Improving the Environmental and Economic Sustainability of Dairy Farming Using Value-added Products Derived from the Anaerobic Digestion of Manure

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ABSTRACT

The aim of this study was to examine how manure-derived value-added products via anaerobic digestion impact the environment and economics of dairy farming. An on-farm anaerobic digester (AD) at Virginia dairy was used in this study. The AD performance evaluated for: (i) biogas production (ii) waste stabilization; and (iii) production of organic fertilizer. Locally available organic waste streams were evaluated for co-digestion with dairy manure to increase biomethane production at the on-farm AD. The effective pasteurization temperature and duration to reduce fecal coliform, *E. coli*, and *Salmonella* concentrations in the AD effluent to acceptable levels for use as an organic fertilizer were determined. A partial environmental and economic analysis was conducted on the AD system to determine its effects on the environmental-economic sustainability of dairy farming. The results showed that the manure-derived value-added products from the AD improved environmental health and had the potential to improve the economic sustainability of the dairy farm. The AD stabilized the manure adequately and produced 400 KW of electricity, enough to power 230 US homes. Blending manure with locally available organic materials increased volatile fatty acid production, suggesting the potential to increase biomethane yields. Pasteurization at 70°C is sufficient to reduce pathogen indicating organisms to acceptable levels for the manure to be used as an organic fertilizer. The payback periods range from 4.6 to 11.8 years for the AD investment costs and reductions in direct manure methane emissions of 2,436 tonnes CO_2e per year.

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1 Introduction

1.1 THE US DAIRY INDUSTRY

Dairy farmers currently face increasing economic and public pressures as they struggle to remain profitable in an economically volatile market (MacDonald et al., 2007; USDA NASS, 2010). Market forces have forced the consolidation and geographic relocation of dairy farms in recent years, often resulting in tensions between farmers and members of surrounding communities (MacDonald et al., 2007; Siegford et al., 2008; USDA NASS, 2010). While the total number of dairy cows in the US has increased in the past several decades, the number of dairy farms declined six fold between 1970 to 2000 (MacDonald et al., 2007). This decrease in the number of distinct dairy operations continued between 2000 and 2006 as operation sizes shifted toward farms of at least 1,000 milking cows to mitigate the effects of marginalized milk profits and to capitalize on economies of scale (MacDonald et al., 2007; USDA NASS, 2010; US GAO, 2004).

The number of large dairy operations with 1,000 to 1,900 cows increased 25% between 2000 and 2006, from 695 to 875. Even more strikingly, dairy operations with more than 2000 cows increased from 280 to 573 farms nationwide (MacDonald et al., 2007). In contrast, the number of operations with less than 500 cows decreased by 35% during the period of 2001 and 2009 (USDA NASS, 2010). These changes in the dairy industry reflect volatile milk prices and increasing feed costs (USDA NASS, 2010; US EPA, 2012; US GAO, 2004). Between 2003 and 2012, monthly milk prices in the United States fluctuated between $0.70 and $1.05 per kg (Bureau of Labor Statistics, 2013). A study conducted by the US Government Accountability Office (US GAO) states that farmers receive 45.9% of the retail price of milk – i.e. farmers were paid $0.32 to $0.48 per kg of milk between 2003 and 2012 (US GAO, 2004); however during this same period the cost of producing milk ranged from $0.40 to $0.53 per kg (USDA ERS, 2012b). Since 2011 the cost of producing milk has continued to increase, with an average of $0.56 per kg milk in 2012 (USDA ERS, 2012a).

The disparity between the costs of production and revenue, also referred to as the cost-return spread, continues to lead to consolidation of dairy production (MacDonald et al., 2007; USDA NASS, 2010). A significant environmental consequence of this industry restructuring is the concentration of manure within fewer localities/geographical zones. If not managed properly, manure may present increased risks to human and environmental health from water, land, and air pollution (MacDonald et al., 2007). On average a mature dairy cow in the U.S. produces approximately 9,160 kg of milk per year and 24,800 kg of manure (ASABE, 2005; USDA NASS, 2009), i.e., for every 1 kg of whole milk delivered to the grocery store, about 2.7 kg of manure is produced and must be managed on-site.

Manure is an excellent source of nutrients and carbon; for that reason, it is used as a fertilizer for cropland managed by the farm. Since crops have varying nutrient needs that vary from manure nutrient concentrations, it is common practice to implement nutrient management plans (NMPs). NMPs are overseen by state and federal regulatory agencies (e.g. US EPA) to ensure that croplands are not fertilized with nutrients in excess of their capacity for assimilation. Currently, NMPs are mandatory for farms with 200 or more milking cows (US EPA, 1999). While NMPs determine and define the amount, frequency, and method of land application for the safe use of manure, the issues of nuisance odors, greenhouse gas (GHG) emissions, and the potential for water/air pollution still remain to be resolved (US EPA, 1999).

1.2 DAIRY MANURE MANAGEMENT

In its raw, freshly excreted form, dairy manure is a matrix of urine, feces, and bedding material that contains nutrients and supports millions of microorganisms (Allen, 1923; MWPS, 2004). Raw manure also contains putrescible organic matter that releases odors and GHGs when degraded by the microorganisms that colonize the cow rumen. Since manure is not completely degraded by the time it is excreted, it remains an energy source for microorganisms and disease vectors like rats and flies. Therefore, although dairy manure can be converted into numerous value added products (e.g. separated solids bedding) some type of treatment is generally required prior to its use (or disposal) to protect public and environmental health.

Like other biological wastes, manure can be treated using aerobic and anaerobic methods to reduce strength (COD), manage nutrient content, and/or inactivate pathogens. Aerobic treatment reduces waste strength over a relatively short period of time and can be used for removal of nutrients like nitrogen and phosphorous (Grady et al., 2011). Anaerobic treatment of high-strength wastes like manure yields benefits that include energy production, minimal energy input (in the form of oxygen-aeration systems), minimal increases in biomass, and production of value-added goods (U.S. EPA AgSTAR, 2011; Cantrell et. al, 2008). Environmental and economic benefits include reduction in manure odors, reduction in GHG emissions, and potential income from the value-added products of anaerobic treatment processes (Yiridoe et al., 2009).

1.2.1 Anaerobic Digestion for Manure Treatment

The goal of manure treatment is the reduction of negative impacts following use as a soil amendment or fertilizer and subsequent release into the environment. Treatment performance can be measured in terms of the reduction of pathogens, COD, biological oxygen demand (BOD), odor, total solids content (TS), or volatile solids (VS) content. A more recently appreciated positive impact of dairy manure treatment and management is the mitigation of GHG release to the atmosphere. The need and

associated benefits of capturing GHGs, specifically, biomethane (CH_4), from dairy operations has led to increased implementation of anaerobic digestion technology on dairy farms since 2000 (U.S. EPA Agstar, 2012b). Anaerobic digestion involves processing manure in an enclosed oxygen free vessel via a series of microbial mediated biochemical transformations occurring over time. The microorganisms degrade the fibrous and soluble organic material in manure, producing CH_4 as a final product. Degradation of the organic matter in the manure during the anaerobic digestion process also reduces putrescible material and odors (Holm-Nielsen et al., 2009; Madsen et al., 2011). Therefore, using anaerobic digesters as part of a manure management system provides a double benefit: preventing the direct release of CH_4 into the atmosphere, and collecting it for use as a renewable energy source (Innovation Center for US Dairy, 2008; U.S. EPA, 2012).

The temperature at which an anaerobic digester is operated affects the amount of biogas produced and level of treatment (Nielsen et al., 2007; Nielsen et al., 2004). Temperature also affects the population dynamics and metabolism of the manure-degrading and methane-producing microorganisms as well as the inactivation of pathogens commonly found in manure, such as *Escherichia coli* and *Salmonella* species (Hooper and Li, 1996; Speece, 1996; Bowman, 2009; Kaparaju et al., 2009). Research indicates that mesophilic anaerobic digestion at about 37^0C, similar to the body temperature of healthy cows, does not significantly reduce pathogen concentrations in manure (Bordeleau and Droste, 2011; Cecchi et al., 1991; Horan et al., 2004; Lang and Smith, 2008; Pandey and Soupir, 2011; Smith et al., 2005). By contrast, thermophilic (between 50^0C and 57^0C) anaerobic digestion achieves reduction in pathogen concentrations in manure and increases biogas production (Cecchi et al., 1991; De Leon and Jenkins, 2002; Metcalf and Eddy, 2003; Lang and Smith, 2008; Lo et al., 1985; Martens and Bohm, 2009). However, it is important to recognize that thermophilic anaerobic digestion requires increased management and higher energy inputs compared to mesophilic digesters, which are more common on dairy farms in the U.S. (AgSTAR, 2012a).

1.3 THE ORGANIC FARMING INDUSTRY

Increased interest in renewable energy has been paralleled by an increased interest in sustainable food production, as evidenced by the past two decades' growth of the organic production and consumer industry. In 1992, the area of land dedicated to certified organic crop production in the U.S. was less than 1.65×10^5 ha (USDA ERS, 2010); by 2008, this had expanded to 1.07×10^6 ha (+600%) (USDA ERS, 2010). According to a poll conducted by Whole Foods Markets (2005), consumers purchased organic foods for a number of reasons, including (in order of importance): avoidance of pesticides (70%), food freshness (68%), and health and nutrition (67%). The sales of organic industry products increased by nearly 20% annually between 1990 and 2006 (Winter and Davis, 2006). In 2011 the estimated annual

organic food sales including milk, fruits, vegetables, grains, and meats was $31.5 billion (Dairy Industries International, 2012).

To meet increased demands for organic food products and benefit from the associated higher prices, many conventional farms have converted to organic production systems, resulting in increased demand for organic fertilizers. In order for a farm and its products to be certified organic by the U.S. Department of Agriculture (USDA), only fertilizers and pesticides approved by the National Organic Standards Board (NOSB) can be used for crop production (Winter and Davis, 2006). The list of NOSB-approved fertilizers includes products like manure and agricultural residues. Because of the limited sources of suitable organic fertilizers, some producers are forced to procure their fertilizers from different regions (i.e. away from their locations) of the United States or from international markets.

Ideally, local organic fertilizer sources, like manure from local dairy farms, would fulfill the nutrient needs of organic farms. However, while dairy manure contains the nutrients needed to support a variety of crops, pathogen concentrations typically exceed recommended standards for use in human food production. To minimize food contamination, the Organic Materials Review Institute (OMRI) defines raw and processed manure fertilizers and the corresponding limitations and restrictions in their use. Raw manure includes "feces, urine, other excrement, and bedding produced by livestock that has not been composted." Raw manure can be used as an organic fertilizer only if it is (i) applied to land used for a crop not intended for human consumption; (ii) incorporated into the soil not fewer than 120 days prior to the harvest of a product whose edible portion has direct contact with the soil surface or soil particles; or (iii) incorporated into the soil not less than 90 days prior to the harvest of a product whose edible portion does not have direct contact with the soil surface or soil particles (OMRI, 1997).

When used in organic production, processed manure is defined as manure that has reached a minimum temperature of either at 66^0C (150^0F) for at least one hour or 74° C (165° F) with a maximum moisture level of 12%; or an equivalent heating and drying process (OMRI, 1997). Additionally, fecal coliform concentrations must be < 1,000 MPN per gram of processed manure and the Salmonella concentrations must be < 3 MPN per 4 grams of processed manure (OMRI, 1997). There are no restrictions on intervals between application and harvest when processed manure is used in organic food production.

1.4 GOALS AND OBJECTIVES

Given heightened concern regarding environmental health and energy security, dairy farms are in a unique position to convert a sometimes troublesome waste into value-added products. Anaerobic digestion of dairy manure produces renewable energy and fertilizers while reducing greenhouse gas

emissions, manure odors, pathogens, and waste strength. While the environmental benefits of anaerobic digestion are easily identified, quantification of production and economic viability varies between farms. This research uses a Virginia dairy farm as a case study site for an in-depth examination of how anaerobic digestion and manure-derived value-added products can affect the environmental and economic impacts of dairy farming.

The overall goal of this study was to evaluate the opportunities to improve the environmental and economic sustainability of dairy production under the following criteria:

a. The farm must have minimal impact on the local environment, including odors, contamination from manure pathogens, and greenhouse gases.

b. The farm must be powered by renewable energy sources.

c. There must be minimal inputs to farm operations and waste products.

d. The farm must be financially solvent.

The specific objectives to achieve this goal were:

1. Evaluate the performance of an on-farm AD with respect to: (i) biogas production (quantity and quality); (ii) waste stabilization; (iii) production of value-added products; (iv) operation and maintenance requirements for the digester; and (v) optimization of anaerobic digester operation.
2. Evaluate the suitability of locally available feedstocks in Virginia for co-digestion with dairy manure to increase biogas production.
3. Determine: (i) the effective combination of pasteurization temperature and duration to reduce fecal coliform, *E. coli*, and *Salmonella* concentrations in anaerobically digested dairy manure to the levels required for use as an organic fertilizer; and (ii) the effects of pasteurization temperature and duration on fertilizer quality.
4. Determine how manure-derived value-added products affect the environmental and economic sustainability of dairy farming using an existing on-farm anaerobic digester as an example.

1.5 THE NEED FOR RENEWABLE ENERGY SOURCES AND MITIGATION OF GHG

The need to establish local renewable energy reserves and evaluate the environmental consequences of utilizing fossil-fuel based energy has been driven by global concerns over fossil fuel depletion and climate changes associated with increased atmospheric carbon (US EPA, 2012). In 1998, the USGS estimated that fossil fuels would be depleted in 88 to 220 years based on the projected use for the year 2000, prompting the need to explore renewable energy sources (Chynoweth et al., 2001). Increasing atmospheric GHG levels resulting from fossil fuel sources have prompted the U.S. government to appropriate more than $90 billion and tax incentives for the development of clean and renewable energy sources focused on preserving energy security and air quality (U.S. DOE, 2012). Greenhouse gases are typically measured in carbon dioxide equivalent (CO_2e) units to normalize the radiative energy (i.e. carbon flux) of any greenhouse gas to that of CO_2, thereby allowing universal comparison (IPCC, 2006). Current estimates state that milk production by the U.S. dairy industry results in annual emissions of 16.2 million tonnes CO_2e; altogether, activities associated with the dairy industry result in the release of 28 million tonnes CO_2e (Innovation Center for US Dairy, 2008). These activities include crop production, milk production, milk processing and packaging, transport, and distribution (Innovation Center for US Dairy, 2008).

As concerns related to energy independence and climate change have heightened, an increasing number of dairy farms in the U.S. have installed anaerobic digesters to reduce GHG emissions and produce renewable energy. In 2011, 15 new anaerobic digesters were commissioned on livestock farms, bringing the total number of operating systems nationwide to 176. According to U.S. EPA (2012), on-farm anaerobic digesters reduced direct methane emissions from manure by 1.2 million tonnes of CO_2e in 2011 and generated 541 million KWH of renewable energy, replacing the use of fossil fuels that would have released 301,000 tonnes of CO_2e (U.S. EPA, 2012).

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2 Evaluation of an On-farm Anaerobic Digester for Manure Treatment and Biomethane Production

2.1 ABSTRACT

Anaerobic digestion of dairy manure has the benefits of reducing greenhouse gas emissions, odors, and chemical oxygen demand while producing biomethane (CH_4). This study explored the performance of an on-farm anaerobic digester (AD) designed to serve a 1200 milking herd dairy farm in Virginia during its first 18 months of its operation. The objective of the monitoring was to assess AD performance with respect to: (i) biogas production (quantity and quality); (ii) waste stabilization; (iii) production of separated solids and liquids; (iv) operation and maintenance requirements for the digester; and (v) optimization of anaerobic digester operation. Samples from the digester influent, three locations within the digester, digester effluent, and separated liquid and solids from the digester effluent were collected at least once a month and analyzed for manure characteristics including pH, solids, chemical oxygen demand (COD), volatile fatty acids, and nutrient content. Biogas quantity and quality were also routinely monitored electronic gas production logs and Fourier transform infrared spectroscopy. Fermentation potential tests were conducted using the manure generated on the farm to determine the appropriate retention time for the manure in the acid chamber of the on-farm digester. Digester performance was comparable to previously published studies. Total COD and soluble COD concentrations were reduced on average by 29% and 57%, respectively. The average total solids and volatile solids reductions were 28% and 35%, respectively. Average daily biogas production ranged from 3,300 to 7,000 m^3, with biogas yields ranging from 0.43 and 0.89 m^3 kg $VS^{-1}d^{-1}$. The average biomethane content in the biogas was 54%. Biogas was converted to electricity by an on-farm generator that produced an average of 360 KWH d^{-1}, which was fed to the local grid. The solids separator generated 13.4 tonnes of separated solids, which were used on-farm as bedding for the dairy cows. Based on lab-scale fermentation potential tests, a hydraulic retention time of 3 d in the acid chamber would maximize total volatile fatty acid concentrations, which is expected to increase biomethane production.

2.2 INTRODUCTION

2.2.1 Anaerobic Digestion: a Manure Management Solution and Renewable Energy Source

On-farm anaerobic digesters can reduce the negative environmental impacts associated with manure, including odors, putrescible waste (generally measured by chemical oxygen demand, or COD), and greenhouse gas emissions (GHG) (Cantrell et. al, 2008; EPA, 2004). Methane (CH_4) emitted from the biodegradation of manure is the second largest source of GHG from dairy operations, accounting for 5.78 tonnes of CO_2e per year (Innovation Center for US Dairy, 2008). In addition to capturing CH_4, which can be used as a renewable energy source, the anaerobic digestion process converts manure into a homogenous, stable, and nutrient-rich product that can be used as a fertilizer and soil conditioner.

The anaerobic digestion process and its major products are illustrated in figure 2.1. Anaerobic digestion involves four major steps: hydrolysis of biodegradable particulate matter into soluble acids, sugars, and fatty acids; fermentation of hydrolysis products into total volatile fatty acids (TVFAs) and acetic acid (acidogenesis and acetogenesis, respectively); and conversion of fermentation products to methane (methanogenesis) (Grady et al., 2011; Speece, 1996). TVFAs are the substrate for acetogenic and methanogenic microorganisms during methane production (Banister and Pretorius, 1998; Coats et al., 2011; Grady et al., 2011); therefore, to maximize biomethane yields from organic feedstock, it is imperative to produce maximal TVFAs within the limits of inhibition. In the context of biomethane production, the fermentation potential of a material is defined as the sum of its inherent TVFAs and the TVFAs produced during active or designed fermentation phase in a process (Coats et al., 2011; Güngör et al., 2009; Lie and Welander, 1997).

As of September 2012, approximately 160 dairy farms in the US have operational anaerobic digesters, which is equivalent to approximately 6% of farms estimated to be appropriate sites for on-farm anaerobic digestion technology (U.S. EPA AgSTAR, 2011). Despite the benefits of on-farm anaerobic digestion listed, the economics of the system remain a significant barrier. The cost of installing a digester depends on both the type of digester and the herd size, as shown table 2.1 (AgStar, 2010), and can range from 780,000 to several million dollars. In general, the cost of anaerobic digester system increases with complexity and the number of moving parts installed.

The average installation costs for on-farm plug flow and anaerobic lagoon ADs reported by Crenshaw (2009) reflect the cost projections suggested by the US EPA AgStar described in table 2.1 (2010). Currently, 500 cows is the perceived minimum herd size required for on-farm anaerobic

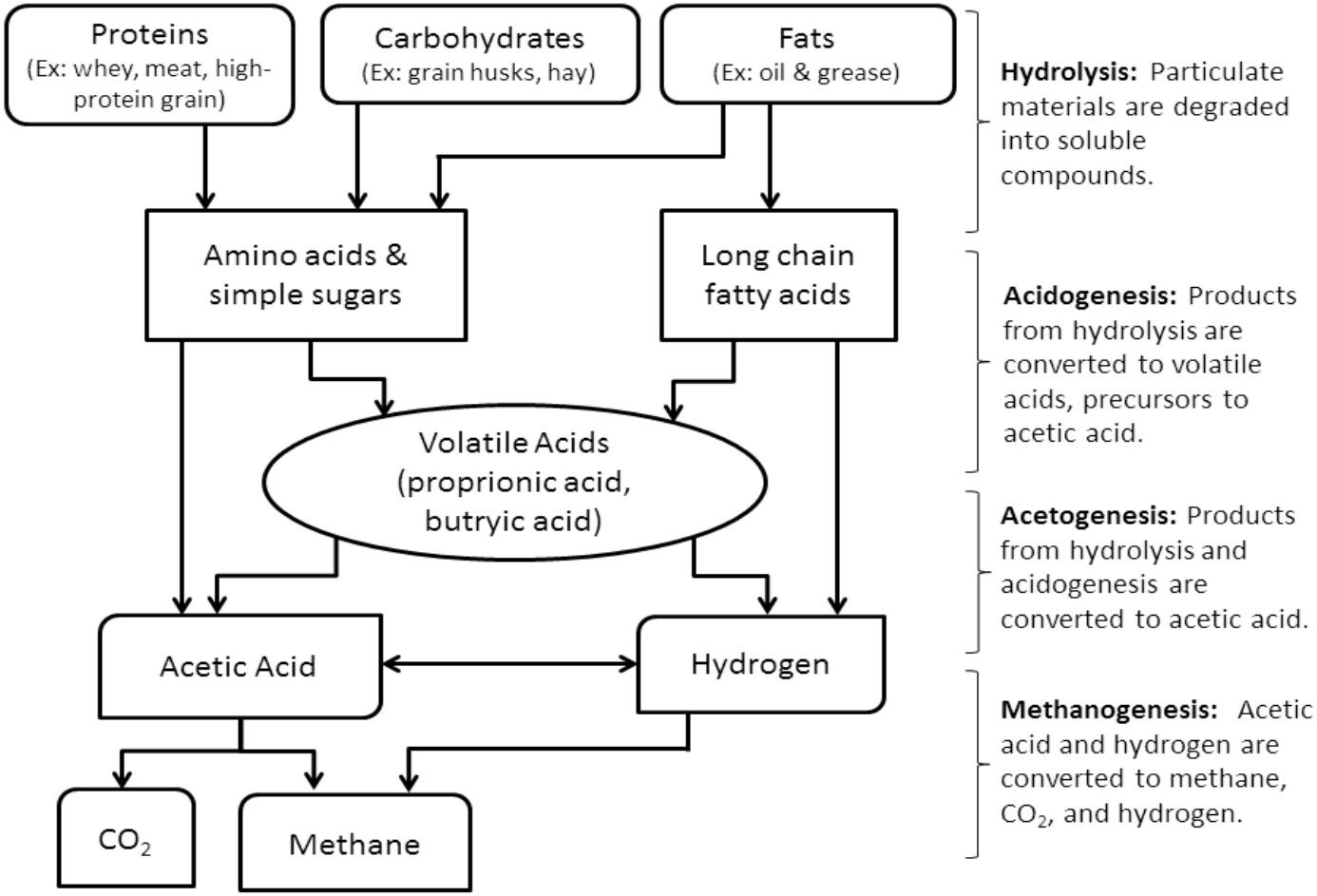

Figure 2.1. Anaerobic Digestion Process Overview (Adapted from Grady et. al, 1999 and Speece, 1996)

Table 2.1. Equations for estimating complete mix, plug flow, and covered lagoon AD system capital costs, adapted from U.S. EPA AgSTAR (2010).

Digester Type	Capital Cost (Sep. 2009 $)	Capital Cost based on Number of Dairy Cows ($)
Complete Mix	$563 \times N + 320{,}864$	$7{,}881 \times N^{-0.3152}$
Plug Flow	$617 \times N + 566{,}006$	$13{,}308 \times N^{-0.3493}$
Covered Lagoon	$400 \times N + 599{,}556$	$68{,}516 \times N^{-0.6074}$

N - the number of dairy cows on a farm

digestion to be economically feasible (US EPA AgStar, 2010). In 2009, the average cost of installing an on-farm plug-flow mesophilic anaerobic digester (MAD) ranged from $800,000 to $4.6 million for dairies with 500 to 4,000 cows (Crenshaw, 2009). In contrast, the average cost of an on-farm covered lagoon AD ranged from $780,000 to $1.2 million for dairies with 500 to 1,600 cows (Crenshaw, 2009).

The success of existing on-farm AD is generally attributed to support from US federal, state, and local governments via policies providing financial incentives such as grants, tax credits, rebates, and low interest loans to offset the prohibitive costs of installing AD systems (AgSTAR, 2013, DSIRE 2013). Incentives vary by locality and are established to promote the installation of renewable energy systems on farm and residential properties in states like New York, North Carolina, Pennsylvania, California, and Wisconsin. Currently, the Commonwealth of Virginia does not have financial or policy incentives through state programs that directly benefit and thereby encourage on-farm AD implementation (DSIRE, 2013). Basing renewable energy credit payments on market prices rather than state-supported supported programs involves considerable uncertainty, which is generally unattractive for producers already subject to anticipated variability in milk prices. These financial pressures often motivate farms with existing ADs to seek additional revenue streams by identifying, utilizing, and selling value-added products from their AD systems (K. VanderHyde, Dairy Energy LLC, personal communication, 3 January 2013).

2.2.2 Value-added Products and Services from Anaerobic Digestion of Dairy Manure

Anaerobic digestion of dairy manure provides several environmental services and value-added products. Services include capturing greenhouse gases that would otherwise be emitted into the environment and reducing nuisance odors associated with untreated dairy manure. Odors have been shown to reduce real estate property values near/adjacent to farm communities and thereby increase tensions between farmers and their neighbors (Palmquist et al., 1997; Smith et al., 2008; Yiridoe et al., 2009). However, anaerobic digestion has been shown to decrease the nuisance manure odors by more than 50% (Powers et al., 1999; Powers et al., 1997).

The value-added product of biogas is produced during the anaerobic digestion of dairy manure. Organic wastes from other industries, which might otherwise be sent to landfills, can also be directed to anaerobic digesters and used to produce biogas. Studies suggest that biogas production and biomethane content can be increased by co-digesting dairy manure with organic feedstock from food (pre- and post-consumption) and industrial food processing plant waste streams (El-Mashad and Zhang, 2010; Zhang et al., 2007). Co-digestion can also improve the nutrient balance within anaerobic digesters, which consequently increases the metabolic activity of microorganisms, treatment efficiency, and the fertilizer quality of AD effluent (Zhang et al., 2007).

Other value-added products from anaerobic digestion of dairy manure include separated manure solids and separated liquid manure. Anaerobically digested separated solids can be used as cow bedding, replacing the need to import bedding materials like wood shavings, straw, or sand onto the farm. Use of anaerobically digested separated solids as beddings may reduce mastitis incidence in dairy cows, thereby reducing treatment and lost milk costs (Dvorak, 2005; K.VanderHyde, Dairy Energy LLC, personal communication, 01 December 2012).

Anaerobically digested and separated liquid manure (ADSLM) can be used as a fertilizer for on-farm crop production or off-farm sale (Cantrell et. al, 2008). However, current restrictions prevent or limit the use of untreated and anaerobically digested dairy manure as a fertilizer in the production of crops for human consumption. Restrictions in the use of manure as fertilizer are enforced to prevent contamination of human food with manure-borne pathogens like *E. coli, Salmonella*, and *Cryptosporidium*. Since raw and anaerobically digested manure have these restrictions, dairy farms use their manure fertilizer for crops intended as dairy feed. Growing interest in organic food production, however, has promoted the development of treatment standards for manure fertilizers used in production of human food crops (OMRI, 1997). Treatment of ADSLM for on-farm use and off-farm sale is described in Chapter 4, Pasteurization of Anaerobically Digested Separated Liquid Manure.

Although the benefits of anaerobic digestion are easily identified, the extent to which manure is stabilized and value-added products are produced varies between farms and digester system types. It is of particular interest to monitor an AD's performance during its early stages of operation to set long-term operational standards that maximize efficiency. The objective of this research was to monitor a recently installed AD at Dairy Energy, LLC with respect to: (i) biogas production (quantity and quality); (ii) waste stabilization; (iii) production of separated liquids and separated solids; (iv) operation and maintenance requirements for the digester; and (v) optimization of anaerobic digester operation.

2.3 MATERIALS & METHODS

2.3.1 Location and Description of the Anaerobic Digester System

The AD was located on a 1,200 milking herd dairy farm in Chatham, Virginia. The reactor vessel was an in-ground two-stage mixed plug-flow designed by DVO, Inc. (Chilton, WI). Construction of the AD began in December 2009 and was completed in December 2010, with the first batch of manure loaded into the digester on December 25, 2010. Evaluation of the AD performance was conducted from May 2011 to August 2012, though the monitoring of biogas production continued through December 2012.

The material flow through the AD system is illustrated in figure 2.2. The digester (figure 2.3) was 48 m long by 23 m wide and 5 m deep with a working volume of 4,700 m^3 and a 660 m^3 headspace for

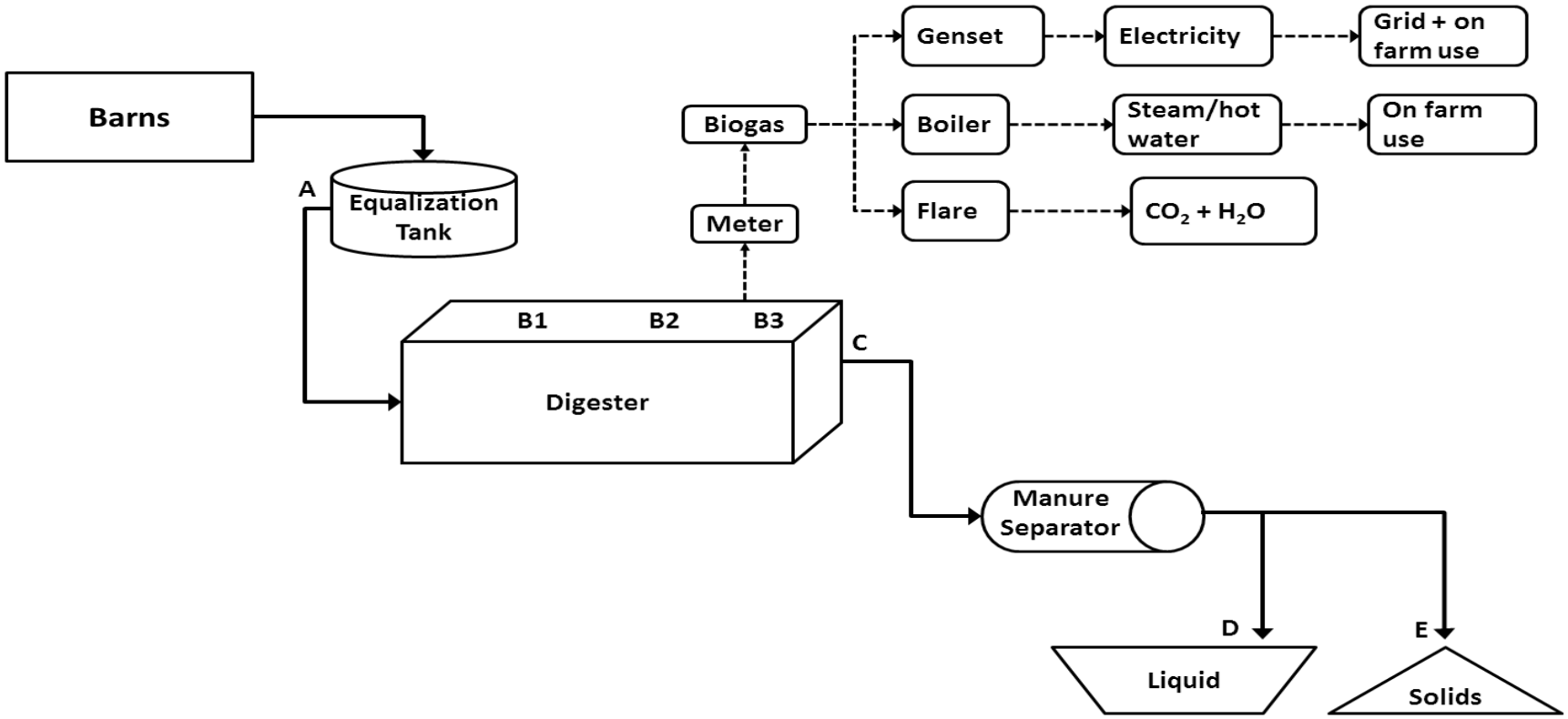

Figure 2.2. Material Flow through the Anaerobic Digester System.

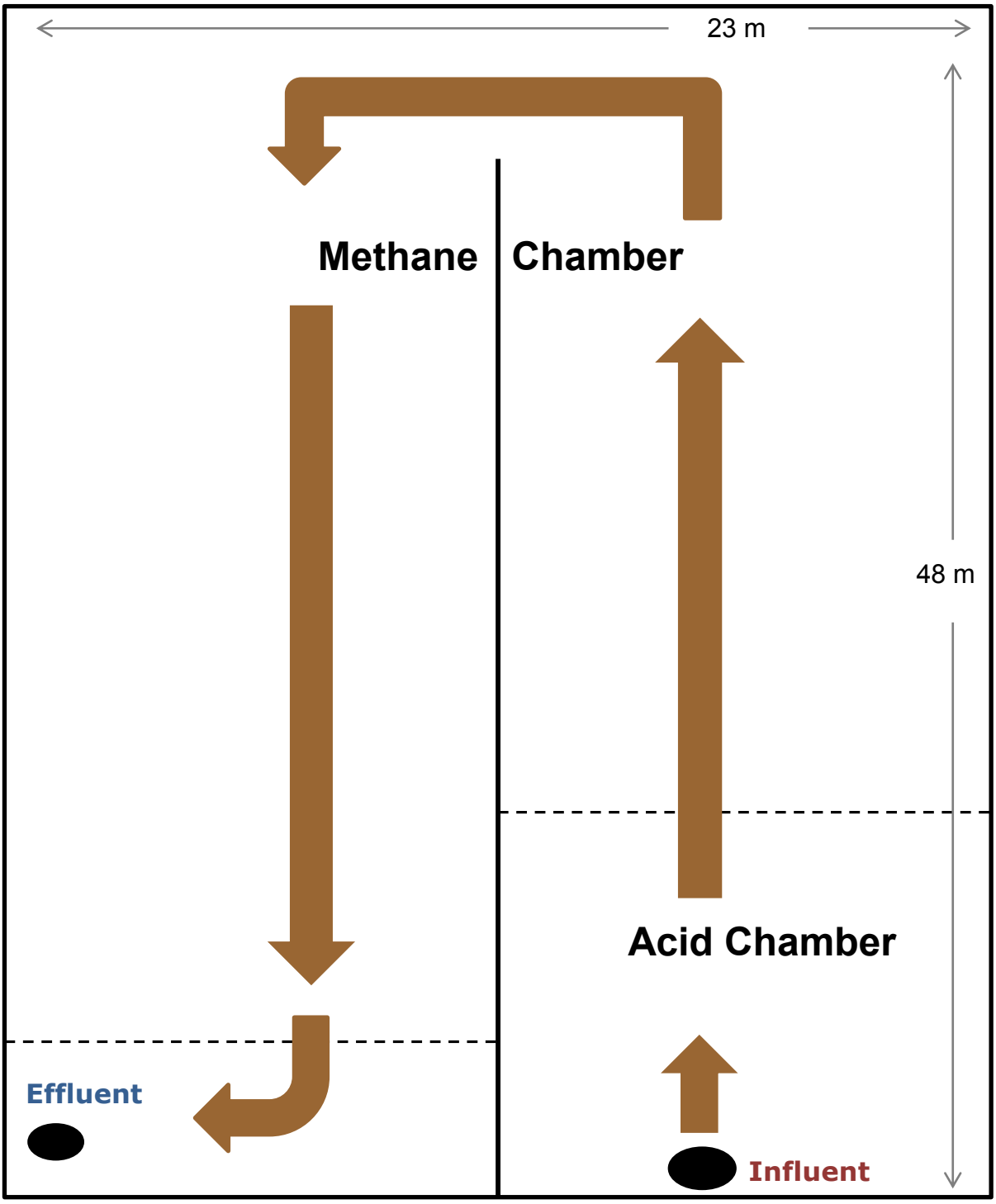

Figure 2.3. Layout inside the two-stage plug flow anaerobic digester.

gas storage. The digester had two chambers: the first chamber, called the "acid chamber," was designed for fermentation of manure to produce volatile fatty acid (VFA), and the second chamber, called the "methane chamber," was devoted to methanogenesis. The design total hydraulic retention time (HRT) was about 28 d. The influent and effluent ports of the digester were located on the same end.

