ABSTRACT

CULTURABILTY OF POTENTIAL PATHOGENIC BACTERIA IN A CO-DIGESTION ANAEROBIC DIGESTER SYSTEM

By: Shannon A. Johnson

Anaerobic digestion (AD) has the potential to reduce organic wastes and produce renewable energy from the degradation of organic materials to produce biogas. However, mixed substrate anaerobic digesters may create a suitable environment for opportunistic pathogenic bacteria. The fate of potential bacterial pathogens has been studied in manure-based digesters but their survival is not well understood when digesters are co-fed with food waste. Mixed substrates may change internal conditions and subsequently pathogen survival, resulting in concerns for general environmental health. The purpose of this study was to introduce and enumerate potentially pathogenic bacteria in a bench-scale AD system while analyzing possible relationships between colony growth with typical AD parameters: temperature, pH, and volatile fatty acids (VFAs). Food waste was utilized as a substrate and percolate (a manure surrogate) was used as an inoculum to examine the survival of five species of bacteria (*Campylobacter jejuni, Staphylococcus aureus, Salmonella enterica, Enterococcus faecalis,* and *Escherichia coli*) that were selected on likelihood of entering a digester or that they are indicators for groups potentially located in digesters. It is hypothesized that incomplete acidogenesis – from mixed substrates – may increase VFAs, lower pH, and therefore decrease pathogen survival. A modified membrane filtration method, with selective and differential media, were used to enumerate the microorganisms. In the *Enterococcus* spp. experimental growth system, linear regression analyses revealed that there is a relationship of colony reduction with: temperature, pH, and total VFAs. The reduction of four of the five organisms, early in AD, indicates that co-fed digesters can reduce pathogens and lower environmental health risks.

CULTURABILTY OF POTENTIAL PATHOGENIC BACTERIA IN A CO-DIGESTION ANAEROBIC DIGESTER SYSTEM

by

Shannon A. Johnson

A Thesis Submitted
In Partial Fulfillment of the Requirements
For the Degree of

Master of Science – Biology

Microbiology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

December 2017

COMMITTEE APPROVAL

Advisor

12-5-17 Date Approved

Member

12-5-17 Date Approved

Member

12/5/17 Date Approved

PROVOST AND
VICE CHANCELLOR

12/6/2017
Date Approved

FORMAT APPROVAL

12/5/17
Date Approved

My thesis is dedicated to Kyle Windsor and Jordalyn Simpson: you both were there as a life saver as I was drowning. I could not have done this work without the help from both of you.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Gregory Kleinheinz, for his support and help throughout this project and graduate school. This project and my time at UW Oshkosh would have been an entirely different experience if I hadn’t taken your advice. I would like to thank Dr. Colleen McDermott for providing cultures and literature for this study as well as allowing me to use her lab space. Thank you, Dr. Sabrina Mueller-Spitz, for your guidance throughout this journey. Your advice and PowerPoint organization always saved me in the nick of time. I always enjoyed our discussions that ranged from microbiology to Dr. Who.

I would like to thank the entire Environmental Research and Innovation Center Lab and Biodigester team for their support, assistance, and facilities. You all always helped me find what I needed and let me take up space with all of my petri plates. A very special thanks to Ethan Poling, Paul Denil, Carolyn Patt, Betina Chaon, Kim Busse, Brian Langolf, Kenny Johnson, and Caitlin Koller, for helping me throughout my project. You all were always willing to give me an extra hand when I was in need.

To Jordalyn Simpson and Chelsea Smies: I would not have been able to (physically) complete this project without you two. It was wonderful working with you both and teaching you new techniques. You both always had a positive attitude even after the long first week of sampling. Your help meant everything to me, especially when I needed it the most.

I would like to thank my friends and family for supporting me through this journey. Every peptalk and adult beverage helped me get to where I needed to be. Thank

you to my roommates, Lauren Hahn and David Wieczorek for keeping me sane, listening to me work out problems, and never letting me drink alone. Kyle Windsor, thank you for always being my partner and reminding me how much I am capable of.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (continued)

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

Introduction

Introduction to Anaerobic Digesters and Organic Waste Materials

Anaerobic biodigesters can be described as contained systems that hold organic waste products that are utilized as substrates for microorganisms to breakdown the substrates into biogas and other products. Organic waste products could be animal manure, food scraps, paper products, and yard scraps (Korres et al. 2013; Kusch et al. 2011). Anaerobic digestion (AD) is comprised of four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In hydrolysis, microorganisms (e.g. bacteria) utilize complex organic materials (e.g. lipids, carbohydrates) to produce fatty acids, simple sugars and amino acids (Korres et al. 2013). In acidogenesis organisms use the products from hydrolysis and break those materials down into different organic acids, ammonia, carbon dioxide, and hydrogen (Korres et al. 2013). In acetogenesis microbes use the organic acids (such as volatile fatty acids) from acidogenesis to form ammonia, acetic acid, carbon dioxide, and hydrogen (Korres et al. 2013). During methanogenesis, methanogenic archaea will utilize acetate, carbon dioxide, and hydrogen to produce methane (Korres et al. 2013). In a digester these four steps can be occurring simultaneously however the steps rely on a dissimilatory microbial food web to occur (Korres et al. 2013). The biogas (i.e. methane) from AD can be utilized for energy (e.g. methane combustion; converted to natural gas) and the remaining organic materials can be used for land application as fertilizer or compost, or treated for animal bedding use, etc. (USEPA 2013). Digesters are widely used in the United States and throughout the world to manage and utilize organic waste materials (e.g. manure, human effluent, industrial wastes). They are an accepted method of treating organic wastes and inactivating potential pathogens (Gunaseelan 1997; Kusch et al. 2011; Sobsey et al. 2006; US EPA 2013; Amani et al. 2010). Common biodigesters in the United States of America are agricultural, food waste-based, industrial based, landfills, and waste water (American Biogas Council 2017). An example of agricultural biodigesters are wet animal manure-based digesters on farms.

Organic waste (e.g. agricultural manure and municipal waste) management is a concern for most developing and developed societies. The process of raising animals and growing agricultural plants for consumption requires critical waste management methods. According to the United States Department of Agriculture (USDA) a typical lactating dairy cow in the United States will produce 80 pounds (approximately 36.3 kilograms) of manure in one day (1995). Utilizing these numbers, a small farm of 100 dairy cows should produce 8,000 lbs. (approximately 3,628.7 kg) of manure in one day and a larger farm of 1,000 cows should produce 80,000 lbs. (approximately 36287.4 kg) of manure in one day (USDA 1995). The USDA estimated that approximately 1.1 billion lbs. of manure were produced by agricultural animals (including fish) in the year 2007 (USEPA 2013). Larger farms, with any type of agricultural animals, are known as Concentrated Feeding Operations (CAFOs). A CAFO is defined as an animal feeding operation with 1,000 or more animal units, where one animal unit is equal to 1000 lbs. of animal (USDA 1995; USEPA 2013). The USDA regulates how CAFOs manage, store, treat, and apply manure (USEPA 2013). The manure needs to be treated appropriately for pathogen content before it can be applied to the land to prevent environmental contamination and health hazards.

In the United States 133 billion lbs. (approximately 60.3 billion kg) of food are wasted from the food supply annually (USEPA 2016). The food waste often is deposited into landfills. Municipal waste includes a variety of organic materials such as yard waste, food waste, and paper products (Composting Council 2011). As of 2005, 245.7 million tons (approximately 222.8 million metric tons) of municipal solid waste were produced in the US (Composting Council 2011). As of 2013, 254.1 million tons (approximately 230.5 million metric tons) of municipal solid waste were produced (USEPA 2015). The United States Environmental Protection Agency (USEPA) have been increasing regulations on the disposal of organic wastes (2016). By the year 2030 the USEPA plans to reduce the 133 billion lbs./year of food waste by 50% (USEPA 2016). According to the US Composting Council (2014), 29 of the 50 states have limitations or complete bans for depositing organic wastes in landfills. Of the 254.1 million tons (approximately 230.5 million metric tons) of municipal organic waste produced in 2013, 87 million tons (78,925,072.38 approximately 78.9 million metric tons) were recycled and composted (USEPA 2015). The organic wastes need to be managed and handled safely as of concern for opportunistic foodborne pathogens.

Farmers and industry have begun combining manure and organic wastes, such as food waste and agricultural waste, into the same biodigester (DeBruyn & Hilborn 2007; ERIC 2017a). However there are safety concerns for the management of manure and co-digestion. There are concerning matters to address for manure management: treatment and storage of manure, safe utilization of waste material, management of ground and surface water contamination from runoff, and overall potential pathogen content.

Manure has been shown to contain pathogens in large concentrations (Sobsey et al. 2006). Anaerobic digestion is a method utilized to reduce or inactive pathogens before the storage or land application of manure materials. Bacterial pathogens in manure slurries, which are applied to the land, can be harmful to agricultural animals as well as to humans via water-run off, wind, direct contract, or ingestion (Kearney et al. 1993; Jones 1979; USEPA 2013). Additionally, untreated manure products with potential pathogens could run-off into streams or into ground water (Sobsey et al. 2006; USEPA 2013). Manure run-off can effect lakes and streams via its nutrient content and potential pathogen content. Compounds from land-applied manure run-off, such as ammonia, can cause a cascade of microbial over-growth in aquatic environments, resulting in low concentrations or no oxygen in the water; the lack of oxygen in these environments can be fatal to living organisms such as fish (USEPA 2013). Pathogens in manure need to be inactivated or reduced before manure can come into contact with the environment.

Anaerobic digestion is an acceptable method to reduce pathogen loads, however, it is uncertain if co-digestion (the combination of digesting two or more different organic waste materials) of manure with other organic materials (e.g. food waste) can adequately reduce pathogens. Since at least 1994 the question of co-digestion has been a safety concern for the health of humans and agricultural animals. The concern is that the final products from an anaerobic digester may introduce new pathogens to agricultural animals

(Larsen et al. 1994). Additionally, food waste and other organics added to manure-based anaerobic digesters may add more nutrients for potential pathogens to utilize during AD as well as food waste could be a source for additional pathogenic bacteria to enter a digester.

Anaerobic digesters are designed to utilize a specific substrate, such as manure or industrial wastes, like paper mill waste products and food waste (Korres et al. 2013; Kusch et al. 2011). The digesters can be considered dry or wet and can be operated at various temperatures. Dry anaerobic biodigesters have greater than 20% solids than liquid material as a substrate (Kusch et al. 2011). Meaning, the ratio of solids must be more than 20% to that of the liquids in the digester. Because dry digesters will have more solid than liquid as a substrate, the substrate materials are digested in ‘batches’. Batch AD means the reactor or fermenter contains the substrates for the entire AD period without replacement of substrate. Substrates with a higher solid to liquid ratio can be difficult to add to a digester during AD. After the AD time period, all materials are removed and new materials are substituted for the old/digested substrate (Kusch et al. 2011). For example, in the University of Wisconsin Oshkosh’s dry anaerobic biodigester (

Figure *1*), half the digested materials, after the AD time period of 28 days, are removed from the fermenter and are mixed with new substrate materials for inoculation and a new AD period (G. Kleinheinz – Personal Communication). The same dry digesters are also found throughout Europe (Kusch et al. 2013; G. Kleinheinz – Personal Communication).

Figure 1. University of Wisconsin Oshkosh Dry Anaerobic Digester (outside view). *Photo Credit: Environmental Research and Innovation Center (ERIC) Laboratory.*

Wet anaerobic digesters have less than 12% solids than liquid as substrates, meaning the substrates in the digester have a higher liquid solids ratio (Kusch et al. 2011). Before substrates can enter a wet digester, they may require a liquidation process and/or pre-treatment in some form to properly undergo AD in this type of digester (Kusch et al. 2011). Wet digesters may be batch or semi-continuous. For semi-continuous digestion, substrate material is fed, or loaded, into the digester at time specific intervals as the waste material or already digested material exists the digester (Kusch et al. 2011). Wet AD can occur in one, two, or multiple stage processes of digestion (Kusch et al. 2011). In batch and in some semi-continuous AD, there is one stage where all AD occurs at one temperature in one reactor or fermenter. In two or multiple stage AD, substrates occur in different reactors/fermenters at different temperatures (Kusch et al. 2011). For example, the first round of AD may be held at a higher temperatures for a short period of time and then held at a moderate temperatures AD for a longer period of time.

Anaerobic biodigesters can be operated at psychrotrophic, mesophilic, and thermophilic temperatures. Psychrotrophic AD, low temperatures < 20° C (Kusch et al. 2011), will not be the main focus of this study. Mesophilic AD is commonly referred to temperatures at or between 30-38° C, however, these digesters can be operated at temperatures ranging from 20-45°C (Kusch et al. 2011). Thermophilic AD are reportedly operated between 48-57° C, but can be run at higher temperatures (60-70° C) for shorter periods of time (Korres et al. 2013; Kusch et al. 2011; Wu et al. 2016). Digesters operate for different time periods and temperatures depending on the type of digester, the material in the digester undergoing AD, and the amount of material. For example, the University of Wisconsin Oshkosh dry anaerobic biodigester runs batches for 28 day periods at a mesophilic temperature of about 38° C (G. Kleinheinz – Personal Communication). Agricultural manure-based digesters are often wet digesters but have different approaches to the treatment of manure.

Manure-based farm digesters are typically operated at mesophilic or thermophilic temperatures. These digesters can be batch and semi-continuous. If the digesters are two or multi-stage, they may be operated at two different temperatures. An example of a small farm manure-based digester is a plug flow digester. Plug flow anaerobic digesters are narrow and long with input and output at opposite ends of the digester; material is pushed through the digester as more substrate is added (Kusch et al. 2011). Local examples of manure-based AD for the Fox Valley area in Wisconsin are the Allenville mixed plug flow digester, operated at a mesophilic temperature of 38° C and the Rosendale complete mix digester operated at a mesophilic temperature of 38° C (ERIC

Lab 2017; G. Kleinheinz – Personal Communication). A safety concern with manure-based anaerobic digesters is contact with potential pathogens before, during, and after AD.

