separated, used softwood bedding producing 39 ± 10 mL methane per g VS added. This confirmed that not only is the softwood bedding non-inhibitory to the anaerobic digestion process, but suggests that separation of the bedding from the manure prior to recovery of bioenergy, a process that could be desirable to reduce reactor volumes or avoid mechanical problems caused by wood particles, would result in a loss of recoverable energy. Whereas it was initially presumed that the increase in methane production from the presence of the manually separated, used softwood bedding was due to small manure remnants that adhered to the wood particles, visual observations indicating particle breakdown suggested the possibility of anaerobic breakdown of the softwood bedding itself. Based on these observations, the amount of softwood bedding that was degrading and its potential for conversion to methane, if any, was further investigated in Exp. 5 (methods described in section 2.2.4 and Table 2.7; results described in section 2.3.4).

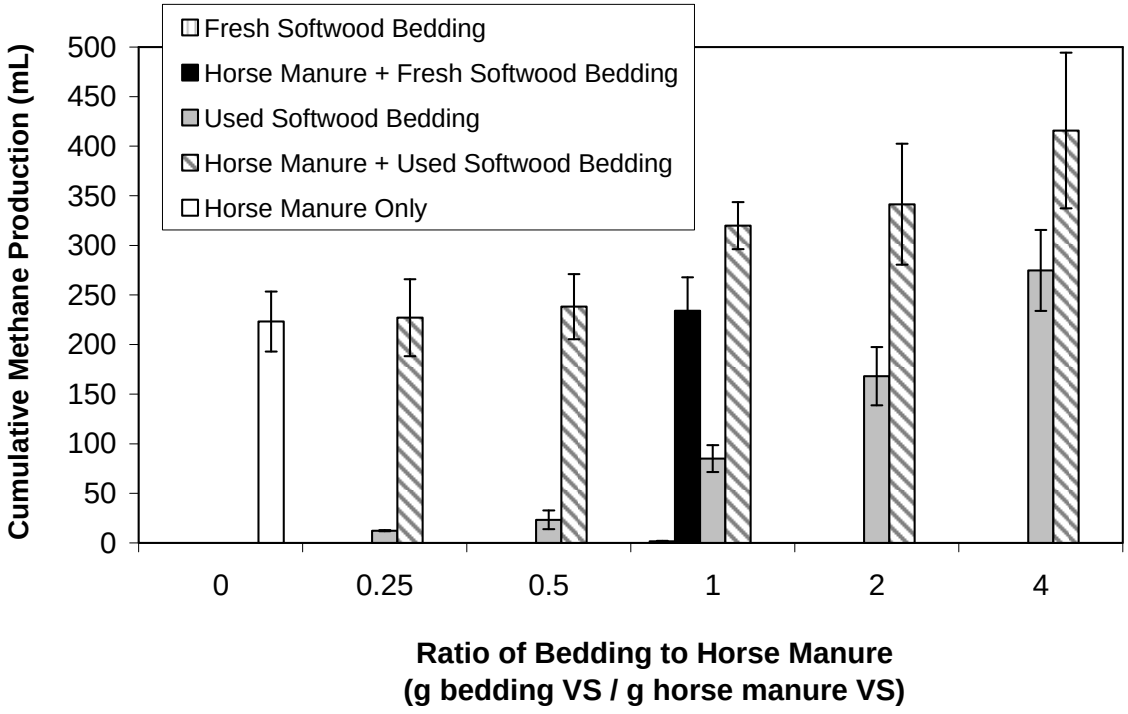


Figure 2.7. Methane production from stall waste as the ratio of used softwood bedding VS to horse manure VS is increased from 0.25 to 4 with comparison to horse manure only and fresh softwood bedding controls, Exp. 4. Values are averages of triplicate bottles and error bars are one standard deviation.

The biogas produced during Exp. 4 had similar methane concentrations regardless of softwood bedding addition (**Figure 2.8.**). It is important to note that methane concentration did not change with increasing concentration of wood, showing again that the presence of wood was not inhibitory to methanogenesis.

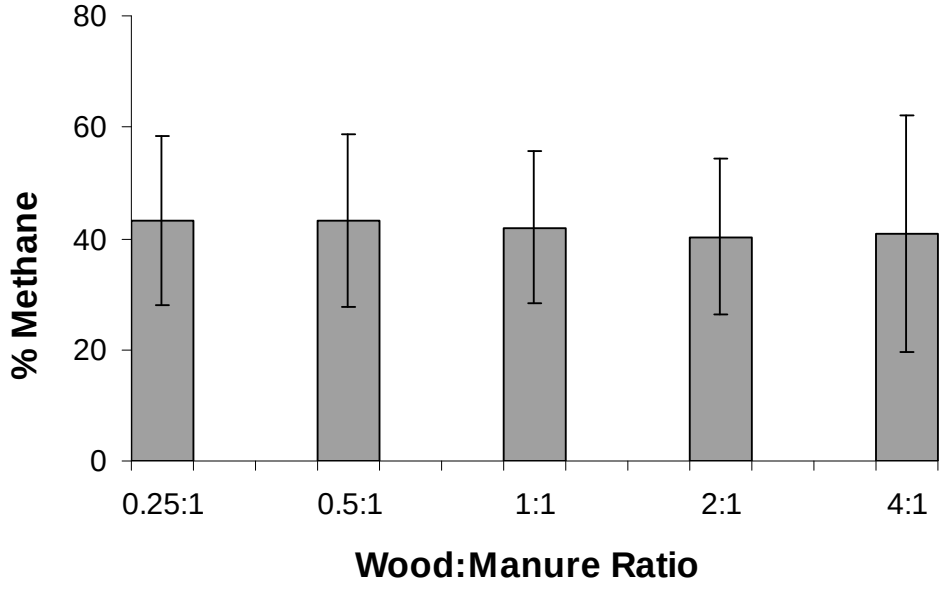


Figure 2.8. Methane content of the biogas at different loading ratios for bottles containing manure and used softwood bedding.

Because the inoculum produced negligible methane (only 0.4 ± 0.35 mL per 10 mL inoculum or per bottle) during each batch experiment, any significant methane produced from bottles containing only inoculum and bedding must come from the conversion of the added bedding. A variety of tests were performed to determine the amount of methane produced from fresh and manually separated softwood bedding, Woody Pet® and straw. In particular, tests were performed to determine whether methane produced from used softwood bedding was produced only from the manure solids adhering to the wood chips or if some of the biogas / methane was being produced from the degradation of the wood itself. Therefore, during Exp. 5 methane production from softwood bedding alone was examined. As can be seen from the results (**Figures 2.9** and **2.10**), a substantial amount of methane relative to the inoculum (control) was produced from those bottles containing only inoculum and fresh softwood bedding. The relative amounts were approximately proportional to the ratios of wood added and

indicated that some wood was being converted anaerobically into methane. The methane production potential of the softwood bedding was 20.0 ± 4.6 mL methane over 33 days of incubation (**Figure 2.9)** or 8.4 ± 1.9 mL methane per g VS added.

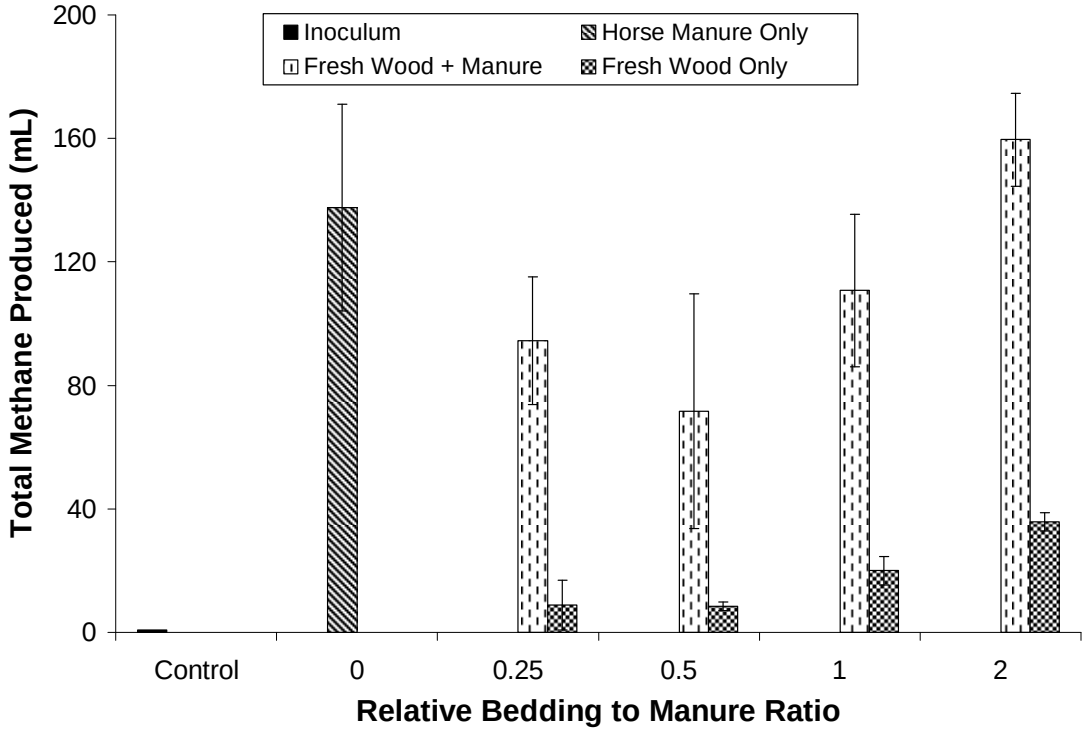


Figure 2.9. Methane production from fresh softwood bedding versus methane production from the mixture of fresh softwood bedding with manure at different bedding amounts relative to manure amount added (VS basis). Values are averages of triplicate bottles and error bars are one standard deviation.

2.3.4. Methane Production Over Time (Exp. 4)

Time progression of methane production was followed for all experiments. Methane production over time during Exp. 4 appears to have peaked shortly after Day 10 for all bottle types, with a decline in methane production evident near Day 30 (**Figure 2.10**).

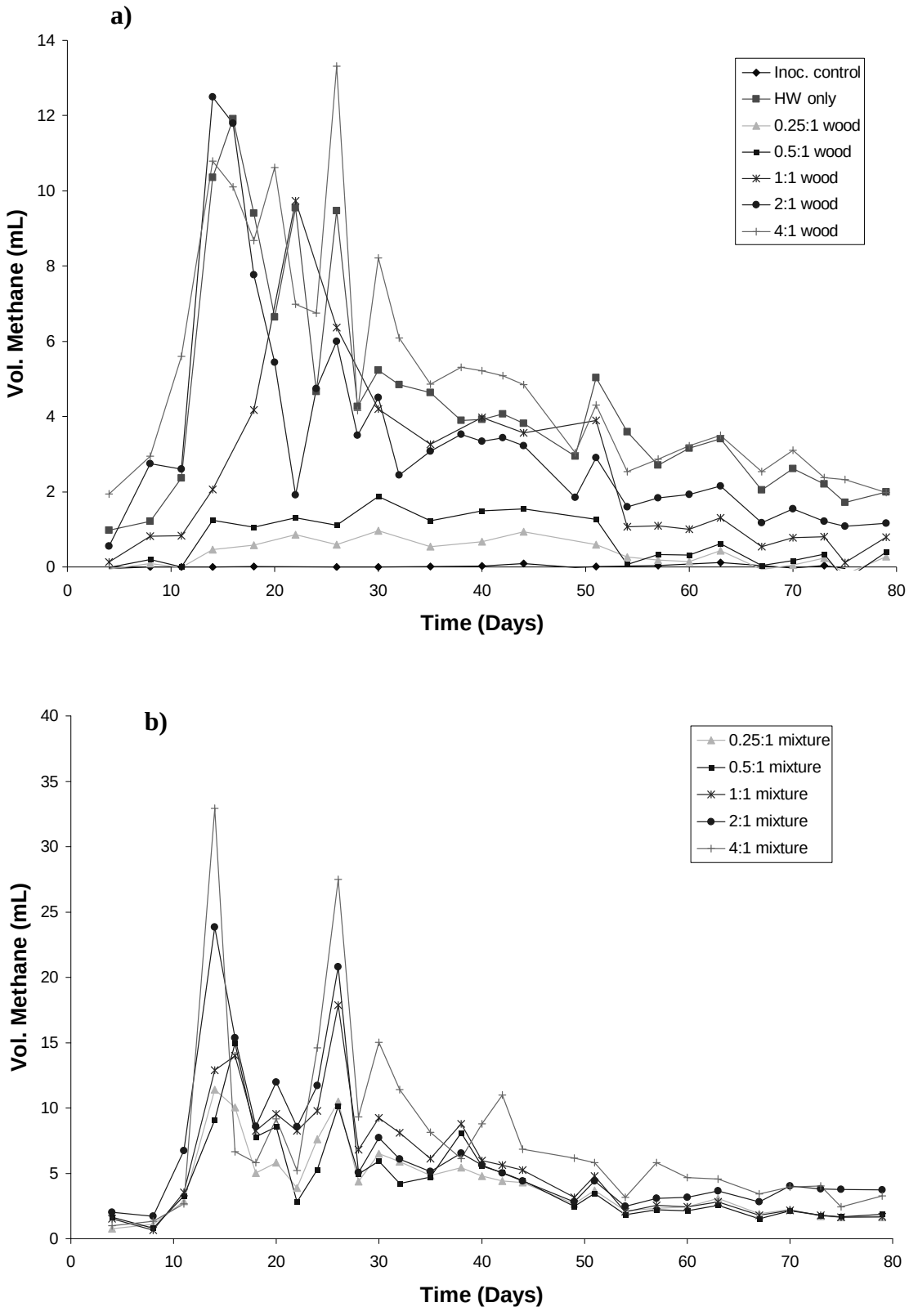


Figure 2.10. Average methane generation over time for batch bottles containing:
a) inoculum, horse waste (HW) only, and (used) wood only
b) horse waste mixed with (used) wood bedding

2.3.5. Anaerobic Digestion of Horse Waste in 125 L Solid State Batch Reactors

Biogas and Methane Production.

Methane production from high solids (41.3 ± 2.5 % TS) stall waste (manure co-mingled with softwood pellet bedding) obtained from Oxbow Stables, Hamburg, NJ was evaluated to determine if the methane production potential realized in batch serum bottle studies as described in sections 2.3.2 and 2.3.3 could be attained at larger scales and in static systems. Biogas production from static piles of waste that were initially amended with inoculum at only 2% volume:volume ratio began almost immediately after reactor startup. The biogas production was initially 30 ± 3 L/d from Days 6 to 12 for digester #1. Operation of digester #2 was not as planned because of a problem with the temperature sensor. The reactor temperature reached approximately 46°C several times prior to switching to a new sensor. Immediately prior to changing the faulty sensor, digester #2 was producing 42 L/d from Days 5 to 7. Following installation of the new sensor, 25 L/d biogas was produced from Days 8 to 9 and then the biogas production decreased gradually over the next 34 days. Beginning on Day 13, biogas production from digester #1 also began a gradual decline over the next 36 days. It was expected that as the waste digested, solids content would decrease and the moisture content of the pile would increase. However, little leachate was observed in the reactors and the dry conditions were confirmed when the lid of each digester was removed and it was observed that only the top layer of the pile (perhaps moistened by initial 2 L of inoculum) had been at least partially degraded. The remaining stall waste appeared to resemble its initial condition. On Days 50 and 43, each digester was reinoculated with 10% volume:volume inoculum. After the addition of inoculum biogas production increased

and was greater than 30 L/d for digester #1 and approximately 30 L/d for digester #2 (**Figure 2.11.**).

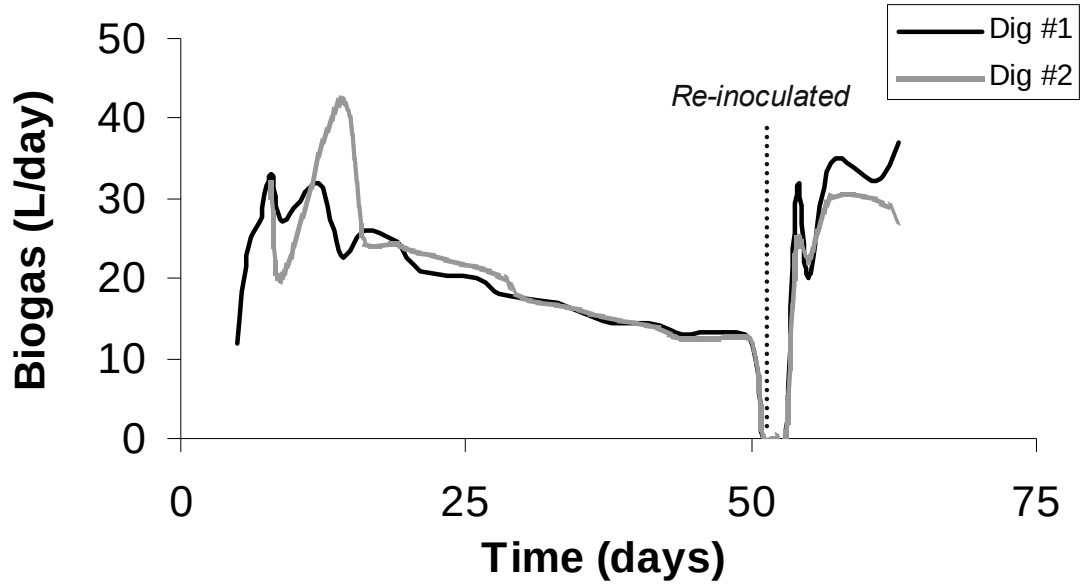


Figure 2.11. Biogas generation rate (L/day) from 125 L high solids batch digesters. The timeline reflects the calendar date with Day 0 as the start day for Digester #1 and Day 7 as the start day for Digester #2.

Methane concentrations exceeded 40% after approximately 12 days into each digester's run (**Figure 2.12**). Methane concentrations approached 50% (for digester #1) between Days 21 to 37, but then a slow but gradual decline in methane content occurred between Days 37 to 47. For digester #2, methane concentrations approached 45% between Days 12 to 21, but then a slow but gradual decline in methane content occurred between Days 21 to 37. It was hypothesized that the relatively low methane production could be a result of the low moisture content.