Manure was scraped from the barns every 30 min and transported by gravity to a 90.9 m^3-capacity equalization tank pit (not shown), equivalent to a loading rate of approximately 6 m^3 manure per 90 min. Influent and effluent rates were equivalent, i.e. discharge was also approximated as 4 m^3/hr. The AD effluent was pumped to the manure separation unit where it was separated into solid and liquid streams mechanically by two screw presses (FAN Separator GmbH, Marktschorgast, Germany) operated in parallel. The screw presses had screens with 0.5 mm slot openings. After separation the liquid stream was pumped to a storage pond and used as crop fertilizer on the farm, while he separated solids were stored in a covered shed with a concrete floor and allowed to dry under ambient conditions. Once dried, the solids were recycled as bedding for the dairy cows in the milking barns or land-applied as a soil conditioner and fertilizer.

The gas produced in the digester was piped and delivered to the engine house to generate electricity. Electricity was generated in a 450 kW Guascor Generator (genset) Model No. MGG-712 genset distributed by Martin Machinery, LLC of Latham, MO (figure 2.4). The genset had an efficiency of 35%. Some of the waste heat was recovered and used to maintain the temperature of the digester contents at 38°C. During the periods of scheduled maintenance of the genset or unexpected shutdown due to power transmission line repairs, the gas was used to power a boiler (figure 2.5). The boiler (Model MPH 60 boiler, Columbia Boiler Co., Pottstown, PA, USA) had an energy input of 2,658 mega joules (MJ) per hour with an efficiency of 83% and a steam output rate of 939 kg per hour. Surplus biogas was flared (figure 2.6) to prevent the escape of CH_4 into the atmosphere. The volume of gas used by the engine, boiler or flare is recorded every 15 min by a data logger with remote access capabilities. Hydrogen sulfide and moisture were removed from the biogas via an in-line filter located before the engine.

2.3.2 Evaluation Criteria for Waste Stabilization and Biogas Production

Association of State Energy Research & Technology Transfer Institutions (ASERTTI) protocol was used to evaluate the performance of the AD (ASERTTI, 2007). Briefly, the ASSERTI protocol recommends monitoring digester performance for a period of at least 12-months after the start-up phase is completed. For a plug-flow or mixed digesters, the start-up phase is considered complete after at least five hydraulic retention times (HRTs) of continuous operation. The waste stabilization parameters

Figure 2.4. The Guascor generator (genset) model no. MGG-712 genset.

Figure 2.5. Model MPH 60 boiler.

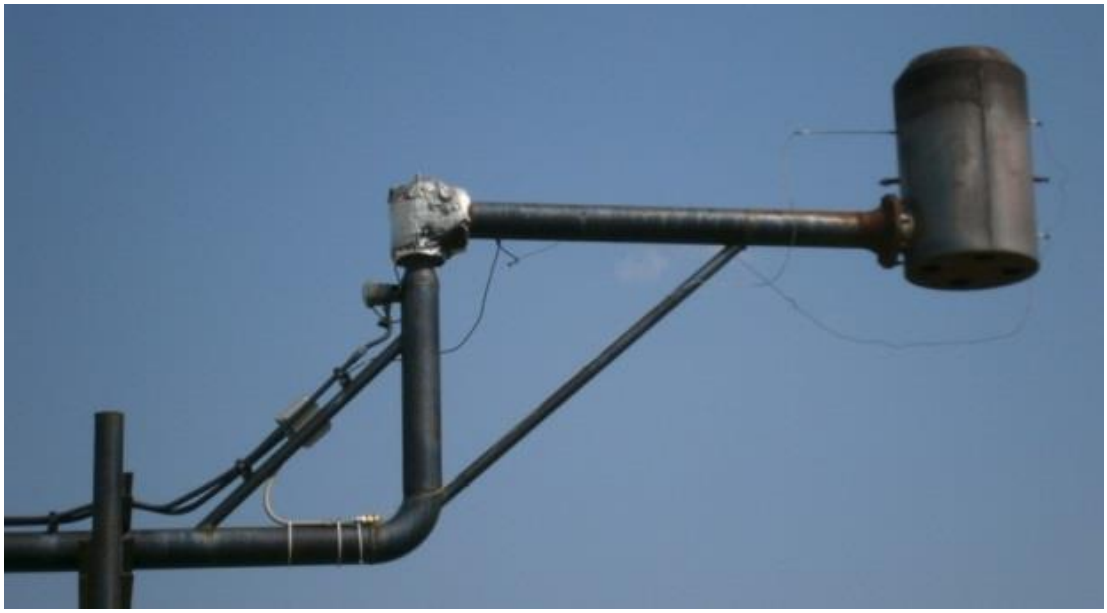

Figure 2.6. Flare for burning excess biogas.

assessed included total solids (TS), total volatile solids (VS), chemical oxygen demand (COD), and total volatile acids (TVFA; the sum of acetic, propionic, isobutyric, butyric, isovaleric and valeric acid). The degree of waste stabilization was assessed by comparing the relevant parameter concentrations in the AD influent and effluent, as described in equation 2.1 (ASERTTI, 2007). Also included in the performance evaluation were the quantity and quality of biogas produced.

$$\text{Waste Stabilization (\%)} = \frac{\text{Concentration}_{\text{Influent}} - \text{Concentration}_{\text{Effluent}}}{\text{Concentration}_{\text{Influent}}} \times 100 \qquad (2.1)$$

2.3.3 Sample Collection

Samples were collected once every two weeks between May 2011 and September 2011 and then once a month between October 2011 and August 2012. Sampling locations are shown in figure 2.2. Samples were collected from the digester influent (A), locations 1, 2, and 3 inside the digester (B1, B2, and B3, respectively), digester effluent (C), separated liquid (D), and separated solids (E). Location 1 was located inside the methane chamber of the anaerobic digester, approximately 5 m beyond the acid chamber. Location 2 was located in the methane chamber, approximately 13 m beyond location 1. Location 3 was also located in the methane chamber, approximately 23 m upstream of the AD effluent.

Prior to collecting influent samples, the manure in the equalization tank was mixed for approximately five min. Samples from the effluent were collected directly from the effluent port of the digester. The separated liquid and solids were obtained after running the manure separators for at least 5 min. Sample volumes of approximately 2 L were collected at each sampling location, bottled, placed on ice, and transported to the Virginia Tech Bioresidues Utilization and Management (BRUM) Lab for analysis. Samples were refrigerated at 4°C and analyzed per the APHA standard methods for wastewater examination (APHA, 2012).

The quality of the gas provided to the generator was analyzed at least once every two months using a CAI 600 Fourier Transform Infrared Spectroscopy (FTIR) gas analyzer (California Instruments, Inc., USA). The gas was analyzed for methane, carbon dioxide, water, and ammonia contents for at least 15 min.

2.3.4 Analytical Methods

Concentrations of TS, VS, and TVFA were analyzed according to the standard methods for analyzing water and wastewater (APHA, 2012) methods 2540B and 2540E, respectively. TVFA were analyzed according to method 5560D. The total COD (tCOD) and soluble COD (sCOD) were analyzed using the HACH Method 8000 (HACH, CO, USA). Samples analyzed for tCOD, sCOD, and TVFA were prepared as described in table 2.2. The TVFAs were analyzed using a gas chromatograph (HP

Table 2.2. Dilutions, pretreatment, and storage of samples for laboratory analysis

Parameter	Dilution	Pre-treatment	Storage
tCOD	100x	Blender-Homogenized (30 seconds)	Refrigeration at 4°C
sCOD	20x	Centrifuged (15000g for 20 min) Filtered (0.45 µm filter membrane)	Analyzed Immediately
TVFA	20x	Centrifuged (15000g for 20 min) Filtered (0.45 µm filter membrane)	Freezer storage at -20°C
TS, VS, pH	1x	None	Analyzed Immediately

tCOD - Total chemical oxygen demand

sCOD - Soluble chemical oxygen demand

TVFA - Total volatile fatty acids

TS – Total solids content

VS - Volatile solids content

5890) equipped with a Nukol-fused Silica Capillary Column (15 m × 0.53 mm × 0.5 µm film thickness) and a flame ionization detector (FID). The injector and detector temperatures were 200°C and 250°C, respectively. The temperature program was set to start at 80°C, hold for 3 min, ramp to 140°C at 6°C min^{-1} and hold at 140°C for 1 min. Helium was used as the carrier gas through the column at a flow rate of 16 $mL \cdot min^{-1}$. Nitrogen was used as the FID makeup gas at a flow rate of 14 $mL \cdot min^{-1}$ for a total flow rate of 30 $mL \cdot min^{-1}$ (makeup flow + carrier flow).

Total Kjeldahl nitrogen (TKN), organic nitrogen (Org-N), ammonia nitrogen (TAN), total phosphorous (TP), dissolved reactive phosphate (DRP), sulfur (S), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), aluminum (Al), and chloride (Cl) values were determined at the Agricultural Services Laboratory, Clemson University.

2.3.5 System Volumetric and Mass Flow Analysis

Flow measurements were conducted to ascertain the digester influent and effluent flow rates in order to determine the actual operating HRT and estimate production rates for separated solids and separated liquids.

2.3.5.1 *Influent, Effluent, and Separated Liquids Volumetric Flow Rates*

The manure volume flow rates into the digester were calculated from measurements of liquid level changes at the equalization tank. The liquid levels were recorded using level logger (In-Situ Inc., Fort Collins, CO, USA) for approximately two months between November 2011 and January 2012. The logger was set to record the manure level at 1-min intervals. The volume of manure fed to the AD was calculated using the changes in the liquid depth and the surface area (length and width dimensions) of the equalization tank. The volume of the effluent was estimated as the sum of the volumes of the separated liquids and solids. To monitor the effluent flow from the digester, the level logger was installed in the pit for storing separated liquids over a 9 d period. The volume of separated liquid was calculated using the same procedure for the equalization tank. The liquid level was recorded once every min. The rate of separated liquids production was calculated from the separated liquid pit length and width dimensions and the recorded liquid level changes.

2.3.5.2 *Separated Solids Production*

The quantity of separated solids produced was estimated by collecting both the solids and liquids coming out of the separators over 5 min periods when the operating at steady state, for a total duration of 60 minutes. The volume and mass of separated solids were measured and the bulk density of separated solids was determined and used to calculate the volume of solids from each separator. The bulk density was determined in the field using a 20-L bucket and a scale. First, the mass of the empty bucket was

measured. Then the volume of the bucket was determined by filling it with water. The bucket was filled approximately to 1/3 of its total volume (equivalent to approximately7 L) of separated solids. The bucket was then dropped 10 times from a height of approximately 0.3 m onto a concrete surface. The bucket was filled with an additional 7 L of solids and compacted as described above. Finally, the bucket was topped with additional solids and leveled off without any compaction. The bulk density was calculated according to equation 2.2.

$$\text{Bulk Density } \left(\frac{\text{kg}}{\text{m}^3}\right) = \frac{\text{Mass of Separated Solids (kg)}}{\text{Volume of Separated Solids } (\text{m}^3)} \quad (2.2)$$

The flow rate of the AD effluent was obtained using the separated solids and separated liquid production rates according to equation 2.3.

$$\text{Flow Rate}_{\text{AD Effluent}} \left(\frac{\text{m}^3}{\text{min}}\right) = \frac{\text{Volume}_{\text{SeparatedLiquids}} (\text{m}^3) + \text{Volume}_{\text{Separated Solids}} (\text{m}^3)}{\text{Time (min)}} \quad (2.3)$$

The AD hydraulic retention time (HRT) was calculated using the working volume and the measured influent flow rate, according to equation 2.4.

$$\text{HRT} = \frac{\text{AD Working Volume } (\text{m}^3)}{\text{Flow Rate}_{\text{AD Influent}} (\text{m}^3\text{d}^{-1})} \quad (2.4)$$

2.3.6 Fermentation Potential of Dairy Manure

To find the optimal HRT based on maximal TVFA production, a series of 9-d fermentation potential tests were conducted in the lab using raw dairy manure obtained from Dairy Energy, LLC. The dairy manure was collected from the equalization tank and transported on ice to the BRUM lab at Virginia Tech. In the lab, the manure was thoroughly mixed and twenty-one 200 ml aliquots were placed in Erlenmeyer flasks (250 ml capacity) and then capped. The flasks were placed in a reciprocating incubator shaker (New Brunswick Scientific Co., Edison, NJ, USA) set at 175 RPM and a temperature of 38°C for a period of 9 d. Sacrificial sampling was completed on days 1 through 5 and on days 7 and 9, by randomly removing three flasks (to provide three replicates) from the incubator shaker without replacement. Samples were analyzed for TS, VS, tCOD, sCOD, TVFAs, and pH as described in section 2.3.3 and table 2.2. This experiment was repeated four (4) times, for a total of 12 observations for each sampling interval.

2.3.7 Statistical Analysis

Statistical analysis was performed using JMP version 10 software (SAS, 2013). The data was tested for normality, and one-way analysis of variance (ANOVA) was used to test the effect of anaerobic

digestion on manure characteristics. The Tukey-Kramer multiple means comparisons was used to test the differences in means of manure characteristics at the AD influent, location 1, location 2, location 3, effluent, and separated liquid. Characteristics of solids at the two solids separators were compared using ANOVA and Tukey-Kramer analyses. For the fermentation study, ANOVA was used to test the effect of time on manure characteristics. The Tukey-Kramer multiple means comparisons was used to test the differences in means of TVFA, sCOD and VS due to time. Differences were deemed significant at $p< 0.05$.

2.4 RESULTS & DISCUSSION

2.4.1 Waste Stabilization

The manure characteristics at various locations of the AD system are presented in table 2.3. The concentrations of the tCOD, sCOD, TVFA, VS, TS, and TAN in the AD influent and effluent were significantly different (ANOVA, $p< 0.0001$). The overall reductions were measured between the AD influent and effluent. Reductions in TS and VS were 29% and 35%, respectively. As expected from a highly fibrous material such as dairy manure, the average tCOD concentration was reduced by just 29%. The overall reduction in sCOD was and 57%. The sCOD accounted for 21% of the tCOD in the AD influent and 13% in the AD effluent, illustrating that the majority of the substrate biodegraded in the AD was in soluble form. This observation is consistent with the understanding that most microorganisms in an anaerobic digester can only utilize substrates that have previously been transformed into a soluble form. Hydrolytic microorganisms degrade complex materials into their simpler, soluble substrates, which fermentative and acetogenic microorganisms use to produce acetate. Manure can be used for methane production only after it has been degraded and converted into acetate for subsequent consumption by methanogens (Grady et al., 2011; Speece, 1996).

The tCOD and sCOD at locations 1, 2, and 3 within the digester were not significantly different, with sCOD-to-tCOD ratios ranging between 12 and 13% at all three locations. This observation suggests that the system was actually operating as a completely-mixed reactor, as opposed to a plug-flow design. The tCOD and sCOD concentrations in separated liquid were not significantly different from the AD effluent (ANOVA, $p<0.0001$). This finding indicates that separation of anaerobically digested manure into solids and liquids does not remove any additional COD for AD effluent or that most of the COD is in the separated liquid.

Table 2.3. Average (±standard deviation) concentrations at various locations of the AD system

Parameter	Location					
	AD Influent	**Location 1**	**Location 2**	**Location 3**	**AD Effluent**	**Separated Liquid**
tCOD ($g \cdot L^{-1}$)	75.44 (±19.47)^{a}	53.90 (±11.19)^{bc}	59.70 (±16.37)^{b}	55.04 (±14.17)^{bc}	53.91 (±11.31)^{bc}	42.39 (±11.20)^{bc}
sCOD ($g \cdot L^{-1}$)	15.70 (±2.5)^{a}	7.00 (±0.99)^{b}	6.82 (±1.04)^{b}	6.81 (±1.10)^{b}	6.79 (±0.82)^{b}	7.38 (±1.37)^{b}
TS (%)	7.4 (±1.0)^{a}	5.3 (±0.5)^{b}	7.1 (±2.3)^{a}	5.9 (±1.0)^{b}	5.3 (±0.5)^{b}	4.1 (±0.9)^{c}
TVS (%)	6.0 (±0.8)^{a}	4.0 (±0.4)^{c}	5.1 (±1.3)^{b}	4.4 (±0.7)^{bc}	3.9 (±0.4)^{c}	2.9 (±0.8)^{c}
TVFA ($mg \cdot L^{-1}$ as COD)	4,354 (±1,158)^{a}	373 (±134)^{b}	325 (±137)^{b}	298 (±124)^{b}	365 (±174)^{b}	417 (±161)^{b}
TKN ($mg \cdot L^{-1}$)	3,223 (±345)^{a}	3,269 (±250)^{a}	3,380 (±283)^{a}	3,413 (±268)^{a}	3,313 (±259)^{a}	3,186 (±381)^{a}
TAN ($mg \cdot L^{-1}$)	1,300 (±162)^{a}	1,614 (±157)^{b}	1,620 (±152)^{b}	1,617 (±156)^{b}	1,667 (±182)^{b}	1,664 (±178)^{b}
TP ($mgP \cdot L^{-1}$)	535 (±95)^{a}	520 (±59)^{a}	577 (±102)^{a}	529 (±77)^{a}	566 (±160)^{a}	505 (±89)^{a}
DRP ($mgP \cdot L^{-1}$ L)	162 (±58)^{a}	71.1 (±40)^{b}	80.0 (±29.4)^{b}	90.5 (±96)^{b}	71.6 (±22)^{b}	49.8 (±20)^{b}
K ($mg \cdot L^{-1}$)	2,257 (±256)^{a}	2,242 (±176)^{a}	2,263 (±154)^{a}	2,177 (±162)^{a}	2,274 (±165)^{a}	2,358 (±204)^{a}
pH	7.53 (±0.05)^{a}	7.81 (±0.05)^{b}	7.79 (±0.06)^{b}	7.81 (±0.06)^{b}	7.79 (±0.05)^{b}	7.94 (±0.05)^{a}
Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	10,437 (±1,990)^{a}	13,127 (±1,466)^{a}	12,422 (±1,492)^{a}	12,556 (±1,155)^{a}	13,493 (±1,536)^{a}	12,877 (±1,349)^{a}

*Concentrations with different superscripts (within rows) are significantly different at $p < 0.5$.

tCOD – Total chemical oxygen demand

sCOD – Soluble oxygen demand

TS – Total solids (% of wet weight)

TVS – Total volatile solids (% of wet weight)

TVFA – Total volatile fatty acids

TKN – Total Kjeldahl nitrogen

TAN – Total ammonium nitrogen

TP – total phosphorus

DRP – Dissolved reactive phosphorous

K - Potassium

Overall, the inherent TVFA concentration in the AD influent was higher and significantly different from the TVFA concentrations at all other sampling locations. The TVFA concentrations accounted for 28% of influent sCOD concentrations, compared to 5.3%, 4.8%, 4.4% and 5.3% of sCOD concentrations at locations 1, 2, 3 inside the digested, and the AD effluent, respectively. These findings show large and significant decrease in TVFAs during the early stages of anaerobic digestion (i.e. influent and location 1). There was minimal decrease and no significant differences in TVFA concentrations at locations 1, 2 and 3, and the AD effluent. However, it was expected that additional TVFAs would be produced in the acid chamber from the hydrolysis and fermentation of organic matter in manure. It was expected that TVFA concentrations would reflect a gradual decrease or differentiated concentrations as the manure moved through locations 2, 3, and the effluent as a result of conversion to CH_4 the methane chamber.

The persistently low TVFA and sCOD concentrations observed starting at location 1 suggests that either hydrolysis and fermentation were not proceeding quickly enough to maintain TVFA and sCOD concentrations, or that the anaerobic digester was actually performing like a complete mixed (also referred to as continuously-stirred) reactor rather than a plug-flow digester. Plug-flow digesters are expected to have uniform properties at all locations within a single "plug" of material. By contrast, all locations within a complete-mix reactor have uniform properties (Grady et al., 2011). The observations in this study suggest that the anaerobic digester was a two-stage compete-mix reactor. The first stage occurred in the acid chamber, which was designed to maximize hydrolysis and TVFA production. The second stage occurred in the methane chamber to maximize biogas production.

As expected with AD systems, there was no significant difference in the nutrient (TKN, TP and K) concentrations between the AD influent and effluent. However, there was a 28.2% increase in TAN and 55.8% decrease in DRP concentrations between the AD influent and effluent. While the cause for the DRP reduction was not investigated, it may have been a result of biomass uptake or chemical precipitation and settling in the digester. There were no significant differences between pH at different points in the system. The pH remained within the range that supports both fermentation and methanogenesis, with an average pH of 7.54 in the digester influent and 7.94 in the effluent (Grady et al., 2011). The total alkalinity of a substrate indicates its buffering capacity, as well as process stability within a system. As described in table 2.3, the alkalinity in the AD system ranged from 10,437 to 13,493 mg as $CaCO_3$ L^{-1}, with no statistical differences in alkalinity between sampling locations.

2.4.2 Biogas Production and Utilization

The average CH_4 concentrations in the biogas measured by the study was 54% by volume. The CO_2 in the biogas accounted for 41% of the total volume. The last 3% of the biogas volume was composed of H_2O (2.42%), NH_3 gas (8.21 ppm), and hydrogen sulfide (200 ppm). The average biogas production volumes ranged from 3,300 to 7,000 $m^3\ d^{-1}$ during the evaluation period (figure 2.7). Biogas production was higher during the colder months (provide listing) compared to the warmer months (June, July, August and September) of the year. During both calendar years, the lowest gas production occurred in July which likely reflects the influence of herd management on the anaerobic digester performance. Typically, starting mid-June through mid-August, the average daily temperatures of southwest Virginia exceed comfortable levels for dairy herds; to maintain health and milk production, cows are usually cooled using a combination of fans and cold water sprays (via misters). The fresh water used in cooling mixes with manure on the barn floor, diluting the manure, which may result in less biogas production potential. In keeping with this hypothesis, observed influent VS concentrations during the months of June and July in 2011 and 2012 were lower than at other times of the year.

In order to further investigate these seasonal variations, the average daily biogas yields normalized to the TVS and SCOD loading rate are presented in figures 2.8 and 2.9. In both cases, the biogas yields remained relatively constant, even during months when manure was diluted. This finding confirms observations in prior studies, in which reducing VS or sCOD loading rates resulted in reduced biogas production rates (Coats et al., 2011; El-Mashad and Zhang, 2010; Kaparaju et al., 2009). Anaerobic digestion of dairy manure at mesophilic temperatures has been shown to produce a biogas yield of between 0.15 and 0.3 $m^3\,kgVS^{-1}\,d^{-1}$ (Braun, 1982; Møller et al., 2004; Thomé- Kozmiensky, 1995). The theoretical biogas production potential of dairy manure is approximately 0.47 $m^3\,kgVS^{-1}\,d^{-1}$ (Møller et al., 2004). Normalization of daily biogas production to average daily VS loading rates from

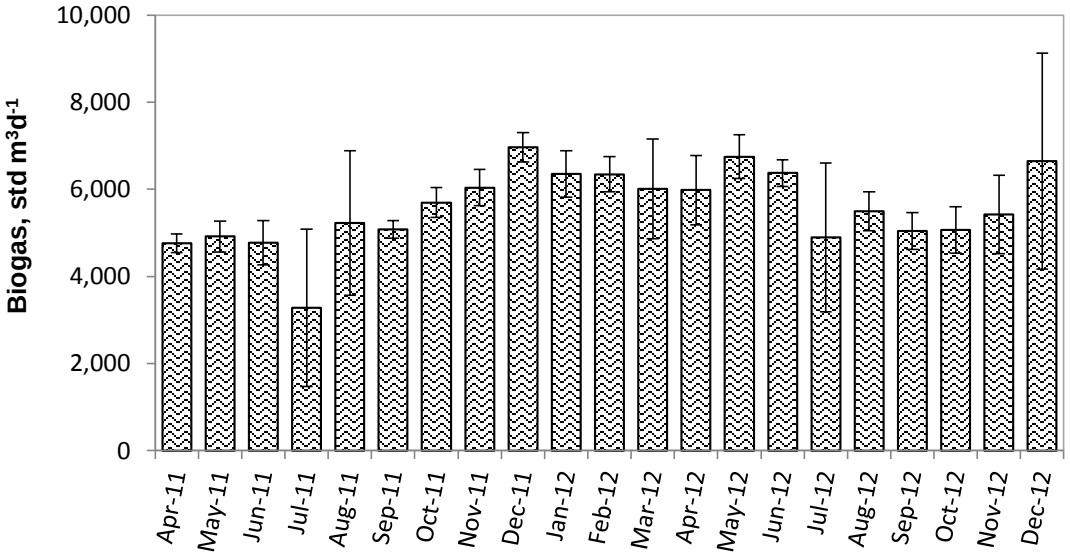

Figure 2.7. Average daily biogas production, by month, with standard deviations.

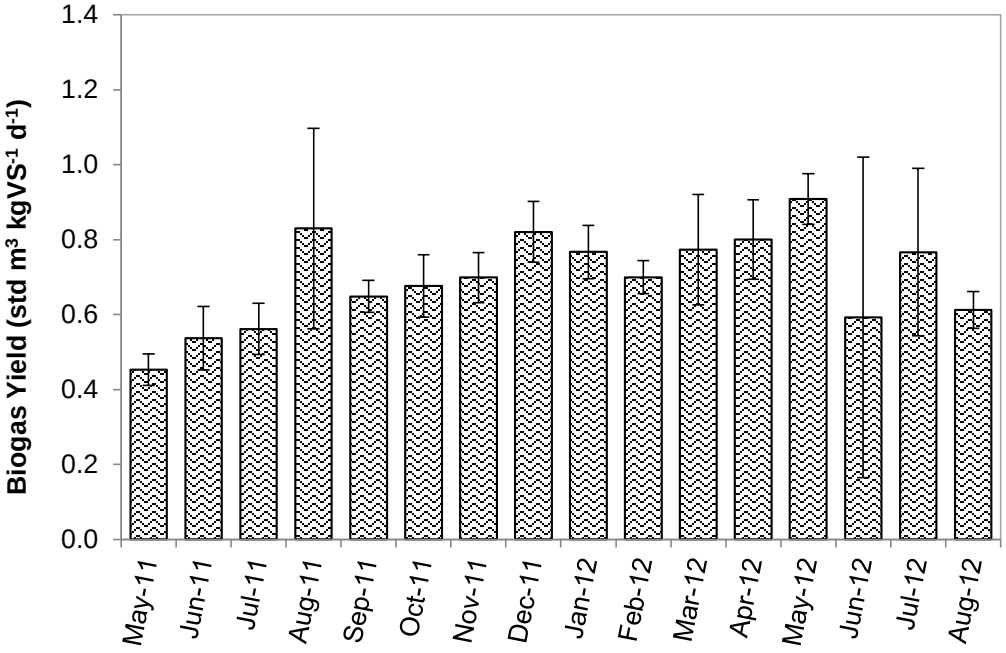

Figure 2.8. Average daily biogas yields by month.

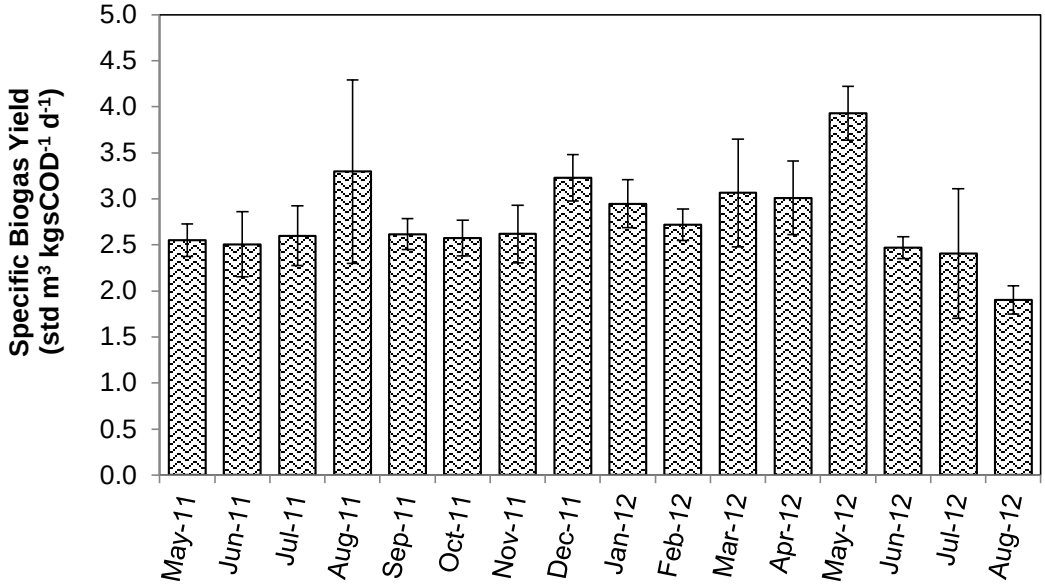

Figure 2.9. Average specific daily biogas yield by month.

the AD in this study showed average biogas yields ranging from 0.43 to 0.89 $m^3 \cdot kgVS^{-1} \cdot d^{-1}$. The highest average daily biogas yields occurred during the months of December 2011, May 2012, and December 2012 when the feedstock was augmented with very high energy materials, including brewery waste and a dairy nutritional supplement, which raised the yield potential. The quantity of feedstock used to augment performance in the AD was not recorded.

Actual field observations of biomethane yields from dairy manure range between 0.11 to 0.23 $m^3 \cdot kgVS^{-1}$ (El-Mashad and Zhang, 2010; Steffen et al., 1998). Assuming an average CH_4 concentration of 54%, the daily biomethane yield in the AD was estimated to range between 0.2 and 0.52 $m^3 \cdot kgVS^{-1}$. Similar to the biogas yield results, the highest biomethane yields occurred when the feedstock was augmented with very high energy materials, including brewery waste and a dairy nutritional supplement.

Biogas utilization is presented in figure 2.10 and shows that the gas was used by the boiler and/or flared prior to August 2011, when the generator was commissioned. Once the generator was in operation, 90 to 99.4% of the biogas was used to generate electricity at an average daily production rate of 360 KWH. Following installation of the generator, biogas was flared during generator maintenance or when biogas production exceeded generator capacity. Flared biogas accounted for 76 to 82% of the monthly average biogas utilized between April and August 2011 and was reduced to 3.9% of the biogas utilized between August 2011 and December 2012 when the engine was operational.

2.4.3 System Mass and Volumetric Flow Analysis

Volumetric flow rates for the system influent, effluent, and separated liquids were derived from manure levels in the equalization pit and the separated solids pit and from the separated solids production rate, as described in equations 2.1, 2.2, and 2.3. The AD influent and effluent had flow rates of 135 $m^3 \cdot d^{-1}$ and 147 $m^3 \cdot d^{-1}$, respectively. Separated liquids were produced with a flow rate of 115 $m^3 \cdot d^{-1}$. Based on the volumetric flow analysis and the digester dimensions, the overall hydraulic retention time (HRT) of the AD is 35.5 d. The acid chamber (i.e. the first chamber) is currently operated at a hydraulic retention time of 6 d.

2.4.4 Production of Value-added Products and Services

The characteristics of the separated liquids and solids are presented in tables 2.3 and 2.4, respectively. Removing solids from the AD effluent resulted in decreases of 22% and 26% in TS and VS

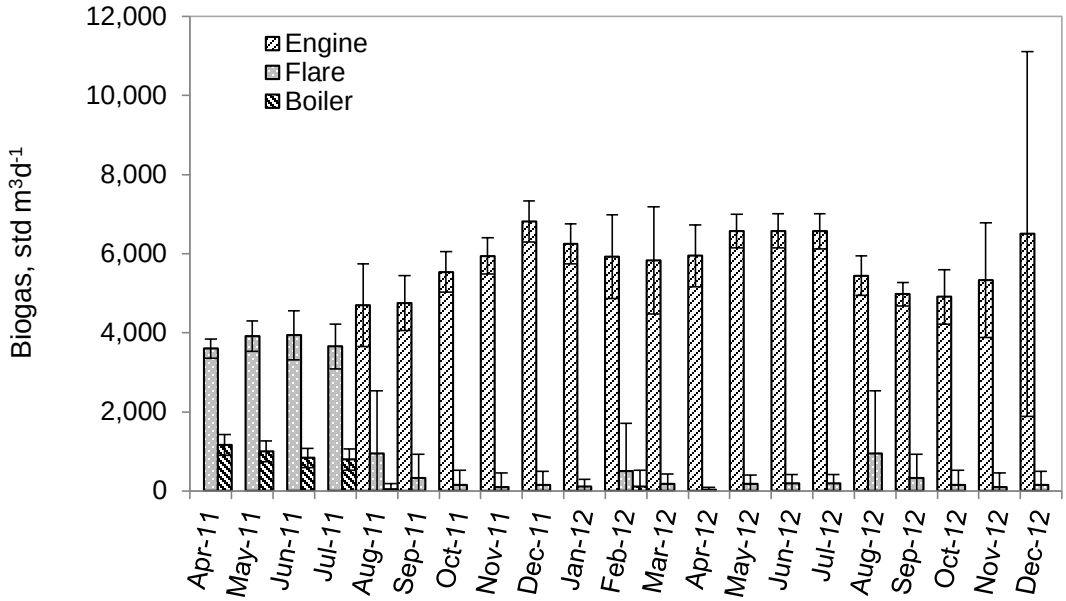

Figure 2.10. Average daily biogas utilization by generator engine, flare, and boiler, according to month.