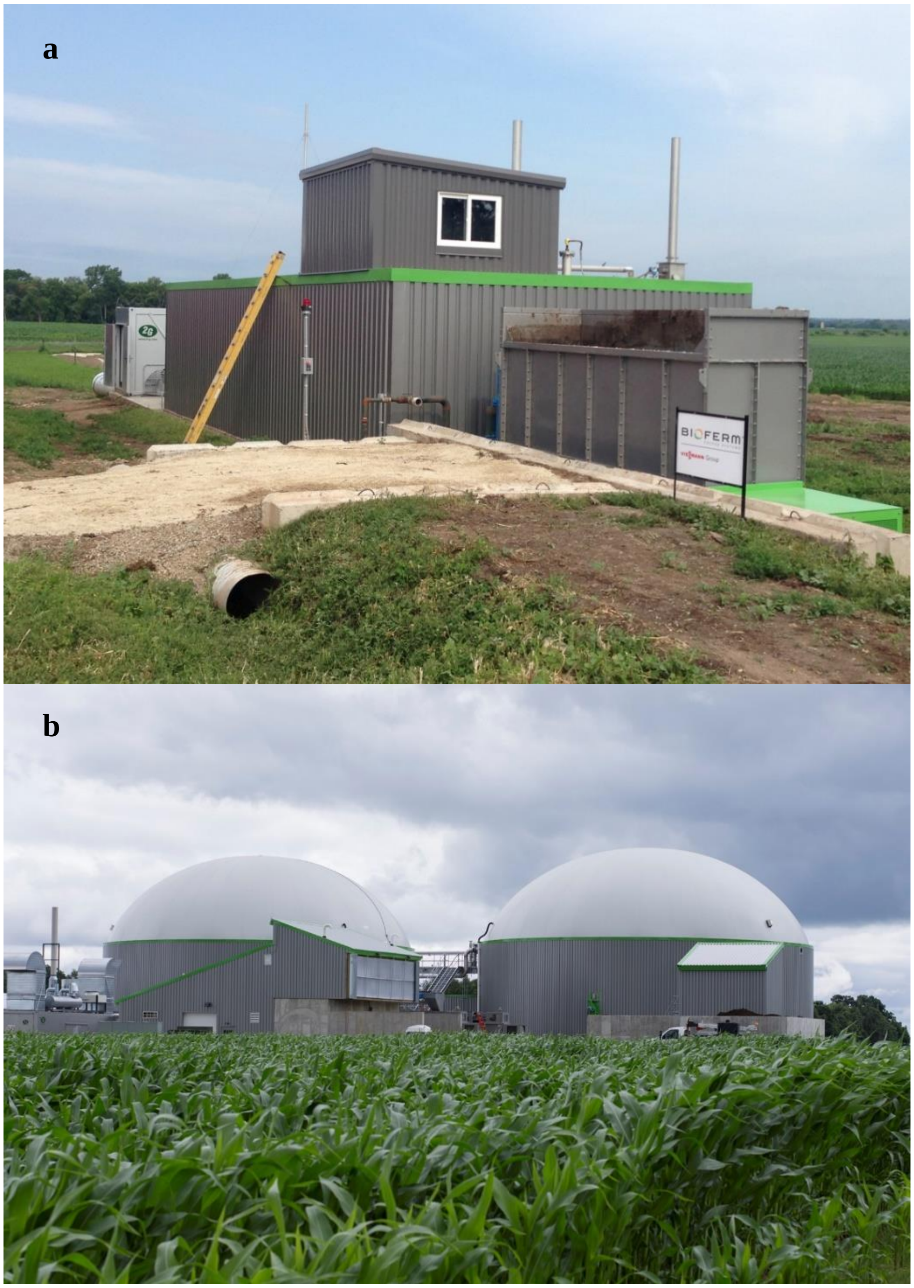

Figure 2. (a) Allenville Farm plug flow anaerobic digester. (b) Rosendale Farm complete mix anaerobic digester. Photo credit: *ERIC Lab.*

Fate of Potentially Pathogenic Bacteria with AD

The fate of potentially pathogenic bacteria during and after AD could impact the health of humans and non-human animals. Potential pathogens in manure can be protected from environmental stressors such as desiccation, however, potential pathogens in manure must interact with high ammonia concentrations and microbial competition for nutrients (USEPA 2013**.** Humans could potentially be infected by handling pre- or post-digested material, by handling agriculture plant products that were grown using digested material, ingesting food/water that has been contaminated by organisms from the digested material, handling or drinking water from improperly treated digested material run off, or by interacting with animals that were re-infected from inadequately post-digested materials (Sobsey et al. 2006; Topp et al. 2009; USEPA 2013). Non-human animals could be re-infected if organisms survives AD and digested material is turned into animal bedding or if digested material is applied to grazing/animal feed growing areas (Sobsey et al. 2006; Topp et al. 2009). Land application of digested materials, along with digested fiber as animal bedding, would likely be main concerns for environmental contamination of potentially pathogenic bacteria from AD (

Figure *3*).

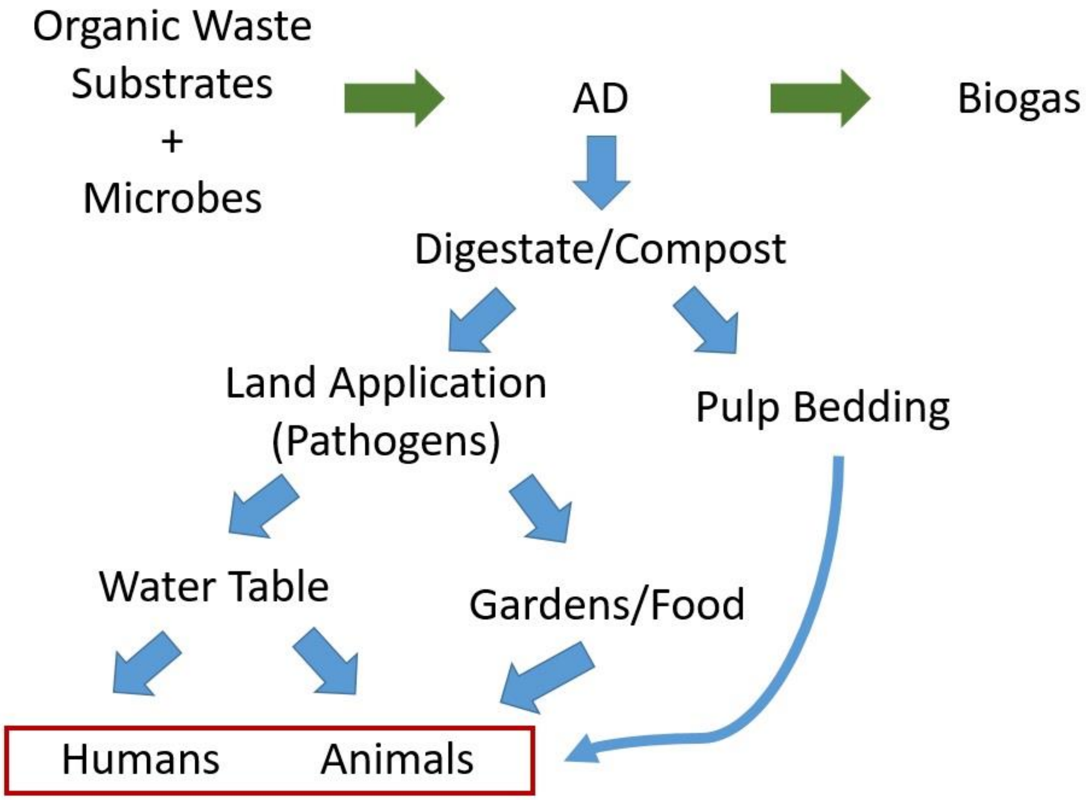

Figure 3. Flow chart of potential pathogen contact and fate in anaerobic digestion (AD) systems and environment (Adapted from Sobsey et al. 2006). Green arrows represent simplified AD towards biogas. Blue arrows represent the possible fate of potential bacterial pathogens after AD.

Rules and regulations for land application of manure-based anaerobic digested material vary on the fecal material type (e.g. human versus non-human animals), seasonality, and local laws/regulations (USEPA 2013). Two main concerns of potentially infected digested material for land application are contamination of the water table and contamination of ingestible agriculture material. After land application of treated non-human animal manure (e.g. cattle slurry), microorganisms should be filtered through the land via grass, dirt, and plants (USEPA 2013). However, areas with karst geology allow for fast water flow through the earth and into the water table. Karst geology is dissolvable bedrock (e.g. limestone, gypsum) with little to no top soil and fissures/cracks/sink holes visible (USEPA 2013). There is little filtration of rain water and the contaminants are not fully filtered through natural means before microbes and other contaminants reach the water table (USEPA 2013). Parts of Wisconsin (e.g. Door and Kewaunee counties) and other parts of the United States have karst or similar geology (Board of Regents of the UW System 2013). Potentially pathogenic microbes that survive AD and land application could travel through the earth into the water table and have been documented in well water which could travel to rivers and lakes (Sobsey et al. 2006; USEPA 2013). Run-off of land-applied manure into local lakes, rivers, and streams are also a concern. Humans may swim in those bodies of water and other bodies of water may be obtained to irrigate crops or agricultural animals (USAEPA 2013). The overall concern is health of those individuals that come into contact with contaminated water from land application of AD treated manure.

Land application of AD treated manure may be utilized for crops or grazing for agricultural animals (Sobsey et al. 2006; USEPA 2013). Microorganisms may accumulate on surfaces of plants or incorporate themselves into plant material. Animals could eat contaminated crops or grazing material where potential pathogens are then reintroduced into herd and the cycle repeats (Sobsey et al 2006**,** Kearney et al. 1993, Jones and Matthews 1975).

Factors Related with Survival in AD

Temperature, pH, and volatile fatty acids (VFAs) are the main factors related to potentially pathogenic bacteria survival. Higher AD temperatures tend to reduce the numbers of introduced and native microorganisms in manure-based digesters. Temperature for AD is normally determined by inoculum material, pathogenic organism concerns, substrate material, or destruction potential (Al Seadi et al. 2008). Temperature is an important parameter to maintain the growth of (native) organisms involved in AD, especially for bacteria that create VFAs and for archaea that produce methane (Korres et al. 2013). Mesophilic and thermophilic temperatures are best for anaerobic organism survival/cellular processes, while greater biogas production occurs at thermophilic temperatures.

Other factors, such as pH and the degradation of substrates, can influence microbial survival in AD (Korres et al. 2013). Environmental pH impacts the growth of all microorganisms, but more so for the growth of methanogenic archaea (Al Seadi et al. 2008; Korres et al. 2013; Willey et al. 2014). The optimal pH for mesophilic AD is between 6.5 and 8.0; AD is hindered if the pH is outside this range (Al Seadi et al. 2008). Some stages of AD need more acidic conditions for the breakdown of substrate materials, while the methanogenesis step requires a neutral pH (Korres et al 2013). pH during AD will fluctuate as a result of microbial activity from the breakdown of substrates, such as the production of VFAs and potentially other metabolic waste products. If pH decreases, as a result of a higher concentration of VFAs, the environment may be favorable for acidogenic bacteria which will subsequently create additional acidic products (Korres et al. 2013). Additionally, bacterial growth in farm anaerobic digesters has been noted to be inhibited by fatty acids in digesters (Abdul and Lloyd 1985). This shift in the microbial community could hinder methane and other biogas production since a more neutral pH is required for methanogenesis along with: acetate, carbon dioxide, hydrogen, and other methane-building compounds. Such an environment could be favorable to potentially pathogenic bacteria that can tolerate or even proliferate under more acidic conditions. Having appropriate temperature and pH ranges are vital for maintaining microorganisms for AD. Should these parameters not be held, the microbial community could be disrupted and therefore biochemical processes may be interrupted.

Volatile fatty acids (VFAs) are organic acid molecules that consist a chain of two to six carbon atoms (Korres et al. 2013). An accumulation of VFAs during AD could cause the pH to lower (Al Seadi et al. 2008). However, depending on the type of manure used, the alkaline nature of manure would buffer the pH effects of high concentrations of VFAs (Al Seadi et al. 2008). However, once the low pH has been detected the acidogenesis/AD process has already been hindered and the low pH indicates problems or unbalanced AD (Korres et al. 2013). Overall, higher concentrations of VFAs can inhibit microorganisms, especially methanogenic archaea (Amani et al. 2010), as a result of a decrease in pH and available compounds to create methane.

Overall, a co-fed anaerobic biodigester with food waste and manure may be feasible for biogas production and potentially pathogenic bacteria inhibition. Manure should be able to buffer the environmental pH that results from the accumulation of VFAs from the breakdown of food waste. To date there are no studies that investigate the relationship between AD parameters (pH, temperature, VFAs) and microbial survival/inhibition in a mesophilic co-fed digester of food waste and manure. One study by Wu and colleagues (2016) monitored a co-fed mesophilic anaerobic digester of food waste and human municipal solids for VFAs and the change of the natural microbial community. The researchers utilized molecular techniques to note the changes in the microbial community but not the direct/visual survival of the community. Their study resulted in a pH range of 5.2 – 6.4, which subsequently inhibited methanogenic archaea.

Introduction to Potentially Pathogenic Bacteria in Anaerobic Digesters

There are a number of pathogens of concern in fecal/manure digesters. These pathogens range from viral, bacterial, and eukaryotic in nature. The focus of this study are potentially pathogenic bacteria. The bacteria of focus are organisms found in fecal material and fecal contaminated food. This study will focus on five fecal/foodborne potential bacterial pathogens: *Campylobacter jejuni*, *Enterococcus faecalis, Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus*. These organisms were chosen due to their prevalence in anaerobic manure digesters.