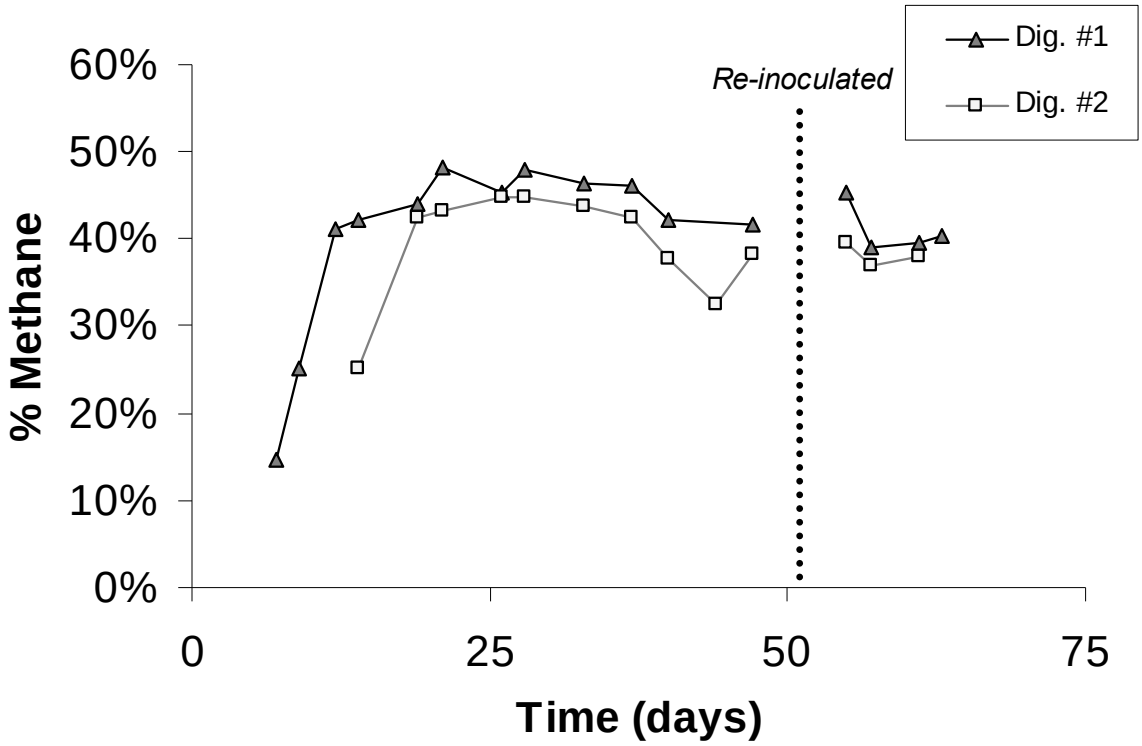


Figure 2.12. Methane content for 125 L high solids batch digesters. The timeline reflects the calendar date with Day 0 as the start day for Digester #1 and Day 7 as the start day for Digester #2.

Following re-inoculation on Day 43, methane concentrations measured on Day 54 were approximately 45% and 40% for digesters #1 and #2, respectively. The methane content dropped about 5% over the next several days, yet shortly afterwards, a gradually increasing trend was noticedobserved. The methane content following re-inoculation could have been influenced by the decay of the inoculum.

After 63 days, the cumulative amount of biogas produced was 1320 L for digester #1 and 1097 L for digester #2 (**Figure 2.13**). The cumulative biogas volume produced was 934 L and 766 L, respectively, for digesters #1 and #2, as ofby Day 50. Since 9.83 kg VS were initially loaded into each digester, 101 L biogas/kg VS and 78 L biogas/kg VS, or an average of 89 L biogas/g VS was produced. The corresponding methane production was 38.5 L/kg VS and 28.5 L/kg VS, for an average of 33.5 L/kg VS.

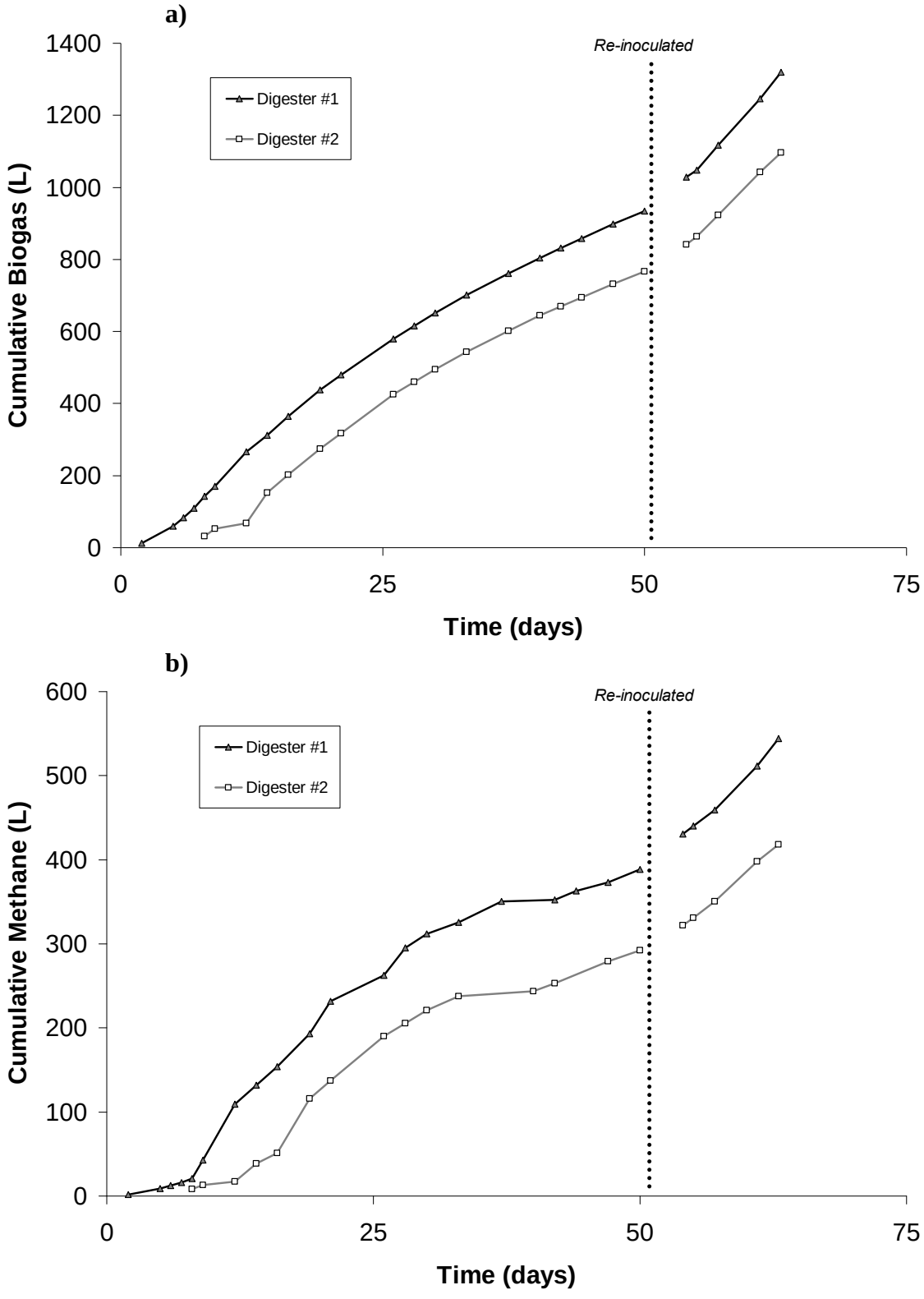


Figure 2.13. Gas production (L) from 125 L high solids batch digesters a) cumulative biogas production; b) cumulative methane production.

Leachate Characteristics.

Leachate samples for digester #1 were taken on Days 13 and 36, and showed an increase in pH from 6.7 to 7.5, respectively, the latter being typical of anaerobic digesters (Hartmann and Ahring, 2005). The inoculum that was added midway through the experiment also had a pH of approximately 7.5, and the digestate, which was measured on Days 55 and 65 had a pH of 7.5 on both occasions. Samples taken from digester #1 on Days 13 and 36 were analyzed for ammonia levels and showed an increase from no detectable ammonia (detection limit ~ 0.1 g TAN/L) on Day 14 to 0.26 g TAN/L on Day 36.

Solids and Water Balance.

Since the total solids of the stall waste was 41.3 %, the percent moisture was 58.7%. Since 29 kg of wet substrate was added, approximately 17 kg, or 17 L of water was added to each reactor. Additionally, the first inoculum added approximately 2 L of water to the reactors. We expected that much of this water would remain absorbed in the waste, but that small amounts would be available for recycle as leachate. There was much less leachate produced than expected (less than 1 L, cumulatively) over the first 40 days from digester #1, and no apparent leachate from digester #2.

Much larger quantities of leachate (digester #1 only) were generated after the second inoculation where 10 L of inoculum was added, compared to prior to re-inoculation (~1 L per re-cycling), although still less than anticipated. This discrepancy was presumed to be due to the high absorbance capacity of wood (Adhikari, et al., 2008), which likely had absorbed much of the water that was added via the inoculum. Digester

#2 continued to produce no leachate and a clog may have been present at the bottom of the digester.

A water balance on the reactors consisted of water in, which was 17 L in the waste originally and 12 L from two inoculum additions; and water out, which assuming a biogas water vapor content of 1.5% at 35°C, was less than 10 mL water lost for each digester. Thus the moisture content of the static waste pile was expected to increase (% TS decreasing) as VS was destroyed. The estimated VS destruction assuming conversion factors of 1.42 g COD/g VS and 0.35 L methane/g COD (Rittmann and McCarty, 2001) was approximately 1.1 kg VS for digester #1 and 0.8 kg VS for digester #2, using total methane produced by Day 63. Digesters were originally loaded with 29 kg waste at 41.3% TS and 82% VS. This corresponded to 12 kg dry solids and 17 kg water. If approximately 1 kg VS were destroyed over the first 63 days, and accounting for the approximately 12 kg water added by inoculum, the TS content at Day 63 would be approximately 27.5% TS or 72.5% moisture. (It is not known how much drainable water would accumulate in the bottom of the digesters at this solids content.)

2.4. Implications: Methane Production Potential of Horse Manure

There has been little published research on anaerobic digestion of horse waste. Kusch et al. (2008) conducted digestion trials under a variety of solid state reactor configurations and utilized stall waste where the manure was mixed with straw bedding. Experiments focused largely on the effect of the moisture content of the waste and inoculum types on methane production in addition to controlling acidification rates. The methane production potential of the manure-straw waste after six weeks was reported to

be approximately 170 L methane/kg VS added (under similar conditions as in this study). (*However, it is important to note that Kusch's experiment (2008) dealt with stall waste with straw as bedding, which was shown through batch experiments that it can produce equal to or greater methane yield per unit weight than the manure itself.)

The methane production for horse manure at 35°C ranged from 45 ± 13 L/ kg VS to 114 ± 73 L/ kg VS over approximately 40 days of incubation to 134 ± 7 L/ kg VS over 79 days of incubation. (Note: one additional experiment produced 215 ± 17 L/ kg VS over 59 days.)

It was determined that the average methane production potential of horse manure alone (from 160 mL batch experiments) was 139 ± 65 L methane/kg VS. In the CFRs, methane production was 148 ± 84 L methane/kg VS under the best operating conditions at a solids retention time of 24 days. Methane production for stall waste (horse manure plus bedding) ranged from 48 ± 5 L/ kg VS to 104 ± 13 L/ kg VS over 79 days of incubation. Finally, in static pile high solids batch reactors using stall waste, the methane production potential over 50 days averaged only 33.5 L/kg VS. These high solids digesters thus produced less methane than shaken batch systems saturated with moisture.

The methane production potential of horse waste is close to reported cattle manure values that ranged from 150 to 240 L methane/kg VS, but far lower than swine waste, for which values ranged from 280 to 360 L methane/kg VS (Liu, et al., 2009). These values are obviously dependent on the run times of the tests, since biogas production was still on-going even after 60 to 90 days in batch tests.

The estimated volatile solids removal efficiencies (methane yields) in the CFRs (35 and 38%) were similar to those reported by (Kusch et al. 2008) of 37 to 48% under

different reactor conditions. Note that Kalia and Singh (1998) investigated mixing horse waste with cow manure as a digester feedstock and reported that 20% replacement of cow manure by horse manure did not substantially reduce methane production or otherwise cause problems in small farm digesters.

With the methane production values obtained from the batch bottle experiments, the energy potential of horse manure alone (per month of peak activity) can be estimated to be 4 to 5 x 10^5 kJ/metric ton wet weight (**Table 2.7**). With the values obtained from our batch bottle experiments, it was estimated that the energy potential of stall waste was 2 to 4 x 10^5 kJ/metric ton wet weight (**Table 2.7**). For the 125 L high solids batch reactors, this value was approximately 1.3 x 10^5 kJ/metric ton wet weight. Since a horse produces approximately 13 wet tons (~ 14.3 metric tons) of stall waste per year (with bedding) (Westendorf et al., 2007; Wheeler and Zajaczkowski, 2002), this amounts to theoretical values of 2.87 to 5.73 GJ (10^6 kJ) generated per horse per year. In comparison, a typical home might consume 50 to 80 GJ per year for heating (Smil, 2005; Zambini, 2006).

Table 2.7 Energy potential (1 x 10^5 kJ/ metric ton wet wt) from methane generated under various conditions and varying bedding ratios [a].

Bedding (VS) to Horse Manure (VS) Ratio	0	0.25	0.5	1	2	4
Horse Manure	4.04 ± 0.72	--	--	--	--	--
Stall Waste	--	3.35 ± 0.56	3.09 ± 0.13	3.40 ± 0.39	2.88 ± 0.69	2.19 ± 0.33
Physically Separated Used (Aged) Bedding	--	1.17 ± 0.06	1.06 ± 0.55	2.35 ± 0.49	2.41 ± 0.46	1.97 ± 0.41
Fresh Bedding	--	0.19 ± 0.17	0.16 ± 0.03	0.30 ± 0.07	0.39 ± 0.03	--
Woody Pet® + Manure	--	--	3.00 ± 0.57	2.21 ± 0.61	2.36 ± 0.51	2.40 ± 0.16
Straw + Manure	--	--	4.51 ± 1.46	6.69 ± 2.81	8.60 ± 1.47	5.66 ± 0.40

[a]*Estimates are derived from 1-month periods of peak methane generation*

2.5. Conclusions

- Initial experiments in semi-continuous-flow reactors indicated that there may be some inhibition of methane production when softwood bedding is mixed with horse manure in stall waste;
- Subsequent batch experiments did not show any inhibition by mixing in fresh, unused softwood bedding, regardless of the relative amount added.
- The methane content of the biogas was also relatively uniform regardless of the relative amount of wood added.
- These results suggest that the presence of fresh softwood chips in mixed horse stall waste does not cause inhibition towards an acclimated anaerobic digestion process; however, this assumption should be applied cautiously.
- Moisture content and proper pre-treatment may be necessary depending on substrate characteristics.
- Stall waste appears to provide less methane per unit wet weight than horse manure alone, but the difference may be minimal compared to removal costs.
- This research should be continued by examining the effect of used bedding, and the acclimation aspects at a microbial community level.
- The softwood chips could cause mechanical clogging of digesters where piping or pumping is utilized. Further research is required to analyze these issues to determine whether it is appropriate to separate bedding from manure prior to digestion.

Chapter III. Thermophilic Anaerobic Co-Digestion of Equine Stall Waste and Food Waste

To be submitted to: *BioCycle*

3.0. Introduction

A 2008 U.S. EPA report (U.S. EPA, 2008) states that Americans throw away approximately 25% of the food they prepare, corresponding to 37 billion kg (100 billion lbs) of food waste produced in the US each year. Approximately 12.5% of municipal solid waste generated by an average US household consists of food scraps, which are organic and readily degradable (U.S. EPA, 2008). As an alternative to landfilling, composting and anaerobic digestion as separate bioprocessing applications have been widely researched and both processes have been implemented to reduce the costs of food waste disposal and to recover beneficial products from the waste (U.S. EPA, 2008; Lou and Nair, 2009).

Composting produces a stabilized product that can be used as a fertilizer and soil amendment, yet not all food waste generated is composted because of the need to collect the material separately from other wastes, and because of lack of food waste composting facilities. (For reviews of food composting literature see Schaub and Leonard, 1996; Fehr et al., 2002; and Lou and Nair, 2009.) Furthermore, composting as a means of waste removal is not ideal because although the process is intended to be aerobic, there is still some anaerobic activity during composting that generates reduced products including methane, ammonia and hydrogen sulfide that create odor and toxicity problems for nearby areas (U.S. EPA, 2008; Lundie and Peters, 2005). Finally, aerobic composting is

an overall energy consuming process, unlike anaerobic digestion. Anaerobic digestion provides a means of food waste disposal with the added benefit of using the methane produced for electricity generation or combined heat and power applications for nearby facilities (U.S. EPA, 2008).

Although most food waste is readily digestible by microorganisms, there are certain limitations that can affect the performance of a food waste digester. In particular, the pH, ammonia concentration and volatile fatty acid concentrations must be controlled to maintain favorable conditions for the anaerobic microbial consortium that carries out the degradation and conversion to methane (Winter and Knoll, 1989; Ward et al., 2008). One of the most common problems in anaerobic food waste digestion is the rapid accumulation of volatile fatty acids (VFAs), e.g., acetic, propionic and butyric acids (Buyukkamaci and Filibeli, 2004). As cellulolytic bacteria (see **Figure 3.1**) degrade complex polymers into monomers and simple substrates, fermentative bacteria convert these substances into short-chained organic acids and VFAs or their deprotonated counterparts, hydrogen (H_2) and carbon dioxide (CO_2) (Winter and Knoll, 1989). The most active fermentative bacteria in food waste digestion are known as acidogenic bacteria, so named because of their ability to produce fatty acid compounds (Kim, 2003). The acidogenic bacteria are able to function at wide pH levels from 4 to 10 and under various conditions that many other microorganisms cannot tolerate (Wang et al., 1999 ; Wu et al., 2009). As acidogenic populations increase, the VFA production increases correspondingly. Among the most common VFAs formed are acetate (acetic acid), propionate (propionic acid), and butyrate (butyric acid) (Buyukkamaci and Filibeli, 2004, Wang et al., 1999). Longer chain fatty acids are subsequently fermented to acetic acid

and H_2 by syntrophic proton-reducing bacteria (Winter and Knoll, 1989). Thus, acetate and H_2 are major end products of anaerobic fermentation and these are readily used by acetotrophic and hydrogenotrophic methanogens, respectively, that convert these substrates to methane (Winter and Knoll, 1989; Wang et al., 1999). Further, acetogenic bacteria may convert H_2 and CO_2 to additional acetate (Valdez-Vazquez et al., 2005). Acetogenic bacteria and methanogenic archaea grow more slowly and are more sensitive to changes in pH and other environmental conditions than the acidogenic bacteria (Valdez-Vazquez et al., 2005). Since acidogenic bacteria grow more rapidly than acetogenic bacteria and methanogenic archaea, they are often present in higher numbers and can degrade complex polymers into fatty acids and H_2 faster than the other two groups of organisms can utilize them. Consequently under some conditions, VFA concentrations can rapidly increase, leading to a drop in pH and a consequent decrease in methanogenic activity (Buyukkamaci and Filibeli, 2004), since methanogens do not function well below a pH of 6.5 (Liu et al., 2008). In general, methanogens are reported to have optimum levels of activity between pH 6.8 to 7.2 (Ward et al., 2008), although some studies have shown good performance levels up to a pH of 8.0 (Taconi et al., 2007; Hansen et al., 1998). After methanogenic activity is suppressed, it may be difficult to recover this activity without dilution and buffering of the digester content and re-inoculation with an active methanogenic population.