Table 2.4. Average (±standard deviation) characteristics of separated solids.

Parameter	Location		
	Separator 1	**Separator 2**	**Combined Average**
TS (%)	29.3 (±2.5)[a]	28.2 (±4.8)[a]	28.7 (±3.8)
VS (%)	25.7 (±2.2)[a]	24.9 (±2.3)[a]	25.3 (±3.4)
TKN (g kg^{-1})	15.9 (±3.5)[a]	15.4 (±3.9)[a]	15.7 (±2.2)
TAN (g kg^{-1})	4.64 (±1.09)[a]	4.72 (±1.20) [a]	4.70 (±0.70)
TP (g kg^{-1})	3.51 (±0.84)[a]	3.21 (±0.10)[a]	3.3.2 (±0.68)
DRP (g kg^{-1})	0.99 (±0.26)[a]	0.94 (±0.33) [a]	1.08 (±0.27)
K (g kg^{-1})	4.20 (±1.10)[a]	4.74 (±1.10)[a]	4.83 (±0.56)
Bulk Density (kg m^{-3})	454 (±16) [a]	408 (±49) [b]	434 (±41)

*Concentrations with different superscripts (within rows) are significantly different at $p < 0.5$.

TS – Total solids content

VS - Volatile solids content

TKN – Total Kjeldahl nitrogen

TAN – Total ammonium nitrogen

TP – Total phosphorus

DRP – Dissolved reactive phosphorous

K - Potassium

concentrations, respectively, in the separated liquids compared to the AD effluent. The average TS and VS contents increased significantly between the AD effluent and separated solids (ANOVA, $p<0.0001$). The separated solids were 28.7% total solids w/w. By contrast, the separated liquids had a total solids content of 4.1%. The average VS contents were 24.9% and 2.9% in separated solids and separated liquids, respectively. From a mass balance perspective, liquids contained higher concentrations in TKN, TAN, TP, and DRP than the separated solids (table 2.5). This nutrition partitioning pattern has been observed in other studies, which observed that the majority of the phosphorus and nitrogen in manure was associated with liquids in dissolved or suspended forms (Chapuis-Lardy et al., 2004; Powers and Horn, 2001; Wu, 2007).

The solids separator generated a daily average of 13.42 tonnes of separated solids at a production rate of 116 kg solids per 1000 L effluent. Solids are used as cow bedding and the quantity generated was sufficient to replace the need for traditional bedding material (wood chips) on the farm. Using separated solids resulted in estimated monthly savings of about $6,000 and reduced mastitis incidence by 50%, as estimated by Dairy Energy, LLC. The reduction in mastitis equated to a monthly cost savings of approximately $15,000 for a 1,000 milking cow herd (K. VanderHyde, Dairy Energy LLC, personal communication, 01 December 2012). Application of separated liquids as fertilizer on the Dairy Energy farmland replaced the need to import fertilizers onto the farm for crop production.

2.4.5 Operation and Maintenance Requirements

Digester system components, such as pumps and mixers, consumed less than 10% of the electricity generated by the AD system. The generator engine required oil change approximately every 500 to 650 hours of engine run time. Routine oil changes took about 90 min. The generator plate cooler required approximately 4 hours of routine maintenance once per year. Routine daily monitoring of the anaerobic digester and its components took approximately 20 min each day.

2.4.6 Fermentation Potential of Dairy

Results from the fermentation potential experiments are presented in figures 2.11 through 2.14. Fermentation of manure yielded TVFA concentrations ranging from 3,905 to 10,740 $mg \cdot L^{-1}$ (as COD). The maximum TVFA concentration occurred on 3 d of fermentation (figure 2.11). The maximum concentration of acetic acid (substrate for methanogens) was also highest following 3 d of fermentation (figure 2.12). The gross fermentation potential of a material describes the inherent and produced (or consumed) TVFAs normalized to unit mass of VS loaded. The net fermentation potential describes only the TVFAs that were produced/consumed during the duration of the test, normalized to unit mass of VS loaded. The gross and net fermentation potentials of the dairy manure are presented in figures 2.13

Table 2.5. Nutrient mass partitioning in separated solids and separated liquids.

Parameter	Location		
	AD Effluent	**Separated Liquids**	**Separated Solids**
Partitioning Volume (m^3)	100	80	20
TS (%)	5.3	4.1	28.7
TVS (%)	3.9	2.9	25.3
TKN (g)	402	259	143
TAN (g)	181	140	41
TP (g)	71	40	31
DRP (g)	13	4	9
K (g)	237	192	45

TS – Total solids (% of wet weight)

TVS – Total volatile solids (% of wet weight)

TKN – Total Kjeldahl nitrogen

TAN – Total ammonium nitrogen

TP – Total phosphorus

DRP – Dissolved reactive phosphorous

K - Potassium

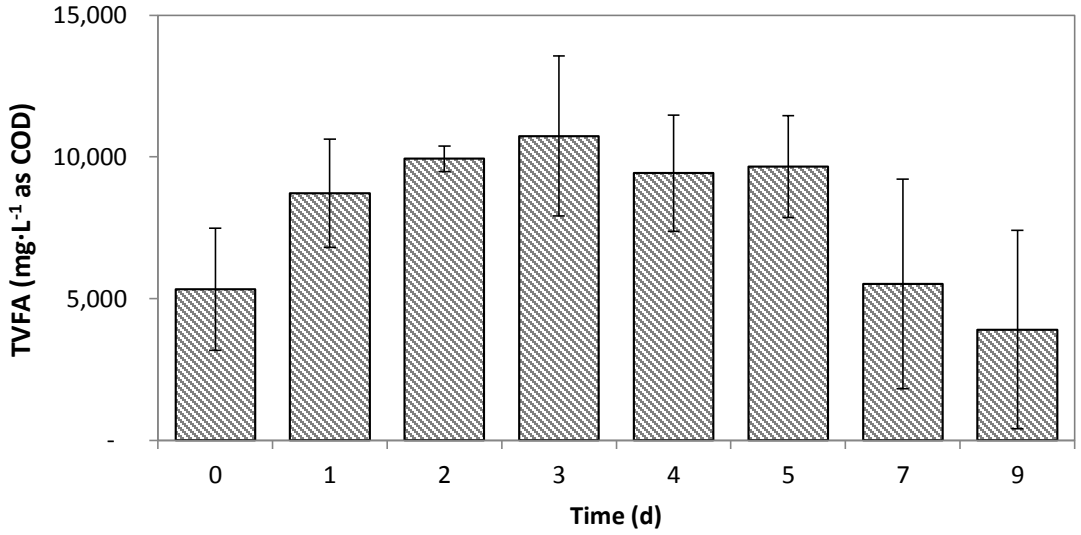

Figure 2.11. Average TVFA concentrations with standard error, over 9 d period.

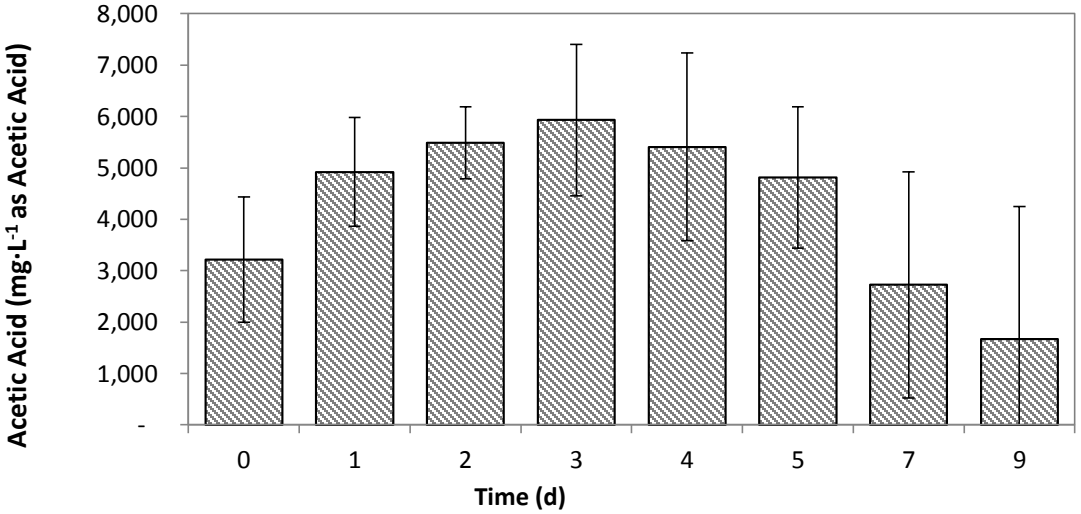

Figure 2.12. Acetic acid concentrations over 9 d fermentation potential test.

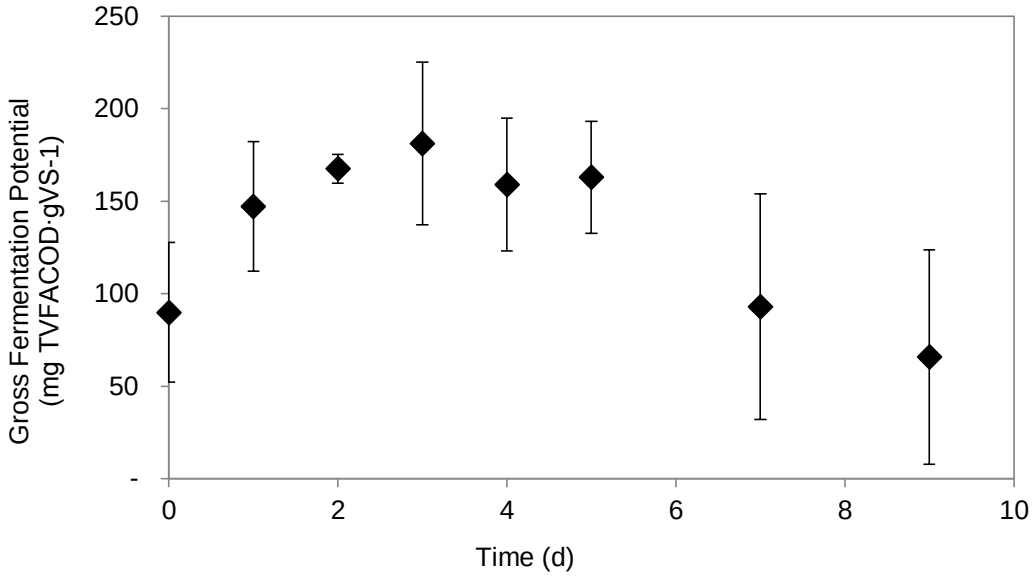

Figure 2.13. Gross fermentation potential of manure over a 9 d period.

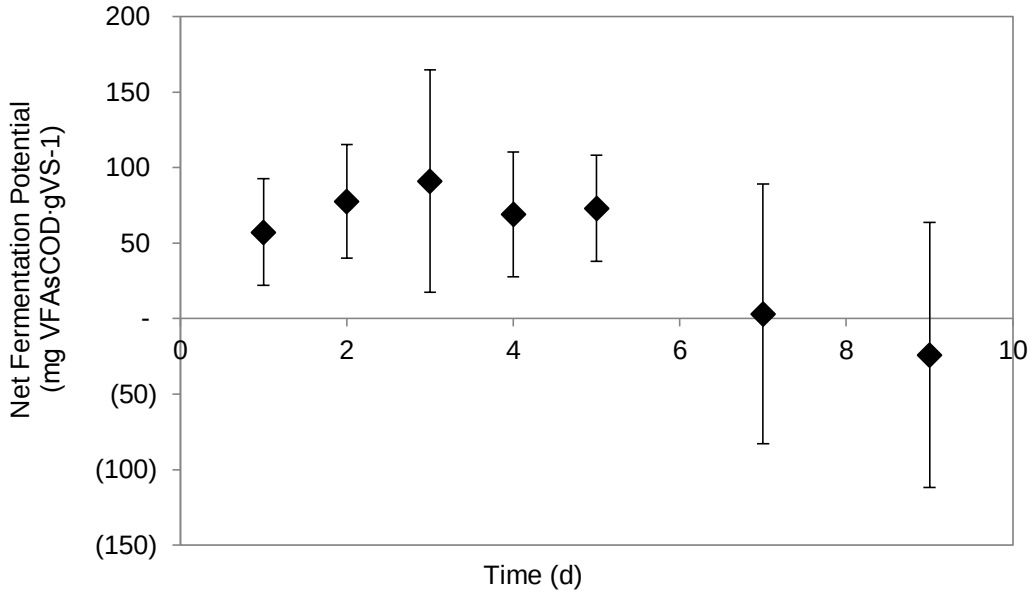

Figure 2.14. Net fermentation potential of manure. Differences in net fermentation potential were not significant between retention times (ANOVA, $p<0.05$).

and 2.14, respectively. The maximum gross and net fermentation potentials of dairy manure occurred on day 3. These results suggest that reducing the acid chamber of the AD to an HRT of 3 d may maximize TVFA concentrations and biomethane production.

Applying the results of the fermentation potential tests to the Dairy Energy Inc. digester with an acid chamber volume of 756 m^3, a 3 d HRT would require a daily volumetric loading of 252 m^3 dairy manure. Consequently, the HRT in the methane chamber would be 15 d for an overall AD HRT of 18 d. The volumetric loading rate would translate to VS and sCOD loading rates of 59.3 kg $m^{-3}d^{-1}$ and 17 g $m^{-3}d^{-1}$, respectively. Coats et al. (2011) found that hydraulic loading rates between 40.7 and 58.1 kg $m^{-3}d^{-1}$ resulted in TVFA concentrations between 7,500 and 9,200 mg L^{-1} in batch reactor bottles that were operated with a 6 d HRT. Increasing the volumetric flow rate to achieve the desirable AD HRT would require additional quantities of manure to be available on the farm. This can be achieved by increasing the herd size at the farm from 1,200 to 2,950 cows or importing manure from other dairy farms with similar characteristics to the Dairy Energy AD.

While the extent to which the Dairy Energy AD would stabilize manure after 18 d of anaerobic digestion may be uncertain, Grady et al. (2011) reported that 15 to 20 d HRTs is generally sufficient to achieve 80% to 90% conversion of biodegradable organic matter to methane. Others have suggested HRTs of greater than 20 d to maximize conversion of dairy manure to biogas and to achieve waste stabilizations (El-Mashad and Zhang, 2010; Holm-Nielsen et al., 2009; NRCS, 2009). Demirer and Chen (2004) studied the effects of hydraulic loading rate and organic loading on volatile solids destruction and biomethane production during two-phase anaerobic digestion. They observed that pre-fermentation of dairy manure with in a reactor with an HRT of 4 d and an organic loading rate of 4 $gVS{\cdot}m^{-3}d^{-1}$ increased biomethane production by 61.2%. However, pre-fermentation of dairy manure in a reactor with an HRT of 1.25 d and an organic loading rate of 30 $gVS{\cdot}m^{-3}d^{-1}$ increased biomethane production by only 14.2% (Demirer and Chen, 2004). The authors suggested that, while pre-fermentation increased biomethane production, the effectiveness of pre-fermentation was reduced when the reactor was operated at high organic loading rates and low HRTs due to washout of fermentative bacteria.

Observations from this study showed that the volatile solids matter in manure was decreased by 33% by the time it the manure reached location 1. However, there was no further decrease in VS content between location 1 and the AD effluent. As previously mentioned, this may be because the methane chamber of the AD is designed as a completely mixed reactor, rather than a mixed plug flow reactor. If the Dairy Energy AD was actually designed for complete mix conditions, the average HRT for the whole AD was 38.5 d, and the HRTs for locations 1, 2, and 3 were equal to each other at approximately 38.5 d.

If the AD was operating under mixed plug flow conditions, the HRT at location 1 was approximately 7.1 d, at location 2 was 12 d, and at location 3 was 25 d.

Prior to implementing any manure management changes on the farm, it is recommended that a complete lab study evaluating the effects of anaerobic digestion on waste stabilization parameters like tCOD, sCOD, TS/VS, and TVFA, biogas yield, biogas quality, and process efficiency when operated with an HRT of 18 d be conducted.

2.5 CONCLUSIONS

Performance measures for the targeted on-farm AD were comparable to those reported in prior studies of anaerobic digestion of dairy manure. The tCOD and sCOD concentrations were reduced by 29% and 57%, respectively. The average daily biogas production ranged from 3,300 to 7,000 m^3 with yields ranging from 0.43 and 0.89 $m^3 \cdot kgVS^{-1}\text{-}d^{-1}$. The biogas had a methane content of 54% methane, with an energy equivalent of 21.45 $MJ \cdot m^{-3}$. It is recommended that long-term monitoring and evaluation activities, such as those described herein, be completed on full-scale anaerobic digesters according to the ASERTTI protocol to evaluate the effects of farm management practices on waste stabilization and biogas production in ADs. At Dairy Energy in particular, further exploration of waste stabilization and biogas production given different HRTs and loading rates would be useful in optimizing system performance.

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3 Evaluating Feedstocks for Co-digestion with Dairy Manure to Increase Biogas Production Volumes

3.1 ABSTRACT

Evaluation of the potential for increasing digester biogas production through the addition of feedstocks is generally based on fermentation potential tests in place of labor- and time-intensive biomethane potential tests; the fermentation potential of a feedstock is thereby defined as the sum of inherent and produced volatile fatty acids normalized to the volatile solids loading of the feedstock. The objective of this study was to identify and assess the suitability of locally available biodegradable waste streams for co-digestion with dairy manure in an on-farm anaerobic digester to increase biomethane production. Dairy manure was anaerobically digested with potato processing waste (PPW) and a dairy nutrient supplement (DNS) for 9 d, during which it was periodically evaluated for total solids, volatile solids, soluble chemical oxygen demand, total chemical oxygen demand, and total volatile fatty acids. The results of this study indicate that co-digestion mixtures containing up to 20% PPW (v/v) yielded higher gross and fermentation potentials than manure alone. Co-digestion mixtures containing up to 10% DNS exhibited increased gross and net fermentation potentials on days 1, 3, and 4 of the test. Although these data do emphasize that changes in influent composition and retention times can significantly shift biogas production, confirmation of these findings via biomethane potential tests prior to field-scale implementation is recommended.

3.2 INTRODUCTION

An estimated 40% of the food produced in the U.S. is wasted and ends up in landfills each year, a loss estimated at more than $168 billion (Hall et al., 2009). Food losses in the U.S. food production chain are attributed to retailers, consumers, producers, and food processors (Gunders, 2012). Losses start at harvest (e.g. unharvested grains, fruits, and vegetables) and continue through food processing (e.g. culling due to undesirable qualities such as imperfect size, color, weight, and blemish levels) (U.S. EPA, 2009). Once at the retailer, consumer and retailer behaviors produce additional food waste, including unsold produce, expired shelf-stable products, and uneaten meal "leftovers" (Parfitt et al., 2010). Food waste, harvest losses, and food processing residues contain readily biodegradable organic matter that, when sent to landfills, contribute to greenhouse gas emissions (Gunders, 2012; U.S. EPA, 2012, 2013).

One technology for resource recovery from organic waste streams like food waste is anaerobic digestion for biomethane production (AgSTAR, 2011; Gunders, 2012; U.S. EPA, 2009). Conversion of organic agricultural residues, food processing wastes, and post-consumer wastes to renewable energy has been extensively studied during the last 15 years (Bocher et al., 2008; Liu et al., 2009; Romano and Zhang, 2011; Steffen et al., 1998; Wang and Banks, 2003; Yue et al., 2013; Zhang et al., 2007). For example, Zhang et al. (2007) showed that using anaerobic digesters to treat food waste collected from the City of San Francisco can yield 0.348 to 0.435 m^3 CH_4 $kgVS^{-1}$ depending on retention time. Food processing wastes, such as whey from cheese making or potato waste from potato chip manufacturing, have been shown to produce higher methane yields as compared to livestock manure. Steffen *et al.* (1998) reported yields of biomethane between 0.54 and 0.76 m^3 $kgVS^{-1}$ from whey compared to 0.11 to 0.23 $m^3 \cdot kgVS^{-1}$ and 0.18 to 0.4 $m^3 \cdot kgVS^{-1}$ from dairy and hog manure, respectively (El-Mashad and Zhang, 2010; Steffen et al., 1998). Biomethane yields from solid potato waste can be as high as 0.40 $m^3 \cdot kgVS^{-1}$, which is about double the yield from dairy manure (Nayono et al., 2012; Parawira et al., 2005).

Biogas yield from dairy manure is typically low, with biomethane content ranging from 50% to 75% (El-Mashad and Zhang, 2010; Møller et al., 2004; Steffen et al., 1998). These relatively low biogas yields have been attributed to inherent manure properties, like high fiber content, which requires long retention times for biodegradation (El-Mashad and Zhang, 2010; Møller et al., 2004). Additionally, as the organic matter in manure has undergone some digestion in the cow's gastrointestinal tract, the most accessible nutrients and biodegradable material have already been removed and are therefore unavailable for biogas production after excretion. While long retention times are good for materials with low biodegradation rates, they are a setback to the use of anaerobic digestion with respect to time and financial investment (Nielsen et al., 2004). Anaerobic digesters with longer retention times require larger reaction vessels, and therefore larger footprints, more construction materials, and more energy for

temperature control compared to digesters with low retention times. These factors result in increased installation and maintenance costs.

One solution for increasing biomethane production is co-digesting organic materials with manure. Co-digestion may enhance the anaerobic digestion process by shifting the nutrient balance to favor microbial metabolism (Speece, 1996; Speece et al., 1983; Wilkie et al., 1986). Also, co-digestion of dairy manure with organic materials can directly increase the quantity and quality of biogas produced (Chen et al., 2008). The variable nature of organic materials and potential for process destabilization from inhibitory substances requires that materials be thoroughly screened prior to their selection and use for co-digestion with dairy manure (Chen et al., 2008).

Typically, biomethane potential tests (BMP) are used to evaluate the suitability of a feedstock for use in anaerobic digestion which record the cumulative amount of biogas collected over a 30 d period; the feedstock that produces the largest volume of biogas is usually selected (Angelidaki et al., 2009; El-Mashad and Zhang, 2010). However, evaluation of feedstocks based on the quantity and quality of fermentation end-products, specifically total volatile fatty acids (TVFAs), is less expensive, and appears a reasonable surrogate as TVFAs the products that are converted to biomethane (Angelidaki et al., 2009; Coats et al., 2011). In this method, the fermentation potential of a feedstock can be defined as the sum of inherent and produced TVFAs, normalized to the volatile solids loading (Coats et al., 2011). The concept of FP has been used in studies to assess the production of volatile fatty acids for use in enhanced biological phosphorous recovery and anaerobic digestion reactors (Güngör et al., 2009; Coats et al., 2011), although it has not previously been used specifically to assess the suitability of feedstocks for co-digestion with dairy manure.

The objective of this study was to identify and assess the suitability of locally available biodegradable waste streams to co-digest with dairy manure to increase biomethane production. The selection of material was focused around an on-farm anaerobic digester operated by Dairy Energy, LLC located in Chatham, VA.

3.3 MATERIALS AND METHODS

3.3.1 Feedstock Identification and Collection

Two organic materials, a dairy nutrient supplement (DNS) and potato processing waste (PPW), were identified and used in this study based on quantities available and proximity to the farm study site. The DNS was liquid while the PPW was a mixture of loosely packed solids of discarded and macerated potatoes and corn generated at a potato chip manufacturing plant. Solids, organics, and nutrients characteristics of the dairy manure (DM), DNS, and PPW used in this study are presented table 3.1.

Table 3.1. Characteristics of dairy manure, dairy nutrient supplement and potato processing waster used in the feedstock blends for fermentation potential tests.

Nutrient	DM	DNS	PPW
TS, $g \cdot L^{-1}$	71.2 (±2.9)	147 (±23)	87.6 (±14.5)
VS, $g \cdot L^{-1}$	59.3 (±2.3)	142 (±21)	70.2 (±14.8)
pH	7.4 (±0.6) [a]	NA	4.4 (±0.0)
tCOD	75,267 (±2,734)	253,067 (±9.0)	134,383 (±10,064)
sCOD	17,019 (±1,479)	2,433 (±16)	60,167 (±12)
TVFA	5,333 (±2,158)	580 (±34)	6,249 (±717)
TAN, $mg \cdot L^{-1}$	1360	0.0	0.00
Org N, $mg \cdot L^{-1}$	1,933	2,670	6,800
Total N, $mg \cdot L^{-1}$	3,293	2,670	6,800
Total P, $mg \cdot L^{-1}$	550	377	611
DRP, $mg \cdot L^{-1}$	164	219	99.0
K, $mg \cdot L^{-1}$	2,315	537	996
Ca, $mg \cdot L^{-1}$	1,923	453	900
Mg, $mg \cdot L^{-1}$	632	113	100
S, $mg \cdot L^{-1}$	365	160	500
Zn, $mg \cdot L^{-1}$	20.7	8.3	10.5
Cu, $mg \cdot L^{-1}$	6.7	1.0	3.29
Mn, $mg \cdot L^{-1}$	19.5	1.4	5.26
Na, $mg \cdot L^{-1}$	613	299	139
Fe, $mg \cdot L^{-1}$	75	15	138
Al, $mg \cdot L^{-1}$	64.9	0.9	82.0
Cl, $mg \cdot L^{-1}$	1,241	672	267

DM – Dairy Manure
PPW – Potato Processing Waste
DNS – Dairy Nutrient Supplement

The organic materials were mixed with dairy manure prior to fermentation to observe TVFA production as a marker of their suitability for co-digestion to enhance biogas production.

The DM, DNS, and PPW were collected on the same day laboratory experiments were initiated. Dairy manure was collected from the Dairy Energy, LLC equalization tank and loaded in 20 L buckets on ice. The DNS was also obtained from Dairy Energy, LLC using 1-L bottles. The PPW was collected in 2-L bottles and transported on ice to the Bioresidues Utilization and Management (BRUM) lab at Virginia Tech.

3.3.2 Experimental Design

The feedstock mixtures used in the fermentation potential studies are described in table 3.2. The feedstocks included 100% DM (control) and 6 blends of DM mixed with varying quantities of DNS or PPW. Feedstock mixtures were prepared on a volume basis. Treatments were identified by the amount of either DNS or PPW added to DM as a percent of the total volume. The PPW was prepared by adding de-ionized (DI) water to make a slurry that had a total solids content of 10% by weight (w/w) prior to mixing with dairy manure.

Each feedstock mixture was added to twenty-one 250-mL Erlenmeyer flasks, capped with rubber stoppers, and placed in a reciprocating incubator shaker (New Brunswick Scientific Co., Edison, NJ, USA) set to oscillate at 175 RPM and a temperature of 38°C. Samples were collected on days 1, 2, 3, 4, 5, 7, and 9 by randomly removing 3 flasks from the incubator shaker without replacement (sacrificial sampling). Fermentation potential testing began on day 0, and subsequent sampling days were identified by the subsequent number of days. Samples were analyzed for total solids (TS), volatile solids (VS), total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), total volatile fatty acids (TVFAs), and pH as described in section 3.3.3.

3.3.3 Gross and Net Fermentation Potential

The gross fermentation potential (gross FP) of a feedstock is sum of the initial TVFAs and TVFAs produced during fermentation, divided by the VS loading on day 0. Equation 3.1 was used to calculate the gross FP for each day of the test.

$$\text{Gross FP} = \frac{\text{TVFA}_i}{\text{VS}_0} \tag{3.1}$$

Where
$TVFA_i$ = total volatile fatty acid concentration (mg L^{-1}) on day i of the test,
VS_0 = volatile solids loading (g L^{-1}) on day 0
.

Table 3.2. Volumes of dairy manure, dairy nutrient supplement, and potato processing waste used in making the feedstock mixtures

Treatment	DM (mL)	DNS (mL)	PPW (mL)
100% DM	200	-	-
3% DNS	194	6	-
5% DNS	190	10	-
7% DNS	186	14	-
10% DNS	180	20	-
10% PPW	180	-	20
15% PPW	170	-	30
20% PPW	160	-	40

DM – Dairy Manure
PPW – Potato Processing Waste
DNS – Dairy Nutrient Supplement

The net fermentation potential (net FP) of a feedstock measures the net TVFAs produced during fermentation normalized to the initial VS loading on day 0. Equation 3.2 was used to calculate the net FP for each day of the test.

$$\text{Net FP} = \frac{\text{TVFA}_i - \text{TVFA}_0}{\text{VS}_0} \quad (3.2)$$

Where
$TVFA_i$ = total volatile fatty acid concentration (mg L^{-1}) on day i of the test
$TVFA_0$ = total volatile fatty acid concentration (mg L^{-1}) on day 0
VS_0 = volatile solids loading (g L^{-1}) on day 0

The specific energy content of feedstocks can be defined as the sCOD concentration, normalized to the volatile solids loading. Specific energy contents were therefore calculated for the feedstock mixtures in this study using equations 3.1 and 3.2, where sCOD concentrations were substituted for TVFA concentrations. Although using sCOD can give an overview on the readily available biodegradable material in the feedstock mixtures, it is not as suitable as presenting the FP data in terms of TVFA when selecting feedstock mixtures to produce biogas. The reason for this is that methanogens are only capable of utilizing TVFAs as a substrate during biomethane production. The sCOD of a feedstock mixture encapsulates several soluble substrates, and is a non-specific to methanogenic microorganisms. The presentation of specific energy content in terms of sCOD was therefore used as a general indicator of the progression of biological activity during the fermentation tests rather than a measure to determine feedstock suitability for co-digestion with dairy manure.

3.3.4 Statistical Analysis

Statistical analyses were performed in JMP version 10 software (SAS, 2013). Results were tested for normality and one-way analysis of variance (ANOVA) was used to compare means of TS and VS content, pH, sCOD, tCOD, and TVFA concentrations, and on gross and net fermentation potentials. The Tukey-Kramer multiple means comparisons was used to test the differences in means. Differences were declared significant at $p < 0.05$.

3.4 RESULTS AND DISCUSSIONS

3.4.1 Feedstock Mixture Characteristics

The characteristics of the feedstock mixtures at the start (day 0) and end (day 9) of the fermentation tests are presented in table 3.3. There were no significant differences in the TS and VS contents of the feedstock mixtures (ANOVA, $p < 0.05$). The organic loading (represented by VS content) for the all mixtures ranged from 56.3 to 65.3 g·L^{-1}. However, the pH and tCOD concentrations of the

Table 3.3. Characteristics of the feedstock mixtures at the start (day 0) and end (day 9) of fermentation potential tests

Treatment	Day	TS ($g \cdot L^{-1}$)	VS ($g \cdot L^{-1}$)	pH	tCOD ($g \cdot L^{-1}$)	sCOD ($g \cdot L^{-1}$)	TVFA ($g \cdot L^{-1}$ as COD)
100% Manure	0	71.2 (±2.9)^{a,b}	59.3 (±2.3)^{a,b,c,d}	7.4 (±0.6)^{a}	75.27 (±2.73)^{a}	17.02 (±1.48)^{a,b}	5.33 (±2.16)^{a,b}
3% DNS	0	70.7 (±4.9)^{a,b}	58.7 (±4.1)^{a,b,c,d}	7.6 (±0.1)^{a,b}	94.47 (±0.05)^{a}	20.14 (±0.04)^{a,b}	7.91 (±0.32)^{a,b,c}
7% DNS	0	67.6 (±1.4)^{a,b}	56.3 (±1.1)^{a,b,c,d}	7.3 (±0.1)^{a,b,c}	78.63 (±0.06)^{a}	21.32 (±0.02)^{a,b}	7.70 (±0.16)^{a,b,c}
10% DNS	0	68.1 (±10.2)^{b}	56.9 (±2.9)^{b,c,d}	7.5 (±0.1)^{a,b}	86.23 (±0.00)^{a}	24.75 (±0.05)^{a}	6.54 (±1.47)^{a,b,c}
10% PPW	0	68.8 (±3.5)^{a}	59.4 (±3.0)^{a,b,c}	6.7 (±0.0)^{b,c}	113.6 (±0.01)^{b}	22.03 (±0.01)^{a,b}	3.05 (±2.12)^{b}
15% PPW	0	71.6 (±7.3)^{a,b}	62.7 (±6.5)^{a,b,c}	6.8 (±0.0)^{b,c}	220.0 (±0.10)^{c}	22.63 (±0.03)^{a,b}	2.88 (±2.35)^{a,b}
20% PPW	0	74.5 (±2.4)^{a,b}	65.3 (±2.4)^{a,b}	6.8 (±0.0)^{b,c}	177.8 (±0.12)^{d}	20.82 (±0.00)^{a,b}	2.95 (±2.21)^{a,b}
100% Manure	9	60.4 (±4.8)^{b}	48.6(±4.2)^{c,d}	8.1 (±0.7)^{a}	72.433(±16.546)^{a}	14.47 (±2.99)^{b}	3.91 (±3.50)^{a,b}
3% DNS	9	64.7 (±2.6)^{b}	52.5 (±2.6)^{b,c,d}	8.1 (±0.2)^{a}	75.733 (±0.07)^{a}	12.17 (±0.12)^{b}	5.09 (±4.49)^{a,b,c}
7% DNS	9	61.1 (±2.2)^{b}	49.7(±1.7)^{c,d}	8.2 (±0.0)^{a}	78.133 (±0.06)^{a}	18.06 (±0.48)^{a,b}	2.19 (±0.51)^{b}
10% DNS	9	59.3 (±1.4)^{b}	47.7 (±1.6)^{d}	8.0 (±0.2)^{a}	79.567 (±0.14)^{a}	14.79 (±0.25)^{b}	4.69(±4.19)^{a,b}
10% PPW	9	58.6 (±3.1)^{b}	49.1 (±2.9)^{c,d}	6.7 (±0.1)^{c}	72.300 (±0.10)^{a}	22.31 (±0.07)^{a,b}	10.44 (±1.23)^{a,c}
15% PPW	9	56.16 (±3.8)^{b}	47.4 (±3.6)^{c,d}	6.6 (±0.0)^{c}	66.933 (±0.06)^{a}	23.09 (±0.04)^{a,b}	11.12 (±0.81)^{a,c}
20% PPW	9	62.1 (±1.5)^{b}	52.835 (±1.0)^{b,c,d}	5.6 (±0.2)^{d}	77.200 (±0.03)^{a}	25.51 (±0.15)^{a}	13.10 (±1.39)^{c}

*Concentrations with different superscripts (within columns) are significantly different at $p < 0.5$.