Campylobacter jejuni. *Campylobacter jejuni has a Gram negative stain reaction and curved, rod shape morphology (Willey et al. 2014). This microbe is microaerophilic, meaning it can only grow in an environment with 2 – 10% oxygen present (Willey et al. 2014). Campylobacter jejuni is often found in the intestinal tracts of birds (e.g. chickens and turkeys) and other non-human animals, such as cattle (Willey et al. 2014). This bacterium can be transmitted from non-human animals to other non-human animals*

(Willey et al. 2014), as well as from non-human animals to humans. Transmission can occur from direct or indirect contact (Willey et al. 2014). All-in-all, C. jejuni is a food (e.g. shellfish, poultry, lettuce) and fecal (e.g. intestinal tracts of poultry) pathogen (Willey et al. 2014). This organism can also be a potable water contaminate (Willey et al. 2014). Campylobacter species are one of the many organisms that can be a part of hydrolysis and acidogensis in AD (Korres et al. 2013). Although anaerobic digesters should be void of elemental oxygen, in the beginning of AD, when fresh substrates have just been added, there could be small amounts of oxygen remaining which could allow for favorable conditions for Campylobacter species to propagate or survive. In some studies of human fecal and non-human manure-based digesters, C. jejuni has been found to both survive and be non-detectable AD. In a continuously fed mesophilic anaerobic digester (35° C), introduced C. jejuni increased at a log rate over approximately 22 days. Secondary digestion (15° C) decreased C. jejuni to 3.85 – 4.21 log (Horan et al. 2004). In another study conducted by Kearney and colleagues (1993), this microbe was introduced into bench-scale mesophilic (35° C) batch and semi-continuous cattle slurry digesters. There was little to no reduction of C. jejuni in a 71-day testing period. The T_{90}*, time when 90% of the original cell inoculum is dead, determined by regression analysis, would have been 793 days (Kearney et al. 1993). In a lab set-up of a thermophilic (50° C) bio-waste (of municipal solids) treating anaerobic digester there was inactivation after the first sampling period (24 hours) and between the second sampling period of seven days (Wagner et al. 2008). Overall, despite requiring microaerophilic conditions, C. jejuni and other Campylobacter species have been able to survive some AD conditions.*

Enterococcus faecalis. *Enterococcus faecalis* has a positive Gram stain reaction with a cocci or oval morphology; this bacterium can ferment carbohydrates and tolerates oxygen (Willey et al. 2014). This microorganism lives naturally in the intestines of humans and other mammals (NCBI 2017). Enterococci, in general, have been used as fecal indicator bacteria (FIB) for other potential pathogens in digesters (Larsen et al. 1994), waste water, and for fecal contamination in surface water and potable water sources (USEPA 2013). At times enterococci have been naturally found in the microbial community in manure-based digesters (Larsen et al. 1994), creating a potential health hazard.

In an extensive study conducted by Olsen and Larsen (1987) *E. faecalis* was introduced into cattle and pig slurry batches and [semi-] continuous lab scale digesters at 35° C and 53° C. In the lab scale cattle slurry batch digester at 35° C the average T_{90}, or the time period to decrease 90% of the initial population, was 2.2 days and the organism was no longer detectable at days 9 and 12. In the lab scale cattle slurry continuous anaerobic digester at 35° C the average T_{90} for *E. faecalis* was 2.1 days. In the lab scale pig slurry batch and [semi] continuous digesters at 35° C the average T_{90} was 1.8 days. In the lab scale cattle slurry batch digester and the cattle slurry [semi] continuous digester at 53° C the average T_{90} was 1.0 hours. In the lab scale pig slurry batch digester at 53° C the average T_{90} was 0.7 hours, while in lab scale pig slurry [semi-] continuous digester at 53° C the average T_{90} was 1.2 hours. Although *E. faecalis* was not detectable for long in the slurries, it is generally considered a hardier organism as a result of its flexible physiology.

Escherichia coli. *Escherichia coli* has a Gram negative stain reaction and is a motile, rod-shaped microbe (Willey et al. 2014). This organism is a food and fecal pathogen (Willey et al. 2014). *Escherichia coli* can be found fecal material that can contaminate foods such as, but not limited to, unpasteurized fruit juices and cider, raw vegetables (e.g. lettuce), and undercooked beef (Willey et al. 2014). This bacterium lives in the gastrointestinal tracks of humans and other non-human animals; this microbe can cause water contamination in surface water and potable water sources (Willey et al. 2014). *Escherichia coli* has been used as an indicator organism for other potential pathogens in digesters (Larsen et al. 1994), waste water, and in surface water and potable water sources (USEPA 2013). Similar to *E. faecalis, E. coli* is widely used as an indicator organism for fecal contamination (e.g. surface water, potable water, food) and can indicate other fecal related pathogens (e.g. bacterial, eukaryotic, viral) that may be present in materials and therefore could be a potential health hazard to humans and non-human animals (Sobsey et al. 2006; USEPA 2013).

Escherichia coli is used more specifically as a FIB because of its consistent presence in fecal matter (Sobsey et al. 2006). This microorganism is often naturally found in the microbial community in manure-based digesters (Larsen et al. 1994; Horan et al. 2004; Rabah et al. 2010). *Escherichia coli* can participate in acidogenesis and produce various VFAs – formate, valerate, isovalerate, propionate, butyrate, and acetate (Amani et al. 2010). Additionally, live *E. coli* has been detected in untreated manure, from various farms, between 56 - 77 days in outdoor barn storage (Mitscherlich & Marth 1984). Because *E. coli* can participate in acidogenesis during AD, there is opportunity for it to survive in a digester. In a study by Horan and colleagues (2004) *E. coli* was introduced into a [semi-] continuously fed waste water treatment sludge mesophilic anaerobic digester (35° C), with an AD time period of 21 days. Log removals, or the reduction of a specific microbial population in logarithmic scale, varied from 1.48 – 1.68 during primary digestion. During secondary digestion at 15° C, there was a log removal of 1.7 after 14 days and > 4.5 log removal over a 31+ days (Horan et al. 2004). In a study by Kearney and colleagues (1993) *E. coli* K12 *str*A was introduced into bench-scale mesophilic (35° C) batch and semi-continuous cattle slurry digesters. In the batch digester the average T_{90} was 0.8 days and was not detectable after day four in AD, while in the semi-continuous digester, the average T_{90} was 1.5 days and was not detectable after eight days in AD (Kearney et al. 1993). In a similar study conducted by Olsen and Larsen (1987), *E. coli* serovars (O8, O147, and O149) were tested in cattle and pig slurry batches and [semi-] continuous lab scale digesters at 35° C and 53° C. In the lab scale cattle slurry batch digester at 35° C the average T_{90} values were: *E. coli* O8 at 2.4 days, *E. coli* O147 at 2.1 days, and *E. coli* O8 was no longer detectable at days 12 and 13 in AD. In the lab scale cattle slurry [semi-] continuous digester at 35° C the average T_{90} values were: *E. coli* O8 at 1.6 days and *E. coli* O147 at 1.9 days. In the lab scale pig slurry batch digester at 35° C average T_{90} values were: *E. coli* O8 at 1.6 days and *E. coli* O147 at 1.1 days. In the lab scale pig slurry [semi-] continuous digester at 35° C the average T_{90} values were: *E. coli* O8 at 2.1 days and *E. coli* O147 at 2.5 days. In the lab scale cattle slurry batch digester at 53° C the average T_{90} values were: *E. coli* O8 at 0.5 hours, *E. coli* O147 at 0.3 hours, and *E. coli* O149 at 0.5 hours. In the lab scale cattle slurry [semi-] continuous digester at 53° C the average T_{90} values were: *E. coli* O8 at 0.6 hours and *E. coli* O147 at 0.5 hours. In the lab scale pig slurry batch digester at 53° C the average T_{90} values were: *E. coli* O8 at 0.3 hours, *E. coli* O147 at 0.1 hours, and *E. coli* O147 at 0.4 hours. In the lab scale pig slurry [semi-] continuous digester at 53° C the average T_{90} value for *E. coli* O8 was 0.2 hours. When *E. coli* serovar O8 was contained in Nylon bags of liquid culture in a full-scale digester cattle slurry at 35° C the average T_{90} value was 2.4 days (Olsen & Larsen 1987). In a study by Wagner and colleagues (2008), *E. coli* was introduced into a lab set-up of a thermophilic (50° C) bio-waste (of municipal solids) treating anaerobic digester there was inactivation after the 24-hour sampling period and between the 7 day sampling period. Overall, *E. coli* is a fecal associated bacterium that can naturally found in digesters but be inactivated or non-detectable after a few weeks into AD.

Salmonella enterica. *Salmonella enterica* has many serovars. This microbe has a Gram negative staining reaction with a motile rod morphology (Willey et al. 2014). This bacterium is found in the intestinal tracts of most animals, notable in poultry and on eggs/egg products (Willey et al. 2014). *Salmonella enterica* can also be found on various fruits and vegetables such as strawberries, melons, celery, and lettuce (Willey et al. 2014). Additionally, this microorganism can cause water contamination (Willey et al. 2014; USEPA 2013). Live *Salmonella* species have been detected in untreated manure, from various farms, for over 77 days in outdoor barn storage (Mitscherlich & March 1984), while *Salmonella* strains have been detected in manure-based digesters (Larsen et al. 1994; Rabah et al. 2010). *Salmonella* species presence in manure could be a potential health hazard before and after AD treatment.

In a study by Larsen and colleagues (1994) almost all digesters, mesophilic and thermophilic cattle and pig slurries, reduced the naturally occurring *Salmonella* species to non-detectable limits through culture methods. Introduced *Salmonella enterica* subspecies *enterica* serovar Senftenberg (a more heat tolerant organism) had a reduction of over two log in a continuously fed mesophilic anaerobic digester (35° C) of waste water sludge for a time period of 21 days (Horan et al 2004). Kearney and colleagues (1993) observed a reduction of introduced *S. enterica* serovar typhimurium into bench-scale mesophilic (35° C) batch and semi-continuous cattle slurry digesters. For batch digestion, the average T_{90} was 0.9 days and *S. enterica* serovar typhimurium was no longer detected after days 20 and 21 of sampling. For semi-continuous digestion, the average T_{90} was 1.1 days and *S. enterica* serovar typhimurium was no longer detected after days six and seven of sampling (Kearney et al. 1993).

In a similar study conducted by Olsen and Larsen (1987), two *Salmonella* serovars (*S. enterica* serovar. typhimurium and serovar dublin) were tested in cattle and pig slurry batch and [semi-] continuous lab scale digesters at 35° C and 53° C. In the lab scale study of a cattle slurry batch digester at 35° C the average T_{90} values were: *S. enterica* serovar typhimurium at 2.5 days and *S. enterica* serovar dublin at 2.2 days, respectively. *Salmonella enterica* serovar typhimurium was no longer detectable at days 14 and 15 of sampling. In the lab scale study of cattle slurry [semi-] continuous digester at 35° C the average T_{90} values were: *S.enterica* serovar typhimurium at 2.9 days and *S. enterica* serovar dublin at 1.9 days. In the lab scale pig slurry batch and [semi-] continuous digesters at 35° C the average T_{90} for *S. enterica* serovar typhimurium was

2.0 days. In the lab scale cattle slurry batch digester at 53° C the average T_{90} for *S. enterica* serovar typhimurium was 0.9 hours and *S. enterica* serovar dublin was 0.6 hours. In the lab scale cattle slurry [semi-] continuous digester at 53° C the average T_{90} values were: *S. enterica* serovar typhimurium at 0.6 hours and *S. enterica* serovar dublin at 0.7 hours. In the lab scale pig slurry batch digester at 53° C the average T_{90} value for *S. enterica* serovar typhimurium was 0.7 hours, while the lab scale pig slurry [semi-] continuous digester at 53° C the average T_{90} for *S. enterica* serovar typhimurium was 0.5 hours. In an additional experiment, Nylon bags of liquid culture of *S. enterica* serovar typhimurium were placed in full-scale cattle slurry digester (35° C). The T_{90} for *S. enterica* serovar typhimurium was 1.6 days. In a study by Wagner and colleagues (2008), in a lab set-up of a thermophilic (50° C) bio-waste (of municipal solids) treating anaerobic digester there was inactivation after the 24 hour sampling period and between the 7 day sampling period.

In a mesophilic anaerobic digester (35-40° C) of vegetable, fruit, and garden waste introduced *S.enterica* serovar typhimurium did not survive during the 21 day digestion period. In one of the six experiments, *S. enterica* serovar typhimurim was not detected at their limit of detection of 1 CFU/mL after 21 days (Termorshuizen et al. 2003). Overall, food waste or agricultural organics added to manure-based digesters may add more nutrients for potential pathogens. In the study conducted by Termorshuizen and colleagues (2003), plant waste organics were not enough to sustain a heavy population of introduced *S. enterica* serovar typhimurium, however, in 1/6 cases there were still cells after 21 days of digestion.

Staphylococcus aureus. *Staphylococcus aureus* has a positive Gram staining reaction and coccus morphology, where cells group together in clusters, and is a facultative anaerobe (Willey et al. 2014). This bacterium has been associated with human and non-human animals in commensal and pathogenic relationships (Willey et al. 2014), while it is also a food borne pathogen. Humans are susceptible to various *S. aureus* infections. As a food borne pathogen, the organism can cause gastroenteritis in humans from pre-formed enterotoxins; in cattle, *S. aureus* can be a causal agent for mastitis (Mitscherlich & Marth 1984). *Staphylococcus aureus* can grow on dairy products, meats, and bakery items (Willey et al. 2014). The major concern surrounding this potential pathogen is that non-human animals could be re-infected if the microbe survives AD treatment and the digested material improperly treated and utilized as animal bedding.