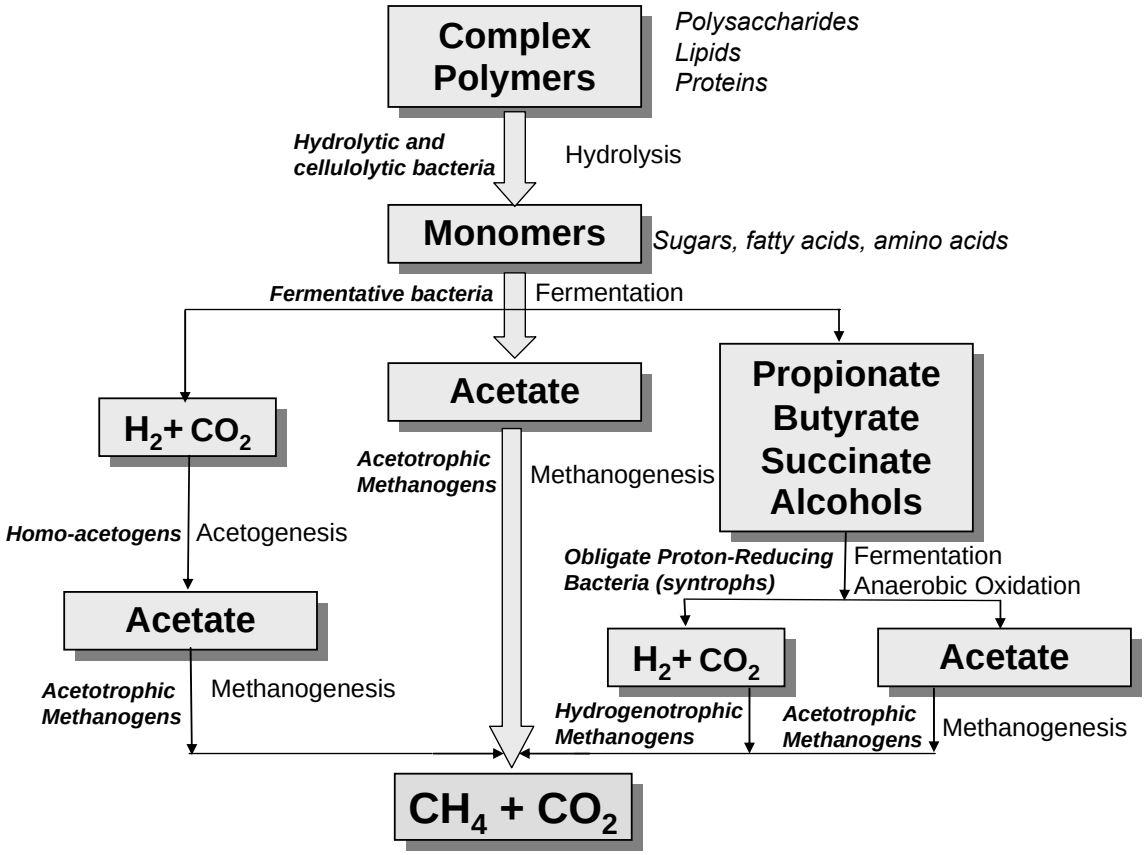


Figure 3.1. Major biodegradation pathways during anaerobic methanogenic degradation of organic material (after Rittmann and McCarty, 2001).

High concentrations of VFAs are often ultimately caused by overloading or rapid startup of digesters (McMahon et al., 2001), and have been a problem for food waste digestion, even when digester conditions and start-up are carefully controlled (Lim et al., 2008). This is because most food waste consists of produce and food products containing complex starches, which can be degraded rapidly by fermentative bacteria (Zhang, 2007). Many operators have tried to remedy this acidification problem by the addition of basic compounds to increase the alkalinity and the pH (Ward et al., 2008). Recently, rather

than the addition of chemicals, co-substrates have been added that either do not degrade as rapidly as food waste or that naturally have a strong buffering capacity (Ward et al., 2008).

Based on the susceptibility to acidification, most large-scale commercial digesters that receive waste food are not fed with this material as a lone substrate. Rather digesters operating in the US and Europe receive a mixture of substrates including waste food, often not more than 20 to 25% of the VS loading (Erdal et al., 2005; PIER, 2008), yard waste, manures, energy crops such as corn and other grain, or other non-food wastes (Minn. Dept. of Ag., 2005). The overarching goal of this part of the study was to examine whether horse manure or equine stall waste could serve as a co-substrate for food waste digestion. Most research pertaining to anaerobic manure digestion targets cattle, swine, and poultry waste, and very few studies have investigated anaerobic digestion of horse waste. The scarcity of research regarding anaerobic digestion of horse waste may be related to the fact that this source of waste biomass is smaller than that from other animal-based production agriculture in most parts of the world; that horse waste is a high solids material well-suited to aerobic composting applications; and because equine facilities tend to range in size from many small (one or a few horses) operations to fewer facilities with large (hundreds of horses) numbers of animals. However, several regions and states are heavily invested in the horse industry and house large numbers of equine animals. This large number of horses produces a large amount of waste that needs to be disposed of properly but also cost-effectively.

Anaerobic digestion of horse waste was investigated by researchers in India (Kalia and Singh, 1998; Mandal and Mandal, 1998), Nigeria (Zuru et al., 2004) and

Germany (Kusch et al., 2008). Additionally, there is unpublished research on anaerobic digestion of horse waste in the USA (Jewell, 2006). Kusch et al. (2008) extensively investigated solid state anaerobic digestion of horse waste mixed with straw bedding and digestion of this material in submerged static piles or in static piles undergoing leachate recycling.

There have been many successful experiments investigating the co-digestion of food or agricultural wastes with various animal manures (Callaghan et al., 2002, Hartmann, 2005). Full-scale systems have been implemented worldwide to provide heat and electricity. For example, such a facility provides energy for an apartment complex in Sweden (CADDET, 2000) and a two-megawatt co-generation system has been designed by Dean Foods and has recently begun operation in Massachusetts (Higgins, 2008). Furthermore, several recent experiments (Song et al., 2004 ; Bouallagui et al., 2008) have shown that co-digestion of other organic substrates with food waste rather than food waste only, led to greater methane yields, both in volume and concentration.

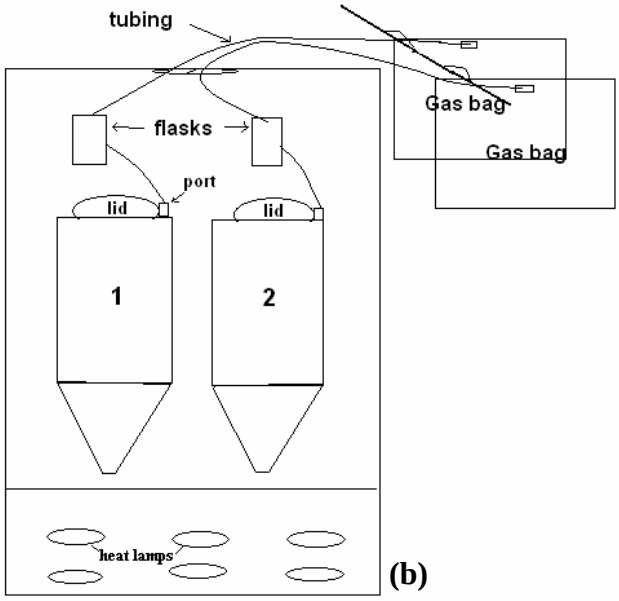
This project investigated co-digestion of equine stall waste and food waste under thermophilic (55°C) conditions. Specific objectives were to determine methane production potential from this co-mingled waste and to document stable performance during steady-state conditions.

3.1. Methods

3.1.1. Digester configuration

Two 20-L Minibrew® conical fermenters (Hobby Beverage Equipment Co., Los Angeles, CA) were utilized as replicate bench-scale digesters (identified as digester #1 and #2) (**Figure 3.2**). The digesters were stored in a constant temperature incubator at 55 ± 2°C. The lid of each digester was fitted with a ChemQuik HFC Series quick-coupling connecter (Cole-Parmer, Vernon Hills, IL), which was inserted through a drilled hole in the top of the fermenter and secured with a rubber and foam washer. Tygon® tubing connected the fitting in the lid of the fermenter to 40 or 80 L Tedlar® gas bags (CEL Scientific, Santa Fe Springs, CA) that were stored at room temperature outside the incubator. A 500 mL vacuum flask was placed in each line connecting the digesters to the gas bags to collect any condensation formed in the biogas collection system.

(a)



(b)

Figure 3.2. The 20 L digesters used for thermophilic co-digestion of food and horse waste; a) photograph of digesters ; b) diagram of digester configuration.

3.1.2. Inocula

Digesters were inoculated with 1.8 L municipal thermophilic digester sludge obtained from the Orange Water and Sewer Authority, Mason Farm Wastewater Treatment Plant, Carrboro, NC and 11 L mesophilic digester sludge obtained from the Joint Meeting of Essex and Union Counties Sewerage Authority, Elizabeth, NJ. In addition to digester sludge, 1.5 L of pre-digested horse manure and a small quantity (100 mL) of pre-digested food waste were added to the digesters, bringing the total volume to 14.4 L, slightly below the maximum initial digester operating level of 15 L.

3.1.3. Feedstocks

The feedstock was a 50:50 mixture of horse waste and food waste on a volatile solids (VS) basis. Stall waste consisting of co-mingled manure and softwood bedding was obtained periodically from horse stalls at the New Jersey Agricultural Experiment Station (NJAES) Animal Care Program on the Cook Campus of Rutgers University, New Brunswick, NJ. Food waste was obtained periodically from Rutgers University dining halls. The waste was either collected from salad bars or consisted of waste meal preparatory material that had been ground to form a slurry. Care was taken to make sure no fish or meat was present in the food waste; however, batches obtained later in the study likely contained proteinaceous material such as eggs, beans and cottage cheese. Feed stocks were stored in separate containers, each waste was mixed thoroughly, sampled for solids analyses, and then stored at 4°C prior to use.

3.1.4. Digester Operation

The digester volatile solids loading rate (VLSR) target was 3 g VS/L-d with a corresponding mixed liquor suspended solids content of 12.5% TS or approximately 100 g VS/L (assuming 80% VS). The estimated solids retention time (SRT) was approximately 45 days. The feeding and wasting protocol based on this original design and assuming 40% VS conversion is shown in Table 3.1. The actual wet weight loadings were adjusted to reflect the actual TS and VS content of a particular batch of feedstocks. The digesters were operated as replicates in a semi-continuous mode and were opened, stirred, wasted and fed according to the target protocol to achieve the VSLR every three to four days. During the wasting and feeding process (draw and fill), the digester lid was removed, the contents were mixed thoroughly, and 150 to 300 mL of digestate was removed for various analytical measurements. The remaining desired amount of digestate was wasted, feedstock was added, the lid was replaced and the headspace was purged with nitrogen to remove air.

The biogas collected in the gas bags was measured using a wet test meter (Precision Scientific, Chicago, IL) every three to four days. The gas volumes produced were measured at atmospheric pressure.

Table 3.1. Target feeding protocol for operation of 20 L thermophilic anaerobic digesters fed stall waste and food waste.

Feed Component Target Loading[a]	Stall waste	Food waste	Water
Wet Mass Loading (g wet wt./d)	70-125	70-484	60-100
Wet Mass Loading (g wet wt./feeding)	210-500	220-1940	150-300
Total Solids Loading (g TS./d)	25-40	50-100	--
Total Solids Loading (g TS./feeding)	75-160	150-400	--
Volatile Solids Loading (g VS/d)	22.5	22.5	--
Volatile Solids Loading (g VS/feeding)	67-90	67-90	--

[a] *Feedstock loadings were calculated based on a design VSLR of 3.0 g VS/L-d, a solids retention time of 45 days, a presumed VS conversion of 40%, a presumed MLVS of 100 g VS/L and a digester volume of 15 L. Feedings were performed every 3 to 4 days.*

3.1.5. Analyses

The methane concentration in the biogas was analyzed via a 0.5 mL gas sample collected at atmospheric pressure using a glass-Teflon®-stainless-steel gas-tight syringe equipped with a side port needle (Valco® Precision Sampling, Baton Rouge, LA) and injected into an Agilent® 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a GS-GasPro capillary column (30 m x 0.32 mm I.D.; J&W Scientific, Folsom, CA) and a flame ionization detector. Helium was the carrier gas at constant pressure 131 kPa (19 PSI). The oven temperature was held at 150°C. The resulting chromatographic peak area was compared to a five-point calibration curve

prepared using mixtures of 0 to 100% methane created by mixing volumes of methane (99% purity; Matheson Tri-Gas, Inc., Montgomeryville, PA) and air in a 0.5 mL gas-tight syringe (Valco® Precision Sampling, Baton Rouge, LA). Volumes of biogas and methane produced were corrected and reported at standard temperature (25°C) and pressure (1 atm) using the ideal gas law.

Digestate samples were obtained at each feeding and measured for pH and alkalinity, total (TS) and volatile (VS) solids content, total ammonia nitrogen (TAN) and volatile fatty acids (VFAs). The pH and alkalinity were measured using an Oakton pH 510 pH/mV/°C meter (Fisher Scientific, Pittsburgh, PA) with an Oakton WD-35801-00 pH probe according to Standard methods (Clesceri et al, 1998).

For TAN determination, 1 mL samples were first centrifuged at 10,000 *g* (Eppendorf Model 5424, Westbury, NY) and then the supernatant was removed and filtered through a 0.45 μm, 25 mm nylon membrane syringe filter (Pall Corporation, East Hills, NY). The filtrate was diluted 1000:1 using milliQ water and analyzed using a Dionex® ICS-1000 Ion Chromatograph (Sunnyvale, CA) with a Dionex® CSRS Ultra II 4-mm cation column. The resulting chromatographic peak areas were compared to a five point curve generated from analysis of standards prepared over a concentration range from 0.0625 to 1.0 mM NH_4^+-N/L, according to standard methods (Clesceri et al, 1998). The corresponding free ammonia (NH_3-N) concentration was determined by equation 3.1, where the pH was the prevailing digester pH at the time of sampling and the pka at 55°C is 8.4.

$$NH_3\text{ - N (mg N/L)} = \frac{TAN}{1+10^{pka\text{-}pH}} \qquad \textbf{Equation 3.1}$$

The samples that were centrifuged and filtered for TAN determination were also used for organic acid analysis. The filtrate was diluted 20:1 using milliQ water and then analyzed on a Beckman Coulter® System Gold™ HPLC (Beckman-Coulter, Inc., Fullerton, CA) using a Bio-Rad® Aminex HPX-87H organic acid analysis column (Bio-Rad Laboratories, Hercules, CA). Detection was by UV at a wavelength of 210 nm. The column was held at 60°C, and the eluent, 5.0 mM H_2SO_4, was configured at a flow rate of 0.6 mL/min. Chromatographic peak areas for samples were quantified by comparison to standard curves over a concentration range from 1 mM to 10 mM for acetic, propionic, and butyric acids (Sigma-Aldrich Co., St. Louis, MO). The total VFA concentration was determined by summing the molar amounts of each individual acid and converting it to a mg acetic acid/L unit.

Samples were taken alternately from one of the two digesters before every feeding. Solids analysis, performed according to standard methods (Clesceri et al, 1998) was by drying known masses of waste overnight in ceramic dishes at 100-105°C, cooling in a desiccator, and subsequently weighing to determine the % TS. Samples were then incinerated in a muffle box furnace at 550°C for approximately two hrs. Samples were cooled in a desiccator and subsequently re-weighed and compared to the TS to determine the %VS.

Periodically, samples of stall waste, food waste, digestate and inoculum were sent to Dairy One Laboratories, Ithaca, NY, and analyzed for % crude protein, (acid detergent) fiber and lignin.

3.2. Results and Discussion

3.2.1. Digester Operational Periods

The digesters were operated for 241 days at a constant VSLR of approximately 3.0 g VS/L-d. Over the first 100 days (~2 solids retention times) acclimation occurred based on trends in digester parameters such as pH, alkalinity, MLVSS and biogas production. Between Days 90 and 142, a steadier operational period occurred and average "steady-state" data are presented for this period. After Day 142, some digester upsets occurred that led to deterioration in digester performance (described in section 3.2.8.)

3.2.2. Feedstock Characterization

Food waste was obtained periodically as available from different Rutgers University dining halls. The particular type of food waste used for feeding the digesters thus changed every two to six weeks, depending on availability. In general, the waste food consisted largely of fruits and vegetables, with lettuce often being the primary component. Other items such as rice and flour were sometimes mixed in as well. The solids contents of the food waste ranged from 5.6 to 31.4% TS and from 81.7 to 94.9% VS, with an average of 12.6 ± 7.6% TS and 89.5 ± 4.5% VS over the course of operation. Solids content of horse stall waste ranged from 22.4 to 39.4% TS and from 79.8 to 91.7 % VS, with an average of 32.6 ± 7.5% TS and 87.1 ± 5.4% VS (**Table 3.2**).

At the start of the "steady-state" runtime beginning on Day 90, the food waste had a TS content of approximately 30% whereas it previously was less than 20% TS (**Table 3.2**). It was also suspected that because this waste was taken from a salad bar, rather than

consisting of ground preparatory materials, it may have contained beans, cottage cheese, and hard-boiled eggs, significant protein sources. Hard-boiled eggs were observed visually. The protein content of the feedstocks was measured twice, on Day 1 and on Day 84 (**Table 3.3**). The crude protein of the food waste was approximately 1% of the dry weight, while that of the stall waste was approximately 4% of the dry weight. However, no further testing was performed and it is not known whether higher protein contents resulting from a new food source may have resulted in less optimal digester performance over time (see section 3.2.9.)

Table 3.2. Results of solid analyses for digester substrates.

Days	[a]Sample	avg TS%	avg VS%	min TS%	max TS%	min VS%	max VS%	days used
1-37	**FW1**	**9.66%**	**93.06%**	8.79%	10.56%	91.94%	93.78%	37
38-59	**FW2**	**6.55%**	**84.59%**	4.72%	10.66%	76.80%	93.54%	22
60-65	**FW3**	**5.93%**	**91.67%**	5.66%	6.20%	91.24%	92.11%	6
66-72	**FW4**	**not tested**	**not tested**	not tested	not tested	not tested	not tested	7
73-83	**FW5**	**16.42%**	**88.42%**	15.55%	17.29%	86.75%	90.09%	11
84-94	**FW6**	**12.79%**	**94.86%**	12.73%	12.85%	94.67%	95.04%	11
94-159	**FW7**	**31.43%**	**81.70%**	28.43%	34.80%	79.08%	84.13%	66
160-181	**FW8**	**19.11%**	**88.89%**	17.90%	20.12%	87.02%	91.59%	22
182-185	**FW9**	**5.58%**	**83.32%**	4.13%	7.14%	81.75%	85.43%	4
188-212	**FW10**	**11.72%**	**92.86%**	10.02%	13.00%	91.75%	94.50%	25
213-219	**FW11**	**not tested**	**not tested**	not tested	not tested	not tested	not tested	7
220-222	**FW12**	**not tested**	**not tested**	not tested	not tested	not tested	not tested	3
223-229	**FW13**	**8.24%**	**91.28%**	7.55%	14.24%	91.24%	93.86%	7
233-241	**FW14**	**10.71%**	**93.96%**	10.70%	10.72%	93.72%	94.20%	9
1-83	**HW1**	**31.98%**	**79.81%**	25.60%	38.42%	73.57%	83.39%	83
84-135	**HW2**	**22.37%**	**90.72%**	21.85%	22.88%	89.85%	91.59%	52
136-229	**HW3**	**36.65%**	**86.06%**	31.28%	40.75%	84.68%	88.28%	94
233-241	**HW4**	**39.42%**	**91.66%**	34.11%	44.73%	90.53%	92.78%	9

[a] *Note: FW = Food Waste; HW = Horse Waste*

Table 3.3. Results of analysis of inoculum, food waste, stall waste and digestate (*Analysis performed by Dairy One Laboratories, Ithaca, NY*).