DM – Dairy manure
PPW – Potato Processing Waste
DNS – Dairy Nutrient Supplement
TS – Total solids content
VS – Volatile solids content
tCOD – Total chemical oxygen demand
sCOD - Soluble chemical oxygen demand
TVFA – Total volatile fatty acids

PPW mixtures were different from those of the DNS mixtures and the 100% DM at the start of the tests (ANOVA, $p < 0.05$). The similarity between the DNS mixtures and 100% DM may have been because the large (90 to 97%) volumes of DM used in making the feedstock. In comparison, the PPW mixtures had tCOD concentrations that were between 19.2 and 144.8 $g \cdot L^{-1}$ higher than the 100% DM and DNS mixtures. The high tCOD concentrations observed in PPW mixtures was likely a result of the readily-biodegradable carbohydrate matter contained in the PPW.

The sCOD concentration in 100% DM was significantly different from the 10% DNS, 10% PPW, and 15% PPW. However, all treatments had sCOD concentrations that were within 5 $g \cdot L^{-1}$ of each other. The TVFA concentrations in the 10%, 15%, and 20% PPW mixtures were significantly lower than TVFA concentrations in the DNS mixtures and the 100% DM.

The characteristics of feedstock mixtures at the end of the fermentation period (day 9) are also presented in table 3.3. When comparing between treatments on day 9, differences in the TS and VS contents were not statistically significant (ANOVA, $p<0.05$). However, the pH of the 100% DM and DNS mixtures were significantly different from the PPW mixtures. The pH of feedstocks have implications for the progression of methanogenesis. Fermentation results in the production of acid products, such as acetic and propionic acid. While acidic environmental conditions select for acidogenic fermentative bacteria that produce the substrate necessary for methanogens, methanogens can only survive in mild pH conditions, generally within the range of pH 6 and 8.5 (Grady et al., 2011). While the PPW mixtures may result in increased acetic acid concentrations than other treatments, the resulting environment may prove inhibitory for methanogens, and consequently may not enhance biomethane production.

There were no differences in tCOD concentration between treatments on day 9. While the sCOD concentrations in the 100% DM and the DNS blends decreased between days 0 and 9, the sCOD concentrations in the PPW mixtures did not change. Similarly, the TVFA concentrations in 100% DM and the DNS blends decreased between days 0 and 9; however, the TVFA concentrations in all three PPW mixtures actually increased between days 0 and 9. These results may suggest that the PPW blends contain more readily fermentable organic matter compared to the 100% DM and the three DNS mixtures. Alternatively, differences in TVFA concentration may have been the result of methanogenic activity. Since dairy manure contains natural populations of methanogenic microorganisms, mixtures with higher DM content would have contained higher concentrations of methanogenic bacteria. Therefore, the differences in TVFA concentrations between treatments may not have been attributed exclusively to the conversion of feedstocks to TVFAs by fermentative bacteria. It is expected that TVFA consumption by

methanogenic microorganisms was occurring simultaneously; therefore, a flux in TVFA concentrations was occurring but not observed during the fermentation potential tests.

3.4.2 Fermentation Potential of the DNS and DM Mixtures

The maximum gross fermentation potential yielded TVFA (as COD) concentrations of 242 and 232 $mg \cdot g^{-1}VS$ after 1 to 2 d of fermentation in the 3% DNS and 7% DNS mixtures, respectively (figure 3.1). The 100% DM achieved its peak gross TVFA concentration on day 3, and the 10% DNS achieved its peak gross TVFA concentrations on days 4. However, even at their peak on days 3 and 4, the 100% DM and 10% DNS treatments were still lower than the 3% DNS and 7% DNS on those days. In general, the 100% DM had gross TVFA concentrations that were lower than the gross TVFAs of all three DNS mixtures on days 0 through 4 of the test.

After attaining a peak gross TVFA concentration of 242 mg (as COD)$\cdot gVS^{-1}$ on day 1 of the test, the gross TVFA of the 3% DNS mixture gradually dropped by 64% to 87 mg TVFA (as COD)$\cdot gVS^{-1}$on day 9. The gross TVFA concentration of the 7% DNS mixture decreased by 83% from its peak on day 3 to 39 mg (as COD)$\cdot gVS^{-1}$ on day 9 of the test. By day 9, the gross TVFAs of the 10% DNS and 100% DM dropped by 58 and 64%, respectively, of their peak values. In contrast to the clear peak and decline of the gross TVFAs of 3% DNS and 7% DNS mixtures, the 10% DNS had a gross TVFA that plateaued between days 1 and 4. This observation may results from sustained fermentation and/or methanogenesis, or inhibition from the DNS feedstock contents at the concentrations in the 10% DNS. The average pH of the 10% DNS during the TVFA test was 7.5, compared to average pH values of 7.9, 7.7, and 7.5 for the 3% DNS, 7% DNS, and 100% DM – ranges that were all within tolerance levels for fermentative and methanogenic microorganisms that suggest that pH inhibition was not limiting (Grady et al., 2011).

While VFA accumulation has been widely accepted to indicate system stress, no single VFA is widely accepted as a sufficient sole stress marker (Ahring et al., 1995; Angelidaki and Ahring, 1994; Gourdon and Vermande, 1987; Hill et al., 1987; Hill and Holmberg, 1988). Hill et al. (1987) suggested that acetate concentrations in excess of 13 mM were indicative of process imbalance. Hill et al. (1982) recommended a propionate-to-acetate ratio lower than 1.4 to maintain process stability due to the inhibitory effect of acetate on propionate (Hill, 1982). Others have suggested that isovalerate and isobutyrate concentrations between 0.06 and 0.17 mM indicate system instability (Hill and Holmberg, 1988). Ahring et al. (1995) studied the effects of increasing acetate, propionate, butyrate, and valerate concentrations on methane production in batch reactors and found that methane production continued to

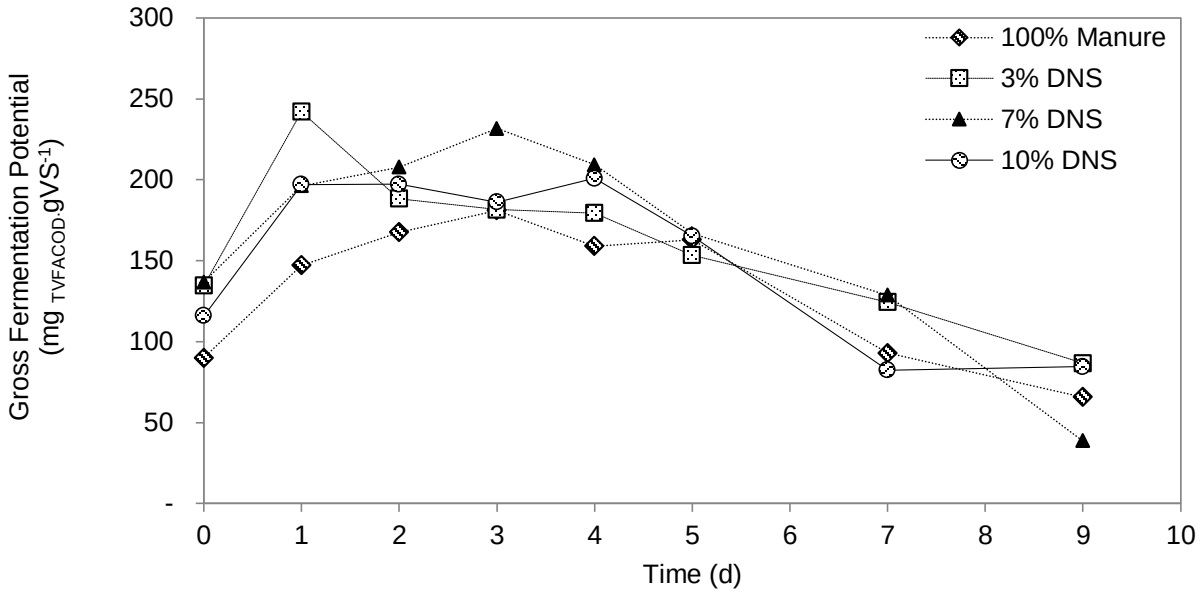

Figure 3.1. Gross fermentation potential of dairy manure & DNS mixtures over time, represented as mg $TVFA_{COD}$·gVS^{-1} loaded.

increase at concentrations of up to 50 mM for each VFA. Review of the VFA concentrations from fermentation potential tests presented in this study indicated that acetic acid, propionic acid, butyric acid, and valeric acid concentrations during the fermentation potential tests fell below the threshold of inhibition suggested by Ahring et al. (1995). Furthermore, in all cases, the ratio of propionate to acetate was considerably lower than the ratio of 1.4 suggested by Hill et al. (1982). While isovalerate and isobutyrate concentrations fell within the 0.6 to 0.17 mM range suggested to indicate process instability Hill and Holberg (1988) during 25% of all days tested, the data did not show any accumulation of isobutyrate or isovalerate. Therefore, when considered as a whole, the VFA data suggest inhibition was not a problem.

Differences in gross TVFA between treatments were only significant on days 1 and 2. The TVFA of the 3% DNS mixture was different than 100% DM on day 1, and the 10% DNS was different than 100% DM on day 2 (ANOVA, $p<0.05$). Based on the gross TVFAs of the 3% DNS, 7% DNS, and 10% DNS mixtures and the 100% DM, a 3% DNS (97% DM) mixture is recommended to maximize TVFA concentrations and biogas production. These data would also recommend a hydraulic retention time (HRT) within the acid chamber of 1 d to maximize TVFA production.

The specific energies of the feedstock mixtures used in this study are reported in terms of sCOD in figure 3.2. When represented as $sCOD \cdot gVS^{-1}$, the 7% DNS and 10% DNS mixtures had higher gross specific energies than the 3% DNS mixture and 100% DM. Like the TVFA concentrations, the 7% DNS mixture reached a peak gross specific energy on day 1, at 457 mg $sCOD \cdot gVS^{-1}$, after which the specific energy decreased gradually. The specific energy in the 3% DNS and 10% DNS mixtures and the 100% DM maintained plateaus for days 0 through 5 and then decreased. As previously mentioned, gross sCOD concentrations (and consequently, specific energy) is merely a snapshot of biological fermentation, rather than a measure for evaluating the biomethane production potential of feedstocks. Based on the results of the gross sCOD concentrations, all three DNS mixtures have the potential to enhance biomethane production as compared to a 100% DM control.

The net fermentation potential of a feedstock distinguishes between the TVFAs present in the feedstock before and those produced during fermentation (Coats et al., 2011) and can be used to evaluate the suitability of a feedstock for biomethane production and design of full-scale anaerobic digestion systems. The net fermentation potential (as TVFA) achieved by each DNS mixture and the 100% DM are presented in figure 3.3. The highest net TVFA concentration of 107 mg (as COD)$\cdot gVS^{-1}$ loaded was achieved by the 3% DNS mixture after 1 d of fermentation. The second highest net TVFA concentration was achieved by the 7% DNS mixture after 3 d of fermentation. The net TVFA concentration of the 3%

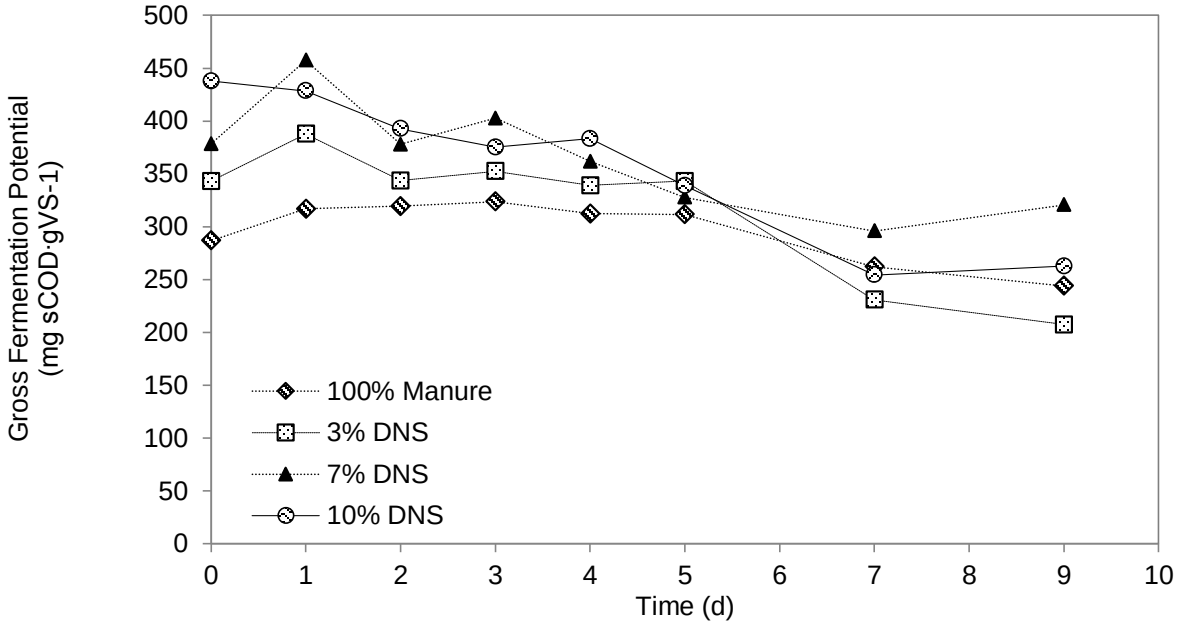

Figure 3.2. Gross fermentation potential of dairy manure & DNS mixtures over time, represented as mg sCOD·gVS^{-1} loaded.

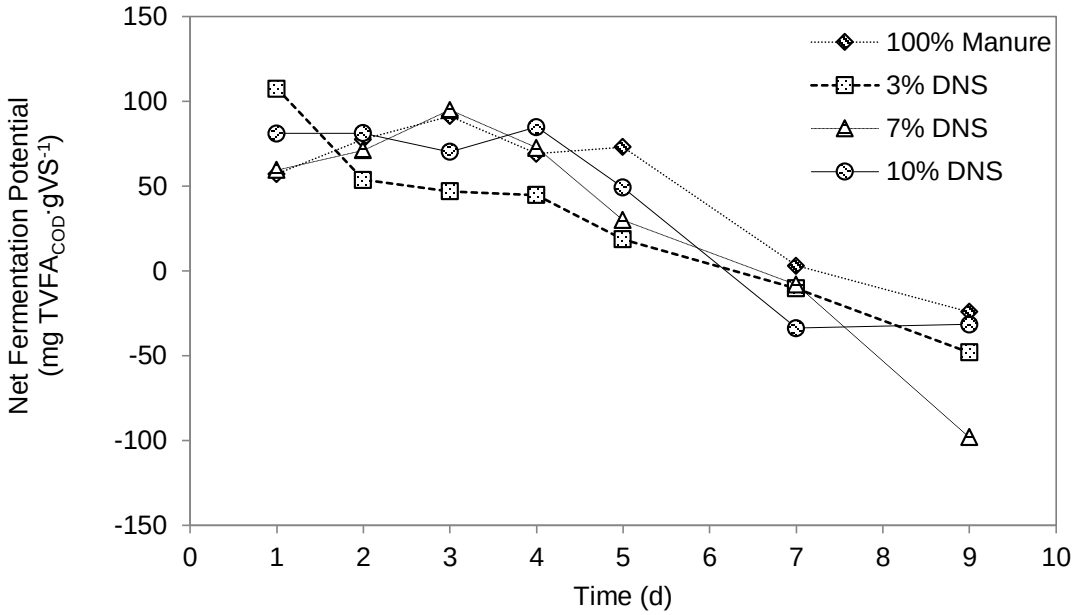

Figure 3.3. Net fermentation potential of the 100% DM and DNS mixtures, represented as mg TVFA$_{COD}$·gVS^{-1} loaded.

DNS was higher than the 100% DM only on day 1, after which the 3% DNS mixture had lower net TVFA concentrations than the 100% DM. The 100% DM had higher net TVFAs than all three DNS mixtures between 5 and 9 d of fermentation. Differences in net TVFA concentrations between treatments were found to be statistically insignificant (ANOVA, $p<0.05$) for all days.

In terms of the net sCOD, the 100% DM again yielded higher specific energy than the DNS feedstock mixtures on most days (figure 3.4). However, the maximum net sCOD concentration was achieved by the 7% DNS mixture on day 1, with a concentration of 79 mg sCOD·gVS^{-1}. All treatments reached their maximum sCOD concentrations on day 1, after which sCOD concentrations declined.

The macronutrient and micronutrients contained in the manure and DNS used in this study are presented in table 3.4. The nutrient content of the DNS were obtained from the manufacturer. Micronutrients like iron (Fe), calcium (Ca) cobalt (Co), nickel (Ni), zinc (Zn), tungsten (W), manganese (Mn), molybdenum (Mo), selenium (Se), and boron (B) are known to stimulate or enhance the anaerobic digestion process, particularly methanogenesis (Murray and Berg, 1981; Speece, 1996; Speece et al., 1983; Wilkie et al., 1986). The DNS used in this study contained Ca, Mg, Fe, Se, and Mo at concentrations known to stimulate methanogenesis (table 3.4). While manure also contains some of the Ca, Mg, and Fe, the concentrations of the other vitamins and minerals contained in DNS and presented in table 3.4 were not determined and therefore comparisons or effects cannot be determined. Prior investigations have only considered the effects of the DNS vitamins and minerals on methanogenesis; therefore, further analysis is recommended to determine the specific effects of these vitamins and minerals on fermentation processes (Murray and Berg, 1981; Speece, 1996; Wilkie et al., 1986).

Based on the results of this study, co-digestion with DNS is recommended at 3% and 10% of the total volume loaded into a two-stage anaerobic digester. If a 3% DNS mixture is used, it is recommended that the acid chamber of an anaerobic digester be operated at an HRT of 1 d. If a 7% DNS or 10% DNS mixture is used, it is recommended that the acid chamber be operated at an HRT of 3 or 4 d, respectively.

3.4.3 Fermentation Potential of the PPW and Manure Mixtures

The maximum gross TVFA concentrations of 242 and 226 mg (as COD)·gVS^{-1} were reached by the 20% PPW mixture during the first two days of the test. The results from the FP test using PPW mixtures and the 100% DM are presented in terms of TVFA and sCOD in figures 3.5 and 3.6, respectively. The 10% PPW, 15% PPW, and 20% PPW mixtures yielded higher gross TVFAs than the 100% DM on days 1 through 9, with the three PPW mixtures maintaining values greater than 180 mg (as COD)·gVS^{-1}. The gross TVFA concentrations of the 100% DM steadily increased toward a peak of 181

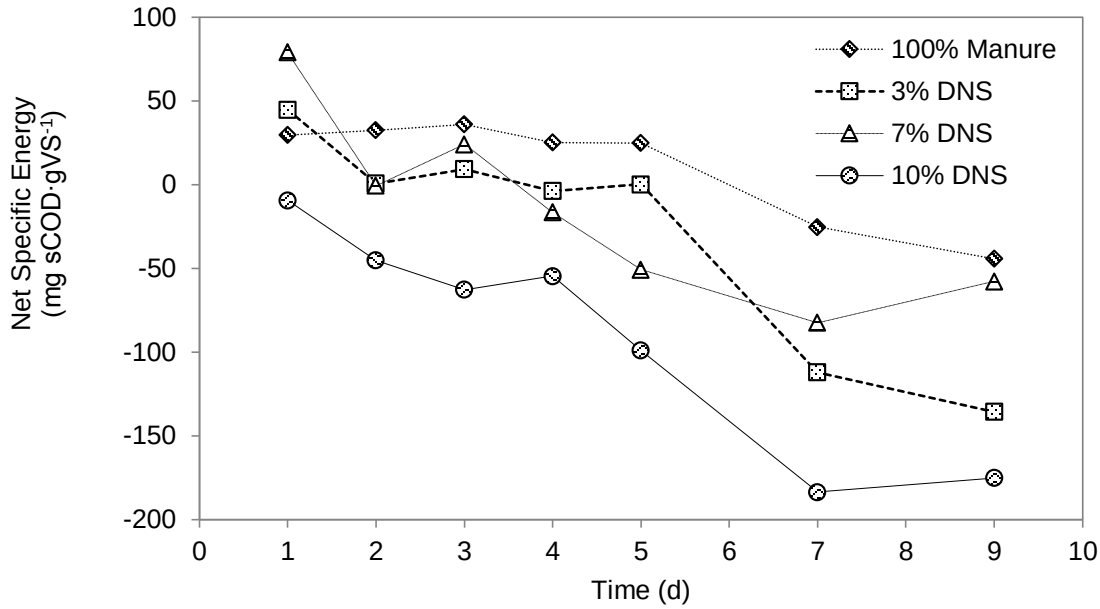

Figure 3.4. Net specific energy of the 100% DM and DNS mixtures, represented as mg sCOD·gVS^{-1} loaded.

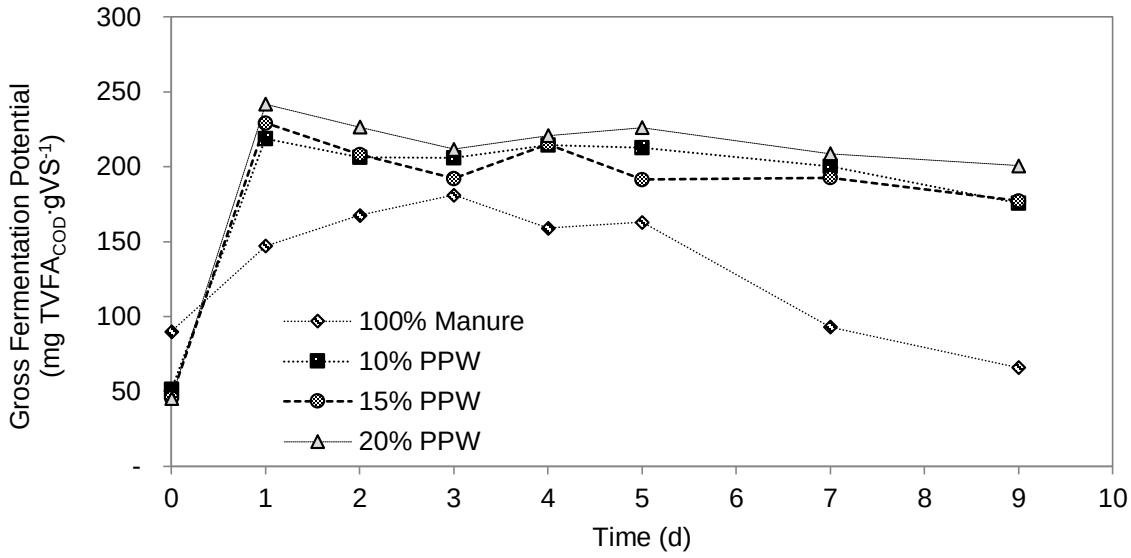

Figure 3.5. Gross fermentation potential of manure and PPW mixtures represented as mg TVFA (as COD)·gVS^{-1} loaded.

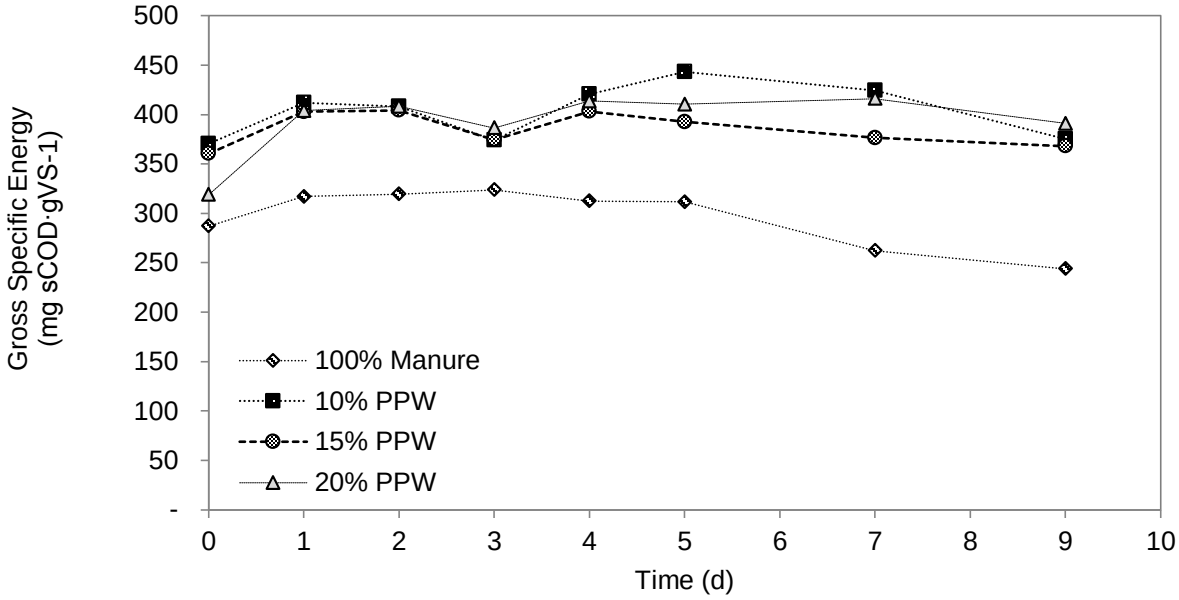

Figure 3.6. Gross specific energy of manure and PPW mixtures, represented as mg sCOD·gVS^{-1} loaded.

Table 3.4. Micronutrient Content in DNS Feedstock

Vitamins & Minerals	Concentration ($mg \cdot L^{-1}$)	Optimum Concentration ($mg \cdot L^{-1}$)	Cited in
Biotin (B-complex)	0.03	-	-
Folate(B-complex)	0.42	-	-
Vitamin A	0.63	-	-
Vitamin B_1 (thiamin)	1.48	-	-
Vitamin B_2 (riboflavin)	1.69	-	-
Vitamin B_3 (niacin)	19.0	-	-
Vitamin B_5 (pantothenic acid)	6.34	-	-
Vitamin B_6 (pyridoxine)	2.11	-	-
Vitamin B_{12} (cobalamine)	0.01	-	-
Vitamin C (ascorbic acid)	159	-	-
Vitamin D (cholecalciferol)	0.01	-	-
Vitamin E (tocopherol)	10.6	-	-
Vitamin K	0.08	-	-
Calcium	1,268	40	(Speece, 1996)
Chlorine	8,623	-	-
Chromium	127	-	-
Copper	2.11	-	-
Iodine	0.16	-	-
Iron	15.9	0.28-50.4	(Speece, 1996)
Magnesium	370	360-4,800	(Speece, 1996)
Manganese	12.7	-	-
Molybdenum	0.16	0.001-0.048	(Murray and Berg, 1981; Speece, 1996; Wilkie et al., 1986)
Phosphorus	1,057	-	-
Selenium	0.04	0.08-0.79	(Speece, 1996; Wilkie et al., 1986)
Zinc	15.9	-	-

mg (as COD)·gVS^{-1} until day 3, after which concentrations decreased to 36% of the peak value by day 9. Comparison of gross TVFA concentrations between treatments showed that the 20% PPW mixture was significantly higher than the TVFA of 100% manure on days 1 and 2. Differences between treatments on days 3 through 7, however, were not significant. On day 9, the gross TVFA concentrations in all PPW mixtures were significantly higher than 100% DM.

Expressed as sCOD, the 10% PPW mixture had the highest gross specific energy on all days of the test, ranging between 370 and 443 mg sCOD·gVS^{-1}. The 100% DM had the lowest gross sCOD concentration on all 9 days of the FP test, ranging between 244 and 324 mg sCOD·gVS^{-1}. The 10% PPW, 15% PPW, and 20% PPW mixtures had gross sCOD concentrations that were within 40 mg sCOD·gVS^{-1} of each other; therefore, there were no significant differences between the PPW mixtures on any days of the test. The only significant differences in gross sCOD concentrations between treatments occurred between the 100% DM and PPW mixtures on days 4 and 5. The gross sCOD concentrations in the 10% PPW and 20% PPW mixtures were significantly higher than in the 100% DM on day 4. The gross sCOD concentrations in the 10% PPW mixture were significantly higher than in the 100% DM on day 5 (ANOVA, $p<0.05$).

The 10% PPW, 15% PPW, and 20% PPW mixtures yielded higher net TVFAs concentrations (as mg COD) gVS^{-1} than the 100% DM on days 1 through 9 (figure 3.7). The highest net TVFA was also achieved by the 20% PPW mixture on day 1, with a concentration of 189 mg TVFA (as COD)·gVS^{-1} loaded. The 15% PPW had the second highest net TVFA concentrations on days 2 and 4, at concentrations of 183 and 169 mg TVFA (as COD)·gVS^{-1}, respectively. The net TVFA concentrations in the 10% PPW mixture surpassed the 15% PPW mixture on days 3 through 9. Differences in net FP were significant between the 100% DM and all three PPW mixtures on days 1, 2, 5, and 9 (ANOVA, $p<0.05$).

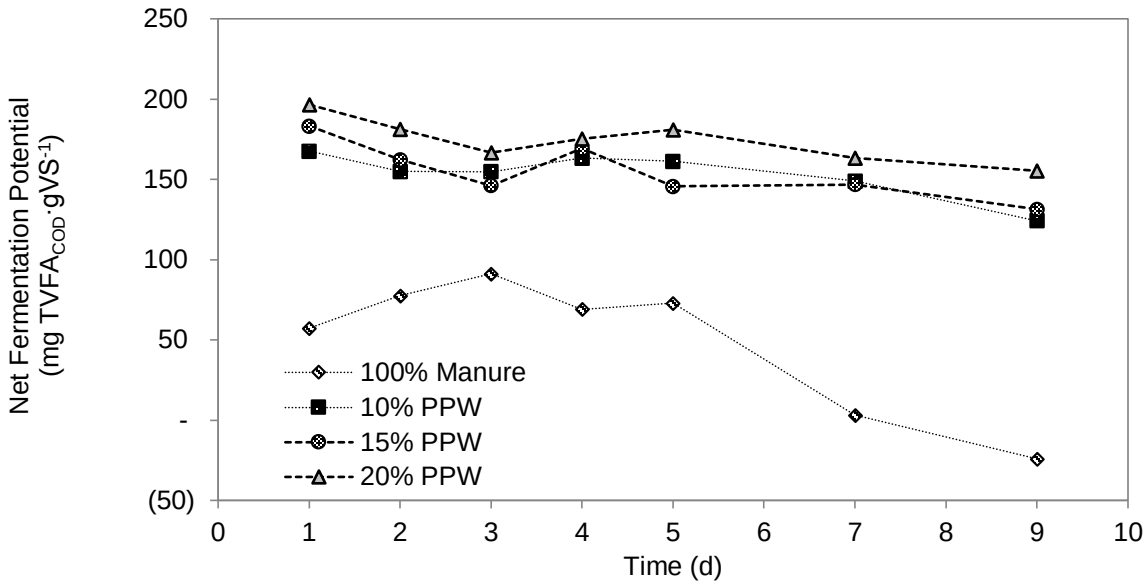

Figure 3.7. Net fermentation potential of the 100% DM & PPW mixtures, represented as mg TVFA(as COD)·gVS^{-1} loaded.

Comparing the 100% DM and PPW mixtures to net specific energy (i.e. mg sCOD·gVS^{-1}), the 10% PPW mixture had the highest net sCOD on all days (figure 3.8). The 20% PPW had the second highest net specific energy on all days. As previously discussed, however, these results do not necessarily suggest that the 10% PPW and 20% PPW mixtures are more suitable for methane production than 100% DM because the sCOD reflects the total soluble biodegradable substrates, while only the TVFAs can be utilized for biomethane production. Altogether, the high net TVFA and as sCOD concentrations in the PPW mixtures compared to the 100% DM, and the reductions in tCOD in the PPW mixtures between days 1 and 9, suggest that the high concentrations of biodegradable content contained within the PPW was readily converted to fermentation products like TVFAs. It can thus be concluded that co-digestion of dairy manure with PPW, where PPW constitutes up to 20% of the loading volume into an anaerobic digester, would results in increased biomethane production compared to dairy manure, alone.

The high gross and net TVFA concentrations observed while fermenting the 10% PPW mixture suggest that PPW may have contained stimulatory compounds in addition to readily degradable organic matter. The nutrient analysis conducted on the PPW and presented in table 3.2 shows that PPW contained higher iron concentrations than 100% manure; however, both mixtures had iron concentrations in excess of the range of 0.28 to 50.4 mg·L^{-1} that has been shown to stimulate methanogenesis (Speece, 1996). PPW contained no ammonium nitrogen, but had higher organic nitrogen concentrations than 100% DM. In the event that the organic nitrogen were to be entirely biodegraded and converted to ammonium nitrogen, it may be possible for the ammonium nitrogen concentrations to reach the methane-inhibitory ammonia concentrations of 4051–5734 mg NH_3–N·L^{-1} (Koster and Lettinga, 1984). However, this range of ammonia concentrations has no known effect on the acidogenic microorganisms involved in fermentation, which is the process analyzed in this study. It would be necessary to consider the effects of ammonia inhibition prior to selecting PPW mixtures for anaerobic digester.

It is recommended that further studies be conducted to determine how much of the organic nitrogen contained in the PPW converts to ammonia nitrogen during anaerobic digestion prior to selecting PPW for co-digestion with manure. However, the results of the gross and net FP concentrations observed in this study suggest that biogas production can be maximized if the acid chamber of a two-stage anaerobic digester were operated with a hydraulic retention time of 1 d and manure were co-digested with PPW in mixtures containing 10% PPW, 15% PPW, or 20% PPW by volume.