Staphylococcus aureus can participate in hydrolysis (producing amino acids and sugars) and acidogenesis (producing acetate and types of fatty acids) during AD (Amani et al. 2010). *Staphylococcus aureus* has been isolated from a cattle manure slurry based digester (Rabah et al. 2010), as well as in untreated manure, from various farms, between 28-70 days in outdoor barn storage (Mitscherlich & Marth 1984). Because *S. aureus* can participate in both hydrolysis and acidogensis, and has been detected in manure, there is a possibility this organism could survive or replicate itself in an anaerobic digester. In a study conducted by Olsen and Larsen (1987) *S. aureus* was introduced into bench scale mesophilic and thermophilic, batch and [semi-] continuous, cattle and pig slurry anaerobic digesters. In the lab scale cattle slurry batch digester at 35° C the average T_{90} was 1.1 days and *S. aureus* was no longer detectable at days five and six during AD. In the lab scale cattle slurry [semi-] continuous digester at 35° C the average T_{90} was 0.4 days. In the lab scale pig slurry batch digester at 35° C the average T_{90} was 1.3 days, while the in lab scale pig slurry [semi-] continuous at 35° C the average T_{90} was 0.4 days. In the lab scale cattle slurry batch digester at 53° C the average T_{90} was 0.5 hours, while the lab scale cattle slurry [semi-] continuous digester at 53° C the average T_{90} was 0.2 hours. In the lab scale pig slurry batch and [semi-] continuous digesters at 53° C the average T_{90} was 0.6 hours.

Overall: AD of Specific Potentially Pathogenic Bacteria

Enteric bacteria, as well as other potentially pathogenic bacteria, are often found in untreated and treated manure slurry and sludge (Horan et al. 2004; Larsen et al. 1994; Mitscherlich & Marth 1984). Batch and semi-continuous mesophilic AD (35° C) has been found to be able to inactivate/reduce to non-detectable: *Enterococcus faecalis* (Olsen & Larsen 1987), *E. coli* (Olsen & Larsen 1987; Kearney et al. 1993; Horan et al. 2004), *Salmonella* serovars (Olsen & Larsen 1987; Kearney et al. 1993; Horan et al. 2004), and *Staph. aureus* (Olsen & Larsen 1987). However, *Campylobacter. jejuni* has inconsistent results for survival in AD systems. Horan and colleagues (2004) discussed the inconsistency of *C. jejuni* survival in AD studies. Destruction of *C. jejuni* appears to depend on the type of digester and contents of digester**.** Mesophilic AD can be effective, however, in the case of *C. jejuni*, more investigation is required.

Thermophilic AD (≥ 50° C) has overall been found to reduce or inactive various potentially pathogenic microorganisms, such as *C. jejuni* (Olsen & Larsen 1987; Wagner et al. 2008). Overall, secondary digestion is best for destruction/inactivation of potential bacterial pathogens to a non-detectable level. Thermophilic AD (as primary or secondary treatment) could be advantageous for inactivating various potential bacterial pathogens (Sobsey et al. 2006).

Objectives

The overarching objective of this research is to evaluate the survival of select microorganisms in laboratory-scale anaerobic digesters. There are two main objectives of this study.

1. To investigate the survival of five potentially pathogenic bacteria
    a. *Campylobacter jejuni*, *Enterococcus faecalis*, *E. coli*, *Salmonella enterica*, and *Staph. aureus* in a mesophilic bench-scale co-fed AD system by traditional culture methods.
    b. These organisms were chosen as a result of their likelihood of deposition into an anaerobic digester (from animal feces and/or a potential food borne pathogen) or are surrogates for other more harmful potential pathogens.
2. The second objective of this study is to evaluate, through statistical analyses, if there are relationships between surviving bacterial concentrations (CFU/mL) and specific physical-chemical parameters of AD:
    a. internal jar fermenter temperature and pH at sampling period
    b. concentration of VFAs (acetic acid, propanoic acid, isobutanoic acid, butanoic acid, isovaleric acid, and valeric acid at each sampling period.

Hypothesis

If co-fed anaerobic digestion systems results in high VFAs and low pH during acidogenesis then potentially pathogenic bacteria survival will be reduced by these stressful conditions.

Materials and Methods

Percolate Collection and Eudiometer Bath Set Up

Percolate was collected from the University of Wisconsin Oshkosh dry biodigester and dispensed into eudiometer jars according to the German DIN 38414 and VDI 4630 methods (Deutsches Institut für Normung 1985; 2006). In brief, the method calls for dispensing 1.9 L of fresh, strained percolate into eudiometer jars. Jars were placed in an insulated warm water bath set to 38° C (Figures 4 &Figure *5*). Jars and eudiometer cylinders were sealed with lab grade high vacuum grease (Dow Corning®) to prevent environmental gas exchange or internal gas escape. Samples of the fresh percolate were collected and analyzed for dry matter (DM), organic dry matter (oDM), and pH according to DIN EN 12880, DIN EN 12879 and DIN EN 12176 (Deutsches Institut für Normung 1998, 2001, 2004). Percolate starvation (i.e., no organics added) occurred for two weeks before food waste was added to the percolate. The eudiometers containing *Salmonella. enterica* or *Staph. aureus* had 31.0 g (+/- 10%) of food waste added and those containing *Campylobacter jejuni*, *Enterococcus faecalis*, or *E. coli* had 32.2 g (+/- 10%) of food waste added. The amount of food waste added was determined based on the DM calculations (

Figure *6*) that followed method DIN EN 12879. As described in the DIN method, positive control (percolate with microcrystalline cellulose, 5.9 g +/- 10%), negative control (only percolate), and experimental (organism with percolate and food waste) were all run in duplicates for a 28 day period of anaerobic digestion.

Figure 4. Eudiometers in water bath. Photo: S. Johnson

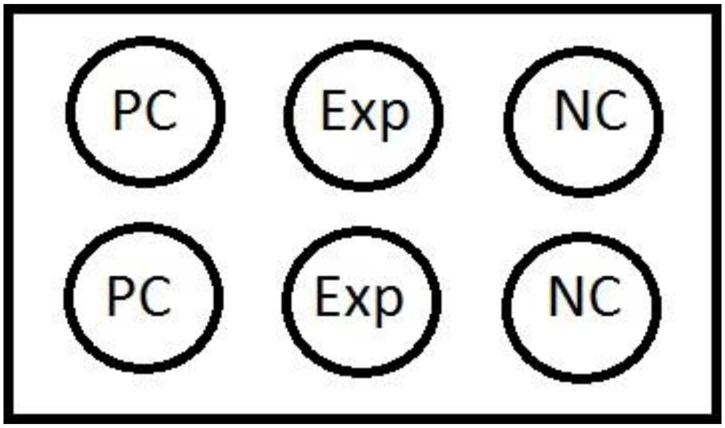

Figure 5. Eudiometer set up in water bath where PC represents positive control; Exp represents experimental; NC represents negative control.

Dry matter

<u>Theoretical Loading Calculation:</u>
First solve for grams/liter (x): 3.0/x = DM%/100
Second solve for Grams (y): y = x(1.9L)

Figure 6. Calculations for dry matter and organic dry matter.

Food Waste Collection, DM, oDM

Food waste was delivered from Sanimax, a food recycling company, to the University of Wisconsin Oshkosh dry biodigester. The delivered food waste was then collected into a five gallon bucket and delivered to the Environmental Research and Innovation Center. The food waste was homogenized in a food processor, dispensed into a clean bin, and mixed to homogenize blended materials (Figure 7). The mixed homogenized food waste was aliquoted into gallon zip lock baggies and stored in a refrigerator between 0 – 6° C. Homogenized food waste was analyzed for dry matter (DM), organic dry matter (oDM), and pH according to DIN EN 12880, DIN EN 12879 and DIN EN 12176 (Deutsches Institut für Normung 1998, 2001, 2004)

Figure 7. Homogenized food waste

Dry matter (DM), otherwise known as total solids (TS), determines the “loading rate” or the amount of sample material that can be introduced into anaerobic digesters (Table 1). DM is determined by the mass of material remaining after water has been removed at 105° C after eight to twelve hours (Sluiter et al. 2008). Organic dry matter (oDM), also known as total volatile solids (TVS), determines the amount of organic material in the sample. The organic material could contribute to methane production via methanogenic archaea. The wet weight, or amount of water, in the sample and inorganic material of the sample cannot contribute towards methane production. The amount of DM, to be loaded into the anaerobic digester, from the sample is determined by the percentage of the whole sample or fresh material (FM).

Table 1. Parameters of materials for anaerobic digestion.

Sample Description	pH	DM (%FM)	oDM (% DM)	oDM (% FM)	Amount of sample added
Percolate (*S. enterica* & *Staph. aureus*)	7.99.	2.8	36.4	1.02	1.9 L
Percolate (*C. jejuni, E. faecalis,* & *E. coli*)	7.65	2.5	47.3	1.19	1.9 L
Food Waste (*S. enterica* & *Staph. aureus*)	4.0	18.4	93	17.11	31.0 g
Food Waste (*C. jejuni, E. faecalis,* & *E. coli*)	4.4	17.7	96.9	17.15	32.2 g
Microcrystalline Cellulose	Not Applicable	96.7	100	96.75	5.9 g

Growth of Organisms for Spiking Eudiometer Jars

Organisms, with the exception of *C. jejuni*, were grown on Brain Heart Infusion (BHI) agar at 35° C for approximately 24 hours. Aseptically, a single isolated colony was inoculated into approximately 40 mL of BHI (Remel) broth contained in a sterile 50 mL conical tube and shaken at 130 rpm at 37° C for approximately 24 hours in duplicates. *Campylobacter jejuni* pure culture was grown on ready-to-use *Campylobacter* Agar with 5 antimicrobials and 10% Sheep Blood (*Campy*-BAP) agar (BD BBL™). The antimicrobials included in the ready-to-use plated medium were amphotericin B, cephalothin, trimethoprim, vancomycin, and polymyxin B. These plates were placed into a sealed gas chamber with one GasPak™ EZ Campy Pouch™ (BD BBL™) for 48 hours at 42° C to create an optimal microaerophilic environment. Aseptically, a single isolated colony was inoculated into approximately 45 mL of Bolton Broth Base + Bolton Broth Selective Supplement (Oxoid) + 10% Laked Horse Blood (Remel) contained in a sterile 50 mL conical tube, to allow for minimal headspace and minimal oxygen introduction, and shaken at 130 rpm at 37° C for approximately 48 hours in duplicates. All cultures were vortexed in their original sterile 50 mL conical tubes at 101.88 *g* for 15 minutes. The culture pellets were centrifuged twice, vortexed to homogenize, and washed three times with 10 mL of sterile phosphate buffer solution (PBS) after centrifugation.

All washed liquid culture solutions were prepared to match a 0.5 McFarland Standard (transmittance of 0.5 at 540 nanometers (nm)). Aquamate spectrometer by Thermo Electron Corporation was utilized for optical density readings. The spectrometer was zeroed with PBS in an appropriate plastic cuvette and the cuvette was rinsed three times between spectrometer readings with PBS. Three milliliters of initial washed culture were aseptically dispensed into a cuvette for an initial reading. Aseptically, a portion of the washed culture was dispensed in a new sterile 50 mL conical tube containing 20 mL of PBS (this became the spiked culture tube). If solution (spike tube) was below 0.5 abs at 540 nm, washed culture solution was aseptically added. If solution (spike tube) was above 0.5 abs at 540 nm, sterile PBS was added to reach an absorbance of approximately 0.5 (Table 2). The cuvette was rinsed at least three times with PBS between readings and different cultures. Once spiked culture tubes reached an absorbance of approximately 0.5, the tubes were vortexed and 4 mL of the spiked culture was added to the appropriate jar. The remaining spiked culture solution was used for dilution series, spread plating method. A dilution series of 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-7}, with sterile PBS were conducted for *Enterococcus faecalis* and *E. coli*. The final dilutions (10^{-4}, 10^{-6}, and 10^{-8}) were plated in duplicate. A dilution series of 10^{-1}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} with sterile PBS were conducted for *Salmonella enterica* and *Staph. aureus*. The final dilutions (10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}) were plated in duplicate. A dilution series of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were conducted for *C. jejuni* and final dilutions plated were 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} (in duplicate). Each organism was spread plated onto appropriate medium, at the appropriate incubation times and temperatures. After incubation, colonies were counted to calculate colony forming units per milliliter, CFU/mL (Table 2, Table 4).

Table 2. Optical density and CFU/mL of spiked cultures.

Organism	Optical Density at 520 nanometers (nm)	Average CFU/mL
Campylobacter jejuni	0.459	7.50E+02
Enterococcus faecalis	0.554	3.89E+08
Escherichia coli	0.503	1.03E+08
Salmonella enterica	0.503	1.10E+07
Staphylococcus aureus	0.495	8.56E+07

Sampling Eudiometer Jars

Before sampling, all eudiometers were measured for biogas volume. If duplicate eudiometers were both greater than or equal to 300 mL in gas volume, they were sampled for biogas quality using a gas meter (Gas Data GMF Series). Environmental air temperature, water bath temperature, and barometric pressure were recorded before gas and sample collection. The eudiometer was opened, separated from the jar and contents were measured for pH and temperature with an Oakton pH Testr 30 portable probe (Eutech Instruments). The probe was calibrated before use and rinsed with Milli-Q water in between each eudiometer jars. After pH and temperature measurements were recorded, jar contents were gently stirred with a sterile serological pipette. Samples from each jar were dispensed into individual 17 x 100 mL sterile polystyrene culture tubes (Fisher*brand*®) for microbial analysis (2.5 mL of sample) and 50 mL screw top centrifuge tubes (Evergreen) for VFA analysis (25 mL of sample). After sample collection, jars were resealed and zeroed for biogas volume. Volatile fatty acid samples were stored at 0 – 6° C until preparation for gas chromatography. Jars were sampled: before jars were spiked with their appropriate organisms (time zero), within 15 minutes of time zero (0.01), and days one, two, three, seven/eight, 14, 21, and 28, the final day, into anaerobic digestion.

Volatile Fatty Acid Analysis with Gas Chromatography

Samples for VFA analysis were processed and prepared for gas chromatography (GC) with a method based on Determination of Volatile Fatty Acids with In-house Method at Schmack Laboratories in Schwandorf, Germany (ERIC 2012) and method development at the ERIC Lab at the University of Wisconsin Oshkosh utilizing an Agilent 6890 with FID detector and autosampler. The volatile fatty acids quantified were acetic acid, propanoic acid, butanoic acid, iso-butanoic acid, valeric acid, and iso-valeric acid. The standards and their ranges for VFA analysis are described briefly in Table 3.