Sample	% Moisture	Crude Protein % dry weight	Acid Digestable Fiber % dry weight	Lignin % dry weight
Inoculum	93.3	1.0	2.6	0.6
Food Waste (Day 1)	91.4	1.8	3	1.1
Food Waste (Day 84)	89.9	2.2	3.2	1
Stall waste (Day 1)	48.1	4.6	30.1	6.9
Stall waste (Day 84)	73.4	4.2	15.1	5.1
Digestate #1 (Days 90-120)	91.4	1.2	3.3	1.3
Digestate #1 (Day 170)	93.3	1.0	2.6	0.6
Digestate #2 (Day 170)	92.4	1.0	3	0.8

3.2.3. Solids Analysis – Digestate

The total solids of the digestate from each digester averaged 10 ± 1.7% with extremes ranging from just above 6% to almost 14%. The volatile solids of the digestate from each digester averaged 71 ± 5.6%, with extremes ranging from just above 57% to almost 80% (**Figure 3.3**). The predicted solids content for the digesters was 12.5% TS and VS was assumed to be 80%. The fact that the TS value was slightly lower indicated that likely more of the feedstock (especially the food waste) was degrading than anticipated. The average VS of the feedstocks was greater than 80%.

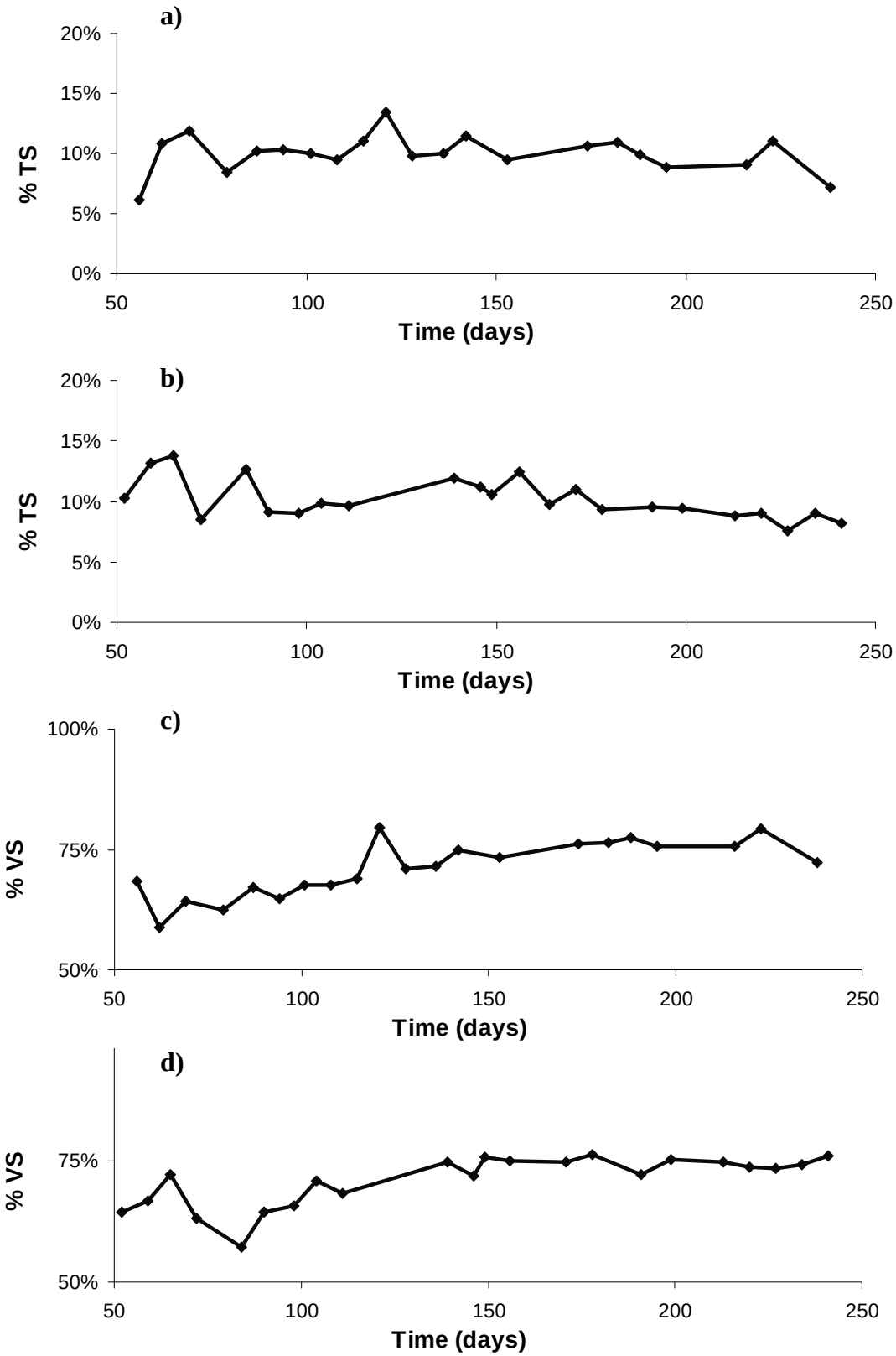


Figure 3.3. Percent total and volatile solids for each digester. **a)** and **c)** show results for digester #1 ; **b)** and **d)** show results for digester #2

3.2.4. pH and Alkalinity

Over an acclimation period of 90 days, pH (**Figure 3.4.a**) ranged from 7.59 to 7.87. During the stable period of operation from Day 90 to 141, the pH ranged from 7.84 to 8.04. Over the final days of operation from Day 142 to 241, the pH averaged 8.0 ± 0.34, with a peak of 8.15 on Day 142 for digester #1 and a peak level of 8.17 on Day 171 for digester #2. For digester #1, the alkalinity (**Figure 3.4.b**) ranged from 9.0 to 11.0 g $CaCO_3$/L over the first 90 days of operation, from 10.0 to 13.3 g $CaCO_3$/L from Day 90 to 141 and over Days 142 to 241, the alkalinity averaged 11.0 to 15.0 g $CaCO_3$/L. For digester #2, the alkalinity (**Figure 3.4.b**) ranged from 6.8 to 11.5 g $CaCO_3$/L over the first 90 days of operation, from 9.7 to 13.1 g $CaCO_3$/L from Day 90 to 141 and over Days 142 to 241, the alkalinity averaged 10.0 to 14.0 g $CaCO_3$/L.

During co-digestion of cattle manure and agricultural food waste, the pH was generally observed to be 7.5, subject to fluctuation, but reached a pH value of up to 8.1 in some studies (Alvarez, 2008; Macias-Corral, 2008; Liu 2009). Optimal performance of anaerobic digestion is prescribed to be at approximately pH 7.4, although some studies show higher methane yields with a pH closer to 7.0. Increased pH could suppress methanogens and lead to increased accumulation of VFAs, which in turn could further inhibit methanogenesis (Chen, 2007).

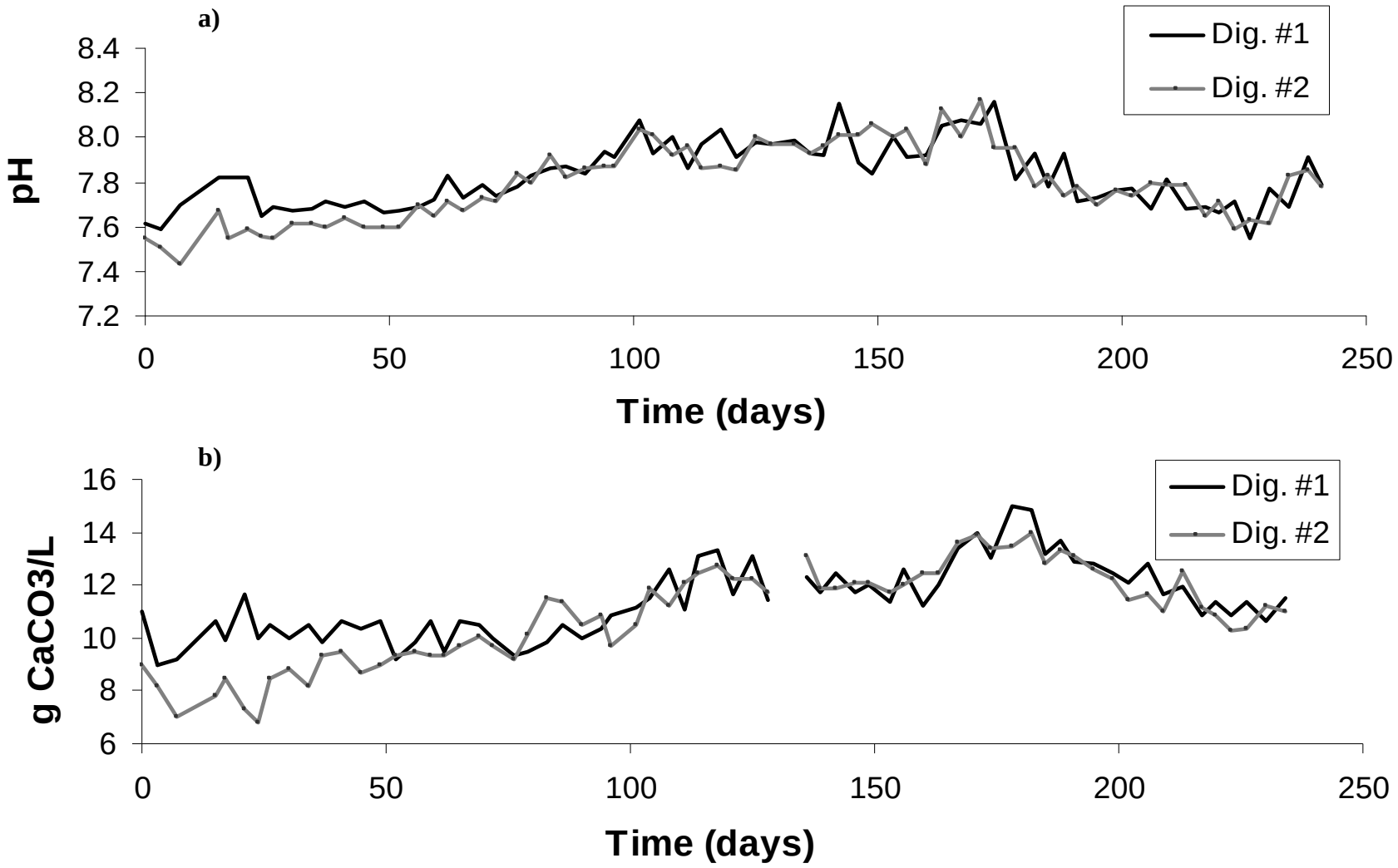


Figure 3.4. Thermophilic digester operational data: **a)** pH values over time for each digester; **b)** alkalinity values over time for each digester.

3.2.5. Biogas and Methane

During the initial part of the acclimation period (Days 1 to 40), the biogas production averaged 12.8 ± 3.7 and 11.8 ± 3.4 L/d, for digester 1 and 2, respectively (data not shown).

Methane contents (**Figure 3.5**) ranged from 27.2 to 64.5% of the biogas and were greater than 60% several times for each digester. During this time, the average methane generation rates were 6.4 ± 2.4 and 6.5 ± 2.1 L/d, for digester 1 and 2, respectively.

Over the remainder of the acclimation period, Days 40 to 90, biogas production averaged 13.3 ± 4.9 and 12.9 ± 2.9 L/d, with methane content gradually increasing from 40% to near 60% of the biogas with average methane contents of 47.3 ± 4.6% and 43.6 ± 7.3%. During this time, the methane generation rates were 6.0 ± 2.4 and 5.6 ± 1.7 L/d.

Peak percent methane values occurred for both digesters on Day 101, reaching 69.0% and 64.7%, respectively, for digester 1 and 2 (**Figure 3.5**). From Days 90 to 142 (the "steady-state" period), the biogas production rate was 16 ± 3.9 and 14.2 ± 1.6 L/d, for digester 1 and 2, respectively, with methane contents ranging from 44.9 to 69.1%, with an average of 56.1 ± 6.5% and 56.1 ± 6.6%. During this time period, the methane generation rate was 8.87 ± 2.1 L/d and 8.4 ± 1.2 L/d.

Over the remainder of the experiment, Days 146 to 241, biogas production was 15.2 ± 3.8 and 14.8 ± 3.8 L/d, for digester 1 and 2, respectively, and the methane content of the biogas dropped sharply, several times below 40% (see section 3.2.9.). During this time, the methane generation rates were 6.3 ± 1.8 and 5.2 ± 1.5 L/d, for digester 1 and 2, respectively.

Methane produced over the course of the entire experiment ranged from 291 to 441 L/ kg VS-d and 255 to 434 L/kg VS-d, with averages of 356 ± 61 L/ kg VS-d and 328 ± 60 L/ kg VS-d, for each digester, respectively (see **Figure 3.6.**)

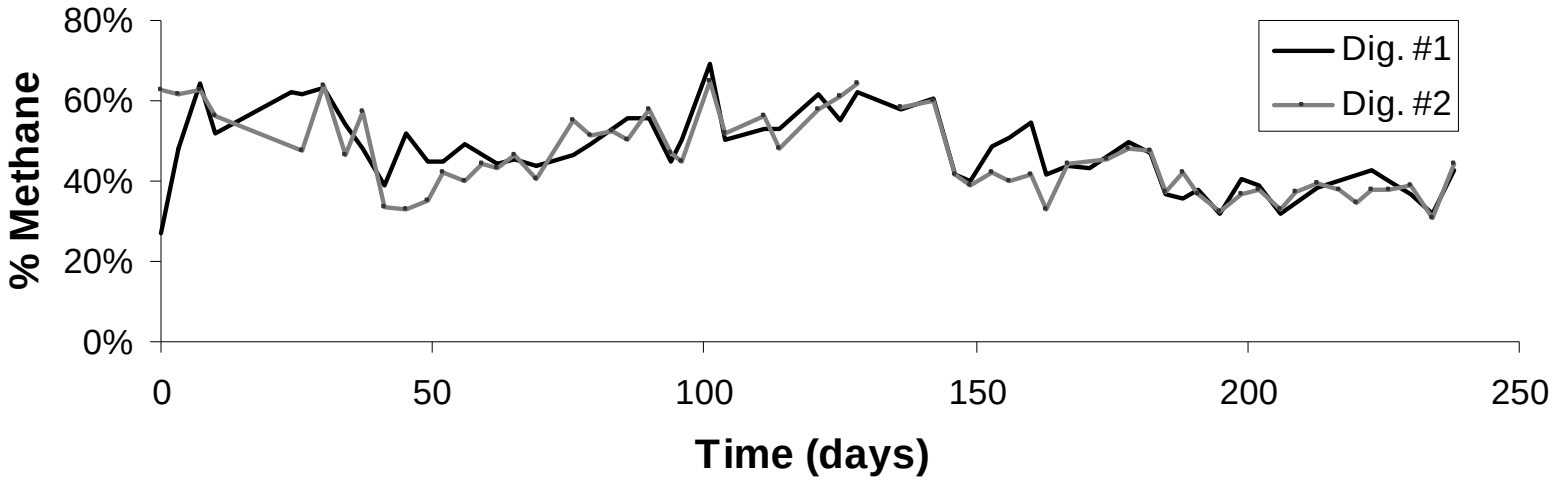


Figure 3.5. Methane content of the biogas for thermophilic anaerobic digesters fed food waste and stall waste.

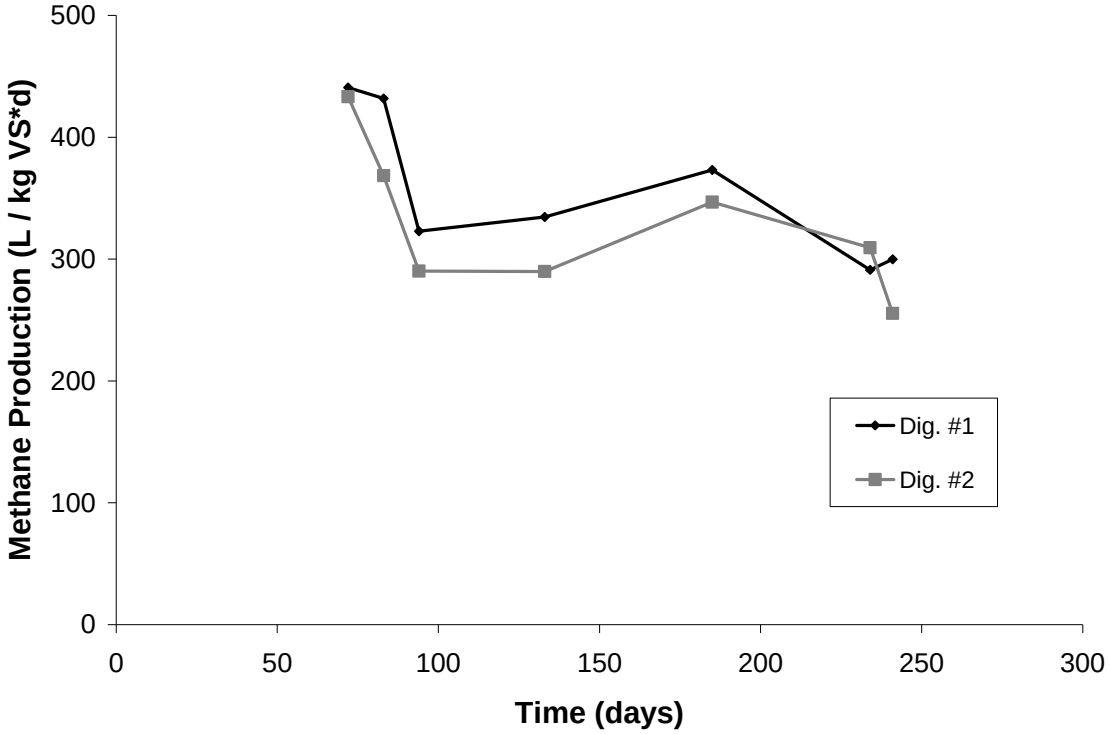


Figure 3.6. Methane generation rates for thermophilic anaerobic digesters fed food waste and stall waste.

3.2.6. Ammonia

Digestate samples from both digesters were analyzed for Total Ammonia Nitrogen (TAN) and free ammonia was calculated according to equation 3.1 (**Figure 3.7**). TAN was first measured on Day 62, near the end of the acclimation period. The TAN was 1.95 g NH_4^+-N/L, which is a normal, non-inhibitory value (Chen et al, 2008), and the corresponding free ammonia concentration was estimated to be 413 mg NH_3-N/L, also generally considered to be in the normal range (Hansen et al., 1998).

On Day 91, at the beginning of the steady-state period, the TAN had increased to 2.66 g NH_4^+-N/L, and the corresponding free ammonia was calculated to be 580 mg NH_3-N/L. Throughout the remainder of the steady-state period (Days 90 to 141), it was

observed that the TAN increased, along with the free ammonia, near the end of this period. Most of the TAN analyses were performed between Days 188 and 241 and averaged 2.41 ± 0.38 g NH_4^+-N/L, corresponding to 473 ± 140 mg NH_3-N/L, with ranges from 1.75 to 3.15 g NH_4^+-N/L and 332 to 797 mg NH_3-N/L.