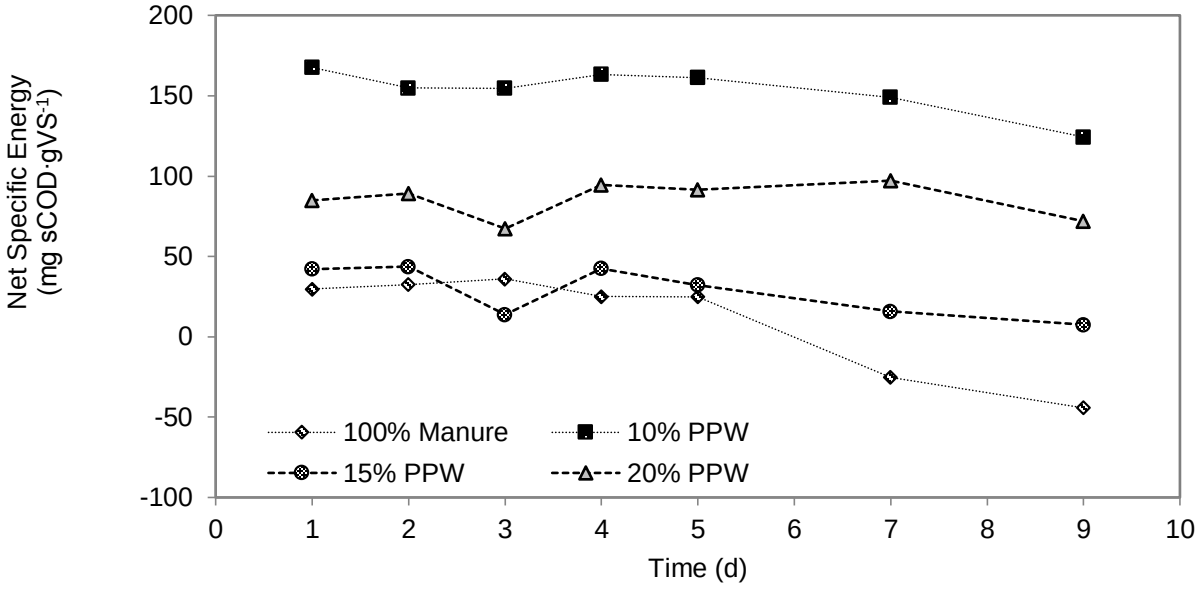

Figure 3.8. Net specific energy of the 100% DM & PPW mixtures, represented as mg sCOD·gVS^{-1} loaded.

3.5 CONCLUSIONS

Based on the results of this study, it is suggested that manure be co-digested with up to PPW, where PPW constitutes up to 20% of the total loading volume. For maximum TVFA production and anticipated biomethane production, the acid chamber of a two-stage anaerobic digester should be operated with a hydraulic retention time of 1 d. The results also indicate that dairy manure can be co-digested with DNS, where the DNS constitutes between 3% and 10% of the total volume, for enhanced biomethane production. For maximum TVFA production using a 3% DNS mixture, it is recommended that the acid chamber of an anaerobic digester be operated at a hydraulic retention time of 1 d. For maximum TVFA production using a 7% DNS or 10% DNS mixture, it is recommended that the acid chamber be operated at a hydraulic retention time of 3 or 4 d, respectively. It is also recommended, however, that the dairy manure, DNS, and PPW feedstocks be further investigated for the nutrient content described in table 3.4, particularly the nutrients selenium and molybdenum. Further research should be conducted to elucidate the effects of DNS, PPW, and manure on individual fermentation and methanogenesis processes.

While the gross and net FPs of a feedstock indicates the progression of fermentation and overall process characteristics, they do not reflect the biogas production potential of feedstocks precisely enough to use as the sole method of evaluating feedstocks for biomethane production potential. In the case of the research presented herein, no known methane-inhibitory compounds were added to the mixtures to prevent the conversion of fermentation products to biogas. Dairy manure has been shown to host both fermentative and methanogenic microorganisms, suggesting that a flux in TVFA production and consumption may have occurred. While the net effects of these changes was captured by the gross and net FP results previously described, the effects of TVFA removal via methanogenesis were not determined. One suggestion to overcome these limitations is the addition of a methane-inhibiting compound to isolate fermentation processes and products. Since the objective of this research is to evaluate feedstock mixtures for co-digestion with dairy manure for increased biogas production, an alternative suggestion is the performance of biomethane potential tests for the feedstock mixtures evaluated in this research. Biomethane potential (BMP) tests determine the biomethane production from anaerobic digestion of feedstocks over a period of time, which is usually 30 d. BMP tests enable initial and final feedstock characteristics to be evaluated, as well as biogas production (quantity and quality) over the course of the test.

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4 The Effects of Temperature and Duration of Pasteurization on Pathogen Inactivation in Anaerobically Digested Separated Liquid Dairy Manure

4.1 ABSTRACT

Mesophilic anaerobic digestion (MAD) is used to treat organic wastes like dairy manure to recovery energy (biogas) and reduce the negative environmental impacts associated with manure, including high chemical oxygen demand, odors, and greenhouse gas emissions (GHG). Products of digestion can be used for energy production as separated liquid fertilizer, and as separated solids bedding for dairy cows. However, recent research has indicated that mesophilic anaerobic digestion does not significantly decrease indicator bacteria or pathogen concentrations. Insufficiently treated manure has the potential to contaminate food and/or water supplies following land application, posing a threat to human and environmental health. The objectives of this study are to: (i) the effective combination of pasteurization temperature and duration to reduce fecal coliform, E. coli, and Salmonella concentrations to the levels required for use as an organic fertilizer; and (ii) the effects of pasteurization temperature and duration on fertilizer qualities in ADSLM. Pasteurization of dairy manure has shown great potential for decreasing pathogen loads in manure. Pathogen die-off during the pasteurization process is a function of temperature and time, but can be affected by factors such as total solids content and pH. Combinations of three pasteurization temperatures (70°C, 75°C, and 80°C) and nine treatment durations (0, 15, 30, 45, 60, 75, 90, 105, and 120 min) were evaluated in the lab via twenty-seven independent trials. Observed reductions in fecal coliform concentrations ranged from 85% to 95%, E. coli reductions ranged from 87% to 96%, and Salmonella bacteria appeared 100% inactivated. The duration of pasteurization treatment did not significantly affect pathogen concentrations; over 85% of inactivation was achieved during the period when manure temperatures were raised to desired pasteurization temperatures. This study contributed greater understanding about pathogen inactivation response in dairy manure. Following treatment, samples from all twenty-seven trials met the minimum requirement for use in organic food production. Given this proof-of-concept, further research at the pilot scale to assess the feasibility of farm-scale implementation is recommended.

4.2 INTRODUCTION

4.2.1 Manure Pathogens

Aerobic heterotrophic plate counts have indicated that average bacterial concentrations in freshly excreted dairy manure range from 7.4 $x10^5$ to 2.1 x 10^9 colony forming units (cfu) per gram manure (Akinde and Obire, 2008; Allen, 1923; McGarvey et al., 2004). Several of the bacterial species frequently isolated from dairy manure are pathogenic to humans, including *Clostridia* spp., *Klebsiella* spp., *Listeria monocytogenes*, *Cryptosporidium parvum*, *Escherichia coli*, *Salmonella enterica*, *Mycobacterium* spp. and *Shigella* spp. (Bowman, 2009; Kirk, 2011; McGarvey et al., 2004; McGarvey et al., 2007). Food and water contamination from manure pathogens can lead to outbreaks of illnesses, including severe cramping, hemorrhaging, bloody diarrhea, dehydration, or, in extreme cases, death (DiRita, 2007a, b). Recent *E.coli* outbreaks originating from spinach and mixed greens in 2012 and from bean sprouts in 2011 are reminders of the importance of observing meticulous sanitary protocols during food production and handling processes (CDC, 2012). Manure pathogens *Listeria*, *Salmonella*, *Shigella*, some *E.coli* strains, *Cryptosporidium* spp., *Mycobacterium avium*, and *Pseudomonas aeruginosa*.

Since dairy manure often contains a range of potential human pathogens, the use of manure as a fertilizer on agricultural lands is regulated by federal, state, and local agencies to protect public and environmental health and to ensure food safety. Dairy manure from concentrated animal feeding operations (CAFOs) is regulated by the US EPA under 40 CFR 412 Subpart C (U.S. Government Printing Office, 2013). Use of dairy manure in human food production is regulated by the USDA under the Organic Foods Production Act of 1990 (USDA, 1990). Regulating the use of manure-based fertilizers prevents contamination of agricultural lands, food, and water sources with pathogens (Cole et al., 1999; Fayer et al., 2004).

4.2.1.1 *Escherichia coli*

Although *E. coli* is notorious for causing well-publicized outbreaks of human illness, the vast majority of strains are non-pathogenic and play an important role in human and ruminant digestive tracts (DiRita, 2007a, b). Some pathogenic *E. coli* strains (e.g. O157:H7) do, however, cause disease in their hosts. If an animal, such as a dairy cow, is infected with *E. coli*, it becomes a host for an extended time period and excretes (i.e. "sheds") virulent *E. coli* in its manure. Since most dairy cows are raised in confinement and in close proximity to each other, bacteria can easily be passed within the herd. Depending on the prevalence of *E. coli* infection within a given herd, and the severity of illness in individual cows, collected farm manure can contain very high pathogen concentrations. Pathogenic *E. coli* strains are classified as enterotoxigenic, enteropathogenic, enterohemorrhagic, enteroinvasive, or

enteroaggregative (DiRita, 2007a). Classification depends upon strain features and mechanisms of attachment and pathogenesis, and is correlated to symptoms of infection (DiRita, 2007a).

Enteroinvasive *E.coli* (EIEC) strains are typically ingested via contaminated food and cause dysentery and diarrhea, resulting in a syndrome identical to Shigellosis (DiRita, 2007b; FDA, 2012). Although infection causes bloody discharge and mucus, it usually results in no complications and is resolved within days (FDA, 2012). Aggregative *E.coli* (EAggEC) strains are seldom associated with contaminated food and water (FDA, 2012). However, the 2011 outbreak associated with German-produced sprouts was in fact an EAggEC strain, O104:H4 (CDC, 2012; FDA, 2012). In this particular case, the strain was incorrectly identified as EHEC due to the production of a shiga-like toxin that caused severe bleeding in the colon, which is typically an EHEC-associated trait (CDC, 2012). Genetic analysis revealed that the EAggEC strain acquired the shiga-toxin producing gene via mobile genetic elements (FDA, 2012). The potential for bacteria to exchange genetic elements is of great interest in modern microbiology research, as it has significant public health implications (Bigot, 2012; Malachowa and DeLeo, 2010; Sherratt, 1995). The recent emergence of EHEC strains has gained much attention due to the widespread and deadly sequelae of infections observed in a number of outbreaks associated with contaminated food. EHEC strains, like the notorious O157:H7 and the recently discovered serotype O104, cause bloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (injury of the kidney caused by toxins) (DiRita, 2007a). A number of studies have isolated *E.coli* O157:H7 from raw dairy manure, and show that it can survive between 25 and 321 d in both pasture and soil (Fenlon et al., 2000; Fremaux et al., 2008; Gagliardi and Karns, 2002; Hutchison et al., 2005). *E.coli* can persist in the environment for prolonged periods; it is therefore important to treat manure for pathogen inactivation prior to its use as a fertilizer in human food production.

4.2.1.2 *Salmonella enterica*

The genus *Salmonella* has only two recognized species, *Salmonella enterica* and *Salmonella bongori*. The species *S. enterica* has six subspecies, each with multiple serovars (antigen combinations) (DiRita, 2007b). *S. enterica* subspecies enterica contains the majority of *Salmonella* serovars that cause human disease (DiRita, 2007b). *Salmonella* is transmitted via the fecal-oral route, and is either contracted directly from feces or from fecal contamination of food and water (DiRita, 2007b). Up to 75% of dairies in California have tested positive for *Salmonella* in feces, and cattle shedding *Salmonella* in their feces usually have concentrations ranging between 20 and 50,000 cfu/gram of manure (Kirk, 2011). *Salmonella* are sensitive to the small, uncharged molecule ammonia (NH_3), which crosses the cell membrane and damages the cell by rapidly shifting the cytoplasm to alkaline conditions (Bolton et al., 2013; Ottoson et al., 2008). If untreated, however, *Salmonella* can survive for as many as 286 d in

manure slurry (Kirk, 2011). The number of *Salmonella* cells needed to cause infection, referred to as the minimal infectious dose, has been reported to range from 10^1 to 10^9 CFU (Greig et al., 2010).

4.2.1.3 *Fecal Coliforms*

The fecal coliforms group is a subset of facultative anaerobic coliform bacteria that includes *Klebsiella* spp., *E.coli*, *Citrobacter* spp. and *Enterobacter* spp (Leclerc et al., 2001). Fecal coliforms are also known as thermo-tolerant coliforms and can be isolated by incubating samples at 44.5 ± 0.2 C (Yakub et al., 2002). Fecal coliform bacteria originate from human and other mammalian feces, and do not proliferate in natural water environments. Therefore, the presence of high fecal coliform concentrations in surface and subsurface waters may indicate recent fecal contamination of water. Since dairy manure contains high fecal coliform concentrations, land application of manure fertilizers results in increased fecal coliform concentrations in soil. Agricultural runoff from soils recently fertilized with manure can potentially contaminate local streams, lakes, and groundwater with fecal coliform bacteria (Brennan et al., 2010; Gerba and J.E. Smith, 2005 ; Goberna et al., 2011; Goss et al., 1998; Mawdsley et al., 1995; Unc and Goss, 2004). Although the vast majority of fecal coliform bacteria are not pathogenic, they may indicate the presence of other pathogenic bacteria of fecal origin, such as *Listeria monocytogenes* or *Cryptosporidium parvum*. Most fecal-borne human pathogens, like *L. monocytogenes* or *C.parvum*, are more difficult and costly to detect than fecal coliform bacteria. Instead of monitoring for these pathogens directly, fecal coliforms are measured in water and manure samples to indicate whether human pathogens may also be present.

4.2.2 Organic Fertilizer Standards for Processed-Manure

According to the Organic Foods Production Act (OFPA) introduced in the 1990 US Farm Bill, a farm can only bear the USDA Certified Organic label if the farm follows an organic farm plan that has been approved by a USDA-accredited organic farm inspector (USDA, 1990). The approved plan must specify the methods and materials used during the food production process. The Organic Materials Review Institute (OMRI) provides a list of approved products for use in certified organic food production, handling, and processing (OMRI, 1997). The Organic Materials Review Institute (OMRI) guidelines stipulate that manure can be used as a crop fertilizer and/or soil amendment if processed at a minimum temperature of 66°C for at least one hour, heating all portions to 74°C and drying the manure to a maximum moisture level of 12%, or an equivalent heating and drying process (OMRI, 1997). Processed manure must contain fewer than 1,000 MPN (most probable number) of fecal coliform bacteria per gram of processed manure. The *Salmonella* bacteria count in the manure must be fewer than 3 MPN per 4 grams of processed manure (OMRI, 1997).

4.2.3 Pathogen Inactivation

Given the prevalence of fecal coliforms and potential for pathogenic bacteria in dairy manure, significant reduction of microbial concentrations in manures used as fertilizer is necessary. All bacteria need certain conditions in order to survive and reproduce. Factors affecting microbial processes include temperature, pH, moisture level, and available substrate (species-specific "food", i.e. carbon and nutrient sources) (Heinonen-Tanski et al., 2006; Smith et al., 2005). Most pathogenic species require neutral conditions between pH 6 and pH 8.5 because extremely low or high pH conditions reduce the stability of microbial cell structures, such as the cell membrane and cell wall (Acquisto et al., 2006; Heinonen-Tanski et al., 2006). Pathogens are also sensitive to the chemical composition of their environment, due to the potential for chemical-cell interactions. For example, if microorganisms are subjected to alkaline chemicals, their cell contents become increasingly alkaline, inhibiting cell activity and causing death (Acquisto et al., 2006). The relative sensitivity of many pathogens enables scientists and engineers to inactivate them through alteration of one or more environmental factor.

Manure can be treated by physical, chemical, or biological processes, or a combination of thereof. The effectiveness of any treatment is influenced by the inherent properties of the manure, including pH, solids content, and volatile fatty acids (VFA). A variety of methods have been shown to inactivate pathogens, the most common being thermophilic anaerobic digestion, chemical treatment (e.g. alkali or lime amendment to raise pH), composting, and heat-drying. Less common treatments include ozone treatment, irradiation (microwave) treatment, and pasteurization treatments (Bowman, 2009). The degree to which each treatment achieves pathogen reduction, nutrient and waste stabilization, and odor reduction varies. These methods are briefly described below.

4.2.3.1 *Anaerobic Digestion*

Anaerobic digestion (AD) has long been used as an effective treatment for stabilizing dairy manure while producing energy (biomethane) and other value-added products like separated solids. While AD can reduce manure odors, capture and thereby reduce greenhouse gas emissions, and stabilize nutrients and organic matter, it does not sufficiently reduce pathogen concentrations in dairy manure or sludge at psychrophilic (20 C) and mesophilic (38 C) temperatures (Horan et al., 2004; Lang and Smith, 2008; Pandey and Soupir, 2011). Pathogen inactivation in anaerobic digesters is affected by temperature, retention time, dispersion, bypass flow, mixing, and dead zones within anaerobic digesters (Smith et al., 2005). Anaerobic digestion at high temperatures (usually 55°C or higher) has been shown to decrease pathogen concentrations compared to psychrophilic or mesophilic temperatures (Aitken et al., 2007; Cecchi et al., 1991; De Leon and Jenkins, 2002; Lang and Smith, 2008; Lo et al., 1985; Pandey and Soupir, 2011; Smith et al., 2005). This is because high temperatures disrupt cell wall bonds,

compromising their structural stability, and increase the fluidity of cell membrane phospholipids, resulting in leakage of cell contents and cell lysis (Madigan et al., 2012a). Heat also denatures cell proteins, such as DNA. If DNA and other proteins are denatured beyond repair, they lose their function, preventing a bacterium from carrying out its metabolic and catabolic processes, eventually causing death (Bauman et al., 2003; Madigan et al., 2012a).

Pandey and Soupir (2011) studied the effect of time and temperature on the reduction of *E. coli* concentrations in dairy manure during psychrophilic (25 C), mesophilic (37 C) and thermophilic (52.5 C) anaerobic digestion. They found that under psychrophilic and mesophilic conditions at least 60 and 40 d, respectively, are required to achieve 90% reduction in *E. coli* concentrations, compared to fewer than 4 d at thermophilic temperatures. The longer treatment retention times associated with psychrophilic and mesophilic anaerobic digestion result in increased reactor sizes and increased construction costs (Grady et al., 2011). Conversely, thermophilic AD reduces reactor size compared to psychrophilic and mesophilic anaerobic digestion, since less time is required for pathogen inactivation (Acquisto et al., 2006; Lang and Smith, 2008). Thermophilic anaerobic digestion also enhances biogas production, and consequently, has gained in appeal among researchers for its ability to maximize microbial inactivation while minimizing initial capital costs (Aitken et al., 2007; Cecchi et al., 1991; De Leon and Jenkins, 2002; Lang and Smith, 2008; Lo et al., 1985; Pandey and Soupir, 2011; Smith et al., 2005). However, thermophilic AD requires a higher energy input to maintain elevated temperatures. Thermophilic AD systems also require additional management, since elevated temperatures promote the formation of methanogenesis-inhibitors like non-ionized ammonia (Bordeleau and Droste, 2011; Cecchi et al., 1991).

Since thermophilic AD are more resource intensive than mesophilic AD with regards to energy and monitoring requirements, mesophilic anaerobic digesters have become the more popular design for on-farm treatment of dairy manure in the US (AgSTAR, 2012). On-farm mesophilic ADs are typically operated with retention times of 10 to 30 d at approximately 38°C (US EPA, 2004). These conditions, however, are insufficient for pathogen inactivation and effluent from mesophilic anaerobic digesters has been shown to contain elevated pathogen concentrations (Horan et al., 2004; Lang and Smith, 2008; Pandey and Soupir, 2011; Smith et al., 2005). The addition of a post- or pre- AD treatment technology specifically for the inactivation of pathogens can alleviate environmental degradation from land-application of dairy manure, including contamination of agricultural runoff receiving waters with manure-borne bacteria (Gerba and J.E. Smith, 2005 ; Mawdsley et al., 1995; Unc and Goss, 2004).

4.2.3.2 *Chemical Treatment*

Pathogens in manure and sewage sludge can also be inactivated by chemical stressors, including alkaline treatment, lime stabilization, ferrate oxidation, and acid treatment (Acquisto et al., 2006). Alkaline treatment, lime stabilization, and ferrate oxidation cause dramatic and rapid changes to the pH of a material. These changes damage bacterial cell walls and change the internal chemistry of bacteria, causing either shock or irreversible inactivation (Bauman et al., 2003; Madigan et al., 2012a). Since the chemical reactions that occur during these applications are also exothermic, the material temperature increases, contributing to pathogen inactivation (Ghiglietti et al., 1997). Ammonia-based treatments, such as those using alkaline or lime substances, release aqueous ammonia that damages pathogen cell structures (Avery et al., 2009; Bolton et al., 2013; Vanotti et al., 2005). The interaction between temperature, pH, and ammonia are believed to contribute to pathogen inactivation more than any single factor alone (Acquisto et al., 2006; Ghiglietti et al., 1997).

Although these methods reduce pathogen concentrations, chemically treated manure and sewage sludge still contains substrates that can support microbial growth (Acquisto et al., 2006). It is therefore necessary to treat manure using methods that maintain unfavorable pH conditions and prevent recontamination over time. The use of strong acids, like sulfuric or nitric acid, can maintain undesirable pH conditions for microbial growth for longer periods due to the dissociation of strong acids into hydrogen and hydroxide ions, which disrupt cell membrane activities (Acquisto et al., 2006). However, downstream uses of the treated products must be considered when evaluating this option, since the dissociated ions from a strong acid can corrode storage vessels and damage plant roots and stems, thereby inhibiting plant growth, (Acquisto et al., 2006; Bowman, 2009). Furthermore, manure has a high buffering capacity and would require the use of large quantities of acid to reduce the pH to a suitable level (Li et al., 2010).

4.2.3.3 *Microwave Treatment*

All biological materials, including manure, have electrical properties like absorbance and conductivity (Metaxas and Meredith, 1988). Because of this, manure molecules subjected to microwave energy will vibrate at the same frequency as the microwaves applied. This vibration results in a heating effect and raises temperatures beyond the survivable limits of many pathogens (Acquisto et al., 2006; Remya and Lin, 2011; Woo et al., 2000). Material properties like solids content, protein and fat content, ionic strength, and viscosity determine how quickly the material can be heated by microwaves (Hong et al., 2006; Metaxas and Meredith, 1988). Effective inactivation of pathogens using microwaves has been demonstrated in a number of studies, with results ranging from complete inactivation of fecal coliforms to approximately 5 log (99.999 %) reductions in *E. coli, Staphylococcus intermedius,* and *Pseudomonas*

aeruginosa in sewage sludge (Hong et al., 2006; Martin et al., 2005; Pino-Jelcic et al., 2006). Materials containing high solids content, like dairy manure, require higher equivalent microwave doses and therefore increased energy inputs (Acquisto et al., 2006; Pino-Jelcic et al., 2006). Microwaves solubilize organic materials more than conventional heating methods, increasing substrate availability and thereby supporting the regrowth of pathogens - particularly those that are heat-resistant (Hong et al., 2006; Remya and Lin, 2011; Woo et al., 2000). To prevent regrowth and proliferation of pathogens, microwave technologies must be paired with additional treatments to completely inactivate pathogen populations (Acquisto et al., 2006). Currently, the cost of installing and operating microwave technology on farm for manure treatment is prohibitively expensive, but with rapid advances in knowledge and technology, microwave technologies may become a viable alternative for manure treatment in the future (Acquisto et al., 2006; Bowman, 2009).

4.2.3.4 *Ozone Disinfection*

Ozone is a powerful disinfectant for liquid wastes because it oxidizes the cell walls of bacteria and protozoa resulting in lysis, and/or damages nucleic acids preventing reproduction (US EPA, 1999a). The effectiveness of ozone treatment depends on the concentration of ozone, contact time between ozone and the waste, and the cell wall structure of the target organism (Acquisto et al., 2006). Despite the benefits of using ozone to treat wastes, it is not suitable for high-strength or high-solid wastes like dairy manure because these qualities inhibit contact between microorganisms and ozone (Acquisto et al., 2006). Ozone technology is also costly and potentially toxic, so its implementation requires management and expertise (US EPA, 1999a).

4.2.3.5 *Ultrasound irradiation*

Ultrasound irradiation, also known as sonication, has been explored in recent years for treatment of wastewaters. Ultrasound causes cavitation – the formation and immediate implosion of bubbles within a liquid – and results in extreme pressure and temperature gradients as well as the formation of "water darts" and free radicals (Drakopoulou et al., 2009; Flynn, 1964). All of these mechanisms contribute to bacterial cell damage through cell lysis and/or damage to cell membranes, DNA, and organelles (1990; Madge and Jensen, 2002; Mason, 1993). Ultrasound irradiation causes cavitation in wastewaters at frequencies ranging from 20 to 100 kHz (Madge and Jensen, 2002). However, bacterial disinfectant efficiencies have been shown to be insufficient when used as the sole treatment source (Drakopoulou et al., 2009; Pitt and Ross, 2003). Research into the technical feasibility and cost efficiency of wastewater treatment using ultrasound irradiation technology is ongoing (Drakopoulou et al., 2009; Madge and Jensen, 2002; Mahamuni and Adewuyi, 2010; Pitt and Ross, 2003). As a result, this technology is not yet feasible for on-farm treatment of dairy manure.

4.2.3.6 *Ultraviolet irradiation*

Ultraviolet (UV) waves inactivate bacteria by transferring electromagnetic energy into their cells. This intense energy transfer damages genetic material, like DNA (Venieri et al., 2011). Irreversible DNA damage prevents bacteria from performing life-sustaining activities and reproducing (US EPA, 1999b). UV irradiation is an effective technology because pathogen inactivation occurs relatively quickly and it does not require the addition of chemical treatments like chlorine, which produces potentially harmful byproducts and has been linked to the emergence of chlorine-resistant pathogens (Chong et al., 2010; Korich et al., 1990; Macauley et al., 2006; Payment, 1999). The presence of total suspended solids (TSS), humic acids, iron, and carbonates decreases disinfection efficiency because these materials also absorb UV radiation (US EPA, 1999b). Optimal pathogen inactivation takes place at wavelengths between 250 and 270 nm and when UV lamps are placed a minimal distance from the wastewater (US EPA, 1999b). Although UV irradiation is currently one of the most effective pathogen disinfectants for wastewater, technical and cost barriers prevent its implementation on farms for treating wastes with inevitable high-TSS and humics content, such as dairy manure (Macauley et al., 2006; Malato et al., 2007). Furthermore, installation of a UV-system is cost-prohibitive for most dairy farms, with the average cost for a UV system around $244,000 and annual operating costs of $19,190 (Hanzon, 1999).

4.2.3.7 *Composting*

Composting is a biological process in which microorganisms consume and degrade organic materials in the presence of oxygen under controlled conditions (Bowman, 2009; Merkel, 1981). During this process, microorganisms generate metabolic heat as they consume the substrates in the composting material. As microbial degradation proceeds, the compost retains metabolic heat because it is accumulated faster than it dissipates (Bowman, 2009). The temperature of the pile increases, resulting in temperatures in that promote the growth of thermophilic microorganisms and the death of mesophilic microorganisms from excess heat exposure. Since most human and zoonotic pathogens are mesophilic, the heating that occurs during composting reduces pathogen loads in manure (Martens and Bohm, 2009; Shepherd et al., 2007; Tirado and Michel Jr., 2010). The effectiveness of composting treatment depends on factors such as oxygen content, temperature, moisture content, pH, and carbon-to-nitrogen ratio within the composting material. Figure 4.1 shows the temperature, oxygen demand rate, and carbon dioxide evolution profile during composting phases (Bowman, 2009).

Composting can be accomplished in enclosed vessels (in-vessel), windrows, or aerated static piles (Merkel, 1981). In composting, oxygen is necessary for aerobic biodegradation of organic matter. Supplying oxygen into compost piles usually requires specialized and/or energy-intensive equipment, such as windrow turners, oxygen blowers, and mixers/turners. Composting requires an “active” period,

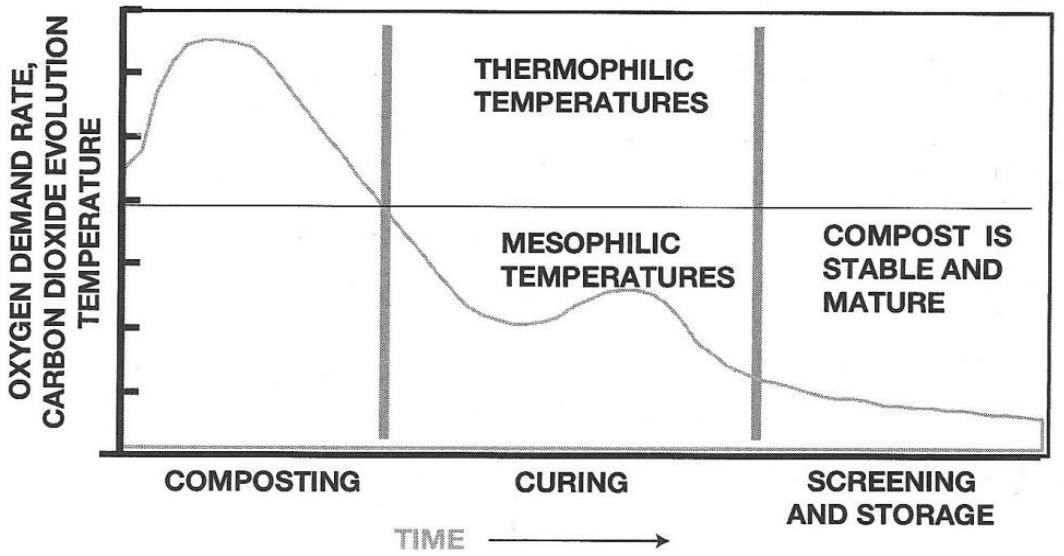

Figure 4.1. Composting phases in relation to temperature, oxygen demand, and carbon dioxide evolution (Bowman, 2009, used under fair use, 2013).

during which microorganisms are highly active and require large amounts of oxygen. This period usually takes 10 to 15 d to completely biodegrade readily available organic matter, and is illustrated by the "composting" phase in figure 4.1. If composting is completed with enclosed vessels, an additional 4 to 16 weeks is required for complete stabilization of the organic matter, as represented by the "curing" phase in figure 4.1, (Merkel, 1981). By contrast, windrow composting takes between 6 to 18 weeks to fully stabilize (Merkel, 1981; Tirado and Michel Jr., 2010). The level to which the composting process is considered to be complete depends on the desired qualities of the finished compost, but composting is generally accepted to be completed when there is little to no further change in nutrients and organic matter. (Bowman, 2009; Tirado and Michel Jr., 2010).

Composting temperature is a large determinate of waste stabilization and pathogen inactivation. Pathogen inactivation starts to occur at temperatures in excess of 55°C. The longer the duration of these temperatures, the greater the pathogen inactivation. This is because many pathogenic bacteria, like *E.coli*, *Salmonella*, and *Klebsiella* spp., have optimal growth at mesophilic conditions (Aitken et al., 2007; Berry and Wells, 2010; Fotadar et al., 2005). As temperatures approach themophilic conditions, metabolic activities cease and DNA starts degrading (Madigan et al., 2012b).

The USDA mandates that static aerated compost systems reach and maintain temperatures between 55°C and 70°C for a minimum of 3 d to sufficient achieve pathogen inactivation for compost to be safely used as a soil amendment in fields used for human food production (National Organic Standards Board, 2002; OMRI, 1997; Shepherd et al., 2007). Windrow compost systems must attain temperatures between 55°C and 70°C for at least 15 d prior to use in organic food production (National Organic Standards Board, 2002; OMRI, 1997; Shepherd et al., 2007; Stentiford, 1993).

4.2.3.8 *Pasteurization*

Pasteurization involves applying heat to a liquid to raise and maintain its temperature at 70° C or higher for a designated time to reduce microbial concentrations (Bagge et al., 2005; Martens and Bohm, 2009; Ugwuanyi et al., 1999). Although pasteurization has historically been primarily associated with the food processing industry since its development by Louis Pasteur in 1862, it is a viable treatment option for any liquid, including liquid manure. The efficiency of pathogen inactivation depends primarily on the temperature and duration of heating, and may also be influenced by material properties such as total solids content, pH, and chemical composition (Heinonen-Tanski et al., 2006; Martens and Bohm, 2009; Ugwuanyi et al., 1999). It has been reported that high organic solids content decreases the efficiency of pathogen inactivation during pasteurization because microbes can be imbedded within the solids,

shielding them from heat and chemical treatments (Acquisto et al., 2006; Smith et al., 2005; Ugwuanyi et al., 1999).

Microbial death rates vary between species that receive identical treatments; some pathogens, like *Salmonella*, are more sensitive to high temperatures than others (Bolton et al., 2013; Bowman, 2009; Department of Veterinary and Food Stuffs, 1995; Kirk, 2011). When attempting to inactivate pathogens in complex matrices like manure, the effectiveness of any treatment can vary between experiments or replicates within experiments. Dairy manure is particularly heterogeneous due to its semi-digested constituents and materials that get integrated during collection. The varying nature of manure affects the efficiency of any treatment for pathogen inactivation. It is therefore necessary to perform pilot scale studies with replicate sampling to reliably determine the treatment duration and temperature needed for pathogen inactivation prior to implementing full-scale treatment systems at farms and wastewater facilities.

A number of studies have evaluated the effect of pasteurization temperature and time on pathogen inactivation in sewage sludge and manures (Bagge et al., 2005; Lang and Smith, 2008; Marañón et al., 2006; Martens and Bohm, 2009; Ugwuanyi et al., 1999). Lang and Smith (2008) investigated the time-temperature relationship by inoculating centrifuged liquid raw sewage supernatant (CLRS) and tryptone soya broth (TSB) with *E. coli* (NCTC9001), *Salmonella typhimurium*, *S. senftenberg*, *E. coli* 0148, *E. coli* 158 and *S. oranienburg*. The CLRS contained 0.2 % solids by weight and the TS content of the TSB was not reported, though it can be assumed to be negligible. The trials were performed by pipetting 1.8 mL aliquots of inoculated media into thermal death tubes, and heating these tubes to a temperature of 70° C in a water bath. They observed that 100% of *E. coli* and *Salmonella* strains were inactivated within 10 seconds of thermal exposure, with the exception of *S. senftenberg*, which was inactivated after 10 seconds in CLRS but 40s in TSB (Lang and Smith, 2008). Marañón et al. (2006) pasteurized dairy manure at 70°C for two hours prior to mesophilic anaerobic digestion to determine the effect of pasteurization on reducing pathogen and microorganism concentrations. Pasteurization for two hours completely inactivated *E. coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Pseudomonas* spp., and fecal coliform bacteria (Marañón et al., 2006).