Table 3. Composition of volatile fatty acid standards.

Standard Name	Composition	Range of Detection
Blank	Milli-Pore water and 20% formic acid	No VFAs should be detected.
100 part per million (ppm) Standard	100 ppm of acetic acid, propanoic acid, butanoic acid, iso-butanoic acid, valeric acid, and iso-valeric acid and 20% formic acid	100 ppm +/- 10% (990-110 ppm of each VFA)
500 ppm Standard	500 ppm of acetic acid, propanoic acid, butanoic acid, iso-butanoic acid, valeric acid, and iso-valeric acid and 20% formic acid	500 ppm +/- 10% (450-550 ppm of each VFA)

Membrane Filtration and Microbial Analysis

Membrane filtration utilized a modified United States Environmental Protection Agency (USEPA) Method 1603 (2002) for recovery of microorganisms from the test systems. Autoclaved filtration base holders were placed in a filtration manifold attached to a vacuum pump (Figure 8). Sterile 47 mm, 0.45 µm mixed cellulose ester gridded filters (Millipore Ltd) were aseptically placed (ethanol and flame sterilized forceps) onto to the filtration base. Autoclaved filtration funnels were placed atop the base and secured with a spring clamp. Funnels were covered with autoclaved tin foil to maintain vacuum and to prevent debris from entering the unit. The filters were pre-wet with 5 mL of sterile PBS, vacuumed through the filter, and then 10 mL of PBS was dispensed.

Each sample tube was vortexed for 10 seconds before 1 mL of sample was dispensed into sterile glass test tube containing 9 mL of sterile PBS creating a 10^{-1} dilution. A dilution series, determined by the organism's growth from previous sampling periods, were made from the 10^{-1} dilution tube. The minimum dilution was 10^{-1} and the maximum dilution was 10^{-7}. The dilution tubes were vortexed for 10 seconds before 1 mL of the diluted sample was dispensed into the filtration unit containing 10 mL of PBS. The diluted sample in duplicate was filtered via vacuum, always with the highest dilution filtered first. Between duplicates the filtration unit, including the bottom of the filtration funnel, were rinsed with at least 100 mL of Milli-Q water. Between organisms, the filtrations unit were rinsed with 100 mL of Milli-Q and then exposed to ultraviolet light for five minutes. Filters were aseptically placed onto a medium appropriate for the organism under investigation, utilizing sterilized forceps (70 – 95% ethanol and Bunsen burner). Plates were incubated upside down for a specific time and temperature for each organism.

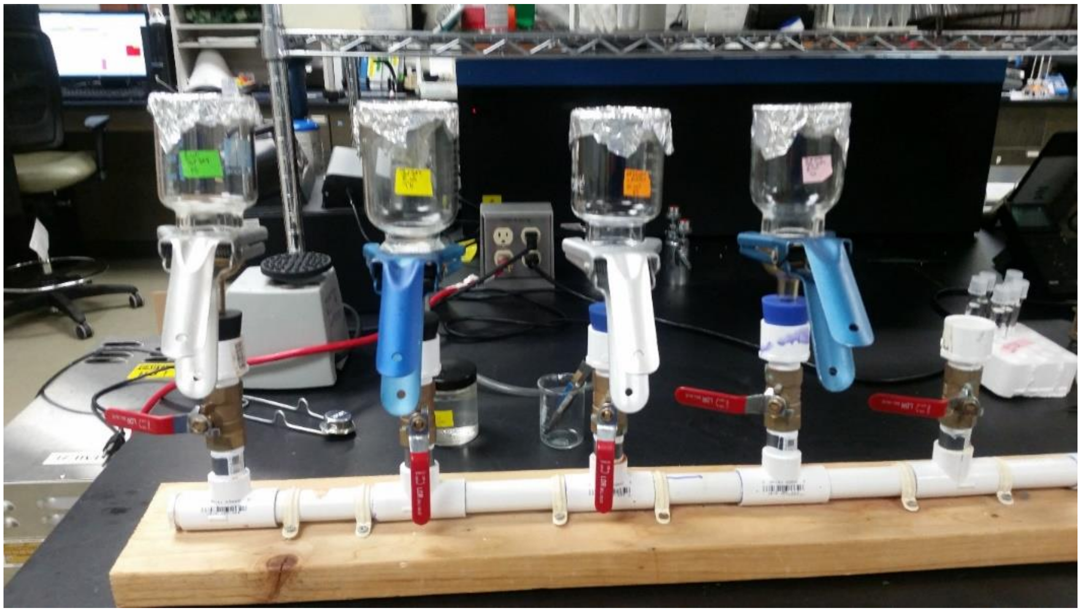

Figure 8. Vacuum filtration set up. *Photo: S. Johnson*.

Campylobacter jejuni**.** Filters from the jars spiked with *C. jejuni*, and negative control sample filters, were grown on Campylobacter Agar with 5 antimicrobials and 10% sheep blood agar (BD BBL™). These plates were placed upside down into a sealed a gas chamber with one GasPak™ EZ Campy Pouch™ (BD BBL™), to create an optimal microaerophilic environment, for 48 hours at 42° C. After 48 hour suspected colonies were counted to calculate CFU/mL. The spiked culture exhibited gray and mucoidal colonies and exhibited swarming on the Campy medium. Suspected colonies were streaked for isolation on Campy medium, placed in a sealed chamber with a gas pack, and incubated for 48 hours at 42° C. Gram stain reaction and cell morphology under a microscope were used to determine if the suspected colonies were *C. jejuni*. *Campylobacter jejuni* have a negative Gram stain reaction and a curved rod morphology (Willey et al. 2014).

Enterococcus **spp.** Filters from the jars spiked with *E. faecalis*, and negative control sample filters, were grown on m-Enterococcus agar (Acumedia Manufacturers, Inc). The plates were incubated for 48 hours at 35° C. After 48 hours, suspected colonies, red and maroon, were counted to calculate CFU/mL. Suspected colonies, one to three per dilution plate, were streaked for isolation and incubated for 48 hours at 35° C. After 48 hours, isolated colonies from these plates were selected for biochemical testing. Aseptically, a single colony was inoculated into 100 mL of a sterile PBS in a sterile bacteriological bottle. Enterolert® enzyme substrate (IDEXX) was dispensed into the bottle, shaken to dissolve, and incubated at 41° C for 24 hours (based on method Active

Standard ASTM D6503). After 24 hours, samples positive for *Enterococcus* spp. fluoresced blue under UV light while negative results did not fluoresce.

Escherichia coli. Filters from the jars spiked with *E. coli*, and negative control sample filters, were grown on modified m-TEC agar (Difco™ & BBL™), pH modified aseptically to 7.3 +/- 0.2. The plates were incubated for 2 hours at 35° C, to help resuscitate stressed or injured cells, and then at 44° C for 22 hours to select for E. coli (US EPA 1603 2002/ Difco™ & BBL™ 2nd Edition Manual). After 24 hours, suspected colonies, purple/magenta, were counted to calculate CFU/mL. Suspected colonies, one to three dilution plate, were streaked for isolation and incubated for 24 hours at 35° C. After 24 hours, isolated colonies from these plates were selected for biochemical testing. Aseptically, a single colony was inoculated into 100 mL of a sterile PBS in a sterile bacteriological bottle. Colilert® or Colilert-18® enzyme substrate (IDEXX) was dispensed into the bottle, shaken to dissolve, and incubated at 35° C for 18 hours if Colilert-18® was utilized or 24 hours if Colilert® was used. Isolates positive for *E. coli* were yellow and fluoresced bluish-green and negative samples did not fluoresce.

Salmonella enterica. Filters from the jars spiked with *S. enterica*, and negative control sample filters, were grown on Hektoen Enteric agar (Acumedia Manufacturers, Inc). The plates were incubated for 48 hours at 35° C. The company recommended 24 hours, however, this strain of *S. enterica* grew slowly and did not exhibit a full biochemical reaction after 24 hours. After 48 hours, suspected colonies, green and blue green with black centers from hydrogen sulfide production, were counted to calculate

CFU/mL. Suspected colonies, one to three per dilution plate, were streaked for isolation and incubated for 48 hours at 35° C. After 48 hours, isolated colonies from these plates were selected for biochemical testing using API 20E biochemical test strip (bioMérieux). Following the company instructions, aseptically, a single colony was inoculated into 5 mL of a sterile 0.85% saline in sterile culture tubes, vortexed to homogenize, and aseptically dispensed into the strip wells. Biochemical results were logged into the APIweb™ service online database.

Staphylococcus aureus. Filters from the jars spiked with *Staph. aureus*, and negative control sample filters, were grown on Mannitol Salt Agar (Acumedia Manufacturers, Inc). The plates were incubated for 24 hours at 35° C. After 24 hours, suspected colonies, yellow with an agar color change to yellow from fermented mannitol, were counted to calculate CFU/mL. Suspected colonies, one to three per dilution plate, were streaked for isolation and incubated for 24 hours at 35° C. Gram stain reaction and cell morphology under a microscope were used to determine if the suspected colonies were *S. aureus*. *Staphylococcus aureus* cells have a positive Gram reaction, are round, and cluster in grape-like groups.

Statistics

Raw CFU/mL data were transformed to $\log_{10}$ CFU/mL. Means and standard deviations were calculated with Microsoft Excel. Statistical analyses for linear regressions were analyzed with R Studio (version 3.2.3). Graphs were made with Microsoft Excel and R Studio; tables were made in Microsoft Word.

Results and Discussion

The overall objective of this work was to expose potentially pathogenic microorganisms to model anaerobic digester (AD) test systems to evaluate their survival in an AD system co-fed with food waste. Linear regressions were utilized to determine relationships between $\log_{10}$ CFU/mL for each pathogen with system parameters: temperature, pH, and Total VFAs. The potential relationships between the growth of these organisms (*Campylobacter jejuni*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus*) with AD parameters could be utilized as ways to manager a digester with pathogen control. If co-digestion can safely and effectively occur, specifically to reduce pathogen load and produce biogas, there are more options for organics that were destined for a landfill.

Table 4. Theoretical inoculation and concentration in eudiometer system for each test organism.

Organism	Average Inoculum	Total Added Inoculum	Concentration CFU/mL after mixing	Log_{10} CFU/mL transformed after mixing
C. jejuni	7.50E+02	3.00E+03	1.58E+00	2.87E+00
E. faecalis	3.89E+08	1.55E+09	8.18E+05	5.91E+00
E. coli	1.03E+08	4.12E+08	2.17E+05	5.34E+00
S. enterica	1.10E+07	4.40E+07	2.32E+04	4.4E+00
S. aureus	8.56E+07	3.42E+08	1.80E+05	5.26E+00

Campylobacter jejuni

The *C. jejuni* starting inoculum did not succeed in establishing itself in the test system (Figure 9). No cells could be cultured through membrane filtration at time zero or any sampling point during the 28 day AD period. The starting inoculum was able to grow on Campy agar in a microaerophilic environment. The growth before introduction into the test system and lack of growth in the test system indicated the starting inoculum was viable but did not have the ability to survive in the test system. In two other experiments (data not shown), *C. jejuni* was not able to grow or be isolated in the same test system set up of AD conditions. In one of the two other preliminary experiments before this experiment, the *C. jejuni* test system samples were enriched and incubated in Bolton Broth + Supplement + Laked Horse Blood before membrane filtration. Another potential explanation for no growth could be that the cells were stressed before entering the system. This organism is microaerophilic (Willey et al. 2014); during the washing procedure the cells could have been exposed to prohibitive amounts of oxygen, which would negatively influence survival and growth. *Campylobacter jejuni* washed inoculum was tested in sterile PBS and autoclaved percolate; samples from the sterile PBS and sterile percolate were isolated using a dilution series and membrane filtration. The organism was able to grow and be isolated from the sterile PBS and autoclaved percolate (data not shown). These instances of no growth from the test system versus growth in sterile systems could indicate that competition could be a factor for survival. However, the three different conditions all had different pH levels (data not shown) while the temperature remained near/approximately 38° C. pH should have a role in survival however, it is likely that stress and potential competition have more substantial roles in *C. jejuni* survival; *Campylobacter jejuni* was exposed to the same amount of initial stress before entering the three different test systems (percolate + food waste, autoclaved percolate, and autoclaved PBS). Therefore competition could be a larger factor in *C. jejuni* survival. Overall, the preliminary experiments, main experiment (Figure 9), and follow up experiment in sterile test environments demonstrate *C. jejuni* most likely cannot survive co-digestion AD systems.