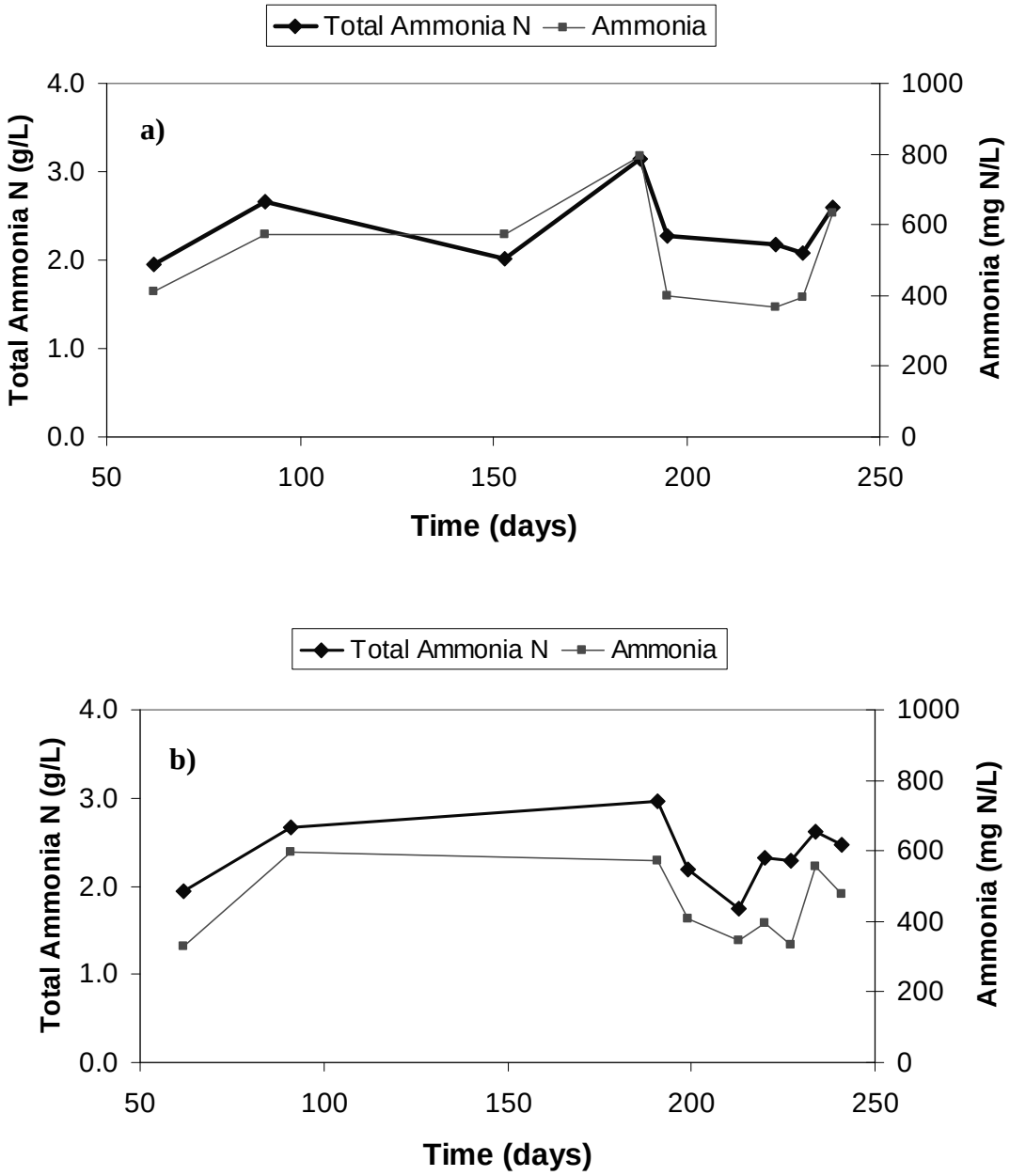


Figure 3.7. TAN and free ammonia in digestate from anaerobic digesters fed food waste and stall waste: a) Ammonia-nitrogen and free ammonia for digester #1; b) Ammonia-nitrogen and free ammonia for digester #2.

3.2.7. Volatile Fatty Acids (VFAs)

Results of the limited number of VFA analyses that were performed for each digester are shown in Table 3.4. VFA concentrations near the start of the steady-state period (Days 90 to 141) were well below inhibitory levels (< 30 mg/L) and no detectable amounts of propionic or butyric acid were found. Over the next 100 days, VFA levels increased only slightly; however, one elevated measurement with propionic, butyric, and iso-butyric acids all present at substantial concentrations, was observed on Day 195. The implications of this increase are discussed further in section 3.2.9.

Since food waste is readily degraded by fermentative bacteria (see section 3.1), it could yield fatty acid concentrations far greater than those produced in wastewater treatment plants or industrial sludge (Lim et al., 2008; Buyukkamaci and Filibeli, 2004). Typical concentrations for frequently-fed high-solids anaerobic food waste digesters range from 5 to 30 g/L total VFAs, depending upon environmental conditions (Lim et al., 2008). Yet most anaerobic digesters are not designed to produce such high VFA concentrations, particularly because methanogens only perform well with total VFA levels below 1000 to 1500 mg/L (Malina and Pohland, 1992). The most dominant fatty acid is either acetic acid or propionic acid, depending on conditions and substrates in the digester. Some studies have shown that even concentrations up to 2 to 3 g/L propionic acid do not inhibit methanogenesis (Pullammanappallil et al., 2001), while other studies (Buyukkamaci and Filibeli, 2004; Wang et al., 1999) have demonstrated the negative effect of any individual VFA even at concentrations less than 1000 mg/L.

Table 3.4. Average lactic acid and VFA concentrations (mg/L) measured during operation

Organic Acid	Digester 2 Day 72	Digester 1 Day 101	Digester 1 Day 153	Digester 1 Day 195	Digester 2 Day 227
Lactic	ND	14.4	16.8	64.8	9.6
Acetic	10.8	12	14.4	518.4	81.6
Propionic	ND	ND	ND	38.4	8.4
Iso-Butyryic	ND	ND	ND	33.6	13.2
Butyric	ND	ND	ND	22.8	ND
Total Organic Acids	10.8	26.4	31.2	678	112.8

ND = Not Detected (below detection limit)

3.2.8. Summary of "Steady Condition" Operation.

A summary of the average operating conditions for each digester during the steady operational period between Days 90 and 142 are shown in Table 3.5.

Table 3.5. Summary of "steady" operating parameters (Days 90 to142) during thermophilic anaerobic digestion of food waste and stall waste mixtures.

	pH	Alkalinity (g $CaCO_3$/L)	Methane Produced (L/kg VS-d)	Methane Concentration (%)	Methane Yield (% VS converted)
Digester #1	7.95 ± 0.06	11.67 ± 1.11	356 ± 61	56.1 ± 6.5	29%
Digester #2	7.93 ± 0.06	11.51 ± 0.95	328 ± 60	56.1 ± 6.6	34%
Average	7.94 ± 0.08	11.59 ± 1.01	342 ± 60	56.1 ± 6.6	31%

The pH values were moderately higher than many reported anaerobic digestion studies (Macias-Corral et al., 2008 ; Ward et al., 2008) and those recommended by Kusch et al. (2008) for digestion of horse waste. It has generally been advised to keep the pH below 7.5 (Ward et al., 2008; Kusch et al., 2008) for effective methanogenesis. Despite the somewhat elevated pH, the methane content of the biogas was well over 50%, making the biogas readily useable for combustion (Earle et al., 1991). The percentage of volatile solids that were converted to methane, 31%, was higher than reported by Bouallagui et al. (2008) for food waste with activated digester sludge and several other co-substrates (10-11%), but somewhat lower than the values reported by Alvarez and Liden (2008) for co-digestion of food and animal wastes (50-67%).

3.2.9. Digester Failure

On Day 142, the pH reached a high in digester #1 of 8.15 (digester #2 peaked at 8.06 on Day 149), and a high alkalinity (12.4 g $CaCO_3$/L) was also observed. On Day 146, the methane content of the biogas dropped by almost 20% for both digesters. The trend of low methane content continued through Day 156 for digester #2 although digester #1 began to recover. On Day 163, the methane content for both digesters decreased again, to 40% and 33%, respectively. It was speculated that this may have occurred because a new batch of food waste was utilized beginning on Day 160. The new food waste had a lower TS content (19%) and a higher VS content (88%) than previous batches, and did not contain any noticeable proteinaceous materials. However, on Day 167, the alkalinity began to climb and peaked between Days 178 and 182 at 15.0 and 14.0 g $CaCO_3$/L for digester 1 and 2, respectively.

As was well documented (Chen et al., 2005; Hansen et al., 1998), high free ammonia levels can greatly affect methanogenic populations. The high pH on Day 142 may have reduced methanogenic activity, allowing a buildup of VFAs to occur, leading to a subsequent drop in pH in digester #1, yet no noticeable change in pH or alkalinity occurred for digester #2 and it did not recover. On Day 185, when the second decrease in methane content occurred, the pH had spiked for both digesters with values of 8.05 and 8.13, respectively, and measurement on Day 163 showed much higher alkalinity levels (~14 g/L $CaCO_3$), which continued until Day 185 when another decrease in percent methane occurred. On this Day (185), the alkalinity levels also dropped, indicating an increase in volatile fatty acids. Consequently, this would have be expected to lower the pH, which it did. It is interesting to note, however, that immediately preceding these events, the feedstock was also changed, and it contained mostly lettuce with a low solids content of 5.9% TS.

The methane content rose slightly during Days 211 to 241, with the pH of digester #2 dropping to 7.65 and 7.59 on Days 217 and 223, respectively. The pH increased for the following two weeks to about 7.8. The pH of digester #1 did not drop until Day 226 (7.55) and fluctuated between 7.69 and 7.91 for the remainder of the experiment.

3.3. Conclusions

The results of this experiment showed that co-digestion of waste food and stall waste is feasible at thermophilic (55°C) temperatures. Wood that was present in the stall waste did not seem to have a negative effect on the anaerobic digestion. Methane content of the biogas was often greater than 50% and the volatile solids conversion rates were

estimated to be between 29% to 34%, comparable to many other co-digestion studies (Alvarez and Liden, 2008; Macias-Corral et al., 2008). The average methane production was 356 ± 61 L/kg VS-d. The VSLR (2.2 kg VS/m^3-d) was relatively low and the SRT (45 days) was relatively long for a thermophilic system. This represents a low rate system. However, this study aimed to determine the success of the operation at a relatively moderate loading rate initially, without risking reactor upsets while trying to reach higher VSLRs.

An important observation was that alkalinity increased over the course of the reactor operation. At the end of the operational period, the methane content of the biogas was less than 50%, indicating potential upset of the methanogens. Unfortunately, with the limited data on ammonia and VFAs, there is limited ability to diagnose the exact causes for the decrease in methanogenesis.

This experiment was followed by the startup of a 6 m^3 pilot scale digester operated in a similar manner. This study (see Chapter 4) was carried out at the Rutgers University EcoComplex in conjunction with EarthPledge and continued to investigate the co-digestion of food and horse wastes.

Chapter IV. Pilot-Scale Anaerobic Co-digestion of Food Waste and Horse Waste

To be submitted to: *BioCycle*

Note: This chapter describes a collaborative study involving the Rutgers University Department of Environmental Sciences, The Rutgers EcoComplex (Bordentown, NY) and EarthPledge (New York, NY). Mr. Daniel Macready obtained feedstocks, operated the pilot scale anaerobic digester, measured alkalinity and pH, and collected operating data under the supervision of Mr. Eugene Reiss and Mr. David Specca. EarthPledge financed the installation of the digester via funding from the United States Environmental Protection Agency, Mitsubishi International Foundation, and the Ittelson Foundation. The digester was designed by Dr. John Ingersol, Eco Corp, Inc. Mr. Greg Loosevelt of EarthPledge provided extensive technical advice and input into digester operation.

4.1. Introduction

In 2007, the New Jersey Agricultural Experiment Station completed a report entitled "Assessment of Biomass Energy Potential for New Jersey" (Brennan et al., 2007). Two goals of this report were to assess (1) the characteristics and quantity of NJ biomass resources; and (2) technologies (commercially or near commercially available) that are capable of producing biopower or biofuels from NJ biomass resources. From this report, it was determined that NJ produces 8.3 million dry tons of biomass that could be used for renewable energy. It was determined that depending upon the conversion

technologies used, this biomass could deliver either ~9 % of the current electricity demand or ~ 5% of the current transportation fuel demand of NJ.

Food and agricultural wastes were reported to be among the most important potential NJ biomass feedstocks for energy conversion. Brennan et al. (2007) reported that as of 2007, about 286,000 dry tons of food waste was recoverable as a biomass source and that approximately 79% of this waste food is currently landfilled. Common means of disposing of food waste other than landfilling include composting, incineration, and gasification. However, because food waste often has a high moisture content, these options may not be practical (Zhang et al., 2007). Technology assessment for the NJ biomass waste stream identified anaerobic digestion as one of the available technologies that could be most useful for converting available wastes to bioenergy (Brennan et al., 2007). Currently, there are no anaerobic digestion facilities in NJ that process agricultural or food wastes.

Anaerobic digestion of waste food and source separated organic materials has been performed on a large scale in European countries for 30 years. (For a review of current technologies see Nichols, 2004.) A variety of digester configurations are marketed including: high solids, horizontal plug flow systems; upright completely mixed systems; and phase separated, two stage systems. Anaerobic digestion facilities in the US are limited primarily to those located at wastewater treatment plants and dairy and other animal manure digesters. According to the EPA AgStar Program, there were 135 animal manure anaerobic digestion facilities in operation in the US as of May 2009 (U.S. EPA, 2009b), but none of these are in NJ. Interest is growing in North America for further recovery of bioenergy from additional biomass sources. For example, recently the East

Bay Municipal Utility District (EBMUD) in Oakland, CA, began digesting food waste for energy recovery (U.S. EPA, 2009c); a two-megawatt co-generation system has been designed by Dean Foods that has recently begun operation in Lynn, MA (Higgins, 2008); and in Toronto, Ontario, Canada, the Dufferin Organics Processing Facility is testing a full scale 3600 m^3 anaerobic digester for treatment of source-separated organic waste gathered from residential and commercial sources (Van Opstal, 2006).

Anaerobic digestion produces methane for electricity generation or combined heat and power applications for nearby facilities, while providing a means of food waste disposal (U.S. EPA, 2008). Although microorganisms can readily digest most food waste, there are certain limitations that can affect the performance of a food waste digester. In particular the pH, volatile fatty acid (VFA) concentrations, and ammonia concentration must be controlled to maintain favorable conditions for the anaerobic microbial community that carries out the degradation and conversion of the organic matter to methane (Winter and Knoll, 1989; Ward et al., 2008). A common problem in anaerobic food waste digestion is rapid accumulation of volatile fatty acids (Buyukkamaci and Filibeli, 2004) resulting in suppression of pH and inhibition of methanogens. (For a more extensive review see **Chapter 3** of this thesis.) After methanogenic activity is suppressed, it may be difficult to recover this activity without dilution and buffering of the reactor content and re-inoculation with an active methanogenic population.

High concentrations of VFAs may be caused by overloading reactors or by attempting rapid startup (McMahon, et al., 2001), and have been a problem for food waste digestion, even when these reactor conditions and start-up are carefully controlled (Lim et al., 2008). This is because food waste contains abundant complex starches,

which can be degraded rapidly by acidogenic, fermentative bacteria (Zhang, 2007). Operators have attempted to remedy this by the addition of basic compounds to increase the alkalinity and boost the pH (Ward et al., 2008). Recently, rather than the addition of chemicals, co-substrates have been added that either do not degrade as rapidly as food waste or that naturally have a strong buffering capacity (Ward et al., 2008).

Based on the susceptibility to acidification, most large-scale commercial digesters that receive waste food are not fed with this material as a lone substrate. Rather, digesters operating in the US and Europe receive a mixture of substrates including waste food (often not more than 20 to 25% of the VS loading (Erdal et al., 2005; PIER, 2008)), yard waste, manures, energy crops such as corn and other grain, or other non-food wastes (Minn. Dept. of Ag., 2005).

While there are many other waste biomass sources in NJ, the largest source of recoverable agricultural livestock waste in 2007 was equine waste at 102,400 dry tons, greater than the amount of all other agricultural livestock wastes combined (Brennan et al., 2007). Equine waste could serve as a buffering co-substrate for food waste digestion in New Jersey. Thus, the overarching goal of this study was to examine whether these two large sources of waste biomass, horse manure or equine stall waste and waste food could serve as a co-substrates for anaerobic digestion. The feasibility of horse waste as a single substrate for anaerobic digestion was shown in **Chapter 2**. Further, in **Chapter 3**, it was shown that co-digestion of food waste and horse waste was successful at the 15 L scale. Here, the implementation of anaerobic digestion of food waste using equine waste as a co-substrate at the pilot scale (6.3 m^3) is described.

In 2007, the Rutgers University EcoComplex in conjunction with EarthPledge initially implemented an anaerobic digester utilizing food waste as a single substrate. The digester experienced upsets characterized by low pH early on and a suitable and likely co-substrate was sought. During the research described in **Chapter 3**, semi-continuous thermophilic anaerobic digestion of a 50:50 (VS:VS) mixture of equine stall waste and food waste was examined at the 15-L scale in anticipation of using a similar combination at the 6 m^3 scale using the EcoComplex digester. The EcoComplex digester is to serve as a prototype long-term semi-continuous feed digester for larger and/or more widespread uses around New Jersey and the US.

4.2 Digester System Description and Operation

[Provided by Mr. Daniel Macready, Mr. David Specca and Mr. Greg Loosvelt.]

4.2.1. Inoculum

The Orange Water and Sewer Authority (OWASA) in Carrboro, NC provided 3.4 m^3 (910 gal) digestate from their thermophilic anaerobic digester at their Mason Farm Wastewater Treatment Plant on June 19, 2008. At the time of delivery, the pH was 7.3, total solids were 2%, total volatile solids were 60%, ammonia-nitrogen was 6%, and total nitrate/nitrite was undetectable. Table 4.1 shows the analyses of the OWASA digestate over the months preceding delivery. An additional 0.6 m^3 (146 gal) of tap water was added to bring the initial volume in the digester to a total of approximately 4 m^3 (1056 gal). The temperature was gradually raised to 57°C (135°F) and held constant thereafter.

4.2.2. Feedstocks

The digester feedstock consisted primarily of a mixture of waste food and horse waste. Waste food was obtained by Mr. Daniel Macready on a biweekly basis from Whole Foods, Inc., Marlton, NJ. From Day 1 to 26 stall waste consisting of horse manure and softwood chip stall bedding at a ratio of approximately 1:1 on a VS basis was used as a co-substrate. The stall waste was obtained from horse stalls at the New Jersey Agricultural Experiment Station (NJAES) Animal Care Program on the Cook Campus of Rutgers University. Because of concern for system components clogging from wood particle buildup, from Day 28 to 344, horse manure alone (with no stall bedding) was added as a co-substrate. Horse manure largely absent of any other contaminating material was obtained from outdoor loafing sheds located at the NJAES Animal Care Program.

The ratio of waste food to horse waste varied over the course of operation as the digester was started up to eventually reach 204 kg (450 lb) wet solids per feeding every two to three days, maintained at a 3:1 ratio of food waste to horse manure on a VS basis. The corresponding volatile solids loading rate (VSLR) was thus slowly increased to approximately 1.87 kg VS per m^3-d over the course of 372 days of operation.

Late in the study, after 287 days of operation, a trial amount of Streufex® pelleted straw bedding (Magna (MEC), Aurora, Ontario, Canada) was added to the digester in addition to a small amount of recycled office paper. The purpose of the addition of these materials was to increase the C:N ratio.

Table 4.1. Results of analysis of the Orange Water and Sewer Authority (OWASA) digestate from a thermophilic anaerobic digester used as inoculum for the startup of the EcoComplex digester (courtesy of OWASA).