Since pasteurization treatment has been shown to have a high efficiency in pathogen inactivation, it has been implemented for treatment of animal manures and sewage sludge in a number of countries, including Denmark, Sweden, Germany, Austria, the UK, and Canada (European Parliament and Council, 2002; Heinonen-Tanski et al., 2006; Lang and Smith, 2008; Marañón et al., 2006; Smith et al., 2005). Implementation of pasteurization systems has been recommended by national and international regulatory

agencies to prevent contamination of food and water with pathogens from manure and sewage sludge (European Parliament and Council, 2002). According to the EU Regulation 1774/2002, manure is classified as a Category 2 Animal By-Product that must be treated at 70°C for at least 60 min before use as fertilizer on agricultural lands intended for human food production (European Parliament and Council, 2002).

A survey of the anaerobically digested separated liquid manure (ADSLM) showed average fecal coliform concentrations of 8,780 MPN g^{-1} manure and average *Salmonella* concentrations of 110 MPN $4g^{-1}$ manure. The average *E. coli* concentrations in the ADSLM were 5,770 MPN g^{-1}manure. These concentrations are greater than 8 and 36 times the OMRI standards for fecal coliforms and *Salmonella*, respectively. Thus, the objectives of this study were to determine (i) the effective combination of pasteurization temperature and duration to reduce fecal coliform, *E. coli*, and *Salmonella* concentrations to the levels required for use as an organic fertilizer; and (ii) the effects of pasteurization temperature and duration on fertilizer qualities in ADSLM.

4.3 METHODS & MATERIALS

4.3.1 Collection and Preparation of Separated Liquid Manure

The manure used in this study was obtained from the effluent of a mesophilic anaerobic digester operated by Dairy Energy, LLC, in Chatham, Virginia. The effluent was pumped to a manure separation unit where it was separated into solid and liquid streams mechanically by two screw presses (FAN Separator GmbH, Marktschorgast, Germany) operated in parallel. The screw presses had screens with 0.5 mm slot openings. Approximately 6 liters of the anaerobically digested separated liquid manure (ADSLM) was collected for each pasteurization test. The ADSLM was placed on ice and transported to Virginia Tech, where it was refrigerated at 4°C. The pasteurization tests were started within 30 min of arrival at the lab for a total of 2.5 hours after collection of the ADSLM from the farm. Preparation and analysis of samples was completed at the Bioresidues Utilization & Management and Environmental Microbiology labs at Virginia Tech. The liquid manure was handled and processed using aseptic techniques to prevent cross-contamination of microorganisms and DNA residues. All study trials were conducted between July and December 2011, with specific trial temperatures alternated to minimize the effects of potential monthly or seasonal variations in pathogen concentrations. As the ADSLM storage pit at Dairy Energy is open to the environment, manure was always collected at least one week following a precipitation event to minimize any effects of dilution. Manure was collected from the farm on the same day the trial was conducted, for a total of 10 d of manure collection.

4.3.2 Experimental Design

The experiment was conducted as a factorial design using two factors: treatment temperature (3 levels: 70°C, 75°C, and 80°C) and treatment duration (9 levels: 0, 15, 30, 45, 60, 75, 90, 105, and 120 min). Each of the possible twenty-seven combinations of these two treatments was evaluated for fecal coliform, *E. coli*, and *Salmonella* inactivation, and for total nitrogen and phosphorous concentrations.

4.3.2.1 *Pasteurization Procedure*

Three independent runs were completed for each pasteurization temperature for a total of 9 trials (e.g.3 trials each 70°C, 75°C, and 80°C). Pasteurization of the ADSLM liquid was performed using a VELP Scientifica DK 20 digestion unit with an aluminum block that provided thermal homogeneity (VELP Scientifica, Usmati, Italy). The VELP DK 20 digestion unit held 20 test tubes, each with a rated capacity of 250 mL, external diameter of 42 mm, and a length of 0.35m. Each of the 20 tubes was filled with a 60 mL aliquot of ADSLM to ensure that the liquid was in complete contact with the heating block.

The tubes were placed into the wells of the block digester and heated to the desired pasteurization temperatures (70°C, 75°C, or 80°C).The block digester temperature was set at 80°C, 85°C, and 90°C to attain the pasteurization temperatures of 70°C, 75°C, and 80°C, respectively. The rate of temperature change (e.g. ramping temperature) in the ADSLM to reach the desired pasteurization temperature was approximately $1.6°C \cdot min^{-1}$. Once the ADSLM reached the target temperature, the sampling procedure was initiated. The first sample was collected at time zero which was defined as the moment the desired pasteurization temperature was attained. Subsequent samples were collected every 15 min, as detailed in table 4.1. Samples were identified by the treatment time, which was defined by how long the ADSLM had been heated at the designated pasteurization temperature.

During pasteurization, the temperature of the liquid in each tube was monitored individually using hermetically sealed insulated thermocouples (Omega Engineering Inc., Stamford, CT, USA) located approximately 2.5 cm below the liquid surface. The temperatures of liquid in each tube were recorded continuously using a ciDAQ data acquisition system (National Instruments Co., Austin, TX, USA). Prior to use in each trial, thermocouples were calibrated at 4°C, 15°C, 25°C, 35°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, and 95°C.

At each designated sampling event, two test tubes were removed from the block digester and composited to make a sample of volume 120 mL. The samples were poured into autoclaved bottles and immediately refrigerated at 4°C to prevent further pasteurization. Samples were stored at 4°C for analysis

Table 4.1. Sample Matrix for 70^{o}C, 75^{o}C and 80^{o}C pasteurization temperature

Treatment	Description
Control	No heat treatment
T0	Designation of time when liquid manure attained the desired pasteurization temperature
T15, T30, T45, T60, T75, T90, T105, T120	Designation of pasteurization duration after attaining the desired pasteurization temperature (15, 30, 45, 60, 75, 90, 105, and 120 min).

of nutrients, physical characteristics, fecal coliform, *E. coli*, and *Salmonella* concentrations. Analysis of fecal coliforms, *E.coli*, and *Salmonella*, total solids, and total dissolved solids was completed within 24 hours of each pasteurization trial. Samples from each trial were composited based on their pasteurization time and temperature and preserved.

4.3.2.2 *Initial Heating Effects*

The pasteurization temperatures of 70°C, 75°C and 80°C could not be achieved instantaneously; thus, a series of additional tests were conducted to assess the reduction of fecal coliforms, *E.coli*, and *Salmonella* as the ADSLM was being heated to target pasteurization temperatures. The block was operated as described previously (set desired digester temperature to attain the pasteurization temp, insert thermocouples into the test tubes, followed by placing the test tubes in the block digester). Samples were collected while ADSLM temperatures increased from 20° C to 70°C, 75°C, or 80°C. For the 70°C trial, samples were collected after 8, 16, 22, and 26 min of heating. For the 75°C trial, samples were collected after 6, 12, 16, 20, 24, and 28 min of heating. For the 80°C trial, samples were collected after 8, 14, 19, 26, 30, and 38 min of heating. Experiments were stopped once ADSLM reached the target pasteurization temperature. Samples were collected in replicates of three, poured into individual bottles, and analyzed for fecal coliforms, *E. coli*, *Salmonella*, and total solids.

4.3.3 Analytical Methods

For each trial, composited ADSLM samples from each treatment (Control, T0, T15, T30, T45, T60, T75, T90, T105, and T120) were analyzed for total solids (TS), total suspended solids (TSS), S*almonella*, *E. coli*, and fecal coliforms, according to the detailed procedures provided below.

4.3.3.1 *Physical Characteristics*

The TS and TSS concentrations were determined via methods 2540B and 2540C as described in the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Briefly, the TS was determined by weighing approximately 20 mL of ADSLM sample (W), placing the sample in pre-weighed crucibles (CW), and drying the sample in an oven set at 104° C for 24 hours. Dried samples were then cooled to room temperature in a desiccator and weighed (DW). The TS was calculated using equation 4.1.

$$\text{TS (\% by wet weight)} = \frac{(\text{DW}-\text{CW})}{(\text{W}-\text{CW})} \times 100\% \qquad (4.1)$$

The TSS was obtained by calculating the difference between TS and TDS. TDS analysis was determined by weighing approximately 20 mL of ADSLM sample (W) and centrifuging at 10,000 rpm for 10 min. The centrifuged supernatant was then filtered through a 1.5 μm glass microfiber filter paper (Whatman Inc, Florham Park, NJ, USA). The filtrate (F) was collected in a pre-weighed crucible (CW) and dried in an oven set at 104°C for 24 hours. Dried samples were then cooled in a desiccator and weighed. TDS was calculated using the following formula:

$$\text{TDS (\% by wet weight)} = \frac{(\text{F}-\text{CW})}{(\text{W}-\text{CS})} \times 100\% \quad (5.2)$$

TSS was calculated using the following formula:

$$\text{TSS (\% by weight)} = \text{TS (\%)} - \text{TDS (\%)} \quad (5.3)$$

4.3.3.2 *Nutrients*

For nutrient analysis, samples from trials were composited according to their treatment. In other words, samples from identical pasteurization temperatures and treatment duration were combined to make one single composite sample. Composited samples were sent to the Agricultural Services Laboratory at Clemson University. Composited samples were analyzed for total Kjeldahl nitrogen (TKN), organic nitrogen (ON), ammonia nitrogen (TAN), total phosphate (total-P), and potassium (K).

4.3.3.3 *Microbial Analysis*

Fecal coliforms and *E. coli* were analyzed using the IDEXX Colilert 2000 method (IDEXX Laboratories, Inc., Westbrook, ME). Samples were serially diluted to 10^{-4} concentration using 10 mL of original sample and sterilized DI water to fall within detection limits of the method (1 to 2,419.6 MPN). The diluted ADSLM samples were poured into an autoclaved 100 mL polypropylene bottle, followed by the addition of a single Colilert substrate packet and 1mL of Tween 20 surfactant (Polysciences, Inc., Warrington, PA, USA). The bottle containing ADSLM, DI water, Colilert substrate, and surfactant was inverted approximately 10 times to homogenize contents and then poured into an IDEXX 2000 Quanti-Tray (IDEXX Laboratories, Inc., Westbrook, ME), and sealed using an IDEXX Quanti-Tray sealer. The samples in the IDEXX tray were incubated at 44.5°C for 24±1 h, after which the number of positive fecal coliforms and *E. coli* wells were counted and recorded. The wells testing positive for fecal coliforms appear dark yellow compared to the standard (e.g. figure 4.2). *E. coli* positive wells fluoresce under 450 nm UV light, as shown in figure 4.3. Concentrations were reported as most probable number (MPN) per 100 mL using the IDEXX MPN calculator. The IDEXX MPN calculator is based on the Hurley & Roscoe MPN formula (Gronewold and Wolpert, 2008; Tillett and Coleman, 1985). The concentrations were normalized to the mass of ADSLM sample, yielding units as MPN g^{-1} wet manure.

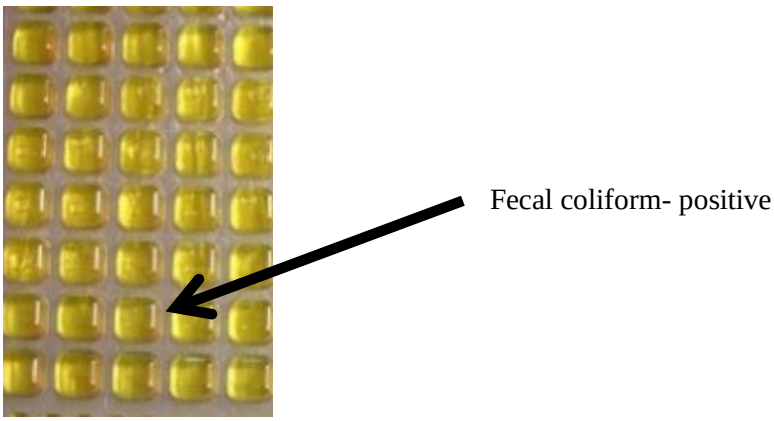

Figure 4.2. IDEXX plate with positive fecal coliform growth (yellow wells).

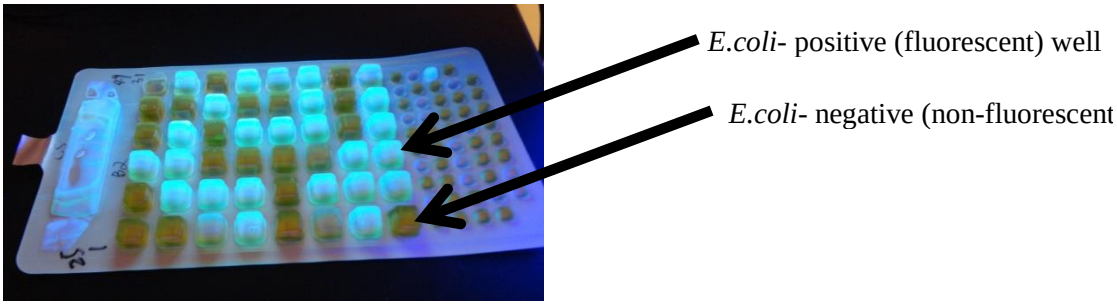

Figure 4.3. IDEXX plate with positive *E.coli* growth (fluorescent wells).

The *Salmonella* content was analyzed using the Rappaport-Vassiliadis (RV) method (Ruiz Gomez et al., 1998). Briefly, samples were diluted to between 1/2 to 1/6 concentrations to stay within the limits of detection of 1 to 1100 MPN. Diluted samples were pre-enriched by inoculating triplicate sample volumes of 0.1, 1, and 10 into vials containing 10 mL buffered peptone water (BPW), for a total of 9 inoculated BPW tubes. The inoculated BPW was incubated at 37°C for 18 to 24 hours. Aliquots of 100 µL BPW were transferred into vials containing 10 mL of RV broth for selective enrichment of *Salmonella*. The inoculated RV broth was incubated at 43°C for 24 hours. After incubation, inoculated RV broth was streaked on *Salmonella-Shigella* agar (SS agar) plates using an inoculum loop. The SS-agar plates were divided into three triangular sections and a sample from each RV vial was streaked onto a single section, providing triplicate samples for each of the three dilutions. Plates were incubated at 37°C for 24 hours and examined for positive *Salmonella* growth. *Salmonella* colonies appear black and circular with a clear zone of hemolysis around the colony. A single colony from each positive section was inoculated into a BD™ BBL™ Enterotube II (BD Diagnostic Systems, Heidelberg, Germany) and incubated at 37°C for 24 hours. Samples were considered positive for *Salmonella* if the Enterotubes had positive reactions in all of the following tests: metabolism of glucose, gas production, presence of lysine decarboxylase and ornithine decarboxylase, hydrogen sulfide production, arabinose fermentation, sorbitol fermentation, dulcitol fermentation, and metabolism of citrate. The Enterotubes also had to be negative for the following tests to be considered positive for *Salmonella*: formation of indole, acetoin production, presence of phenylalanine deaminase, and presence of urease. *Salmonella* concentrations were determined for each sample according to the Hurley & Roscoe MPN formula (Gronewold and Wolpert, 2008; Tillett and Coleman, 1985), for which each *Salmonella*-positive plate section represented a sample replicate. Figure 4.4 shows a *Salmonella-Shigella* agar plate with positive *Salmonella* growth. Figure 4.5 shows an example of a *Salmonella*-positive Enterotube.

4.3.4 Statistical Analysis

Statistical analysis of the complete block design was performed using JMP version 10 software (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was used to compare sample means between pasteurization temperatures and treatment times. The Tukey-Kramer Honestly Significant Difference (HSD) method for multiple means comparisons was used to detect significant differences in sample means between pasteurization temperatures and treatment times. Differences were declared significant at $p < 0.05$.

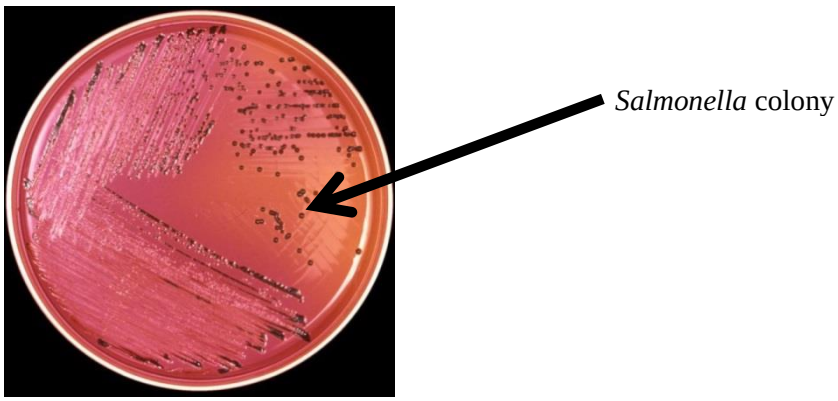

Figure 4.4. Positive *Salmonella* growth on *Salmonella-Shigella* agar (Todar, 2012, used under fair use, 2013)

Figure 4.5. Positive *Salmonella* growth on a BD™ BBL™ Enterotube II.

4.4 RESULTS & DISCUSSION

4.4.1 Manure Characteristics

The mean TS, TSS, pH, fecal coliform, *Salmonella*, and *E. coli* concentrations of ADSLM prior to pasteurization treatment are presented in table 4.2. Although ADSLM used in the study were collected on three separate times, there were no significant differences (ANOVA, $p<0.05$) in TS, TSS, pH, fecal coliforms, and *E. coli*, and *Salmonella* concentrations. The average TS content ranged from 3.78% to 3.87% by wet weight (wet w/w) and the average pH of ADSLM ranged from 8.01 to 8.05. It was therefore assumed that the effects of initial TS and pH contents of the ADSLM were insignificant, and any differences in pathogen concentrations were a result of pasteurization temperature and treatment duration.

4.4.2 Pasteurization Process

The temperature profiles for the ADSLM during pasteurization are shown in figure 4.6, 4.7, and 4.8 for the 70°C, 75°C, and 80°C treatments, respectively. On average, the ADSLM temperature attained the desired pasteurization temperatures of 70°C, 75°C, and 80°C within 35, 40, and 45 min of heating.

4.4.3 Inactivation of Fecal Coliform Bacteria

4.4.3.1 *Effects of Pasteurization Trials on Fecal Coliforms*

The ADSLM fecal coliform concentrations are shown as a function of pasteurization temperature and treatment duration in figure 4.9. At 70°C, the average ADSLM fecal coliform concentrations were reduced by 85% to 96% (i.e. 0.83 $\log_{10}$ and 1.39 $\log_{10}$ reductions) compared to the control within 120 min of reaching 70°C. Pasteurization at 75°C reduced fecal coliform concentrations by 91% to 96% of original concentrations (equivalent to 1.04 $\log_{10}$ and 1.39 $\log_{10}$ reductions, respectively). Fecal coliform concentrations were decreased by 92% to 95% (equivalent to 1.07 $\log_{10}$ and 1.31 $\log_{10}$ reductions, respectively) during pasteurization at 80°C. All temperature treatments reduced the fecal coliform concentration of manure to below the OMRI standard of 1,000 MPN·g^{-1} of processed manure.

Table 4.2. Characteristics of the ADSLM used for each pasteurization temperature trial

Parameter	Temperature (°C)		
	70	**75**	**80**
TS (%)	3.86 (±0.02) [a]	3.87 (±0.15) [a]	3.78 (±0.10) [a]
TSS (%)	2.73 (±0.12) [a]	2.81 (±0.15) [a]	2.79 (±0.15) [a]
pH	8.01 (±0.33) [a]	8.05 (±0.15) [a]	8.01 (±0.10) [a]
Fecal coliforms (MPN g^{-1})	9,306 (±2,100) [a]	8,586 (±4,164) [a]	8,252 (±3,685) [a]
E.coli (MPN g^{-1})	5,744 (±2,809) [a]	6,342 (±2,795) [a]	5,784 (±2,258) [a]
Salmonella (MPN g^{-1})	202 (±126) [a]	151 (±130) [a]	138 (±144) [a]

* Comparisons are across pasteurization temperatures. Means in the same row with same superscripts are not significantly different at $p < 0.05$

TS –Total solids (% of wet weight)

TSS – Total suspended solids (% of wet weight)

MPN g^{-1} – Most probable number per gram wet sample

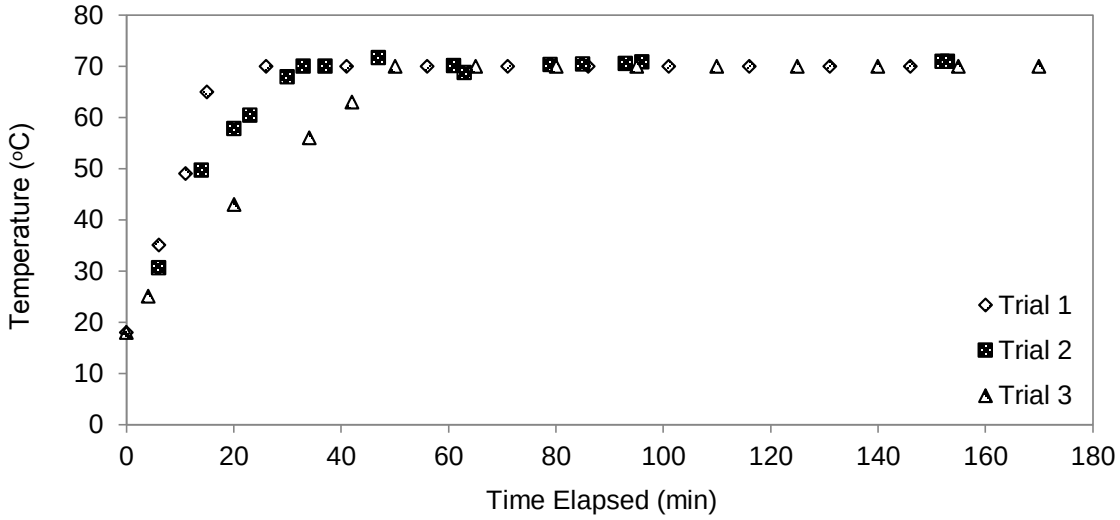

Figure 4.6. Temperature profiles for the three pasteurization trials conducted at 70°C. The temperature of manure reached 70°C within an average of 35 min following initial heating of the Velp tubes.

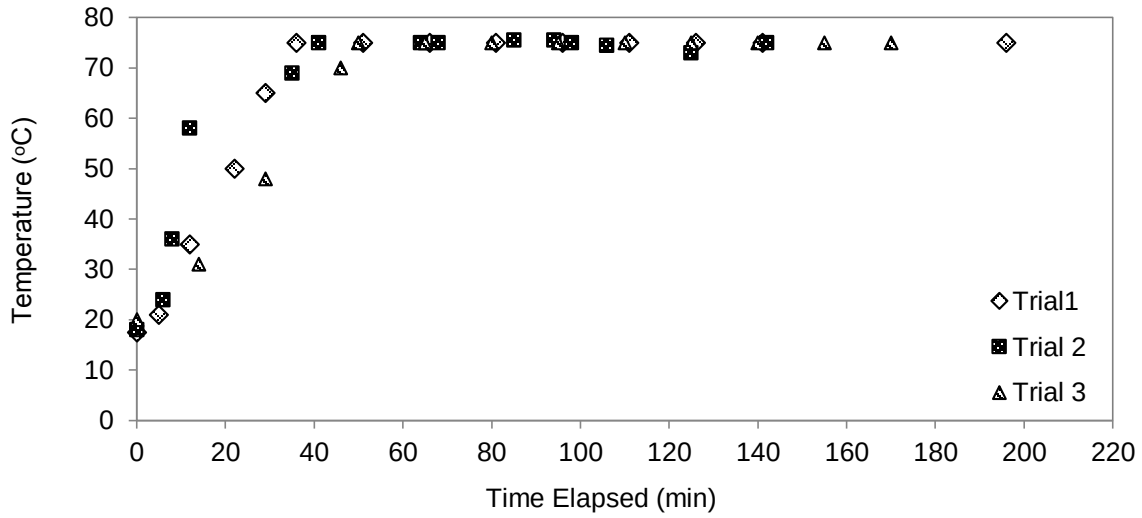

Figure 4.7. Temperature profiles for the three pasteurization trials conducted at 75°C. The temperature of manure reached 75°C within an average of 40 min following initial heating of the Velp tubes.

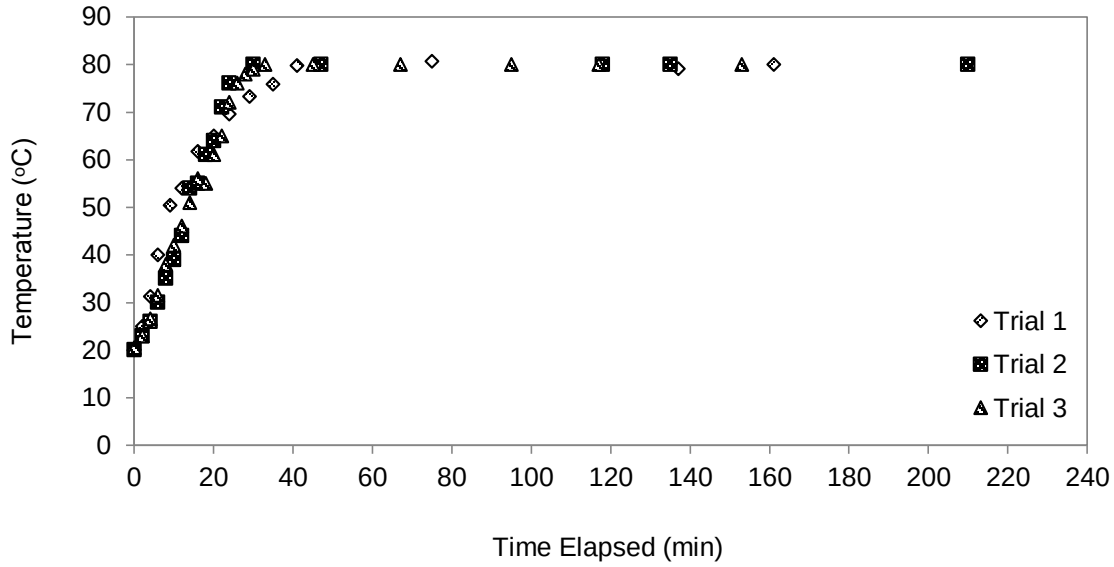

Figure 4.8. Temperature profiles for the three pasteurization trials conducted at 80°C. The temperature of manure reached 80°C within an average of 45 min following initial heating of the Velp tubes.

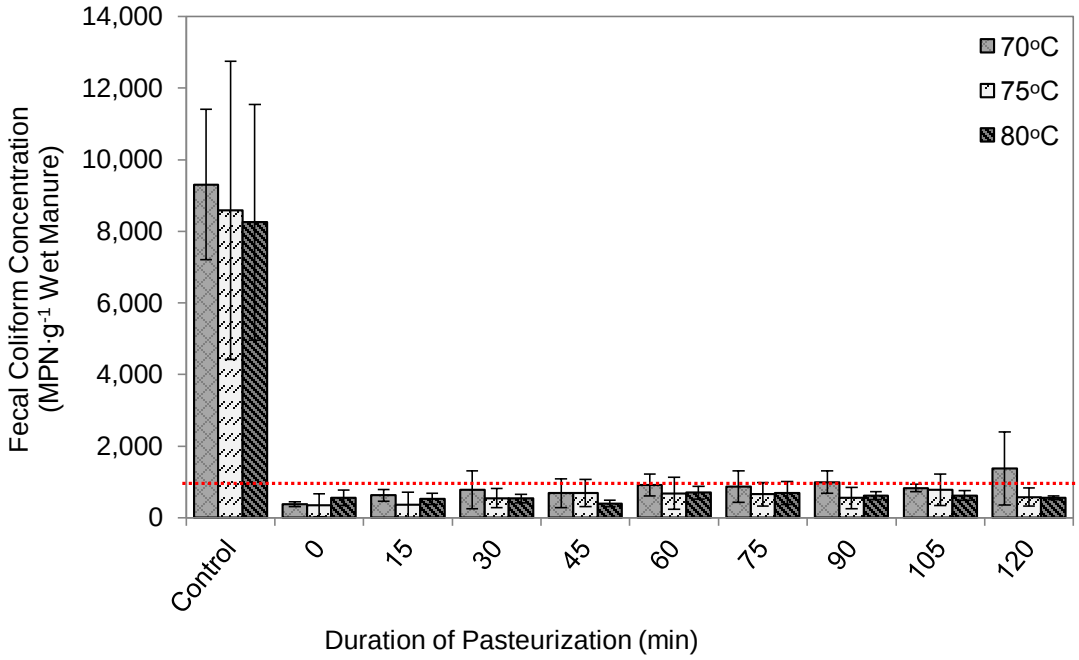

Figure 4.9. Fecal coliform concentrations during pasteurization at 70°C, 75°C, and 80°C. ▪▪▪ OMRI standard for fecal coliforms in processed manure (1,000 MPN·g^{-1}).

A comparison between the control and ADSLM pasteurized at 70°C, 75°C, and 80°C showed that the fecal coliforms in the control was significantly different from fecal coliform concentrations at all nine treatment durations (p-value <0.0001). However, there were no significant differences in fecal coliform concentrations between pasteurization temperatures. This finding suggests that increasing the temperature of an on-farm pasteurizer from 70°C to 80°C would not result in significantly enhanced treatment of fecal coliform bacteria. Comparison of fecal coliform concentrations between treatment times showed that, beyond the time at which the target pasteurization temperature was reached (T0), treatment time did not significantly affect fecal coliform concentrations (ANOVA, $p<0.05$). Comparison of the pasteurization temperatures and treatment durations yielding the greatest reductions in fecal coliform concentrations showed no significant difference between treatments (ANOVA, $p<0.05$). These findings suggest that once manure reaches the target temperature, it is unnecessary to hold manure at that pasteurization temperature for an extended duration of time.

The results from the 70°C, 75°C, and 80°C trials showed that 85% to 99% of fecal coliforms were inactivated by the time manure had reached the target treatment temperature. It was hypothesized that most fecal coliforms were inactivated as a result of raising the ADSLM temperature from 20°C to between 70°C and 80°C. This hypothesis was tested as described in Section 5.3.2.2 and results from these tests are presented in Section 5.4.3.2.

4.4.3.2 *The Effects of Initial Temperature Increase on Fecal Coliforms*

The fecal coliform concentrations were significantly reduced as the ADSLM temperature rose from 20°C to pasteurization temperatures. However, although fecal coliform concentrations decreased as ADSLM temperatures increased, differences between samples of varying temperatures and heating durations were found to be insignificant (ANOVA, $p<0.05$). Averaged concentration data from all the treatment temperatures (70°C, 75°C, and 80°C) are shown in figure 4.10. The results show a drastic reduction in fecal coliform concentrations when the ADSLM temperature reached 48°C, which occurred 6 min after the onset of heating. Average fecal coliform concentrations decreased between 81% and 99% (corresponding to $\log_{10}$ 3.93 and $\log_{10}$ 4.02 reductions, respectively) during the 26 min when manure was heated from 20^0C to 70^0C. Fecal coliform concentrations decreased between 93% and 100% (corresponding to $\log_{10}$ 3.99 and $\log_{10}$ 4.02 reductions, respectively) during the 30 min when the ADSLM was raised to 75^0C. Fecal coliform concentrations decreased between 90% and 100% (corresponding to $\log_{10}$ 3.98 and $\log_{10}$ 4.02 reductions, respectively) during the 38 min when the ADSLM was heated to 80^0C. Statistical analysis showed that fecal coliform concentrations in the control

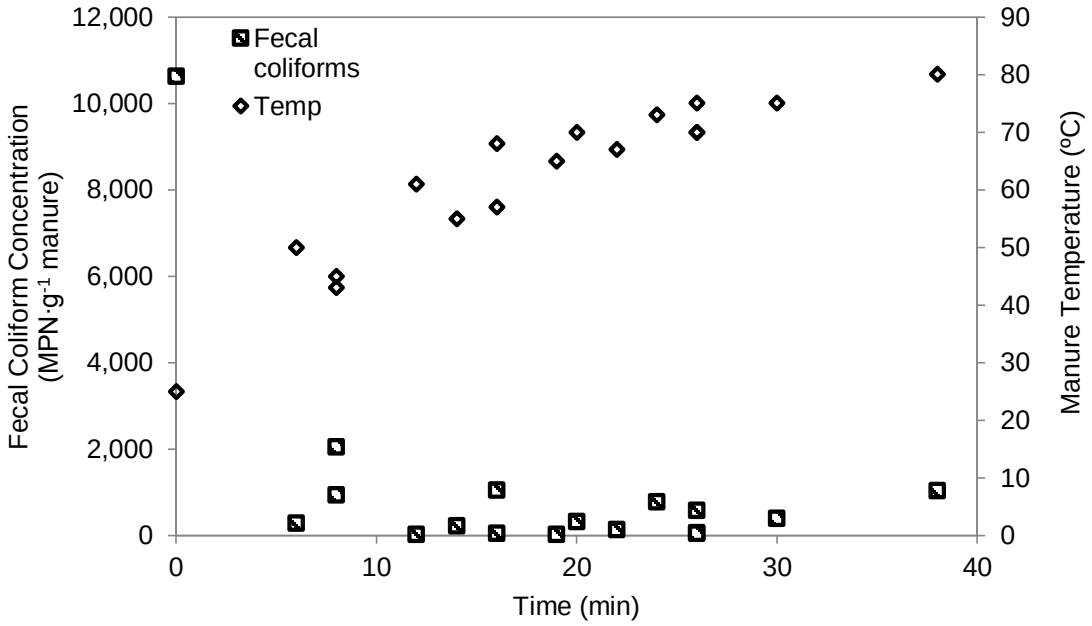

Figure 4.10. Fecal coliform concentrations and corresponding temperatures as ADSLM was heated from 20°C to the desired pasteurization temperatures of 70°C, 75°C, or 80°C.

were significantly different from fecal coliform concentrations in ADSLM that underwent any heat treatment (ANOVA, $p<0.0001$).