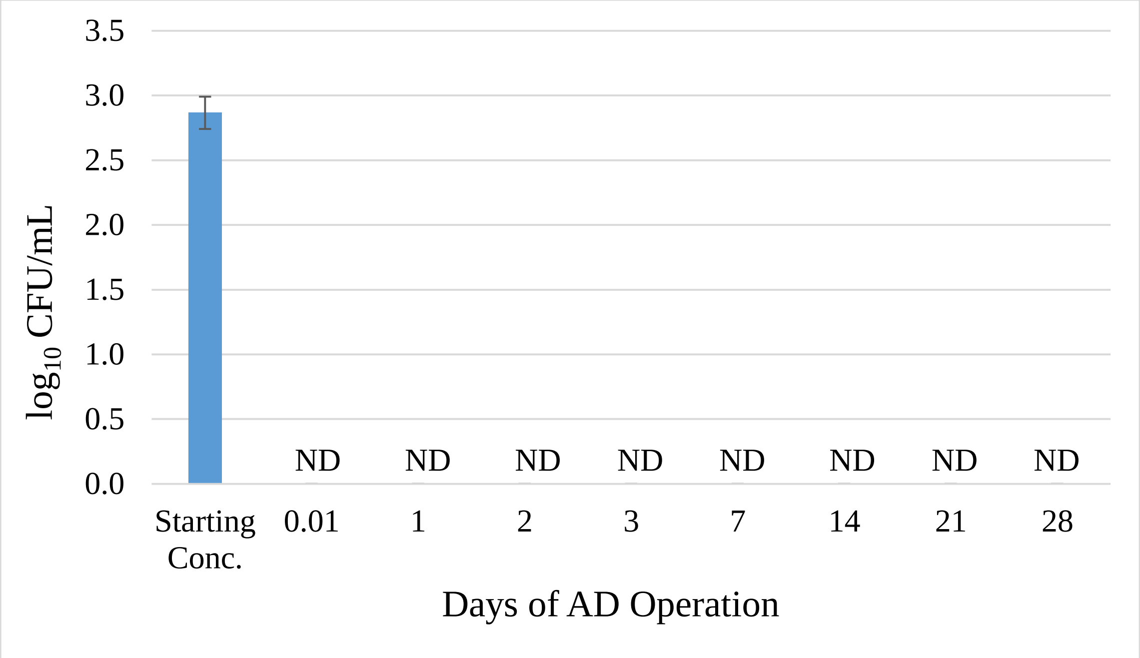

Figure 9. *Campylobacter jejuni* reduction in test system eudiometer eudiometers. Growth is average log10 transformed CFU/mL. Time zero sampling time is not included because *C. jejuni* is not a native organism to percolate. Error bars are +/- standard deviation. “ND” represents non-detect, where ND is < 1 CFU/mL.

Enterococcus faecalis

The starting inoculum of *E. faecalis* was able to establish itself in the test system. *Enterococcus* species (spp.) are native to percolate and were able to continue a population in the negative control system, throughout most of the AD period (Figure 10). Time zero represents the average colony growth before the experimental eudiometers were inoculated with *E. faecalis* and the *Enterococcus* spp. average growth before the negative control eudiometers were sampled more frequently (Figure 10). *Enterococcus* spp. were not detected in the negative control eudiometers on days two and 28 of AD. The cells may not have been detectable on day two of AD as a result of sampling error, cellular stress, or perhaps a change in the population. However, since *Enterococcus* spp. were detected on day three of AD, the lack of CFU/mL is possibly a sampling error. *Enterococcus* spp. were not detected in the experimental eudiometers on days 21 and 28 of AD. Experimental enterococci may have dropped to a level of ND after day 14 as a result of a pH spike (Figure *11*). The experimental test systems (percolate + food waste + *E. faecalis* inoculum) were able to reduce the introduced and native *Enterococcus* species to non-detect after 21 days of AD (the detection range is between 0 and 250 colonies on a 47 mm filter).

There was background flora on *Enterococcus* spp. sample plates. The manufacturer instructions stated that *Enterococcus* colonies should be round red to maroon in color. The starting inoculum for *E. faecalis* were round maroon colonies. Over the course of the experiment, colonies that tested positive as an *Enterococcus* species changed colors (from red, dark maroon/purple, maroon, pink, and maroon with pink halos). According to the manufacturer (Acumedia®/Neogen®), maroon and light/dark red colonies are considered enterococci. The different colored colonies were streaked for isolation and tested using Enterolert®. Reasons behind the color change could be: a change in population species, a change in native and non-native populations, and/or a change in population biochemical reactions/metabolism of Triphenyl Tetrazolium Chloride (TTC) in the medium. The exact answer would be difficult to determine without further testing, however, environmental isolates may behave differently than do standard laboratory strains.

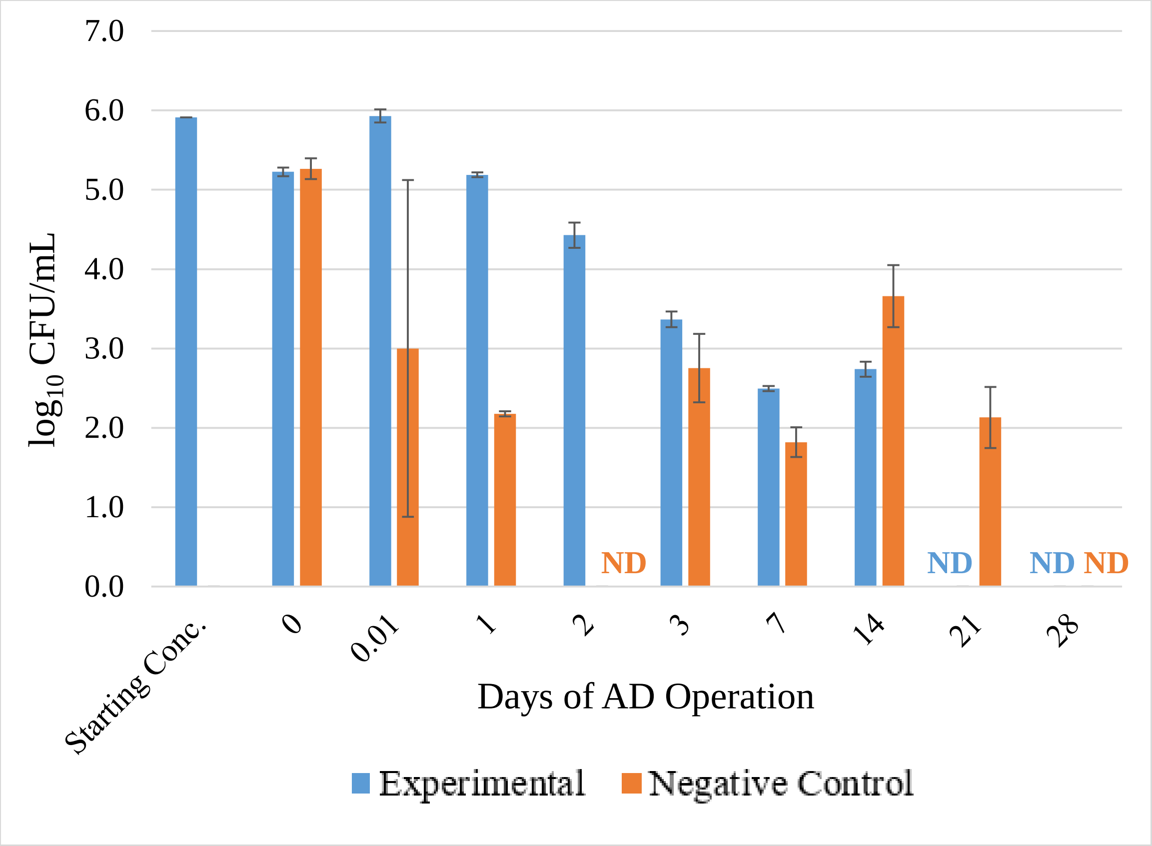

Figure 10. *Enterococcus* species reduction in test system eudiometer eudiometers. The starting inoculum “Starting Conc.” is placed in graph for reference. Experimental *Enterococcus* reduction is displayed in blue while the negative control *Enterococcus* reduction is displayed in orange. “ND: represents non-detect, where ND is < 1 CFU/mL. Growth is the average $\log_{10}$ transformed CFU/mL. Error bars are +/- standard deviation. Time Zero represents the sampling time before the starting concentration of *E. faecalis* was introduced into the experimental eudiometers (*E. faecalis* + homogenized food waste + percolate).

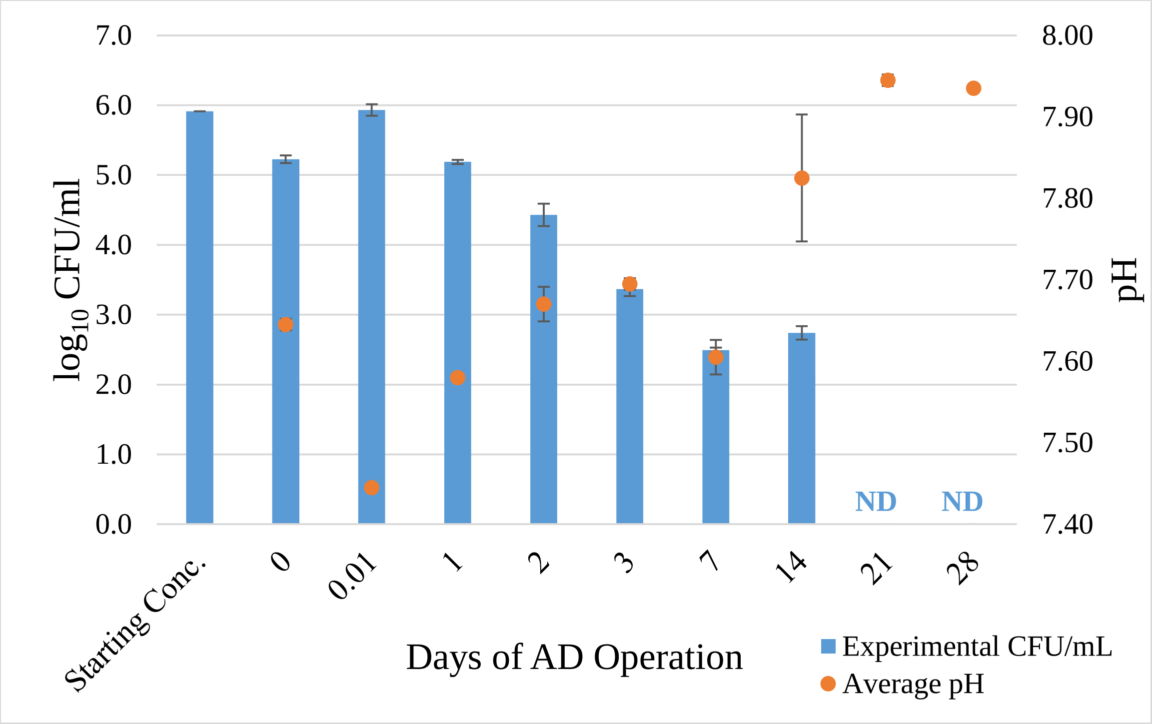

Figure 11. Average log$_{10}$ transformed CFU/mL and average pH for experimental *Enterococcus* species and test system at sampling time points. *Enterococcus* CFU/mL is displayed by blue bars with the average pH of the system displayed as orange dots. Error bars are +/- standard deviation. “ND” represents non-detect, where ND is < 1 CFU/mL.

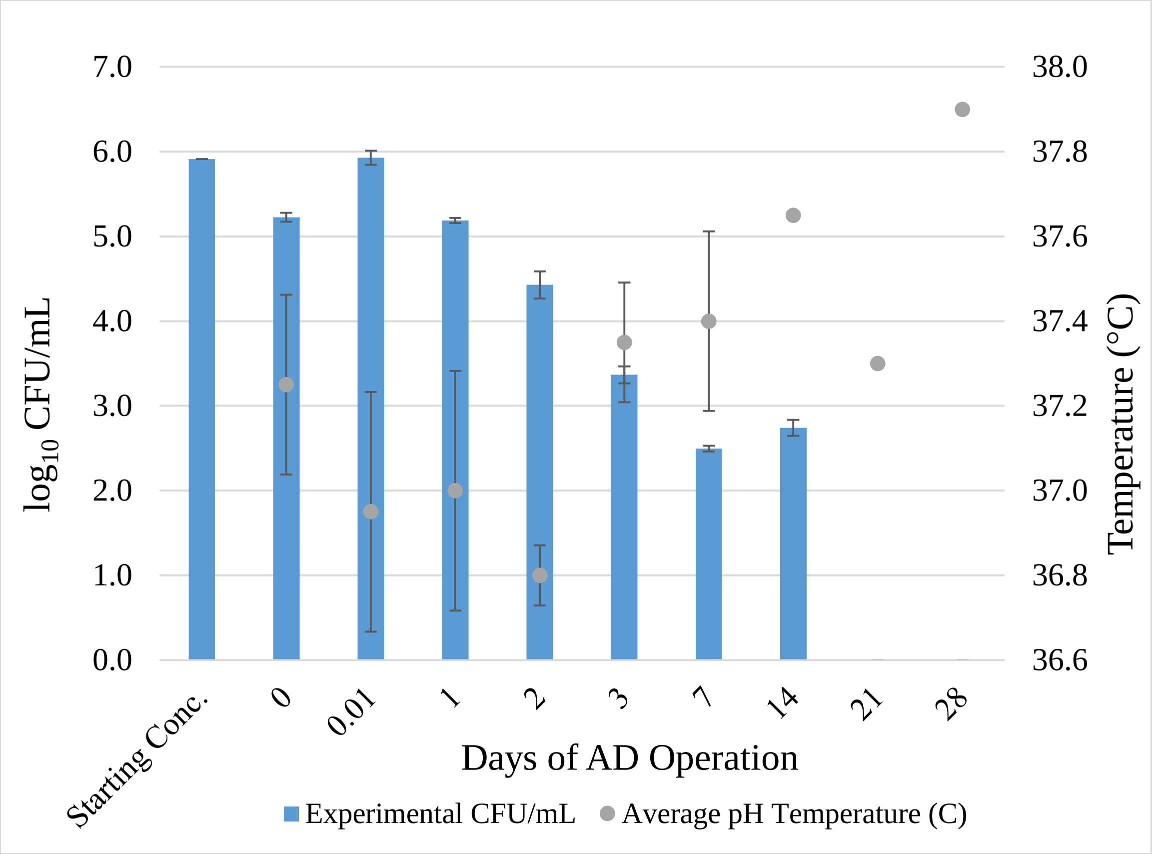

Figure 12. Average log10 transformed CFU/mL and average temperature for experimental Enterococcus species and test system at sampling time points. Enterococcus CFU/mL is displayed by blue bars with the average temperature of the system displayed as grey dots. Error bars are +/- standard deviation. “ND” represents non-detect, where ND is < 1 CFU/mL.