	Sample or Composite Date	1/07/08	3/03/08	5/13/08
	Percent Solids	2.11%	2.13%	2.05%
Units in (mg/kg) unless denoted as (%)	Arsenic	<4.74	<4.69	<24.4
	Cadmium	0.711	0.47	<2.44
	Chromium	43.3	44.1	39.4
	Copper	359	418	361
	Lead	12.6	22.1	18.4
	Mercury	0.758	0.892	0.966
	Molybdenum	11.1	15	<48.8
	Nickel	13.6	15	<24.4
	Selenium	10.3	<4.69	<24.4
	Zinc	815	869	756
	Total Kjeldahl Nitrogen	10.60%	11.90%	12.40%
	Ammonia-Nitrogen	5.47%	8.17%	6.25%
	Nitrate and Nitrite	0%	0%	0%
	Total Phosphorus	4.68%	3.66%	5.37%

4.2.3. Digester Configuration and Operation

Photographs of the EcoComplex digester system (courtesy of Professor A.J. Both) are shown in **Figure 4.1**. The system consists of: an external feed hopper with two-way auger; a Rotacut RC 1500 E in-line macerator (Vogelsgang, Ravenna, OH) with electric motor; a polyethylene pre-mix tank mixed by a stainless steel 90 GPM submersible sewage pump (Meyers Pentair Water, Ashland, Ohio) and heated by a Process Technology Derated Triple Metal L-Shaped stainless steel heater (Process Technology, Mentor, Ohio); a Vogelsang V100 QHD reversible rotary lobe feed pump, powered by a Nordbloc helical gearbox (Nord Gear Corp., Charlotte, NC); an upright cylindrical

digester tank; and a polyethylene waste tank (Snyder Industries, Inc., Lincoln, NE). The system was operated as a semi-continuously fed reactor with feedstock added every two to three days. The upright cylindrical stainless-steel digester tank held a maximum of 6.3 m^3 (1675 gal). The temperature inside the digester was maintained at 57°C (135°F) via the heat trace and insulation. The heat was supplied by a Chromalox SRM/E self-regulating, energy efficient heating cable (Chromalox, Inc., LaVergne, TN). The Chromalox cable was aluminum taped to the exterior of the digester tank. The entire tank was insulated with a minimum of 5 cm (2 in.) of Enamo-Grip spray-on polyurethane coating. The temperature was monitored via thermostats at depth in the top and middle of the tank contents. The heating cable was operated by an existing Argus environmental control system (Welland, Ontario, Canada).

The upright digester tank had no capability for mixing and was originally intended to be operated in a semi-continuous plug flow manner. The mixing regime (for example that might be imparted by biogas production) that actually occurred in the digester was not characterized and thus the operational status and the flow regime was unknown.

The waste levels in the pre-mix and waste tanks were measured with a tape measure (to establish volume) prior to beginning the feeding process. Duplicate 500 mL samples were taken from the digester via a sample port located on the side of the digester tank.

To commence feeding, pre-weighed feedstock was added directly to the external stainless steel hopper and an auger internal to the hopper was run in reverse while water was added, until an appropriate feedstock consistency (assessed visually) was achieved.

The auger was then switched to the forward direction and the feedstock was forced through a six inch stainless steel pipe toward a Vogelsang Rotacut RC 1500 E in-line macerator with electric motor. The particle size of the feedstock was reduced to approximately 15 mm (maximum) by the macerator.

After exiting the macerator, the feedstock was forced into an insulated 0.95 m^3 (250 gal) pre-mix tank, and a single 500 mL sample was taken as the feedstock entered the pre-mix tank. The level inside the pre-mix tank was again measured (to determine volume) after the flow ceased. Valves were then manually positioned to allow flow from the bottom of the digester tank into the pre-mix tank. The Vogelsang V100 QHD reversible rotary lobe pump powered by a Nordbloc helical gearbox was used to fill the pre-mix tank. The valves were then repositioned to allow flow from the bottom of the digester tank to the waste tank. A volumetric amount of digestate equal to the volumetric amount of new feedstock/water mixture added to the pre-mix tank was then wasted into the waste tank. This waste volume was determined by the dimensions of the pre-mix and waste tanks and the physical water level measurements of each. Duplicate 500 mL samples were taken as the digestate entered the waste tank. The level in the digester tank was periodically checked via the upper-level sample port sensor on the digester tank.

A Meyers stainless steel 90 GPM submersible sewage pump mixed the feedstock and digestate in the pre-mix tank in order to inoculate the new feed material with the methanogenic microbial community. Throughout mixing, a Process Technology D3L-series stainless steel L-shaped heater heated the mixture of feedstock and inoculating digestate in the pre-mix tank to approximately 38°C. A Process Technology NR-series non-indicating thermostat controlled the heating process.

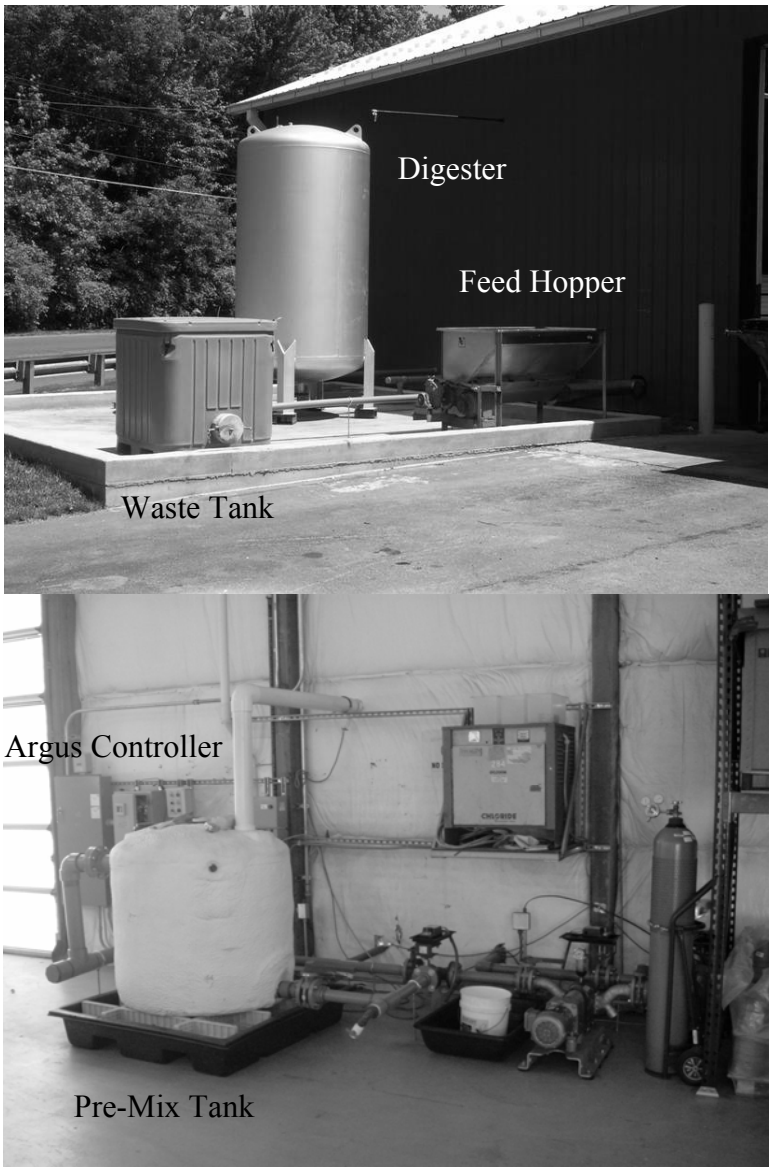


Figure 4.1 Photograph of the Rutgers EcoComplex anaerobic digester (photographs courtesy of Professor Arend-Jan Both, Rutgers University).

pipeline. The combined biogas was sent to a boiler to generate heat or to a microturbine to create and export electricity to the grid. Valves were installed late in the study to isolate the digester biogas flow and direct any created gas through a rotameter. The

biogas flowing through the rotameter was periodically collected in 3 L Tedlar® gas sampling bags for gas chromatography analysis to determine the methane content.

4.3. Analytical Methods

4.3.1 Biogas and Methane

Biogas produced by the digester was periodically sampled from the reactor headspace via connection to a 3L Tedlar® gas bag. Biogas samples were transported to the Rutgers University Environmental and Natural Resources Sciences Building in New Brunswick, NJ. The methane concentration in the biogas was analyzed via a 0.5 mL gas sample collected at atmospheric pressure using a glass-Teflon®-stainless-steel gas-tight syringe equipped with a side port needle (Valco® Precision Sampling, Baton Rouge, LA) and injected into an Agilent® 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a GS-GasPro capillary column (30 m x 0.32 mm I.D.; J&W Scientific, Folsom, CA) and a flame ionization detector. Helium was the carrier gas at constant pressure 131 kPa (19 PSI). The oven temperature was held at 150°C. The resulting chromatographic peak area was compared to a five-point calibration curve prepared using mixtures of 0 to 100 % methane created by mixing volumes of methane (99 % purity; Matheson Tri-Gas, Inc., Montgomeryville, PA) and air in a 0.5 mL gas-tight syringe (Valco® Precision Sampling, Baton Rouge, LA). Volumes of biogas and methane produced were corrected and reported at standard temperature (25°C) and pressure (1 atm) using the ideal gas law. To determine the rate of loss of methane from the sampling bags, the biogas samples were stored in the Tedlar® bags at room

temperature and pressure and the methane content was re-measured several days later. Losses from the gas bags determined in this way were found to be 4 to 6% per 24 hr period or approximately 0.15 to 0.25% per hr.

4.3.2. Chemical analyses

Both "top" (middle of digester) and "bottom" (wasted digestate) samples were obtained periodically and analyzed for pH and alkalinity, total and volatile solids content, total ammonia nitrogen (TAN) and volatile fatty acids (VFAs). Top samples were taken from a sampling port located approximately in the center of the digester and bottom samples were taken when digestate from the reactor was partially recycled and wasted.

Samples for pH and alkalinity were analyzed on site by Mr. Daniel Macready according to Standard Methods (Clesceri et al, 1998) and using an Accumet® Basic AB15 pH meter (Fisher Scientific, Pittsburgh, PA) and a BRAND™ Digital Buret™ III Bottle Top Burette (BrandTech, Essex, CT).

Samples for solids, TAN and VFA analyses were brought to the Rutgers University Environmental and Natural Resources Building in New Brunswick, NJ and stored at 4˚C until analyzed.

For TAN determination, 1 mL samples were first centrifuged at 10,000 *g* and then the supernatant was removed and filtered through a 0.45 μm, 25 mm nylon membrane syringe filter (Pall Corp., East Hills, NY). The filtrate was diluted 1000:1 using milliQ water and analyzed using a Dionex® ICS-1000 Ion Chromatograph (Sunnyvale, CA) with a Dionex® CSRS Ultra II 4-mm cation column. The resulting chromatographic peak areas were compared to a five point standard curve generated from analysis of standards prepared over a concentration range from 0.0625 to 1.0 mM NH_4^+-N/L, according to

standard methods (Clesceri et al, 1998). The corresponding free ammonia (NH_3-N) concentration was estimated using equation 4.1, where the pH was the prevailing digester pH at the time of sampling and the pka at 55°C is 8.4.

$$NH_3\text{ - N (mg N/L)} = \frac{TAN}{1+10^{pka\text{-}pH}} \qquad \textbf{Equation 4.1}$$

Samples used for TAN determination were also used for organic acid analysis. The filtrate was diluted 20:1 using milliQ water and then analyzed on a Beckman Coulter® System Gold™ HPLC (Beckman-Coulter, Inc., Fullerton, CA) using a Bio-Rad® Aminex HPX-87H organic acid analysis column (Bio-Rad Laboratories, Hercules, CA). Detection was by UV at a wavelength of 210 nm. The column was held at 60°C, and the eluent, 5.0 mM H_2SO_4, was configured at a flow rate of 0.6 mL/min. Chromatographic peak areas for unknown amounts were quantified by comparison to standard curves over a concentration range from 1 mM to 10 mM for acetic, propionic, and butyric acids (Sigma-Aldrich Co., St. Louis, MO). The total VFA concentration was determined by summing the molar amounts of each individual acid and converting it to a mg acetic acid/L basis.

4.3.3. Solids Analyses

Samples were taken during the course of every feeding. Solids analysis was performed according to Standard Methods (Clesceri et al, 1998), by first drying overnight in ceramic dishes at 103 to 105°C, cooling in a desiccator, and subsequently weighing to determine the percent total solids. Samples were then incinerated in a muffle box furnace at 550°C for approximately two hrs. Samples were cooled in a desiccator and

subsequently reweighed and compared to the total solids to determine the percent volatile solids.

Periodically, samples were sent to Dairy One Laboratories (Ithaca, NY) and analyzed for crude and soluble protein (**Table 4.2**).

4.3.4. Biochemical Oxygen Demand (BOD) Analysis

On Day 236, samples were analyzed for BOD. BOD analysis was performed according to Standard Methods (Clesceri et al, 1998) at dilutions of 0.0005, 0.001, and 0.003.

4.4. Results

4.4.1. Feedstock Characteristics

As described in Section 4.2.2, ratios of manure to food waste fed varied over the course of the digester start-up and operation. Due to the large particle size, complexity and heterogeneity of the mixture, the food waste could not be analyzed for solids content directly. Horse manure was analyzed on several occasions and had an average TS content of 40.3 ± 4.1 % and an average VS content of 82.3 ± 4.8 %. The feedstock, the macerated mixture of both wastes plus water, prior to entering the holding tank, was analyzed for solids frequently (**Table 4.3**). Between Days 0 and 79, the feedstock mixture was approximately 13.6 kg (30 lb) horse waste to 13.6 kg food waste on a wet weight basis. After Day 79, the food waste loading was increased and the feedstock mixture of food waste to horse manure eventually reached a ratio of approximately 3:1 on a kg VS basis, which corresponded to a ratio of approximately 2:1 on a wet weight basis.

Ultimately, the digester loading target was about 204 kg (450 lb) wet solids per feeding maintained at a 3:1 ratio of food waste to horse manure on a VS basis (**Table 4.3**).

As seen in Table 4.2., the feedstock characteristics, with respect to protein content, changed between Day 54 and Day 147, and was also noticeably different on a visual and olfactory basis. The protein content of the feedstock increased from 1% dry weight to 5% dry weight during this time and remained high throughout the course of this study. Since the food waste used in this study contained no or very little meat, fish, eggs, or dairy products, it was surprising to find that the food waste had protein content greater than 5%. The manure, in contrast had a protein content of approximately 1% dry weight. It was hypothesized that the waste obtained from Whole Foods may have had substantial amounts of soy (and possibly other bean) products, containing protein, and thus contributed to the general protein accumulation and subsequent conversion to ammonia-nitrogen (section 4.4.5.).

Table 4.2. Characteristics of EcoComplex Digester Macerated Feedstock in Combined Form as It Entered the Pre-Mix Tank.

Day of Operation	**54**	**147**	**151**	**163**
% TS	6.4	17.7	20.0	18.1
% Protein	1.0	5.9	4.4	5.5
% Soluble protein	58.0	59.0	39.0	58.0

Table 4.3. Total and Volatile Solids Contents of Feedstock and Corresponding Feedstock Ratios on a (lbs.) Wet Weight Basis.

Day	% TS	% VS	Manure:Food
53	4.85%	79.15%	*40/10*
54	3.97%	83.08%	*40/10*
138	21.48%	81.39%	*20/100*
148	22.99%	86.11%	*20/130*
153	19.67%	82.84%	*20/120*
155	16.53%	82.70%	*20/125*
159	19.89%	88.35%	*20/125*
160	22.93%	88.37%	*20/125*
164	23.50%	92.20%	*20/125*
173	26.63%	89.42%	*20/125*
177	18.83%	88.26%	*30/125*
187	16.97%	82.62%	*40/125*
190	13.48%	82.02%	*30/125*
199	14.05%	87.75%	*40/125*
201	23.92%	89.91%	*40/125*
204	17.55%	87.81%	*50/125*
236	13.55%	84.27%	*50/125*
238	16.42%	89.16%	*50/125*
269	16.84%	87.67%	*40/105*
290	14.21%	88.29%	*40/105*

4.4.2. Reactor performance results

Results from the pilot-scale anaerobic digester operated at the Rutgers EcoComplex are presented for up to 372 days of operation, although after 329 days, the system was operated only intermittently, as explained in section 4.5. The goal of operation was to increase the VSLR over time to approach a maximum design loading of up to 6 kg VS per m^3 reactor-day, while avoiding acidification or other reactor upsets, and to monitor results to compile reactor operational outputs. At the final time point presented for VSLR, 344 days, the VSLR was 1.87 kg VS/m^3 reactor-day. The mass of waste food and horse waste added at each feeding and the ratio of food waste to horse

manure on a kg VS: kg VS basis is shown in **Figure 4.2.** Note that stall waste (horse manure plus softwood bedding) was added from Day 0 to Day 26, and thereafter horse manure alone (no bedding was added). The average VSLR (see **Figure 4.3.**) correspondingly increased from 0.47 to 1.87 kg VS/m^3 reactor-day over the course of operation, with some higher loadings occurring transiently.

The effluent solids were measured periodically and the effluent solids concentrations are shown in **Figure 4.4**. From Days 137 to 277, samples obtained from the middle of the digester ranged from 1.5 to 2.25% for total solids and from 62% to 70% for volatile solids. Digestate samples, obtained during re-cycling, were at moderately higher solids concentration of 3.0 to 4.5% for total solids and 72% to 77% for volatile solids.

Digestate solids concentrations were always lower than was expected based on the original digester design. It was expected based on the loading rates and an estimated VS removal efficiency of approximately 40% that the prevailing solids content of the digestate would be from 8 to 12 percent total solids. Instead, the TS of the digestate was usually < 5%, and this value decreased even further around Day 279. It was hypothesized that a liquid channel had formed through the center of the digester and that most of the solids had collected by sedimentation on the bottom and on the sides of the tank. On operational Day 372, after reactor operation had been discontinued, this was confirmed when the tank hatch was opened and visual observation confirmed that solids had accumulated in the digester through sedimentation and a lack of agitation.

4.4.3. Biogas and Methane Analyses

Measurement of the biogas volumetric flow rate was difficult because the biogas was plumbed directly into a landfill biogas pipeline and the flow rate was affected by operation of the landfill gas combustion system, so that accurate readings could not be obtained. Methane content of the digester biogas (**Figure 4.5**) was measured beginning on Day 154. The methane content was initially 64.1%, and remained at approximately 60% until day 197, when the percent methane decreased over several readings. There are several potential reasons for the low methane content readings. The first reason is that a landfill gas combustion system came online and apparently disrupted the pressure in the biogas discharge line from the digester. This could have resulted in the mixing of landfill biogas (containing 35 to 40% methane) with the digester biogas. The second potential reason is increasing ammonia concentrations in the digester (section 4.4.5). Prior to Day 200 when the lowest methane readings were observed, the TAN in the upper reaches of the digester had increased from 2.47 to 3.46 mg NH_4^+-N/L from Day 163 to Day 197 and peaked at 3.92 on Day 189 with a corresponding increase in free ammonia as high as 600 mg NH_3-N/L, high enough to potentially inhibit methanogenesis (Vidal et al., 2000).