4.4.4 Inactivation of *E. coli*

4.4.4.1 *Effects of Pasteurization Temperatures on* E.coli

The effects of pasteurization temperature and treatment duration affected *E. coli* concentrations in ADSLM are presented in figure 4.11. Pasteurizing separated liquid manure at 70°C reduced *E.coli* concentrations by 87% to 96% (equivalent to 0.9 $\log_{10}$ and 1.38 $\log_{10}$ reductions, respectively) compared to the control. At 75°C further decreases in E. coli concentrations of 91% and 96% (equivalent to 1.04 $\log_{10}$ and 1.39 $\log_{10}$ reductions, respectively) were observed compared to the control. Pasteurization of separated liquid at 80°C decreased *E. coli* concentrations by 90% to 93% (equivalent to 1.07 $\log_{10}$ and 1.31 $\log_{10}$ reductions, respectively) compared to the control. Although there are no explicit OMRI standards for *E. coli* standards in processed manure, it is desirable to minimize *E. coli* concentrations to minimize the human health risks associated with pathogenic *E. coli* strains; in recent years, environmental legislation, especially with respect to surface waters, have increasingly focus on E. coli as more indicative of human health risk than the broader fecal coliform group (US EPA, 2003). Pasteurization at all temperatures reduced *E. coli* concentrations to fewer than 750 $MPN \cdot g^{-1}$ processed manure. The *E. coli* concentrations in the control were statistically different from each time and temperature combination at all pasteurization temperatures (p-value <0.0001). However, comparison of *E. coli* concentrations of varying pasteurization temperatures showed no significant differences (p-value <0.05). These findings suggest that increasing treatment temperature from 70°C to 80°C does not improve the reduction of *E. coli* bacteria in ADSLM.

Heat treatment has been reported to cause heat-shock and stress in bacteria like *E.coli* (Ron et al., 2000; Shapiro and Cowen, 2012). When temperature changes occur faster than a bacterium's regulatory system can respond, it dies or becomes irreversibly damaged (Madigan et al., 2012b). Lang and Smith (2008) observed that pasteurization of sludge supernatant and tryptone soya broth (TSB) inactivated *E. coli* strain NCTC 9001 in less than 10 s (Lang and Smith, 2008). This particularly short inactivation time is likely because Lang and Smith used batch sizes of 0.1 mL, which increased the surface-area to volume ratio compared to larger batch sizes, like the 60-mL batches used in this study. It is presumed that having an increased surface-area to volume ratio minimizes temperature differentials between the heat source and the center of the matrix, thereby reducing the amount of time needed to inactivate pathogens in an entire

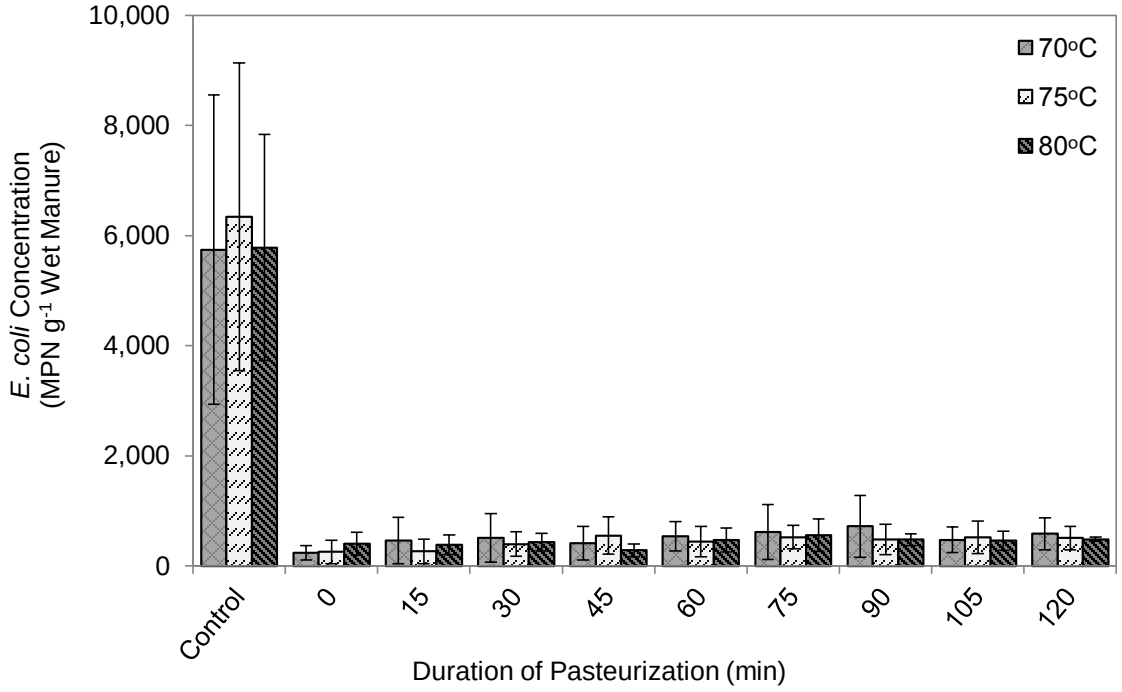

Figure 4.11. *E. coli* concentrations during pasteurization at 70°C, 75°C, and 80°C.

sample volume. In recognition of the fact that on-farm pasteurizers need to treat large volumes of manure at a time, the volume used in this study is expected to reflect pathogen inactivation expected from on-farm batch pasteurizers. The present study also used ADSLM, which has a higher TS content than the sludge supernatant and TSB pasteurized by Lang and Smith (2008). It is believed that manure solids protect microorganisms from the effects of heat treatment; therefore manures with elevated TS and TSS content are likely to require increased treatment durations to achieve sufficient pathogen inactivation (Acquisto et al., 2006; US EPA, 1999a, b).

4.4.4.2 *The Effects of Initial Temperature Increase on* E.coli

Heating manure from 20°C to 70°C, 75°C, or 80°C resulted in significant reductions in *E. coli* concentrations. Figure 4.12 illustrates that *E. coli* concentrations, like fecal coliform concentrations, significantly decreased after 6 min of heating the ADSLM, when the ADSLM temperature reached 48°C. *E. coli* concentrations decreased by 88% to 100 % (corresponding to $\log_{10}$ 3.80 and $\log_{10}$ 3.85 reductions, respectively) during the 26 min when ADSLM was heated to 70°C. *E. coli* concentrations decreased by 93% to 100% (corresponding to $\log_{10}$ 3.82 and $\log_{10}$ 4.85 reductions, respectively) during the 30 min it took for the temperature of manure to rise to 75°C. *E. coli* concentrations decreased by 89% to 100% (corresponding to $\log_{10}$ 3.80 and $\log_{10}$ 3.85 reductions, respectively) as manure was heated to 80°C. Statistical analysis showed that *E. coli* concentrations were significantly different between the sample control and heat-treated ADSLM samples (ANOVA, $p<0.0001$). Although *E. coli* concentrations generally decreased as ADSLM temperatures increased, differences between samples at different pasteurization temperatures and treatment durations were not significant (ANOVA, $p<0.05$).

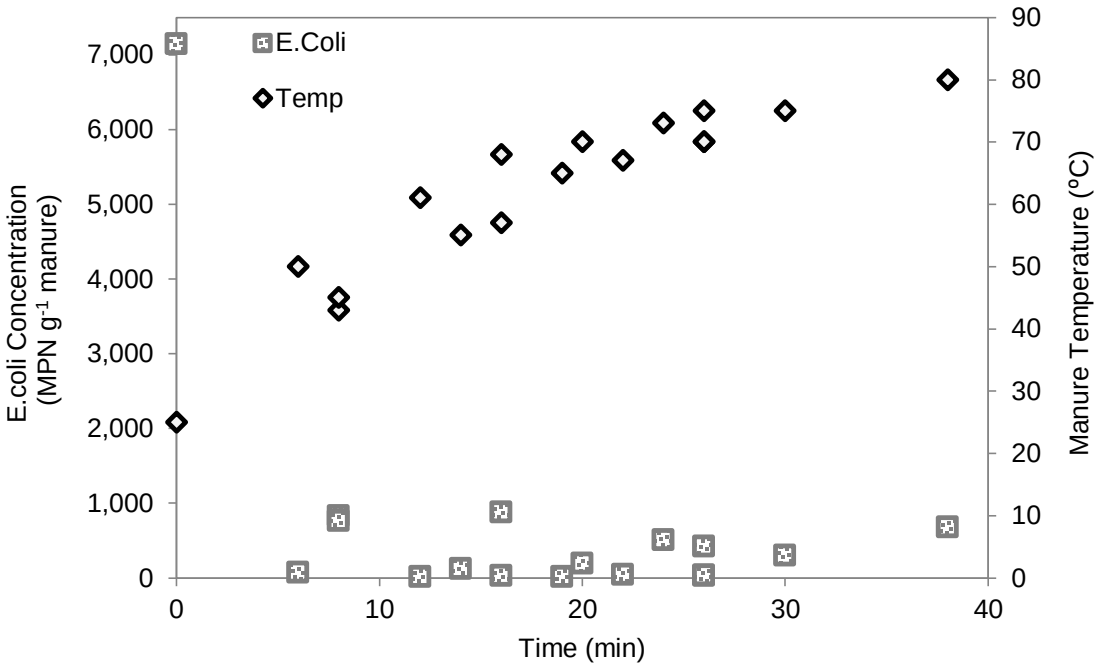

Figure 4.12. *E. coli* concentrations and corresponding temperatures as ADSLM was heated from 20°C to 70°C, 75°C, or 80°C.

4.4.5 Inactivation of *Salmonella*

4.4.5.1 *Effects of Pasteurization Temperature*

Salmonella concentrations during pasteurization at 70°C, 75°C, and 80°C are presented in figure 4.13. Pasteurization of ADSLM at 70°C reduced *Salmonella* concentrations by 100% (equivalent to a ~1.99 $\log_{10}$ reduction) for all treatment durations. At 75°C and 80°C the *Salmonella* concentrations were reduced by 100% (equivalent to a 1.87 $\log_{10}$ and 1.48 $\log_{10}$ reductions, respectively) compared to the control for all treatment durations**.** The *Salmonella* concentrations in the control were significantly different from treated ADSLM for all three pasteurization temperatures (p-value <0.0001). These results suggest that pasteurizing manure at 70°C, 75°C, and 80°C for any duration of time will reduce *Salmonella* in separated liquid dairy manure to required levels, perhaps removing it completely. This finding is supports the finding of Lang and Smith (2008) who observed that pasteurization of sludge supernatant and tryptone soya broth at 70°C completely inactivated *Salmonella typhimurium* (strain NCTC 74) in less than 10 seconds and inactivated heat-resistant *Salmonella senftenberg* 775W (strain NCTC 9959) within 40 seconds (Lang and Smith, 2008). As previously discussed, the inactivation rates observed in the small (0.1 mL) batches and low-TS content media tested by Lang and Smith (2008) yielded results that may not represent the inactivation kinetics achieved by on-farm pasteurizer units.

4.4.5.2 *The Effects of Initial Temperature Increase*

Salmonella concentrations decreased as ADSLM was heated from 20°C to 70°C, 75°C, or 8°C. As shown in figure 4.14, *Salmonella* concentrations decreased by 78% following 6 min of heat treatment, and *Salmonella* concentrations decreased further when the ADSLM temperature reached at least 65°C, after 19 min of heat application. *Salmonella* concentrations decreased by 39% to 100% (equivalent to 1.55 and 1.95 $\log_{10}$ reductions) during the 28 min when ADSLM was heated from 20°C to 70°C. *Salmonella* concentrations decreased by 78% and 100% (equivalent to 1.84 $\log_{10}$ and 1.95 $\log_{10}$ reductions, respectively) compared to the control during the 35 min it took for the temperature of ADSLM to rise to 75°C. *Salmonella* concentration were reduced by 79% to 100% (equivalent to 1.85 $\log_{10}$ and 1.95 $\log_{10}$ reductions, respectively) compared to the control as ADSLM was heated to 8°C. As shown in figure 4.14, the ADSLM did not reach OMRI standard of fewer than 3 MPN·$4g^{-1}$ processed manure until ADSLM had been heated for at least 12 min. However, at 14 min (56°C), *Salmonella* concentrations met the OMRI standard. Only after 19 min (67°C) were *Salmonella* concentrations consistently fewer than 3 MPN·$4g^{-1}$of processed manure. Statistical analysis revealed that differences between *Salmonella* concentrations in the control and heated ADSLM were not significantly different from each other (ANOVA, $p<0.05$).

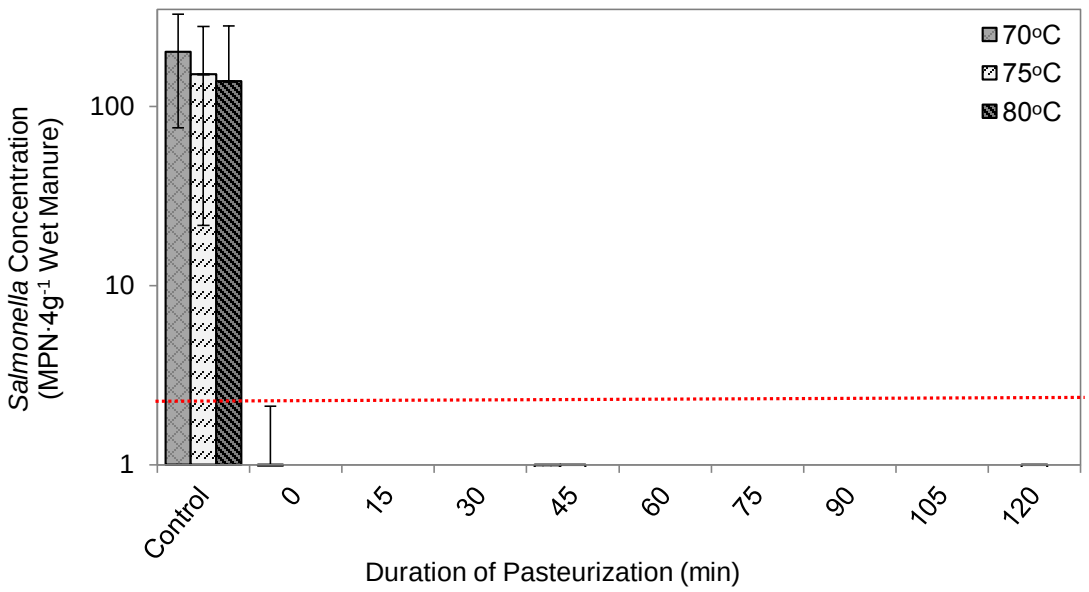

Figure 4.13. Fecal coliform concentrations during pasteurization at 70°C, 75°C, and 80°C. ▪▪▪▪ OMRI standard for *Salmonella* in processed manure (3 MPN *Salmonella* per 4g).

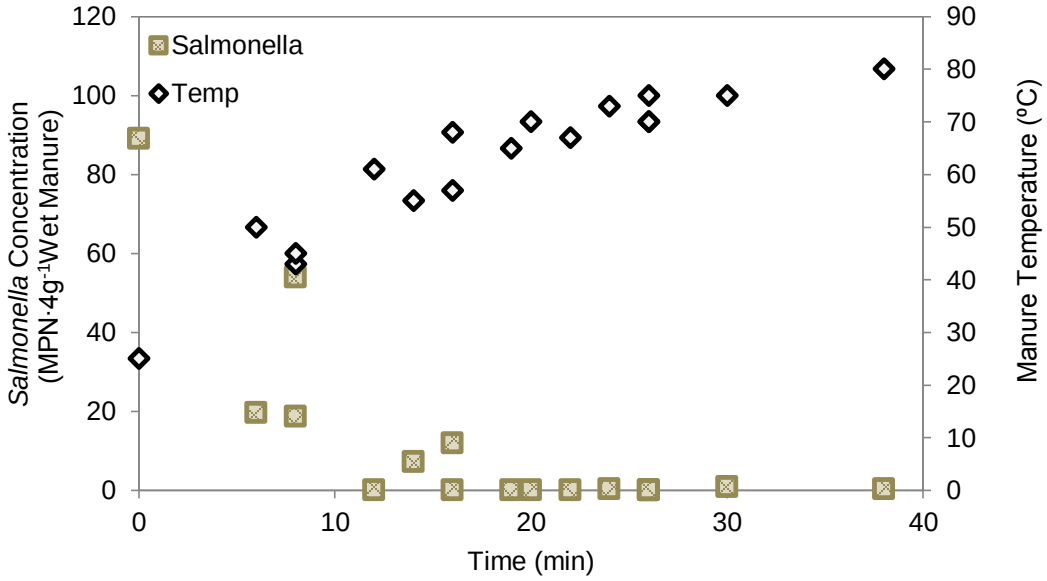

Figure 4.14. Salmonella concentrations and corresponding temperatures as ADSLM was heated from 20°C to 70°C, 75°C, or 80°C.

4.4.6 Effects of Pasteurization on Fertilizer Quality

Analysis of nutrient data in the control and pasteurized ADSLM showed that the Total-N (as total Kjeldahl nitrogen) and the TAN-to-Total-N ratios remained relatively unchanged following pasteurization at 70°C, 75°C, and 80°C (figures 4.15 and 4.16). Since samples were composited, statistical data was not available to indicate the significance of any differences in nutrient data.

4.5 CONCLUSIONS

Pasteurizing anaerobically digested separated liquid manure (ADSLM) at 70°C reduced fecal coliform concentrations by between 85% and 96% (0.83 $\log_{10}$ and 1.39 $\log_{10}$ reductions, respectively). Maximum inactivation of fecal coliforms occurred following only 25 min of heat treatment, while manure temperatures rose from 20°C to the target temperature of 70°C. Pasteurization of ADSLM at 75°C and 80°C yielded similar results in fecal coliform inactivation. *E. coli* and *Salmonella* concentrations were reduced to fewer than 750 MPN·g^{-1} and 1 MPN·$4g^{-1}$ manure, respectively, at all pasteurization temperatures and treatment durations. These findings suggest that heating anaerobically digested separated liquid manure to 70°C and holding it for a brief duration of time at 70°C reduces fecal coliform and *Salmonella* concentrations to the extent that ADSLM can be used as fertilizer in fields used for human food production, according to the OMRI standards for processed manure (OMRI, 1997). Heating manure to 70°C for a brief duration of time has the additional benefit of minimizing the external energy required to heat and hold manure at high temperatures.

Despite the observation that sufficient decreases fecal coliforms and *Salmonella* can be achieved with minimal holding duration at 70°C, it is of interest of human and environmental health to treat manure at 70°C for a duration of time to incorporate a margin of error due to the dangers of food contamination by manure pathogens. It is therefore recommended that anaerobically digested separated liquid manure be pasteurized at 70°C for at least 60 min prior to use as fertilizer for human food production.

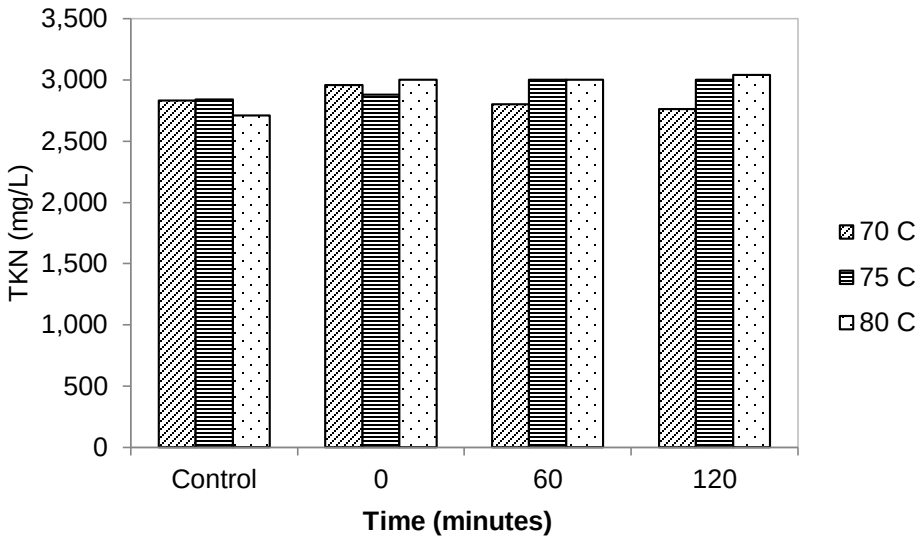

Figure 4.15. Total Kjeldahl nitrogen concentrations as a function of pasteurization temperature and duration.

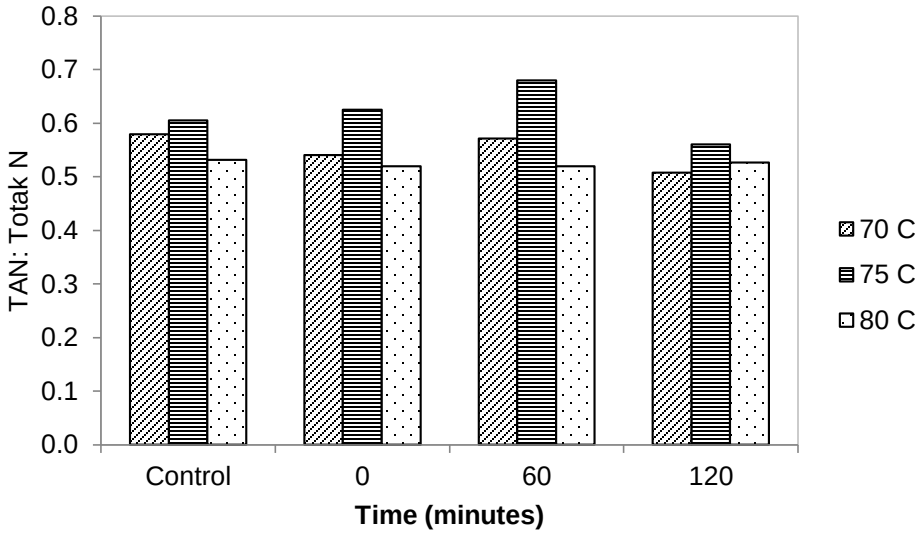

Figure 4.16. Ratios of TAN-to-Total Nitrogen (as TKN) as a function of pasteurization temperature and duration.

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5 Environmental and Economic Analysis of an On-farm Anaerobic Digester and Value-added Products

5.1 ABSTRACT

All anthropogenic activities, including agricultural production, have economic and environmental impacts and/or consequences; however, these can be difficult to assess for complex agricultural production systems, such as dairy farms. To better understand the consequences of operating an on-farm anaerobic digester (AD) and utilizing its value-added products, the environmental and economic activities associated with the AD were quantified. A linear model was used to determine to the payback period for the initial AD investment based on varying sale prices for electricity, separated liquid fertilizer and carbon credits produced by the on-farm AD. A partial environmental analysis was conducted on the AD by calculating the carbon footprint of operating the AD and utilizing or selling its value-added products. The results indicated that the payback period for the initial AD investment ranged from 4.6 to 11.8 years. Converting the manure management system at from a storage lagoon to an anaerobic digester resulted in an annual methane emissions savings estimated at 2,436 tonnes of CO_2e per cow per year. Electricity generation from biogas replaced the need to combust fossil-fuel energy sources that would have emitted between 1,460 and 2,609 tonnes of CO_2e per year. Analysis of the carbon footprint of transporting pasteurized anaerobically digested manure to an organic horticulture farm indicated an annual footprint of 15.15 kg CO_2e. These findings suggest that installing an AD on dairy farms has net positive impacts on the environmental and economic sustainability of dairy farming. Overall, this study has enhanced the understanding of the environmental and economic impacts of integrating an on-farm AD into dairy production operations.

5.2 INTRODUCTION

All anthropogenic activities, including agricultural production, have economic and environmental impacts and/or consequences. For example, producing sweet corn at a farm in Oklahoma for consumers in New York involves several economic activities. Farm level activities include purchase of seeds, fertilizer, and herbicides/pesticides; labor; planting/harvesting equipment; and petroleum to run equipment. The corn gets to the consumer through distribution networks that involve capital exchanges for freight trucks, drivers, loaders/unloaders, and petroleum gas. What are often disregarded, however, are the environmental impacts of such systems, like the consumption of energy to produce fertilizers for food production, the use of fresh water resources for irrigation, and the consumption of non-renewable and carbon-emitting fossil fuels for harvesting and transporting agricultural goods to consumers. The intricacy and interconnectivity of food production systems with water and energy systems make it difficult to elucidate the environmental and economic impacts of any single product or activity. Figure 5.1 illustrates activities in the dairy production chain that exemplify the nexus of food, water, and energy systems.

It is difficult to valorize services performed by natural systems such as wetlands or the atmosphere. However, some methods have been developed that can convert the environmental benefits of any activity into economic values. The three most common methods are (i) calculating the replacement value of services (e.g. if you fill a wetland, how much will it cost to install structures that perform the same services); (ii) calculating the remediation value for a system (e.g. the cost of utilizing carbon sequestration when the atmosphere has too high a concentration of CO_2); and (iii) estimating a market value for the service (e.g. selling credits or permits for the use of an environmental service) (Alexander, 2005; Bayon, 2009; Chen et al., 2009; Zander and Garnett, 2011).

The technique of developing market values for environmental services has been used by the U.S. and European governments to respond to concerns over global climate change and acid rain (Bayon, 2009; U.S. EPA, 2012). As an example, emissions trading markets, also referred to as cap and trade systems, have helped limit air quality degradation from atmospheric emissions of compounds like sulfur dioxide (SO_x), nitrogen oxides (NO_x), and greenhouse gases (GHGs) (Bayon, 2009; U.S. EPA, 2012). Concerns over rising GHG concentrations have led to the establishment of markets like the Chicago Climate Exchange. The premise of a carbon exchange market is that a governmental agency like the U.S. EPA caps the quantity of GHG, which are measured in carbon dioxide equivalents (CO_2e), that can be emitted by individual firms or industries (Bayon, 2009; IPCC, 2006; U.S. EPA, 2012). Firms that exceed their allocated carbon emission limits must purchase carbon credits from firms with unused credits. Renewable energy

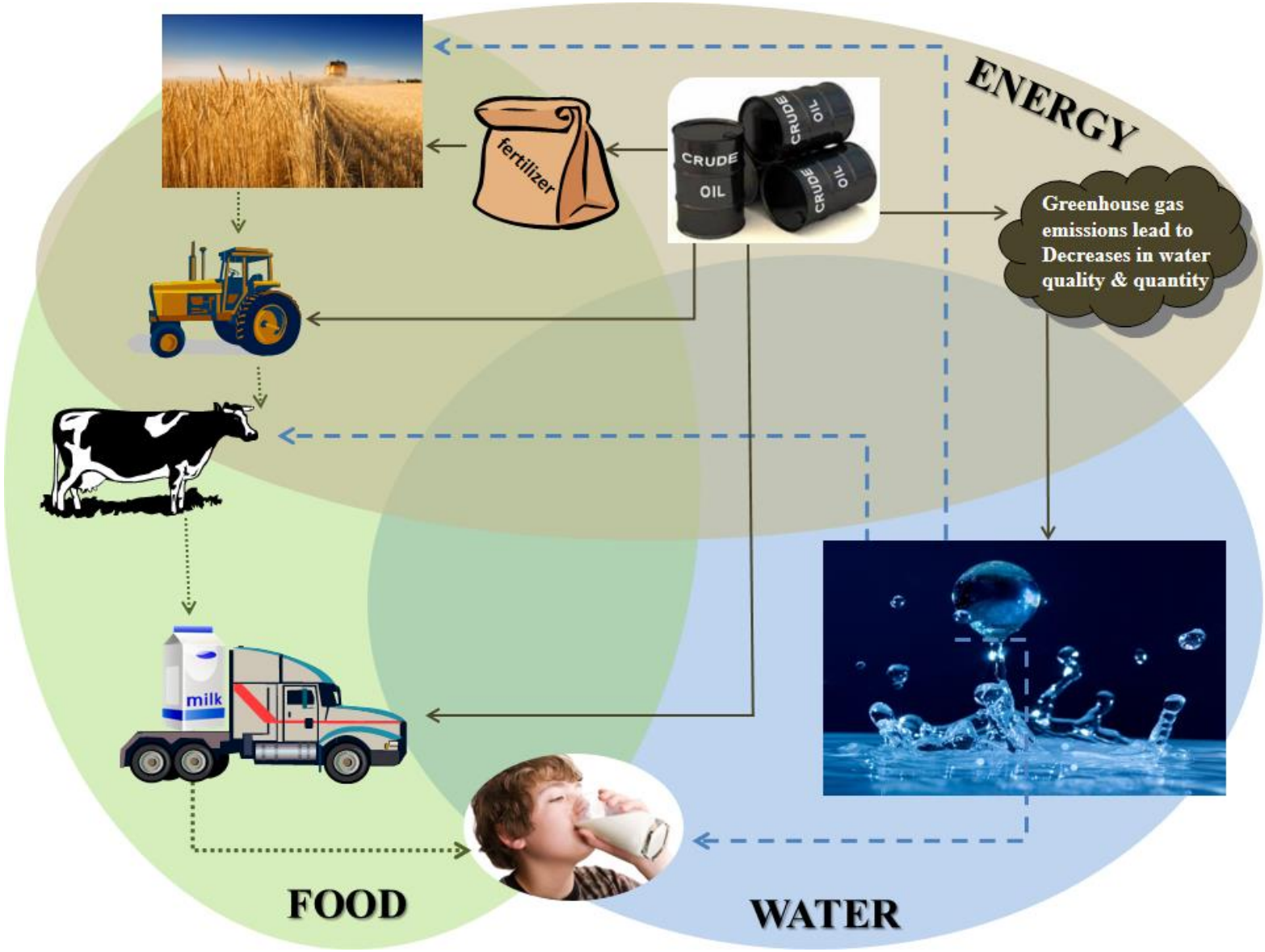

Figure 5.1. Activities in the dairy production chain that demonstrate the nexus of food systems, water, and energy systems.

producers can also sell carbon credits if certified by a carbon credit aggregator. The installation of anaerobic digesters on dairy farms, for example, enables carbon credits that can be sold on carbon exchange markets. In contrast to the perception that carbon credits from anaerobic digesters stem from the amount of renewable energy produced, carbon credits are actually based on the replacement of methane-emitting manure management systems, referred to as "baseline" scenarios, with systems that capture and destroy methane (U.S. EPA, 1999). Processing manure in anaerobic digesters provides the opportunity to capture the methane that would otherwise be emitted into the atmosphere compared to using open manure storages or lagoons. The CH_4 captured by anaerobic digesters is subsequently used to generate electricity, produce hot water or steam, or flared. The result of combustion of CH_4 is the emission of CO_2 (a less potent GHG). Carbon credits are typically calculated based on the number and type of livestock head on a farm, the type of manure storage system used prior to the installation of an anaerobic digester, the material properties of the manure, and the farm's regional climate conditions (IPCC, 2006; U.S. EPA, 1999, 2012).

5.2.1 Environmental and Economic Sustainability

Quantifying the value of ecosystem services provided by natural and engineered biological systems has been recognized as a step towards sustainable agricultural food production. However, the definition of environmental sustainability has long confounded governmental agencies, scientists, and consumers, resulting several, and often very different, definitions (Kreiser, 2012; Smith, 1997; U.S. EPA). The following criteria were used in evaluating the environmental and economic sustainability of dairy farming activities:

1. Minimal or reduced environmental degradation from local farm operations compared to conventional farming techniques.
2. Renewable energy use on the farm and its operations.
3. Minimal or reduced inputs to farm operations and waste products from farm operations compared to conventional dairy farming techniques.
4. The farm is financially solvent with regards to manure management practices.

5.2.2 Evaluating the Environmental-economic Sustainability Using the Dairy Energy LLC Anaerobic Digester as an Example

A conventional dairy farming system involves a myriad of activities, including but not limited to, the following: purchasing and raising cows; cow reproduction; producing dairy feed; purchasing and maintaining equipment; feeding, bedding, and cleaning calves, heifers, and lactating cows; milking; testing milk; treating and managing cow ailments; maintaining farm structures and equipment, and

managing finances. Farms with anaerobic digesters have the added activities of system operation and maintenance. With such a complex system and the confounding uncertainties associated with livestock production, it is difficult to assess the how economics of dairy farming. Nonetheless, careful management and documentation has led to the development of average industry estimates of financial costs of various facets of dairy production, usually expressed as a per-activity basis (e.g. cost of cow care) or a systemic basis (e.g. total cost to produce a liter of milk) (Capper and Cady, 2012; Capper et al., 2009; CEAS Consultants (Wye) Ltd, 2007; USDA NASS, 2010). Although the environmental costs of dairy farming is difficult to assess, a number of recent studies have documented and compared the environmental impacts of dairy production (Belflower et al., 2012; Capper and Cady, 2012; Capper et al., 2009; CEAS Consultants (Wye) Ltd, 2007; de Boer, 2003; Osei et al., 2000; Thomassen and de Boer, 2005).

The information gathered to evaluate the environmental and economic impacts of the anaerobic digester included the potential for sale or on-farm use of several value-added products from the anaerobic digester, digester operation and maintenance. The economic benefits considered were:

1) Sale of electricity from the conversion of biogas to the electrical grid;
2) Sale of pasteurized anaerobically digested separated liquid (ADSLM) fertilizer to an organic horticulture farm in Virginia;
3) Cost savings through replacement of sawdust cow bedding with anaerobically digested separated solids;
4) Cost savings from the 50% reduction in mastitis incidence following the replacement of purchased sawdust bedding with anaerobically digested separated solids bedding from the anaerobic digester; and
5) Sale of renewable energy and carbon credits from installation of the anaerobic digester on a carbon exchange market.

The environmental impacts related to the five economic items listed above were:

1) Reduction in greenhouse gas emissions from the dairy farm resulting from installation of the anaerobic digester
2) Reduction in greenhouse gas emissions resulting from replacement of fossil fuels with renewable biomethane consumption
3) The carbon footprint of pasteurizing and transporting pasteurized ADSLM to a Virginia organic horticulture farm.

The objective of this study was to evaluate the environmental and economic implications of operating an on-farm anaerobic digester and utilizing its value-added products. A partial environmental-economic analysis was used to test the hypothesis that integration of anaerobic digestion and utilization of its value-added products into a dairy production system improves the environmental and economic sustainability of dairy operations.