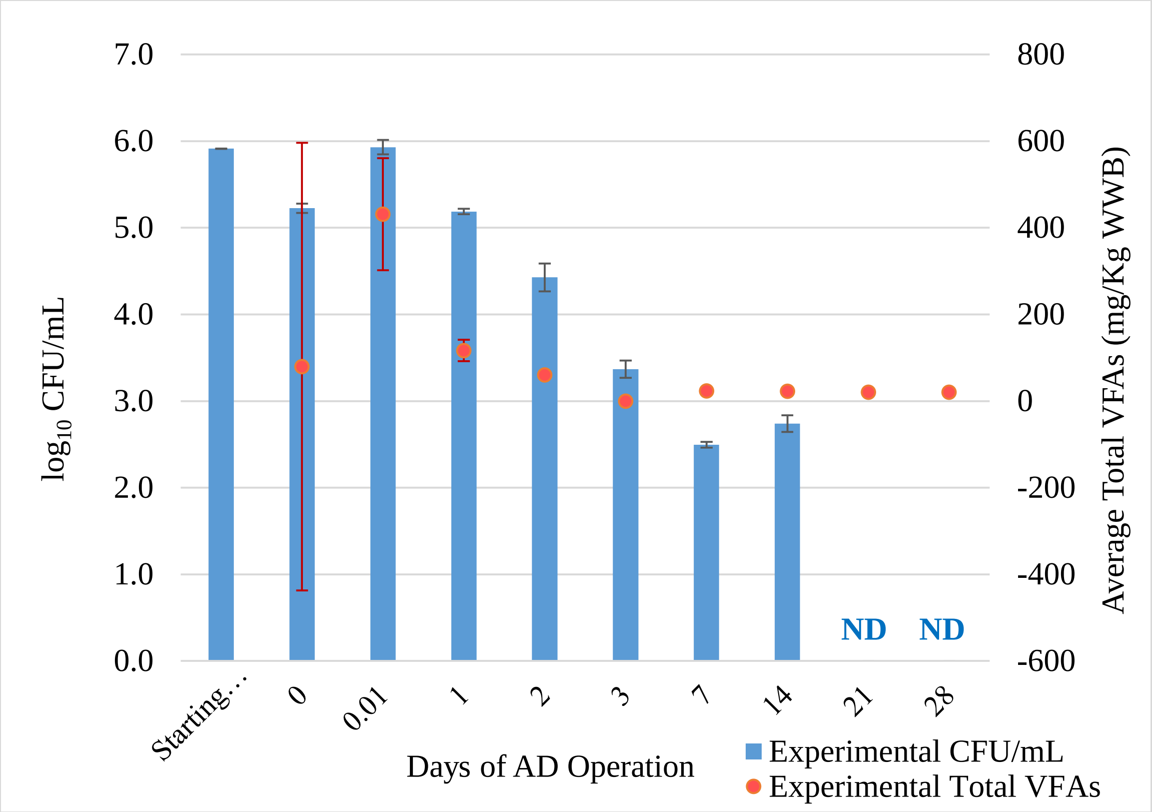

Figure 13. Average $\log_{10}$ transformed CFU/mL and average Total VFAs for experimental *Enterococcus* species and test system at sampling time points. The starting inoculum "Starting Conc." is placed in graph for reference. *Enterococcus* CFU/mL is displayed by blue bars with the average Total VFAs of the system displayed as orange dots. Error bars are +/- standard deviation. "ND" represents non-detect, where ND is < 1 CFU/mL.

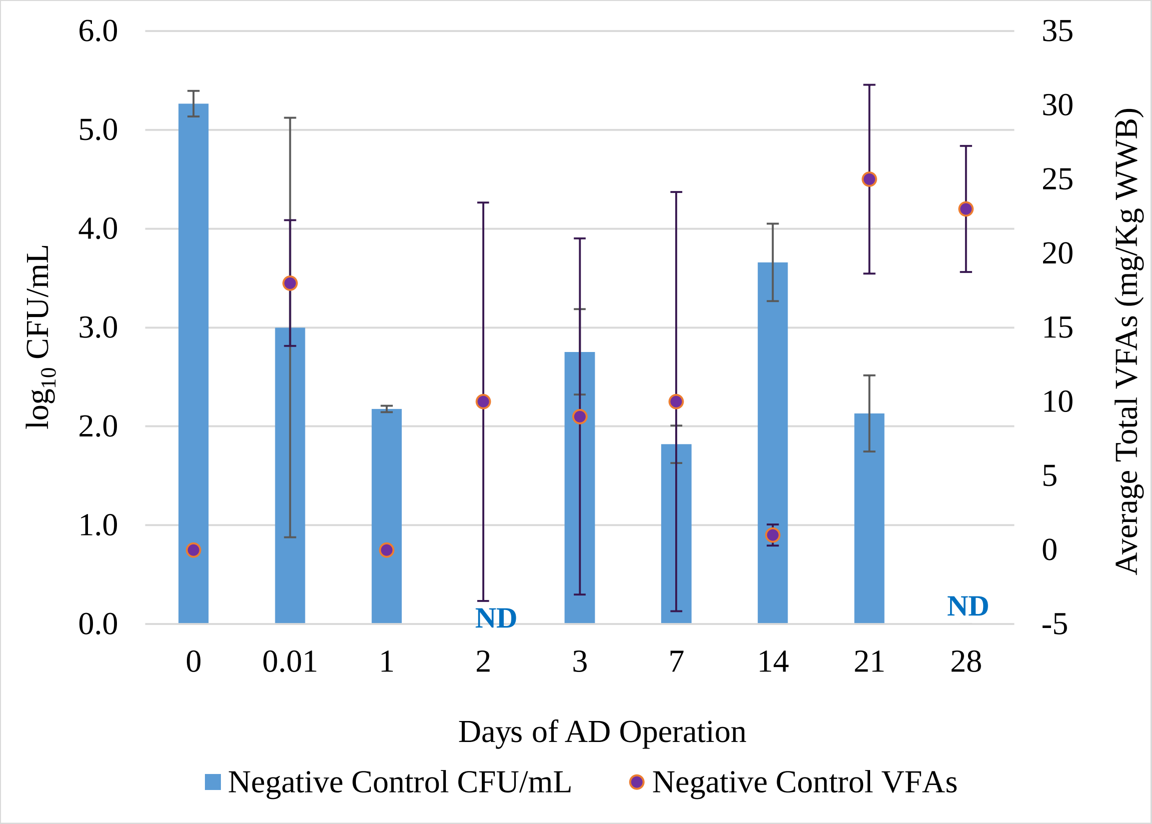

Figure 14. Average $\log_{10}$ transformed CFU/mL and average Total VFAs for negative control *Enterococcus* species and test system at sampling time points. *Enterococcus* CFU/mL is displayed by blue bars with the average Total VFAs of the system displayed as purple dots. Error bars are +/- standard deviation. “ND” represents non-detect, where ND is < 1 CFU/mL.

Enterococci are relatively hardy organisms. They can survive temperatures ranging from 10 – 45°C up to a pH of 9.6, have multiple metabolic options, and are aerotolerant; they have been observed as commensals in mammals and can be opportunistic pathogens in humans (Ramsey et al. 2014; Willey et al. 2014; Byappanahalli et al. 2012). Their ability to survive and replicate in various environmental conditions demonstrate why they can survive both experimental and negative control test systems. Overall, in both experimental set ups and negative controls, *Enterococcus* spp. were reduced to non-detectable over a 28 day period of AD (Figure 10).

Escherichia coli

The *E. coli* inoculum in the experimental eudiometers were detectable through culture methods through two days of AD; the organism was not detected days three through 28 of AD (

Figure *15*). In two past experiments, *E. coli* was a native in the percolate. It is thought that the lack of presence may be due to seasonal changes in the percolate itself. *Escherichia coli* has been detected in manure-based digesters and has the potential to be a part of AD (Larsen et al. 1994; Horan et al. 2004; Rabah et al. 2010). However, as discussed in the introduction, non-native *E. coli* strains have been reduced in other AD systems (Olsen & Larsen 1987; Kearney et al. 1993; Horan et al. 2004; Wagner et al. 2008). Overall, three days of AD co-digestion of percolate and food waste was sufficient to reduce the *E. coli* inoculum to non-detect.

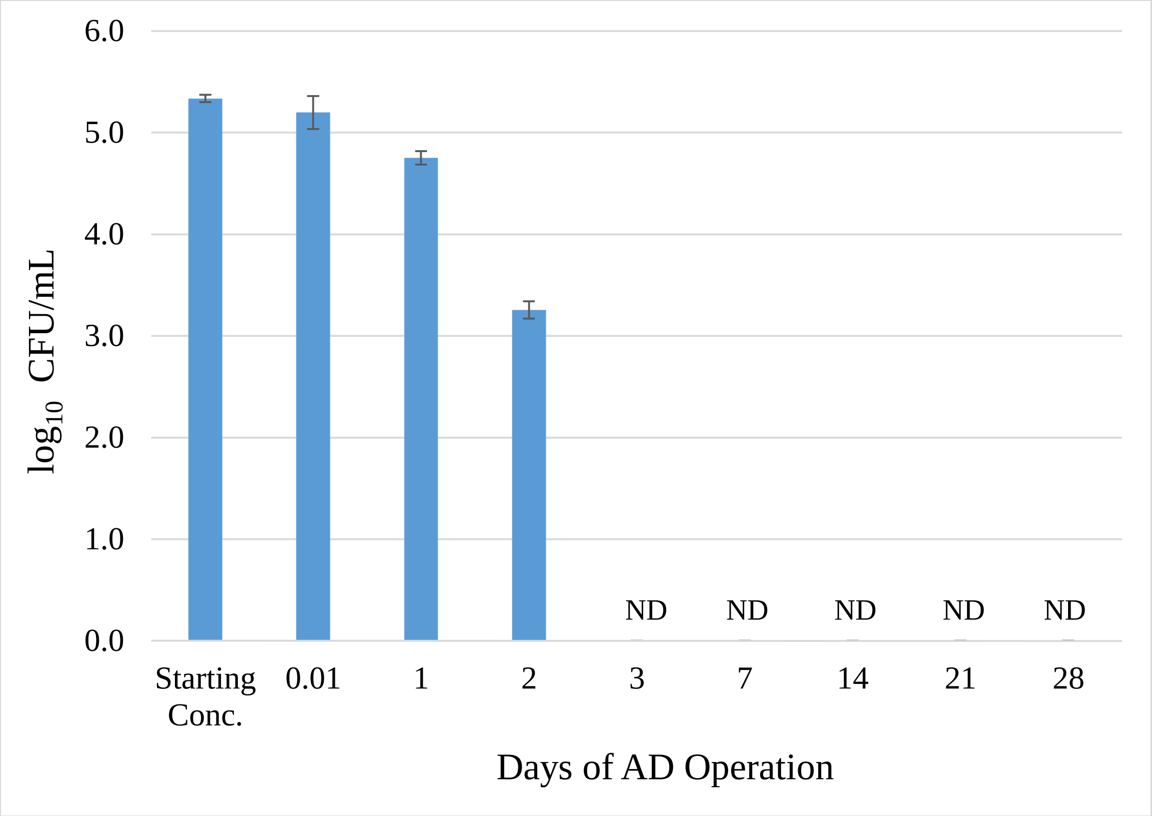

Figure 15. *Escherichia coli* reduction in test system eudiometer eudiometers. The starting inoculum “Starting Conc.” is displaced for reference. Growth is average $\log_{10}$ transformed CFU/mL. Error bars are +/- standard deviation. Time zero sampling time is not included because *E. coli* is not a native organism to percolate. “ND” represents non-detect where ND is < 1 CFU/mL.

Salmonella enterica

The *S. enterica* inoculum in the experimental eudiometers were detectable through culture methods through one day of AD; the organism was not detected days two through 28 of AD (

Figure *16*). Various serovars of *Salmonella* have been detected in manure based AD (Larsen et al. 1994; Rabah et al. 2010). In this system, two days of AD co-digestion of percolate and food waste was sufficient to reduce the *S. enterica* inoculum to non-detectable.

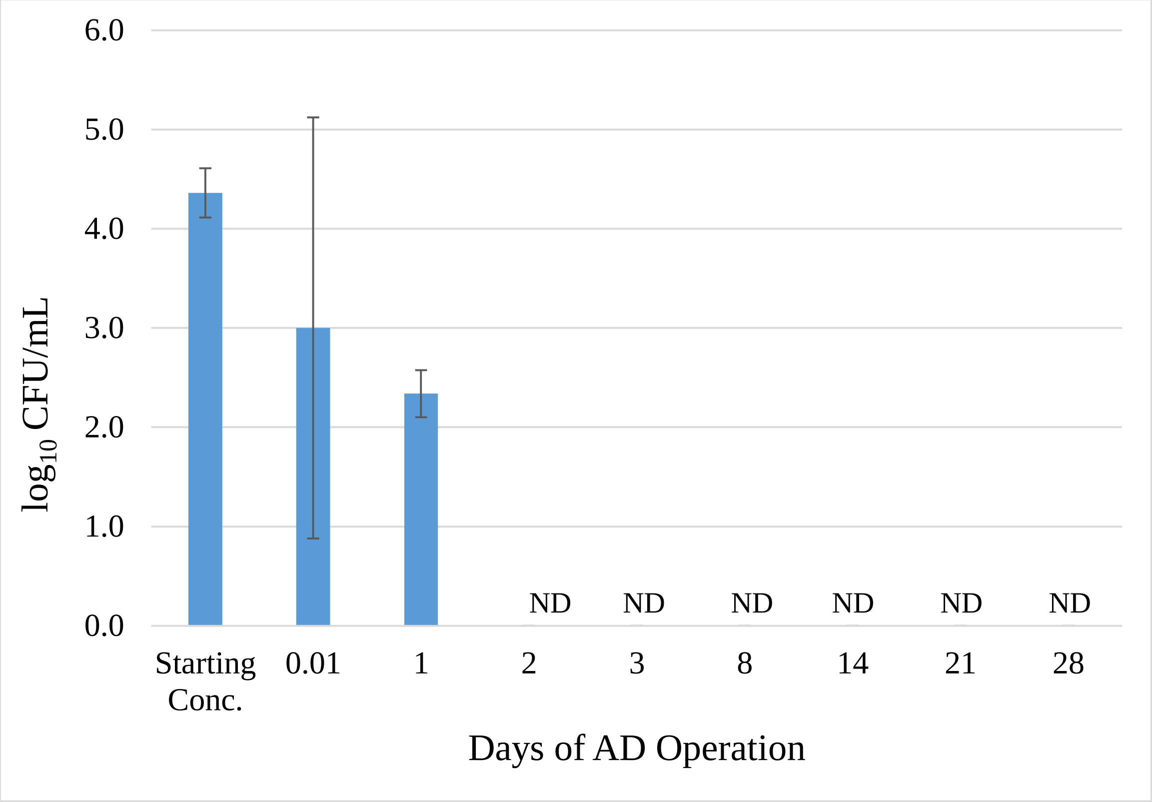

Figure 16. Reduction of *S. enterica* in eudiometers. The starting inoculum “Starting Conc.” is displaced for reference. Growth is average $\log_{10}$ transformed CFU/mL. Error bars are +/- standard deviation. Time zero sampling time is not included because *S. enterica* is not a native organisms to percolate. “ND” represents non-detect, where ND is < 1 CFU/mL.