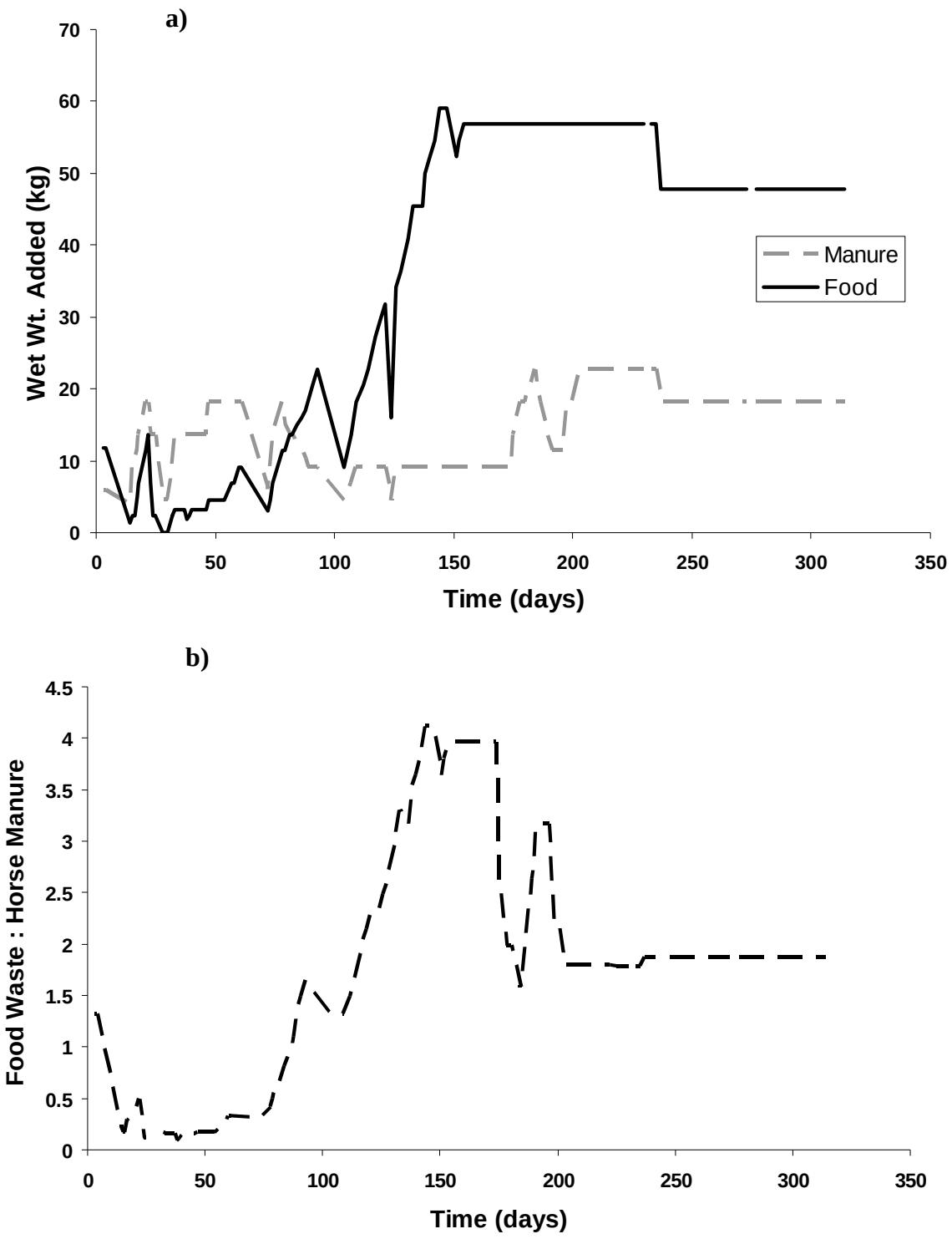


Figure 4.2. Food and horse wastes added at each feeding to the Rutgers EcoComplex digester: a) on a wet weight basis, and b) as a ratio of food waste: manure on a volatile solids basis.

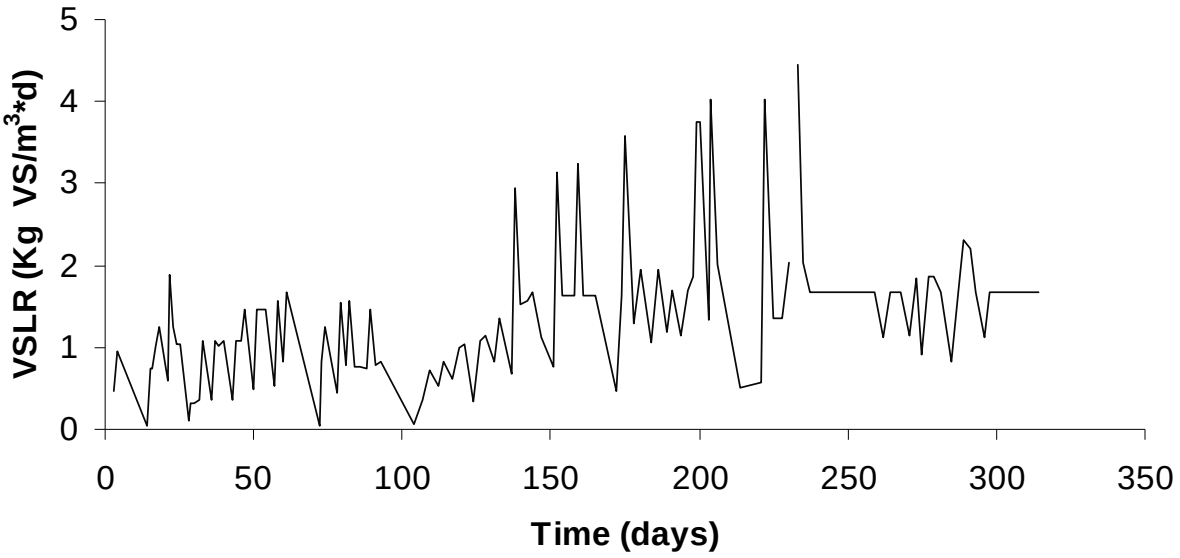


Figure 4.3. Volatile solids loading rate for the Rutgers EcoComplex digester fed a mixture of food waste and horse manure.

From Days 233 to 279, the biogas methane content again ranged from 50 to 60%, and was measured once more shortly after halting feeding (Day 357) to determine if the methane content was still above 50%, despite the bulking solids and channeling inside the digester. The resulting measurement was 51% methane. Thus, overall, the digester biogas methane content was 50 to 60 %, as would be expected, with some unexplained periods when the content were <50%.

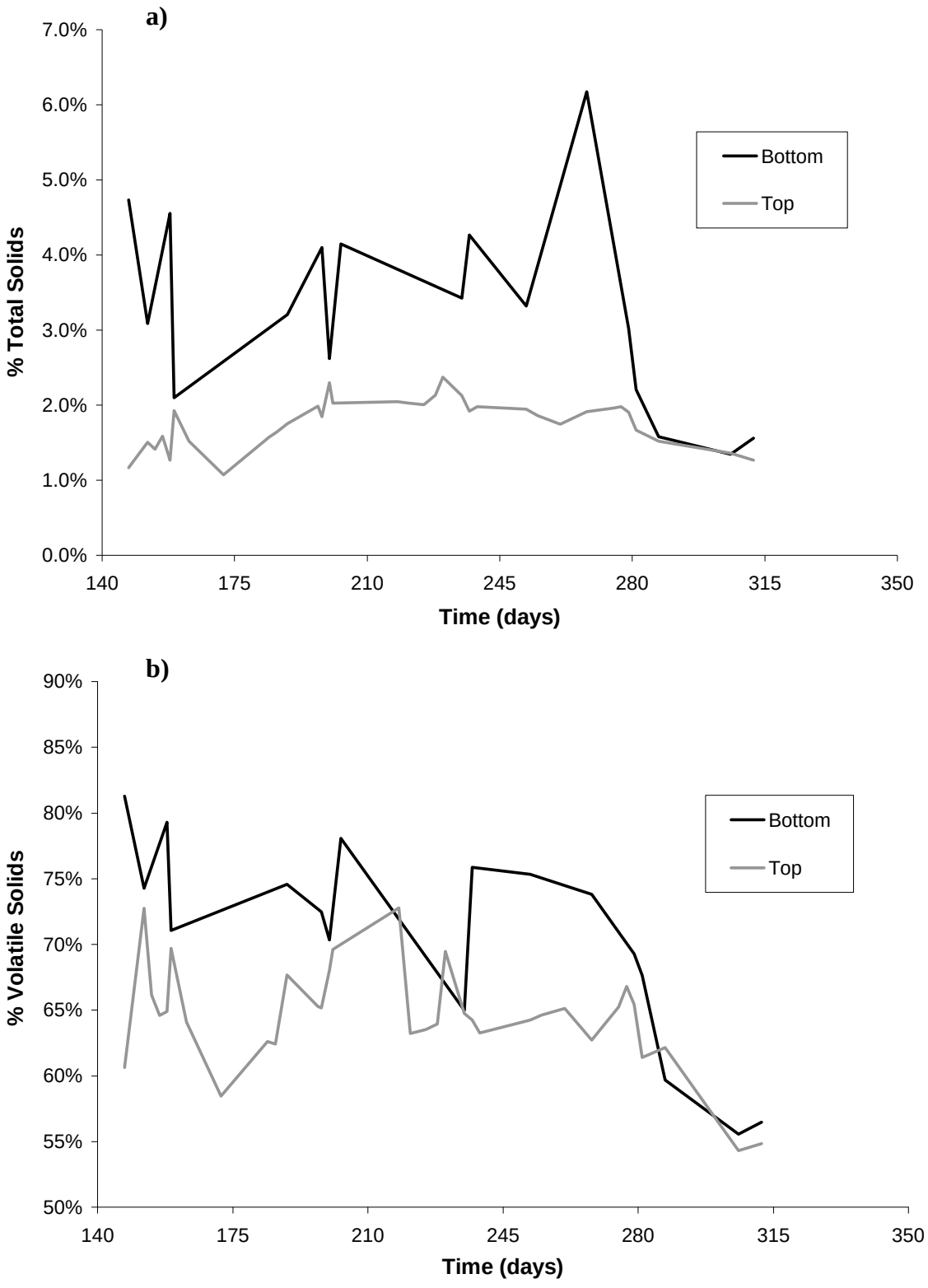


Figure 4.4. Digestate solids content from bottom and top sampling ports of the Rutgers EcoComplex digester: a) percent total solids; b) percent volatile solids.

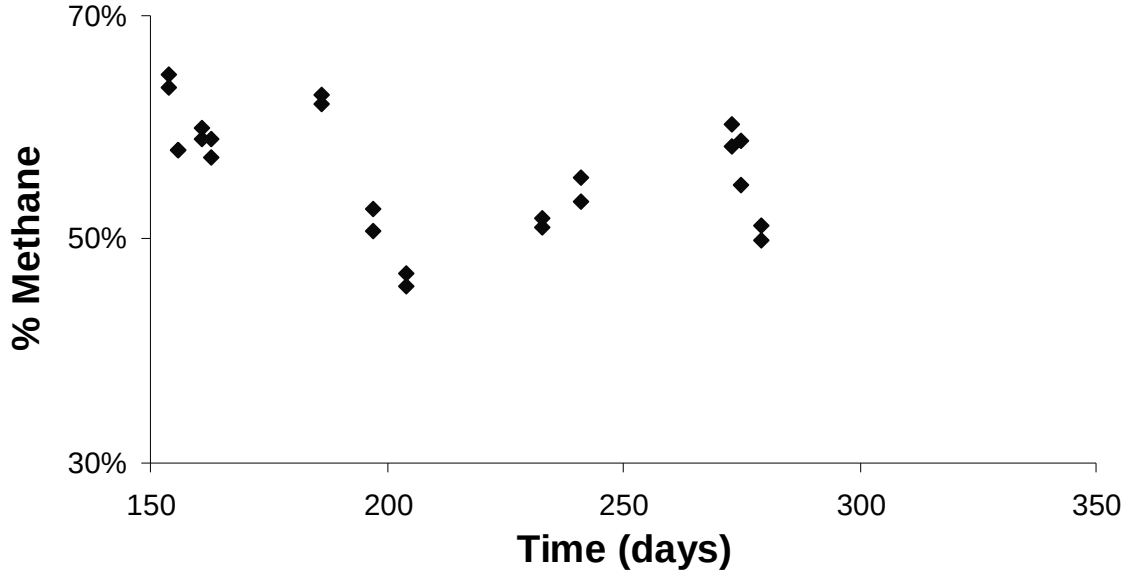


Figure 4.5. Methane content in the Rutgers EcoComplex digester biogas. Replicate measurements are plotted at each time point.

4.4.4. pH and Alkalinity

The feeding rate and pH reached a steady-state on day 154, with a feed ratio of 9.1 kg manure : 56.8 kg food waste (20 lbs : 125 lbs) on a wet wt. basis, which is approximately 1:4 on a volatile solids basis. The pH at this point was 7.74 and remained approximately the same until Day 186, whereupon, after two weeks of experimentally raising the manure loading, the pH began to drop slightly and fell to 7.55 on Day 191. The feed amount was temporarily decreased and the contents of the digester were partially recycled. The pH stabilized around 7.7 and the alkalinity stabilized on Day 237 at about 9.0 g $CaCO_3$ /L. On Day 237, a long-term equilibrium was established at 18.2 kg manure: 47.7 kg food waste (40 lbs : 125 lbs). Despite a constant feed rate, the alkalinity increased again beginning on Day 266, ultimately reaching a level of 10.2 g

$CaCO_3$ /L by Day 281. Alkalinity continued to be slightly above 10 through Day 289.

The alkalinity from that point remained relatively constant with a range between 9.0 and 10.0 g $CaCO_3$ / L. The general increase of alkalinity, particularly from days 90 to 289, was thought to be caused by an increase in TAN over time.

Although the top and bottom were of the reactor were not apparently well-mixed and fluctuations were expected, the general trends could be followed and the evolution of the operation of the digester was apparent.

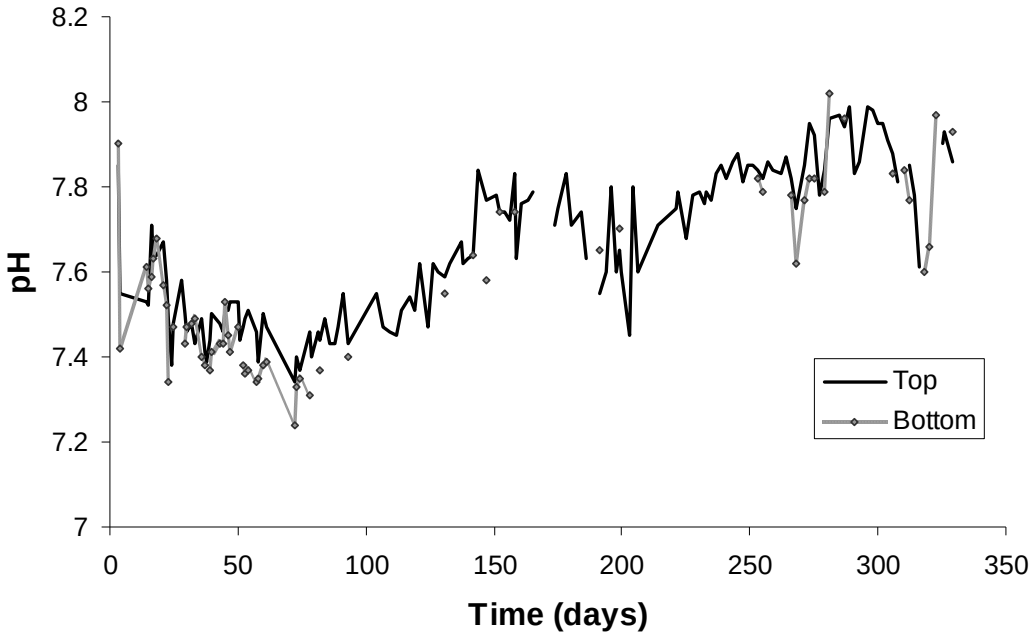


Figure 4.6. The pH of digestate removed from the top (middle) and bottom (digestate) sampling ports of the Rutgers EcoComplex digester.

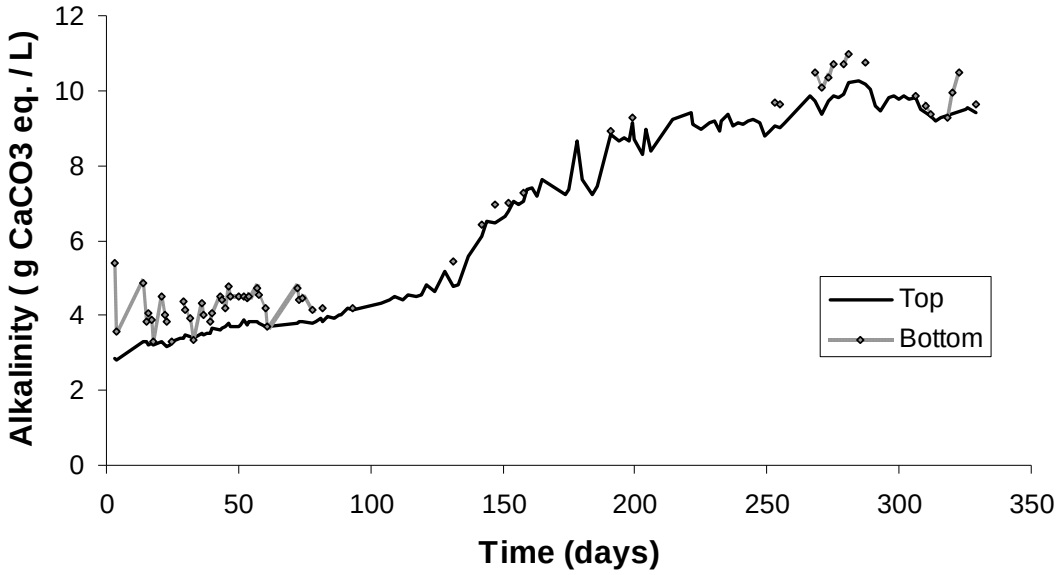


Figure 4.7. Alkalinity of digestate removed from top and bottom sampling ports of the Rutgers EcoComplex Digester.

4.4.5. Nitrogen and Ammonia Analyses

TAN and free ammonia concentrations of digestate taken from the top and bottom sampling ports of the digester are shown in **Figure 4.8**. Nitrogen and free ammonia levels began at low concentrations during the startup of the digester and were relatively stable until Day 170. A large increase in nitrogen levels occurred between Days 172 to 202, corresponding to a gradual increase in the food waste loading rate as well as the largest food to manure ratio leading up to and at the onset of this period. At the time, free ammonia was 291 mg NH_3-N/L and the pH was near 7.6. Because the TAN remained elevated, over time, the alkalinity and pH increased, leading to a more substantial fraction of TAN occurring in the form of free ammonia, according to equation 4.1, ultimately culminating at free ammonia levels close to 1000 mg NH_3-N/L for both sampling locations on Day 296.