5.3 METHODS & MATERIALS

5.3.1 Economic Sustainability of Anaerobic Digestion and its Value-added Products

Each economic activities associated with the anaerobic digester were quantified to better understand the consequences of operating an anaerobic digester and utilizing its value-added products on a Virginia dairy farm using a linear model. The model was used to determine to the payback time on the initial investment for the anaerobic digester (AD). The cost and other financial estimates including installation and start-up costs, annual operation and maintenance expenses, labor for the managing the value-added products, and extraneous annual expenses (e.g. increase in annual property taxes due to installation of AD, grid connection fees from the utility, and certification carbon credits) were obtained from Dairy Energy LLC. The system boundaries for the economic analysis performed in this study is illustrated in figure 5.2.

The quantities of biogas and electricity produced were monitored over a two year period (April 1, 2011 to April 1, 2013). The economic benefits of operating the AD and using or selling the derived value-added products were modeled using the price points presented in table 5.1, which were developed based on conversations with Dairy Energy (K. VanderHyde, Dairy Energy LLC, personal communication, 10 November 2012 and 25 January 2013). Pricing scenarios were compared for different payback periods (4.6 to 11.8 years) based on initial investment (turnkey cost) of approximately $2 million with an annual return on investment (ROI) of 10%.

The turnkey cost covered the costs of property, equipment, property taxes, and startup expenses. The annual costs of operating the system were subtracted from the revenue generated from the sale or use of value-added anaerobic digestion products on the farm to predict the annual net income (ANI) from the

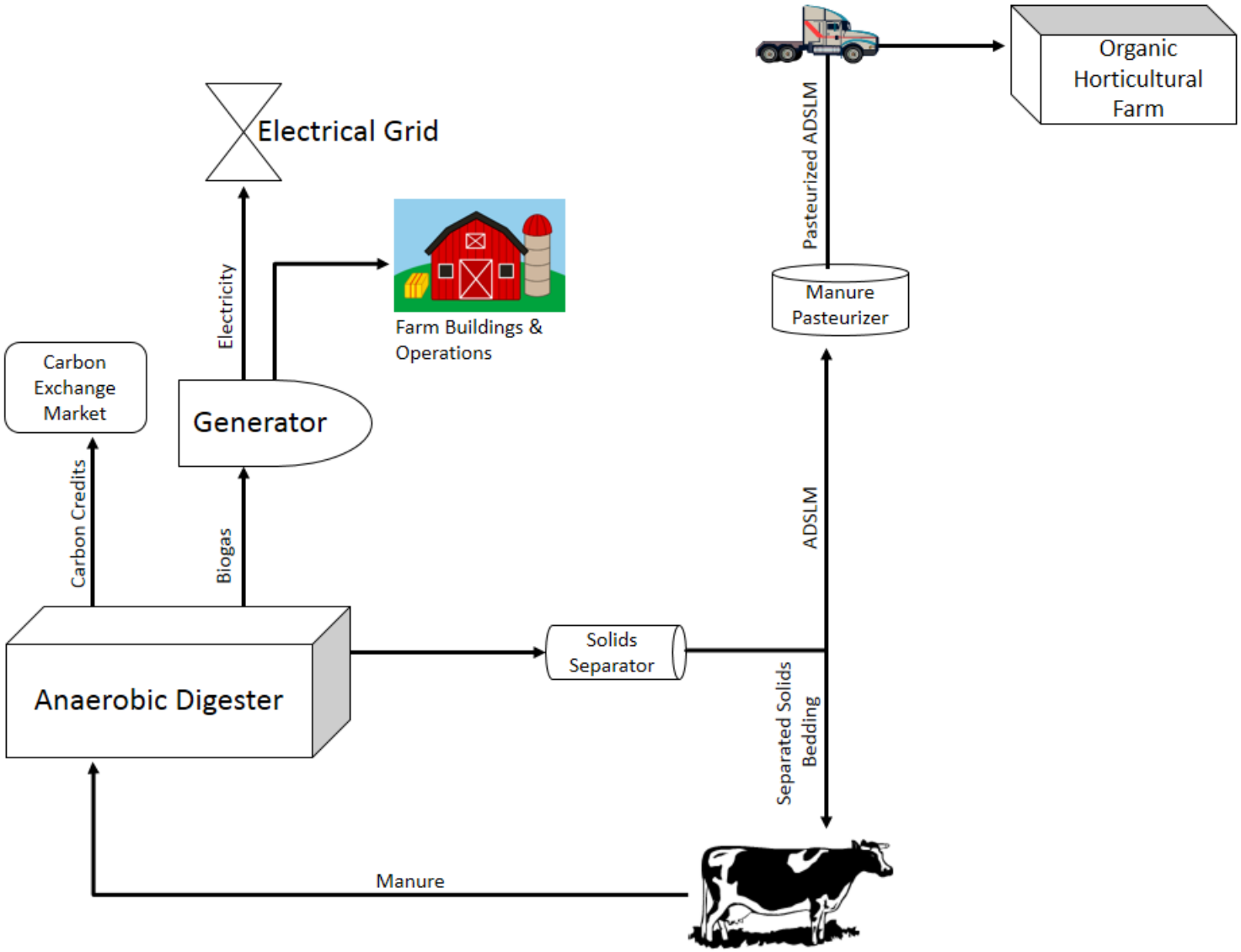

Figure 5.2. System boundaries for economic analysis.

Table 5.1. Economic analysis price points used in modeling.

Activity	Price Range
Electricity sold to the grid	$0.034 to $0.12 per kilowatt-hour (KWH)
Sale of anaerobically digested separated liquids (pasteurized) as an organic fertilizer	$0.11 to $0.26 per liter
Avoided cost when anaerobically digested separated solids is used as cow bedding to replace sawdust	fixed based on proprietary information from Dairy Energy, LLC
Cost savings from reduced mastitis incidence in the herd when separated solids is used bedding	fixed based on proprietary information from Dairy Energy, LLC
Renewable energy and carbon credits	$1 to $3 per credit

system. The annual costs included charges by the electric co-operative to maintain connection to the electrical grid, operation and maintenance of the anaerobic digester and associated value-added products, accreditation for the sale of carbon credits on the carbon market spread over five years, labor for the operation, maintenance and management of the digester and its value-added products, the energy consumed by the AD equipment, and the annual marginal increases in property taxes and insurance for the AD . The annual income from the anaerobic digester and its value added products the operation, maintenance and management of the digester and its value-added products, the energy consumed by the AD equipment, and the annual marginal increases in property taxes and insurance for the AD (ASERTTI, 2007). The annual income from the anaerobic digester and its value added products included the electricity produced, carbon credits sales, avoided cost of sawdust bedding, the avoided mastitis treatments savings, and off-farm sale of fertilizer. Equations 5.1, 5.2, and 5.3 were used to estimate the payback period, annual income (AI), and annual costs (AC) of operating the anaerobic digester and associated value-added products (Newnan et al., 2011).

$$\begin{aligned}\text{AI (\$)} = {} & (\text{Annual electricity production in KWH})(x) \\ & +(\text{Annual carbon credit production})(y) \\ & +(\text{Annual volume of pasteurized fertilizer sold})(z) \\ & +\text{AC savings}_{\text{mastitis treatment}} + \text{AC savings}_{\text{sawdust bedding}} \\ & +\text{AC savings}_{\text{fertilizers}}\end{aligned} \tag{5.1}$$

$$\begin{aligned}\text{AC (\$)} = {} & (\text{annual consumption of electricity by AD equipment})(x) \\ & +\text{AC}_{\text{carbon accredation,spread over 5 years}} + \text{AC}_{\text{Electrical grid charges}} \\ & +\text{AC}_{\text{labor expenses}} + \text{AC}_{\text{O\&M}} + \text{AC}_{\text{property taxes}} \\ & +\text{AC}_{\text{insurance}}\end{aligned} \tag{5.2}$$

$$\text{ANI (\$)} = \text{AI} - \text{AC} \tag{5.3}$$

$$\text{Payback period (years)} = \frac{\ln\left(1-\frac{(2{,}000{,}000)(0.1)}{(\text{ANI})}\right)^{-1}}{\ln(1+0.1)} \tag{5.4}$$

Where
x = price of electricity per KWH and assuming a generator uptime of 90% at 365 KWs.
y = price of a single carbon credit
z = price per L of pasteurized ADSLM sold to the Virginia organic horticulture farm.

The annual cost savings in cow health (e.g. mastitis treatment) and sawdust bedding were a result of replacing sawdust bedding with separated solids from the anaerobic digester. Using anaerobically digested separated solids for dairy bedding resulted in a 50% decrease in mastitis incidence of the milking cows compared to using sawdust (K. VanderHyde, Dairy Energy LLC, personal communication, 01 December 2012). The reduced mastitis occurrences were equivalent to about $15,000 savings per cow or per year. The value of the fertilizer in the manure after anaerobic digestion was equivalent to replacement of synthetic fertilizers required for corn production on an 8 ha field located within 2 km of retail and food businesses. Previously, only synthetic fertilizers could be used on this field to grow crops because of complaints about manure odors. The avoided synthetic fertilizer cost by using by using the digested manure was approximately $34,000 not counting the avoided litigation potential due the abatement of nuisance odors.

Not considered in the annual operating costs were (i) the cost of pasteurizing the dairy manure; and (ii) the value of the fertilizer removed from the farm for sale to the organic horticulture farm. Heat produced from the generator was used to heat the manure to pasteurization temperatures.

5.3.2 Environmental Sustainability of Anaerobic Digestion and its Value-added Products

The environmental sustainability of anaerobic digestion was evaluated using a partial analysis on the carbon footprint of activities associated with operating the AD and utilizing its value-added products. The environmental sustainability of activities were calculated and compared in terms of greenhouse gas savings as carbon dioxide equivalents (CO_2e). Figure 5.3 illustrates the system boundaries of the partial environmental analysis presented in this study.

The carbon footprint of the AD was calculated based on the quantity of greenhouse gases that would have been emitted from dairy manure in a storage pit had the AD not been installed according to equation 5.5. For localities in Southern Virginia, the methane emissions factor is 58 kg CH_4 per dairy cow per year for a dairy facility with a storage pit and an average annual temperature of 13.5°C (ASERTTI, 2007; IPCC, 2006).

$$CF_{AD}(CO_2e) = F_M \times CU \times F_{CO_2} \div 1000 \quad (5.5)$$

Where
F_M = methane emissions factor per dairy cow per year, in kg CH_4 per cow unit
CU = number of cow-units on the farm
F_{CO_2} = GHG equivalence factor for methane

The carbon footprint of utilizing the biogas from the anaerobic digester to produce electricity was calculated using the quantity of greenhouse gases that would have been emitted had that same energy

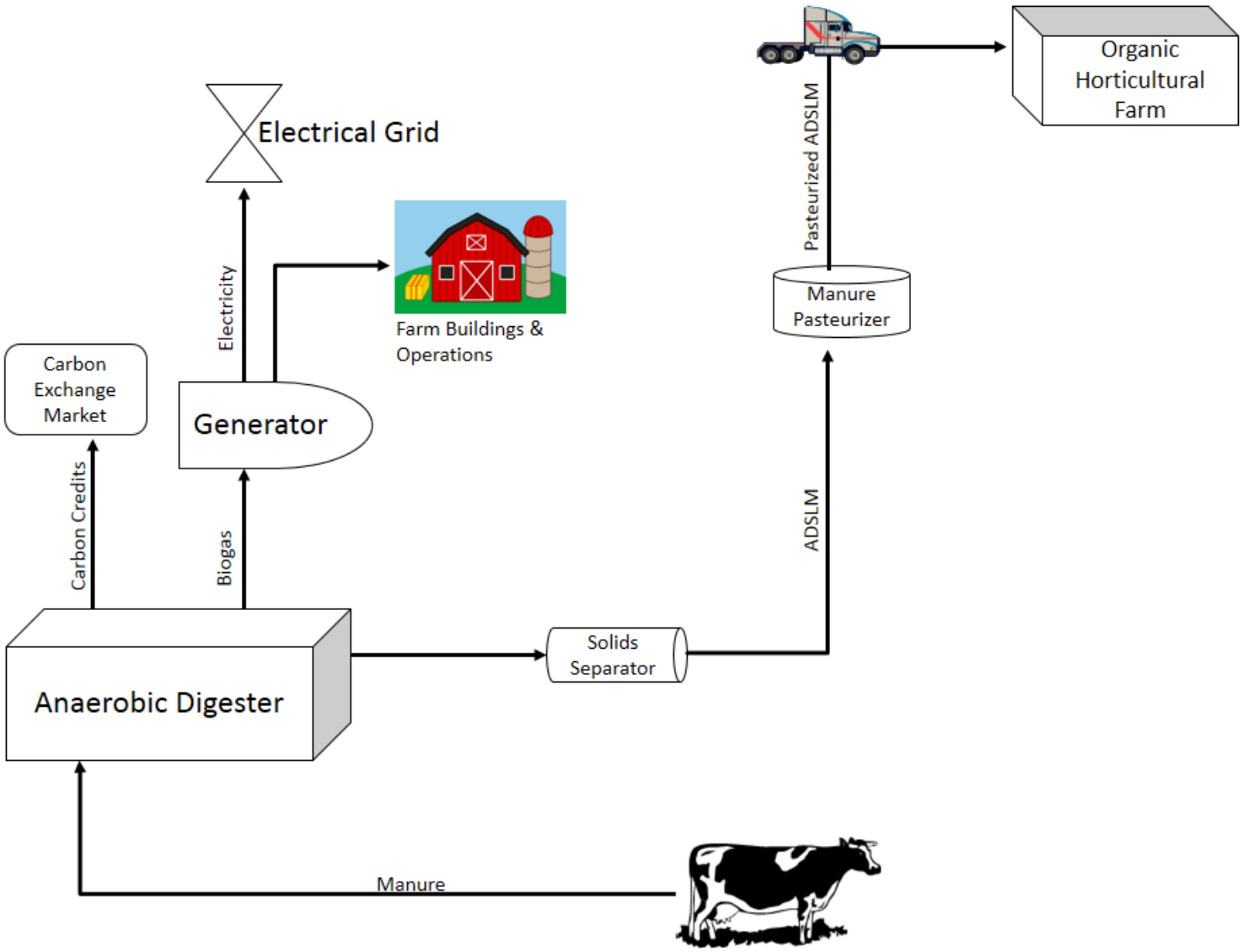

Figure 5.3. System Boundaries for the Partial Environmental Analysis

been supplied using fossil fuel-based energy sources. Values of 0.55 kg CO_2e/KWH and 0.99 kg CO_2e/KWH were used to calculate the carbon emissions of producing electricity from natural gas and coal, respectively (U.S. Energy Information Administration, 2013). The carbon footprint of operating the anaerobic digester was calculated based on the amount of energy consumed by anaerobic digester components, which was estimated to be 10% of the total energy produced during electricity generations. The energy consumed by the anaerobic digester components was subtracted from the annual carbon savings from biogas production to produce a net carbon emissions savings (Net CES_{Biogas}) through use of the biogas from the AD, according to equation 5.6 (ASERTTI, 2007). The carbon footprint of producing pasteurized ADSLM was considered to be 0 because the manure was pasteurized using waste heat generated by the AD generator.

$$\text{Net } CES_{Biogas}(CO_2e) = 0.9 \times P_E \times F_C \quad (5.6)$$

Where
PE = annual electricity production, in KWH
FC = carbon emissions factor for producing electricity from natural gas (0.55) or coal (0.99) in $CO_2e \cdot KWH\text{-}1$

The carbon footprint of transporting pasteurized ADSLM to an organic horticultural farm was calculated using the distance and travel method to transport the pasteurized ADSLM from the Dairy Energy LLC to the organic horticultural farm as depicted in equation 5.7. This carbon footprint was also compared to that of a fertilizer product sourced in the Netherlands that can potentially be used by the organic horticultural farm. Initial interest in the ADSLM by the organic horticultural farm stemmed from the assumption that the carbon footprint of the ADSLM produced by Dairy Energy LLC was smaller than the former imported fertilizer product from the Netherlands. The carbon footprint of transporting the former fertilizer product to the organic horticulture farm was calculated based on information provided by the organic horticultural farm. Based on this information, it was estimated that the fertilizer was shipped via a cargo freight ship from Rotterdam, the Netherlands to Wilmington, USA and via freight truck from the plant in the Netherlands to Rotterdam, Netherlands and from Wilmington in the USA to the organic horticulture farm in Virginia. The carbon footprint of transporting the fertilizer via freight ship was 13.5 g CO_2 per tonne of cargo transported over each km ($gCO_2 \cdot tonne^{-1}km^{-1}$) (McKinnon, 2007). A value of 62 $gCO_2 \cdot tonne^{-1}km^{-1}$ was used to calculate the carbon footprint of using a freight truck on land in the Netherlands and in the U.S.A. (McKinnon, 2007).

$$CF_{ADSLM}(CO_2e) = D_M * M * AU + D_R * R * AU \quad (5.7)$$

Where DM = maritime transport distance, in km
M = carbon expenditure for maritime transport using a cargo ship liner, in gCO_2 per tonne-km
AU = annual fertilizer usage, in tonnes
DM = road transport distance, in km
R = carbon expenditure for road transport using a trailer truck, in gCO_2 per tonne-km

5.4 RESULTS & DISCUSSION

5.4.1 Economic Sustainability of Anaerobic Digestion and its Value-added Products

The economic analysis of operating the anaerobic digester and using or selling its value-added products for a large dairy (1200 milking herd) show ANIs ranging from $295,738 to $560,524. In general the payback period for the initial investment ranged from 4.6 to 11.8 years depending on the ANI. The influence of the price of pasteurized ADSLM is shown in figure 5.4.The payback period on the investment ranged from 5.15 to 10 years when the sale price of pasteurized ADSLM $0.10 L^{-1} and the sale prices of electricity varied from $0.036·$KWH^{-1}$ to $0.12·$KWH^{-1}$ and carbon credits varied from $1·$credit^{-1}$ to $3·$credit^{-1}$. Figure 5.5 illustrates that the payback period ranged from 4.7 to 11.8 years when the sale price of carbon credits was held constant at $2 $credit^{-1}$ and the sale prices for electricity ranged from $0.036·$KWH^{-1}$ to $0.12·$KWH^{-1}$ and the sale price for pasteurized ADSLM ranged from $0.10 L^{-1} to $0.26/L. Figure 5.6 illustrates that the payback periods ranged from 10.2 to 14.5 years when the sale price of electricity was held constant at $0.036 KWH^{-1} and the sale price of carbon credits ranged between $1·$credit^{-1}$ and $3·$credit^{-1}$ and the price for pasteurized ADSLM ranged from $0.10·$L^{-1}$ to $0.26·$L^{-1}$.

The results from modeling payback periods as a function of the maximum, minimum, or median sale prices for each of the value-added products are presented in figure 5.7. Spheres with larger diameters corresponded to longer payback periods. When the payback period was modeled using the maximum sale prices for electricity, pasteurized ADSLM, and carbon credits ($0.12·$KWH^{-1}$, $0.26·$L^{-1}$, and $3·$credit^{-1}$, respectively), the payback period was 4.6 years. By contrast, 11.8 years was required for payback on the investment when the minimum sale prices for electricity, pasteurized ADSLM, and carbon credits ($0.036·$KWH^{-1}$, $0.10·$L^{-1}$, and $1·$credit^{-1}$, respectively) was used. Analysis using the median sale prices of $0.08·$KWH^{-1}$, $0.18·$L^{-1}$, and $1.75·$credit^{-1}$ for electricity, pasteurized ADSLM, and carbon credits, respectively, indicated that payback period would be 6.6 years.

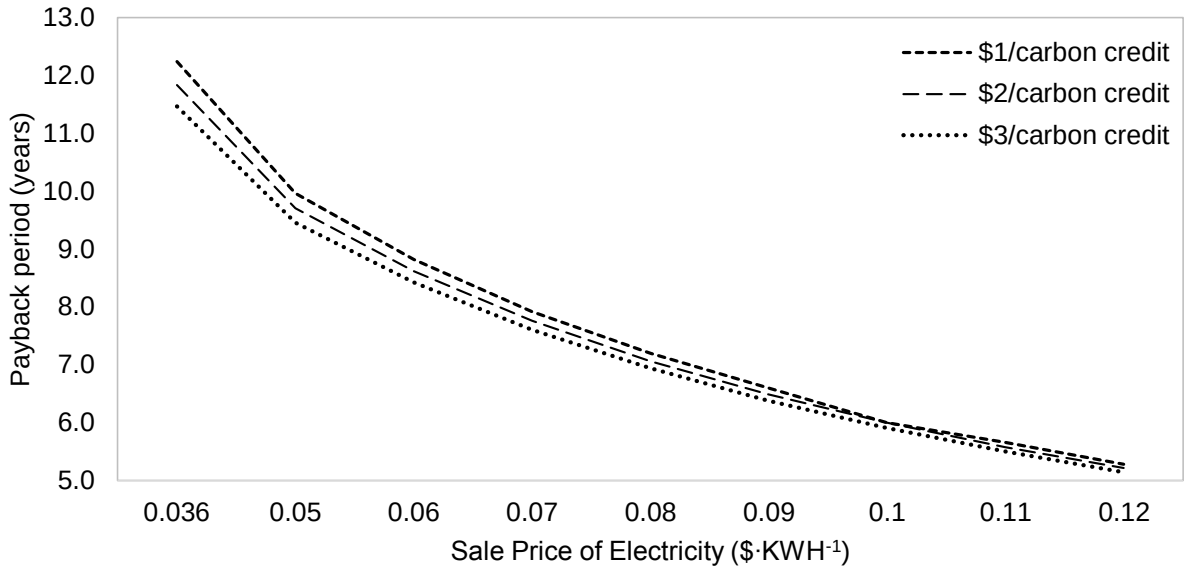

Figure 5.4. Payback period as a function of carbon credit and electricity sale prices. The sale price of pasteurized ADSLM was held constant at and a $0.10·L^{-1}.

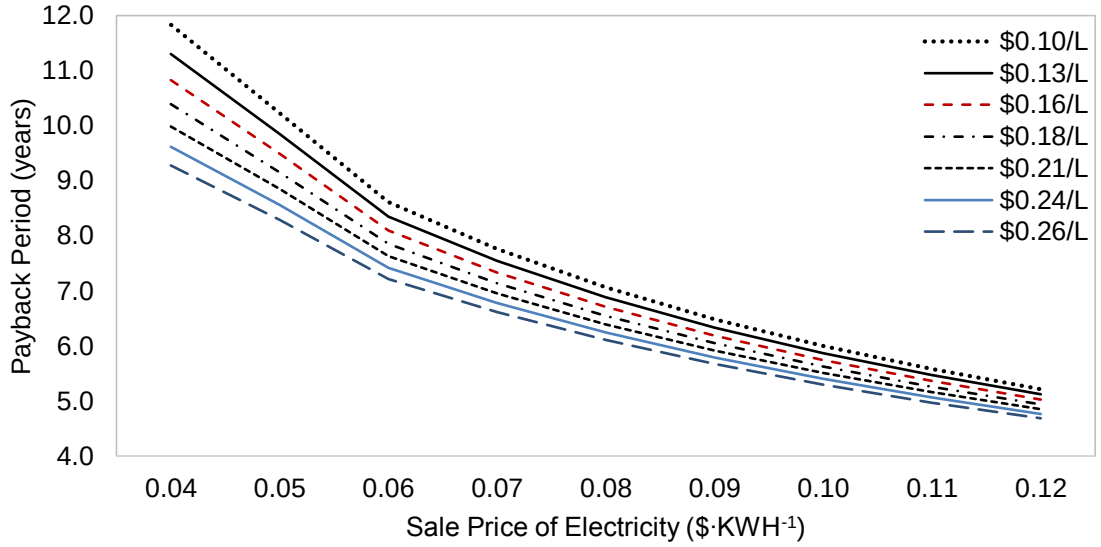

Figure 5.5. Payback period as a function of electricity and ADSLM sale prices. The sale price of carbon credits was held constant at $2·credit^{-1}.

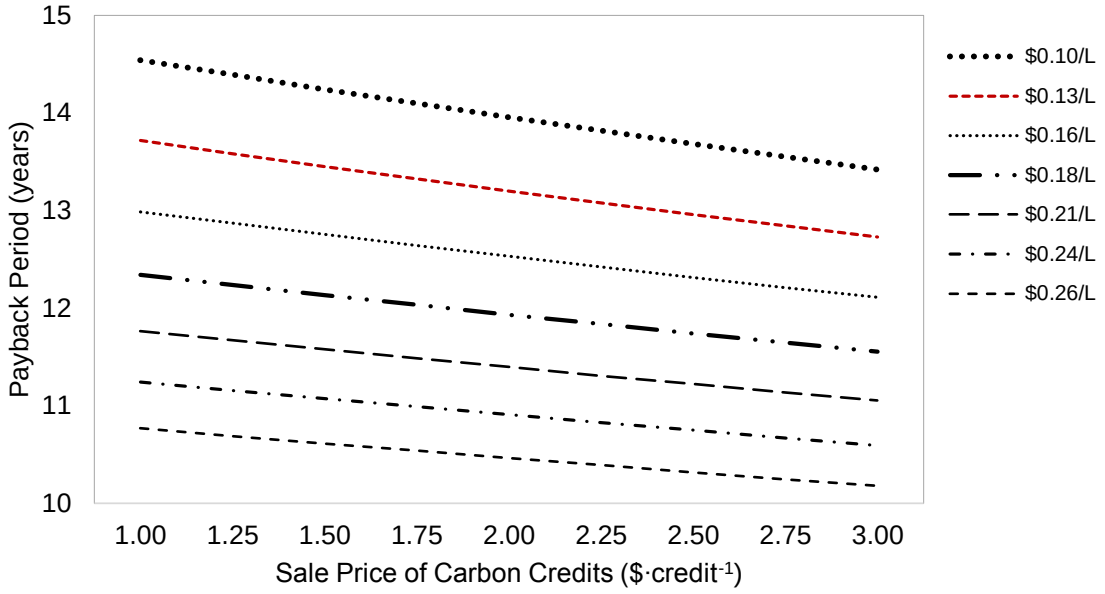

Figure 5.6. Payback period as a function of carbon credit and ADSLM sale prices. The sale price for electricity was held constant at $0.036·KWH^{-1}.

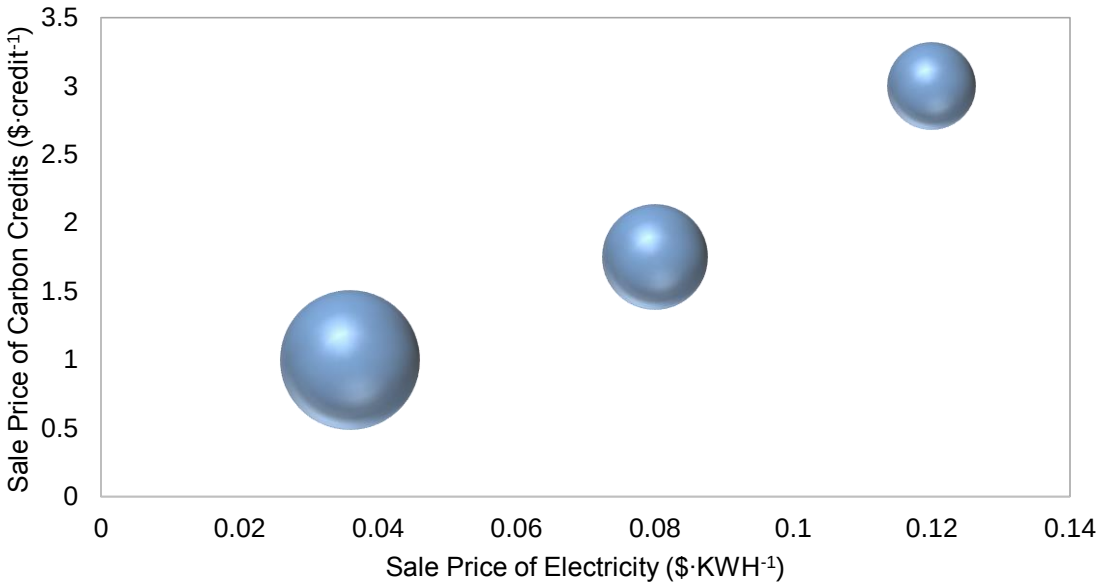

Figure 5.7. Payback periods when the maximum, minimum, or median sale prices for each of the value-added products were modeled. Spheres with larger diameters corresponded to longer payback periods.

5.4.2 Environmental Sustainability of Anaerobic Digestion and its Value-added Products

Converting the manure management system from a storage pit to an anaerobic digester resulted in annual methane emissions savings estimated at 2,436 tonnes of CO_2e per cow per year. Combustion of the biogas from the AD for the produced an average 2.93 GWH per year, replacing the need for fossil-fuel energy sources which emit between 1,622 and 2,899 tonnes CO_2e per year. Energy consumed to operate the anaerobic digester consumed approximately 10% of the energy produced by the AD system. As a result, the net annual savings in carbon emissions achieved by operating the AD system was between 1,460 and 2,609 tonnes of CO_2e per year.

The annual carbon footprint of transporting pasteurized ADSLM to an organic horticulture farm used in this study located 140 km away from the dairy farm was estimated to be 15.15 $kgCO_2e$. By contrast, the transport based annual carbon footprint of acquiring (importing) fertilizer from the current vendor was 7.90 $kgCO_2e$. The carbon footprint of transporting pasteurized ADSLM was nearly 2 times that of the imported fertilizer. First, this study only included the carbon footprint of transporting fertilizers to their destination; the carbon footprint of the production process to generate the imported fertilizer was not considered. Although it is possible that the production process for the imported fertilizer required considerably larger amounts of energy than production of the pasteurized ADSLM, this information was unavailable because of proprietary reasons. Another reason for the difference in carbon footprints was the low nitrogen concentration of the pasteurized ADSLM compared to the imported fertilizer. The nitrogen concentration in the imported fertilizer was more than 13 times higher than the nitrogen concentration in pasteurized ADSLM. According to the organic horticulture farm, it would require 17 times the volume of pasteurized ADSLM to meet the same nutrient and soil qualities as the imported fertilizer. Finally, transporting cargo across the Ocean via freight ship uses only 21.8% of the equivalent energy needed to transport cargo over land via freight truck. Because the majority of transport for the imported fertilizer was via over the ocean and in smaller quantities, the carbon footprint of transporting the imported fertilizer was significantly lower than the pasteurized ADSLM fertilizer.

The organic horticultural farm, or any organic farm receiving pasteurized ADSLM, would need to be located within 104 km of Dairy Energy, LLC for transportation of pasteurized ADSLM to have a smaller carbon footprint than the imported fertilizer. Alternatively, the organic horticulture farm would need to be located greater than 2,320 km from the port at Wilmington, NC.

5.5 CONCLUSIONS

The analysis conducted in this study indicated that the payback period for the initial $2 million investment in the anaerobic digestion system ranged from 4.6 to 11.8 years. Converting the manure

management system at Dairy Energy LLC from a storage lagoon to an anaerobic digester resulted in an annual methane emissions savings estimated at 2,436 tonnes of CO_2e per cow per year. Combustion of the biogas from the AD produced a net average of 2.55 GWH per year, replacing the need to combust fossil-fuel energy sources that would have emitted between 1,460 and 2,609 tonnes of CO_2e per year. The annual carbon footprint of transporting pasteurized ADSLM to the organic horticulture farm in Virginia used in this study was determined to be 15.15 kg CO_2e. By contrast, the annual carbon footprint of transporting the former fertilizer to the organic horticulture farm was 7.90 kgCO_2e. The organic horticulture farm would need to be located within 104 km of Dairy Energy, LLC for transportation of ADSLM to the organic horticulture farm to have a smaller carbon footprint than the imported fertilizer. Alternatively, the organic horticulture farm would need to be located greater than 2,320 km from the port at Wilmington, NC.

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6 Conclusions

The results indicated that the on-farm AD evaluated in this study performed as expected from prior studies on anaerobic digestion of dairy manure. The average tCOD and sCOD concentrations decreased by 29% and 57%, respectively. The average TVFA concentrations were reduced by 92%. Average daily biogas production ranged from 3,300 to 7,000 $m^3 \cdot d^{-1}$ with biogas yields ranging from 0.43 and 0.89 $m^3 \cdot kgVS^{-1} \cdot d^{-1}$.

Results from co-digestion of potato processing waste (PPW) and dairy nutrient supplement (DNS) with dairy manure indicated that biomethane production can be increased compared to digestion of manure, alone. It is suggested that manure be co-digested with up to PPW, where PPW constitutes up to 20% of the total loading volume. For maximum TVFA production and anticipated biomethane production, the acid chamber of a two-stage anaerobic digester should be operated with a hydraulic retention time of 1 d. The results also indicate that dairy manure can be co-digested with DNS, where the DNS constitutes between 3 and 10% of the total volume, for enhanced biomethane production. For maximum TVFA production using a 3% DNS mixture, it is recommended that the acid chamber of an anaerobic digester be operated at a hydraulic retention time of 1 d. For maximum TVFA production using a 7 or 10% DNS mixture, it is recommended that the acid chamber be operated at a hydraulic retention time of 3 or 4 d, respectively. It is also recommended, however, that the dairy manure, DNS, and PPW feedstocks be further investigated for the nutrient content, particularly the nutrients selenium and molybdenum. Further research should be conducted to elucidate the effects of DNS, PPW, and manure on individual fermentation and methanogenesis processes.

Pasteurization of anaerobically digested separated liquid manure (ADSLM) at 70°C to 80°C resulted in observed in fecal coliform concentration reductions ranging from 85% to 95%, *E. coli* reductions ranging from 87% to 96%, and *Salmonella* reductions of 100%. The duration of pasteurization treatment did not significantly affect pathogen concentrations; over 85% of inactivation was achieved during the period when manure temperatures were raised to desired pasteurization temperatures. This study contributed greater understanding about pathogen inactivation response in dairy manure. Following treatment, samples from all twenty-seven trials met the minimum requirement for use in organic food production, enabling its off-farm sale as a fertilizer for human food production. Given this proof-of-concept, further research at the pilot scale to assess the feasibility of farm-scale implementation is recommended.

Economic analysis performed on the AD system and value added products indicated that the payback period for the initial investment in the AD system ranged from 4.6 to 11.8 years. Environmental analysis

on the AD system showed that the AD reduced direct manure methane emissions by 2,436 tonnes of CO_2e per year and replaced the need for fossil fuels that would have emitted between 1,460 and 2,609 tonnes of CO_2e per year. The results of these analyses suggest that on-farm anaerobic digesters and their value-added products yield a net improvement to the economic viability of dairy farming and to environmental health.