There was a concern that increasing ammonia levels may have inhibited methanogenesis, as was hypothesized in bench scale experiments (Chapter 3). However, if TAN or free ammonia played a significant role in the decrease in the methane concentration of the biogas, this relationship appeared to be delayed and decreased methane concentrations did not exactly coincide with high TAN concentrations. Starting from Day 233, the readings of 50 to 55% methane concentrations were considered to be accurate, based on testing of bag leakage. There may have been fluctuation, but significant changes within a matter of several days were not reasonable. Therefore, the average methane content of the biogas starting on Day 197 was 50 to 55%. This percentage indicated that the gas is readily useable as biogas for combustion purposes, but is not as high as some systems have reported. Methane contents expected from thermophilic digesters can vary from 50 to 65%, depending on pre-treatments, substrates utilized, and environmental conditions (Ward et al., 2008; Kim et al., 2006). Several researchers have reported methane contents from thermophilic digesters of close to, and occasionally above, 70% (Song et al., 2004; Zhang et al., 2007).

b)

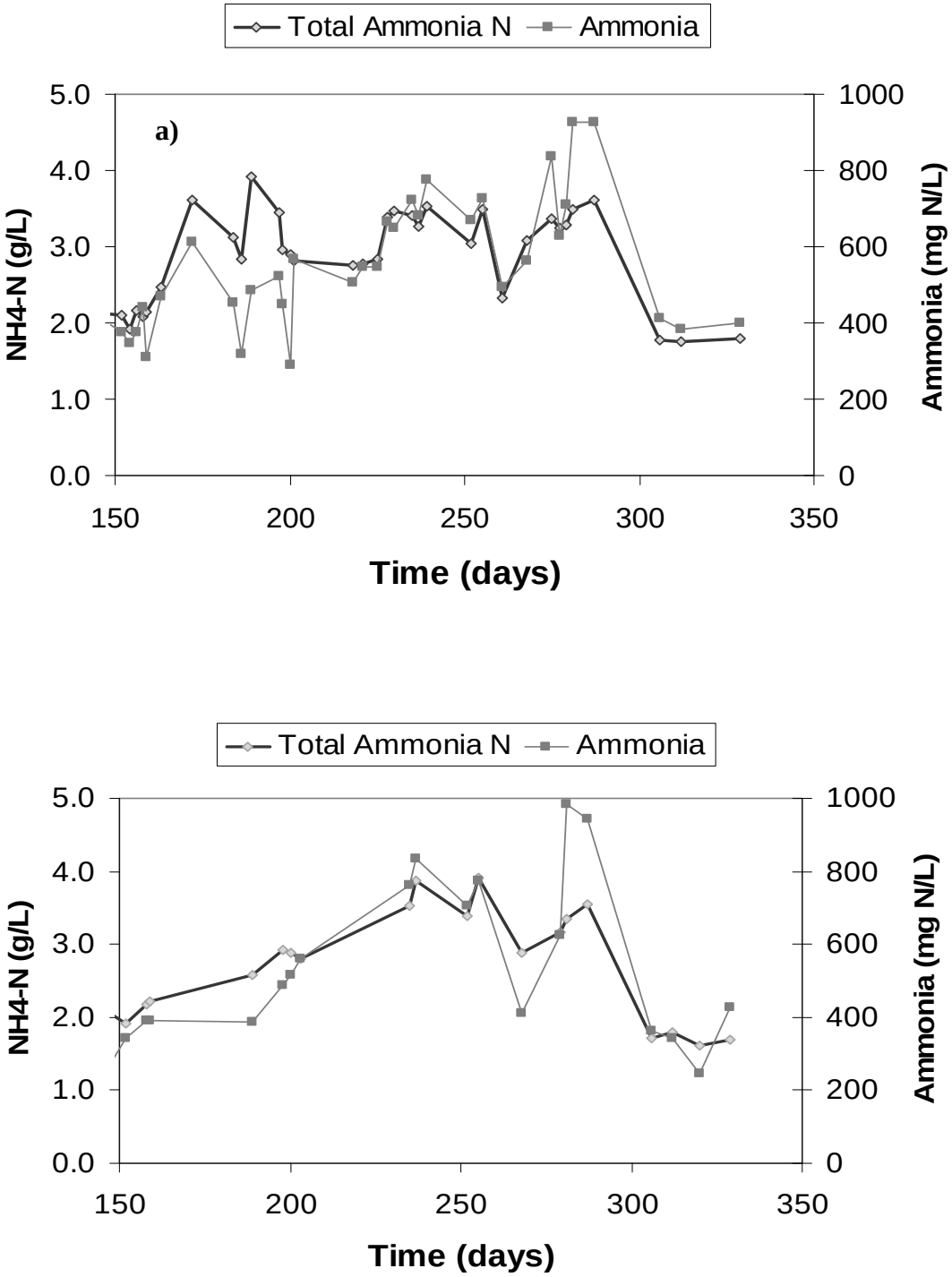


Figure 4.8. Total measured ammonia-nitrogen and calculated free ammonia levels over time for: a) top samples; and b) bottom samples of the Rutgers EcoComplex Digester.

4.4.6. Volatile Fatty Acids (VFAs)

Volatile fatty acid concentrations were analyzed only near the end of the operation of the thermophilic digester. Of particular concern were propionic and butyric acids, which are known to be inhibitory or toxic to methanogens (Wang et al., 2009). From Days 218 to 228, concentrations of acetic and propionic acids fluctuated from 700 to 1400 mg/L and 300 to 550 mg/L, respectively (**Figure 4.9**). During this time, there was no detectable concentration of butyric acid. On Day 239, VFAs began to drop sharply and continued to minimal levels on Day 261. On Day 268, VFAs sharply increased to previous levels, corresponding to a slight drop in pH (7.78 to 7.62). Immediately, thereafter, pH and alkalinity both began to increase steadily as mentioned in section 4.4.4.

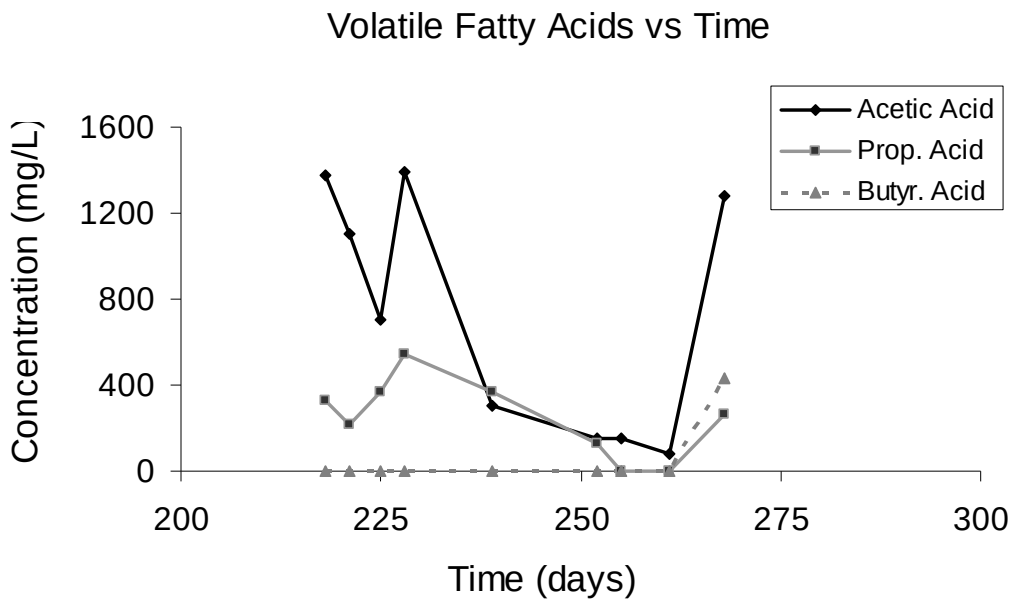


Figure 4.9. Volatile fatty acid (VFA) concentrations in digestate removed from the top sampling port of the Rutgers EcoComplex Digester.

4.4.7. Biological Oxygen Demand (BOD) Test

A biological oxygen demand analysis was performed on samples collected on Day 237 from both sampling locations. Results for both sampling locations were determined to be 2.1 ± 0.3 g BOD/L.

4.5. Shut Down of Reactor

Regular digester operation was interrupted on Day 314, briefly resumed and ultimately halted on Day 329, when the TS of the recycled leachate dropped below 2% (**Figure 4.4**). It was hypothesized that a liquid channel had formed through the center of the digester and that most of the solids had collected by sedimentation on the bottom and sides of the tank. This was confirmed when the tank hatch was opened (Day 384) and visual observation indicated that solids had accumulated in the digester through sedimentation.

Feeding of the digester was resumed intermittently after a pump out of the digester, and wasting of half the solids. Concurrently, a small amount of digestate was removed from the digester tank, placed in batch 160 mL serum bottles and tested for methanogenic activity. Bottle sets were performed in triplicate and contained one set with no substrate added and one set with 2.5 g of sucrose added to each bottle. Both bottles immediately produced biogas, which increased over time to methane contents of above 50%, indicating a healthy methanogenic population and an active microbial community. A solution to impose mixing in the digester is currently being sought.

4.6. Conclusions

The results of this study confirmed that co-digestion of waste food and horse manure is feasible. Despite fluctuations of pH and increases in ammonia levels, the methane content of the biogas remained above 50% for the majority of the experiment. Unfortunately, there was no reliable biogas production data to confirm the efficiency of VS removal.

The ability of horse waste to effectively compliment food waste digestion still has some remaining issues. The C:N ratios must be maintained high enough throughout the digestion process to avoid ammonia toxicity. Measurements of the protein content of horse waste and food waste indicated that protein was 1 to 5% of the dry weight. The digester TAN was as high as 5 g/L with a corresponding free ammonia concentration of up to 800 mg NH_3-N/L. Anaerobic digestion at thermophilic temperatures generally releases more ammonia because of improved protein hydrolysis. There have been many studies documenting ammonia inhibition during anaerobic digestion (Borja et al. 1996; Calli et al. 2005; Gallert et al. 1998; Gallert and Winter 1997; Hansen et al. 1998; Lu et al. 2008; Lu et al. 2007; Pechan et al. 1987; Sung and Liu 2003), although thermophilic microorganisms have also been shown to be more tolerant of ammonia (Gallert and Winter, 1997). Sung and Liu (2003) showed 40 to 60% inhibition of methanogenesis at 5 to 6 g/L TAN, and complete inhibition of acclimated thermophilic digesters at 8 to 13 g/L TAN. Methanogenic populations became acclimated as TAN increased. Calli et al. (2005) showed shifts in populations of methanogenic archaea and acetogenic fatty acid-degrading bacteria using detection of 16S rRNA genes during anaerobic digestion at nitrogen loadings up to 6 g/L TAN with corresponding free ammonia nitrogen

concentrations of 0.8 g/L, indicating that the microbial community adapts to the presence of the ammonia. Borja et al. (1996) reported ammonia toxicity at TAN concentrations greater than 5 g/L, but were able to maintain stable, though reduced methane production at 7 g/L TAN. However, pre-acclimation of cultures at lower concentrations of ammonia (<0.8 g/L TAN) resulted in systems tolerant of ammonia up to 7.8 g/L TAN in continuous flow systems.

Based on the results described here, additional biomass sources with a higher C:N ratio may be needed to accomplish stable food waste/horse waste digestion to maintain lower TAN concentrations. One possible solution is use of stall waste from horses bedded with Streufex®, a pelleted straw stall bedding, which came on to the US market recently and which was not utilized for the work described in this thesis. A higher C:N ratio should reduce TAN and provide better overall stability.

Currently, eliminating the settling and bridging of the solids in the digester tank is the immediate goal. Various options such as an internal impeller, biogas injection and an external recirculation pump are being considered.

The success of this experiment can serve as a prototype for more widespread and larger anaerobic digestion applications, ranging from food preparation facilities to crop and livestock farms throughout NJ.

Chapter V. Overall Summary and Conclusions

The overall objective of studies carried out as part of this thesis was to determine the feasibility of utilizing horse waste for anaerobic digestion and production of biogas. The methane production potential of horse manure was determined to be 139 ± 65 L (average ± standard deviation) methane per kg VS.

Initial experiments in continuous-flow reactors had indicated that the presence of commonly used softwood bedding mixed with horse manure in stall waste may have led to inhibition of the methanogenic process. However, subsequent batch experiments did not show any inhibition by mixing in fresh, unused softwood bedding, regardless of the relative amount or ratio added (see **Chapter 2**). Further, the methane content of the biogas appeared relatively uniform in spite of increasing wood concentrations. These results suggested that the presence of fresh softwood chips in mixed horse stall waste should not cause inhibition to an acclimated anaerobic digestion process.

This research was continued by examining the effect of used bedding on anaerobic digestion of horse manure, since the process of aging through exposure to urine or aerobic degradation could result in changes in the bedding properties leading to greater toxicity. Used softwood bedding was added at ratios up to 4 g bedding VS to g horse manure VS. Again, results showed no inhibition caused by the presence of used softwood bedding, regardless of the amount added (**Figure 2.7**). Not only was there no indication of inhibition, but the presence of the bedding appeared to have contributed positively to methane production with the manually separated, used softwood bedding producing 39 ± 10 mL methane per g VS added. This amount was substantial and

accounted for about 20 % of the methane production potential produced by horse manure alone.

It was initially presumed that the increase in methane production from the presence of the manually separated, used softwood bedding was due to small manure remnants that were adhered to the wood particles. However, the visual observations indicating particle breakdown suggested the possibility of anaerobic breakdown of the softwood bedding itself and partial conversion to methane. Based on these observations, the amount of softwood bedding that was degrading and its potential for conversion to methane, if any, was further investigated (see **section 2.3.4**). It was determined that a substantial amount of methane relative to the inoculum (control) was, in fact, produced from those bottles containing only inoculum and fresh softwood bedding, indicating that some wood was being converted anaerobically into methane (**Figures 2.9** and **2.10**). The methane production potential of the softwood bedding was 19.98 ± 4.6 mL methane over 33 days of incubation (**Figure 2.9)** (about 10% of the methane production potential of horse manure) or 8.4 ± 1.9 mL methane per g VS added.

Overall, this study has confirmed that not only is the softwood bedding non-inhibitory to the anaerobic digestion process, it partially degrades and produces some methane bioenergy. Re-examining the original semi-continuous flow reactor (CFR) studies where toxicity was thought to be a problem, it is possible that acclimation of the microbial process to the presence of the softwood bedding may be an important factor. In the original CFRs, the anaerobic inoculum was not originally exposed to softwood bedding from the beginning of the study. Rather softwood bedding was added after 82 days of operation. Thus in future operation of equine waste digesters if may be important

to make sure that the microbial community is acclimated to the softwood bedding from the beginning of operation. Further study and examination of the specific microbial community members present would be needed to confirm this.

A second major finding from this study is that separation of the bedding from the manure prior to recovery of bioenergy, which could be desirable to reduce reactor volumes or avoid mechanical problems caused by wood particles, would result in a loss of substantial recoverable energy. Since mechanical separation of bedding from the waste would consume both labor and energy, an economic study is needed to determine which operational scenario and reactor design would be most energy and cost effective.

In an effort to reproduce these results at a large scale, a batch experiment was conducted in two 125 L mesophilic digesters. Initial product of biogas was at approximately 30 L/d but quickly began to decrease, as did the methane concentration, which had reached 49% and 45%, for each digester, respectively. The decrease in biogas and methane production was presumed to be caused the high solids and dryness of the material in the digester. The digesters were re-inoculated with a larger volume of inoculum (10 L each) and began again producing near 30 L biogas/d, for each digester, respectively. These results suggest that capture of biogas energy from simple batch reactors wherein waste is emplaced as collected from stalls, inoculated and allowed to digest could produce substantial energy for on-farm use. No separation of bedding would be needed. This system could be utilized with a mobile containerized system wherein waste is digested and later hauled away for disposal or further processing as first suggested by Kusch et al. (2008) for equine waste with straw bedding.

With horse waste digestion seeming a realistic possibility, we also wanted to determine its suitability as a co-substrate. The Rutgers University Eco-Complex in conjunction with EarthPledge initially implemented an anaerobic digester utilizing food waste as a single substrate, which experienced upset characterized by low pH early on and a suitable and likely co-substrate was sought. We therefore examined semi-continuous thermophilic anaerobic digestion of a 50:50 (VS:VS) mixture of equine stall waste and food waste at the 15-L scale in anticipation of using a similar combination at the 6 m^3 scale using the EcoComplex digester. The EcoComplex digester is to serve as a prototype long-term semi-continuous feed digester for larger and/or more widespread uses around New Jersey and the US.

The initial 15 L study (see **Chapter 3**) investigated co-digestion of equine stall waste and food waste under thermophilic conditions. The digester volatile solids loading rate (VLSR) target was 3.0 g VS/L-d with a corresponding mixed liquor suspended solids content of 12.5% TS or approximately 100 g VS/L (assuming 80 % VS). The estimated solids retention time (SRT) was approximately 45 days. The results of this experiment showed that co-digestion of waste food and stall waste is feasible at thermophilic (55°C) temperatures. Wood that was present in the stall waste did not seem to have a negative effect on the anaerobic digestion, as noted in the previous experiments. Methane concentrations in the biogas were often over 50% and the volatile solids conversion rates were estimated to be between 29% to 34%, which is similar to other co-digestion studies (Alvarez and Liden, 2008; Macias-Corral, et al., 2008). The average methane production was 356 ± 61 L/kg VS-d. The VSLR (2.2 kg VS/m^3-d) was relatively low and the SRT (45 days) was relatively long for a thermophilic system. An important observation was

that alkalinity and pH increased over the course of the reactor operation. Further, total ammonia nitrogen levels reached just above 3.0 g/L with corresponding free ammonia concentrations of 800 mg/L. At the end of the operational period, the % methane in the biogas was less than 50%, indicating potential upset of methanogens. Unfortunately, with the limited data on ammonia and VFAs it was not possible to diagnose the exact causes for the decrease in methanogenesis.

Mixtures of horse waste and waste food were also utilized in a 6 m^3 pilot scale digester. This study (see **Chapter 4**) was carried out at the Rutgers University Eco-Complex in conjunction with EarthPledge and continued to investigate the co-digestion of food and horse wastes. The results of of digester operation confirmed that co-digestion of waste food and stall waste is feasible. Despite fluctuations of pH and increases in ammonia levels, methane concentration in the biogas remained above 50% for the majority of the experiment. Nevertheless, the ability of horse waste to effectively compliment food waste digestion still has some remaining issues. As was observed at the 15 L scale, free ammonia approached concentrations (1000 mg/L) that could have caused toxicity. Therefore the C:N ratios must be controlled properly throughout the continuation of the experiment, especially since anaerobic digestion at thermophilic temperatures generally releases more ammonia because of improved protein hydrolysis. Additional biomass sources that impart a higher C:N ratio in the digester feedstock may be needed to accomplish stable food waste/horse waste digestion to maintain lower TAN concentrations. One potential solution is the use of stall waste from horses bedded with Streufex®, a pelleted straw-based stall bedding. This bedding is relatively new to the NJ area but has been adopted by a number of equine operators. In addition to increasing the

C:N ratio of the waste, the straw pellets should be highly biodegradable and increase the energy potential of the stall waste. A higher C:N ratio should reduce ammonia and provide better overall stability of digester operation.

The success of this research forms the basis for more widespread and larger anaerobic digestion applications for equine waste in conjunction with other wastes generated from food preparation and dining facilities and crop and livestock farms throughout NJ. The ultimate impetuous for incorporation of anaerobic digestion in waste processing is two-fold: 1) to allow for efficient and energy-producing means of disposing or utilizing horse waste on farms and other equine facilities ; 2) to provide a stable and easily manageable co-digestion process for agricultural and food-processing industries.

Anaerobic digestion is only one step in the process of treatment and disposal of wastes such as equine stall waste. Ultimately the digestate must be further stabilized perhaps through composting, and the nutrients which largely remain in the waste must be managed to meet the needs for water quality protection. Future work must incorporate a systems and economic approach to examine whether application of anaerobic digestion technology in NJ could lead to true energy offsets and environmental protection.

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