Staphylococcus aureus

The *S. aureus* inoculum in the experimental eudiometers were detectable through culture methods through 0.01 days (approximately 15 minutes) of AD; the organism was not detected days one through 28 of AD (

Figure *17*). Although *S. aureus* has the potential to establish itself in an AD system (Amani et al. 2010; Rabah et al. 2010), one day of AD co-digestion of percolate and food waste was sufficient to reduce the *S. aureus* inoculum to non-detect in this AD system.

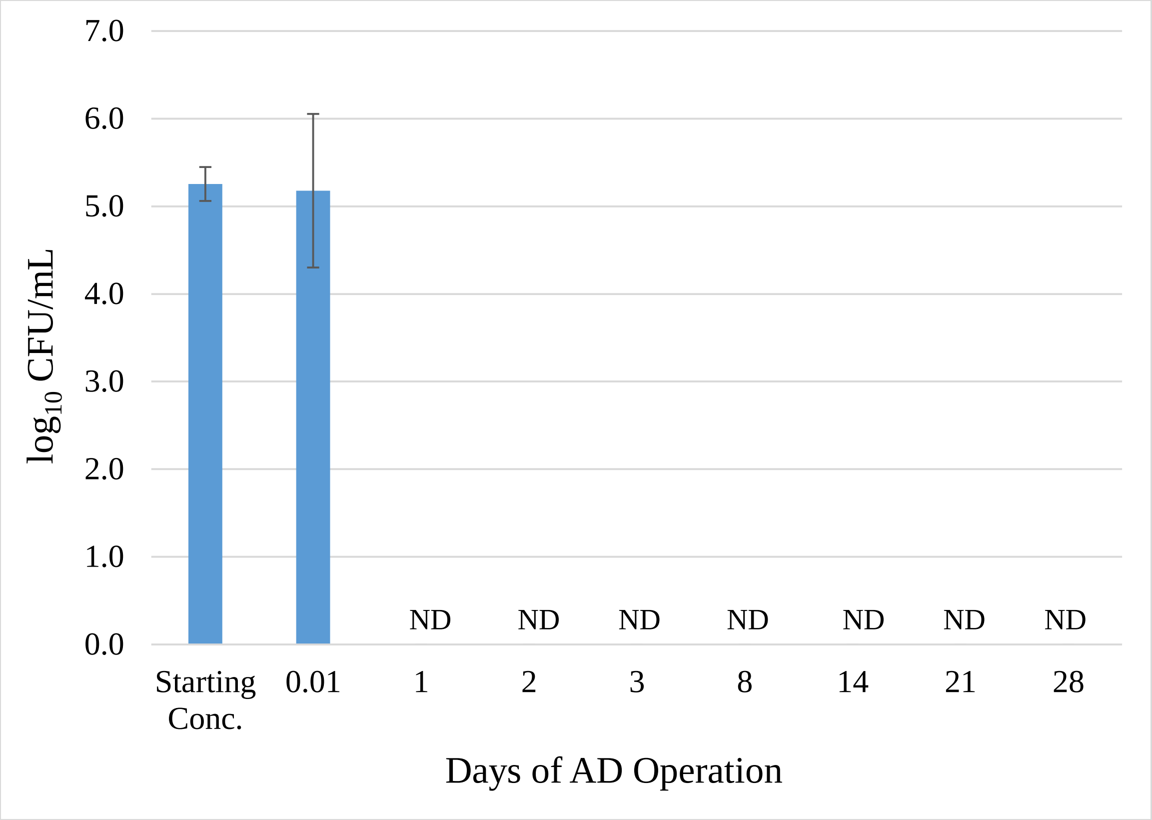

Figure 17. Reduction of *S. aureus* in eudiometers. The starting inoculum “Starting Conc.” is displaced for reference. Growth is average $\log_{10}$ transformed CFU/mL. Error bars are +/- standard deviation. Time zero sampling time is not included because *S. aureus* is not a native organism to percolate. “ND” represents non-detect, where ND is < 1 CFU/mL.

***Enterococcus* Viability and System Environmental Parameters**

Linear regressions of $\log_{10}$ colony forming units per milliliter (CFU/mL) of *Enterococcus* species in the experimental and negative control systems were analyzed with R Studio (version 1.0.136). These linear regressions (Table 5-9) were conducted to determine if there was a relationship between *Enterococcus* spp. $\log_{10}$ CFU/mL in the experimental and negative control systems with: pH, temperature, and Total VFAs. The F statistic (Appendix C) was obtained using methods described in Biostatistical Analysis fourth edition by Jerrold H. Zar (Zar 1999). The null hypotheses are that there are not relationships between $\log_{10}$ CFU/mL and: time, pH, or temperature for experimental and negative control samples. The alternative hypotheses are that there are relationships between $\log_{10}$ CFU/mL and: time, pH, or temperature for experimental and negative control samples. Linear regressions were only appropriate for *Enterococcus* experimental and negative control because there were more sampling points with growth to conduct more statistical analyses. The other organisms were no longer detectable early within the AD time period, resulting in a smaller data set, and those data points would be interpreted as zeros in the statistical program.

The actual F statistic, a value used in statistical analyses to determine the probability that a null hypothesis is true, for all three experimental *Enterococcus* spp. linear regressions were greater than the calculated F statistics (Zar 1999). Therefore, the null hypothesis was rejected, meaning there were statistically significant relationships ($p < 0.05$) between/with $\log_{10}$ CFU/mL and: time, pH, and temperature individually.

The actual F statistic for all three negative control *Enterococcus* spp. linear regressions were not greater than the calculated F statistics. Therefore, the null hypothesis was not rejected, meaning there were no significant relationships ($p < 0.05$**)** between $\log_{10}$ CFU/mL and: time, pH, and temperature (Table 5-9).

Linear regressions for *Enterococcus* spp. experimental $\log_{10}$ CFU/mL and: time, pH, and temperature all displayed a statistically significant relationship ($p < 0.05$) relationship between $\log_{10}$ and the other three variables (Table 5). All linear regressions for *Enterococcus* spp. Negative Control $\log_{10}$ CFU/mL and: time, pH, and temperature did not show a statistically significant relationship (Table 5). Experimental $\log_{10}$ CFU/mL with pH displayed the smallest p value at 2.232E-07 with the second highest R^2 value at 0.8213. Meaning, $\log_{10}$ CFU/mL and pH likely have a strong significant relationship with each other. Experimental $\log_{10}$ CFU/mL and Time displayed the second smallest p value at 1.028E-08 with a highest R^2 value at 0.8779.

Table 5. Linear Regressions for Experimental and Negative Control *Enterococcus* spp. $\log_{10}$ CFU/mL with: time, temperature, and pH.

	p value **(alpha = 0.05)**	R^2 value	(Estimated) Relationship
Experimental $\log_{10}$ CFU/mL and Time	**1.028E-08**	**0.8779**	-0.19299 (**Negative)**
Negative Control $\log_{10}$ CFU/mL and Time	0.244381	0.08366	-0.04382 (None/negative)
Experimental $\log_{10}$ CFU/mL and Temperature	**8.687E-04**	0.5102	-4.0725 (Negative)
Negative Control $\log_{10}$ CFU/mL and Temperature	0.4304	0.03929	-0.9874 (Negative)
Experimental $\log_{10}$ CFU/mL and pH	**2.232E-07**	**0.8213**	Negative
Negative Control $\log_{10}$ CFU/mL and pH	0.3676	0.05098	-3.013

Table 6. Linear regressions for Experimental and Negative Control *Enterococcus* spp. eudiometer systems for pH and temperature.

	Experimental	Negative Control
p value (alpha = 0.05)	**0.003852**	0.7067
R^2 value	0.416	0.009087
Estimated Relationship	0.2930 (positive)	0.03559 (positive)

Table 7. Linear regressions for Experimental and Negative Control Enterococcus spp. eudiometer systems for pH and Total VFAs.

	Experimental	Negative Control
p value (alpha = 0.05)	**0.04439**	0.2384
R^2 value	0.2293	0.08571
Estimated Relationship	-0.0004252 (Negative)	0.002998

Table 8. Linear regressions for the average Enterococcus spp. $\log_{10}$ CFU/mL as a function of Total VFAs in Experimental and Negative Control systems.

	Experimental	Negative Control
p value (alpha = 0.05)	0.05766	0.06618
R^2 value	0.2072	0.1955
Estimated Relationship	0.005073 (None)	-0.06042

Table 9. Linear regression models for the average Enterococcus spp. log10 CFU/mL as a function of Time with Total VFAs as an additional variable in Experimental and Negative Control systems.

	Experimental	Negative Control
p value for model (alpha = 0.05)	**1.702E-08**	0.1871
p value for Time (alpha = 0.05)	**2.08E-08**	0.769
p value for Total VFAs (alpha = 0.05)	**0.0429**	0.160
R^2 value for model	**0.9079**	0.2003
Estimated Relationship of $\log_{10}$ CFU/mL with Time	-0.1813549 (Negative)	-0.01225 (Negative)
Estimated Relationship of $\log_{10}$ CFU/mL with Total VFAs	0.0020313 (Neutral/Positive)	-0.05466 (Negative)

Experimental and Negative Control *Enterococcus* spp. do not have a statistically relationship with Total VFAs alone (Table 8). However, experimental *Enterococcus* spp. linear regression with $\log_{10}$ CFU/mL plus Total VFAs and Time show a statistically significant relationship (Table 9) whereas Negative Control *Enterococcus* spp. do not. Meaning, Total VFAs alone will likely not negatively affect the survival of *Enterococcus* spp. in co-digestion or percolate. However, exposure time plus co-digestion of percolate and food waste will affect the survival of *Enterococcus* spp.

Overall, these data demonstrate that *Enterococcus* spp. $\log_{10}$ CFU/mL that are in a co-digested AD environment with likely have a negative relationship with pH, exposure time in the test system, and temperature. Consequently, the Negative Control

Enterococcus spp. with only percolate (a manure slurry) did not have a statistically significant relationship with the reduction of $\log_{10}$ CFU/mL.

As discussed in the introduction, temperature has been shown to play a major role in survival of organisms in manure-only AD. In pure culture all of the test organisms can grow at 38° C. Thermophilic conditions in co-digested AD systems would likely show a stronger relationship between $\log_{10}$ CFU/mL and temperature; the relationship would likely be negative (an increase of temperature leads to a decrease of $\log_{10}$ CFU/mL) as a result of cellular stress (e.g. denatured proteins). In co-digestion, temperature does play a significant role but combined with time, pH, and total VFAs, $\log_{10}$ CFU/mL can be reduced to non-detect at mesophilic temperatures before a typical 21-28 day cycle. *Enterococcus* spp. are potential pathogens that can tolerate conditions other fecal related organisms cannot (i.e. *E. coli*). Co-digestion of manure and other organics may be a an overall safer way to reduce hardy potential bacterial pathogens while reducing waste, reducing environmental hazards of releasing potential pathogens, and obtaining an alternate energy source (i.e. combustion of methane).

Overall, co-digestion with food waste and percolate (a manure surrogate) in AD conditions reduced almost all organisms (*C. jejuni*, *E. coli*, *Salmonella enterica*, and *Staph. aureus*) to non-detectable culture limits within the first three days of AD. *Enterococcus* species are native to this percolate and may have an advantage over non-native organisms. The potential advantage may be why *Enterococcus* species were able to be detected longer throughout the AD time period. In the *Enterococcus* experimental eudiometers there was a statistically significant relationship between $\log_{10}$ CFU/mL and:

time, pH, and temperature. Because there was a statistically significant relationship between $\log_{10}$ CFU/mL and time for experimental and not the negative control system could indicate that co-digestion is viable method for the reduction of *Enterococcus* species. The native *Enterococcus* species required more than 21 but less than 28 days to no longer be detectable.

Biogas

Methane production was not a main focus of this study, however, volume and composition of biogases were measured before each sampling period and at various time intervals when duplicate eudiometers had ≥ 300 mL of biogas. Although experimental and negative control eudiometers all produced biogas throughout the 28-day cycle, all eudiometers produced less biogas than the positive control eudiometers (percolate and microcrystalline cellulose). This is not atypical of some feedstocks. System interruption is one reason less biogas may have been captured in the system. The experimental and negative eudiometers were opened, after biogas measurement, for a time period of five to fifteen minutes to sample the system and measure pH and temperature (Appendix D). Opening the system for frequent (i.e. at each sampling event) and for extended periods of time (i.e. ≤ 15 minutes) allowed biogas to escape the system, thus more biogas were more likely produced than what were recorded. In general, biogas readings suggested that the test systems operated normally and provided an AD environment that would be typical of this test system.

Conclusion

This study demonstrated that co-digestion of percolate with another organic (i.e. homogenized food waste) will reduce some potential bacterial pathogens, while still producing biogas.

All potential bacterial pathogens, with the exception of *Enterococcus* spp., were reduced to a nondetectable limit (ND < 1 CFU/mL) during AD. *Campylobacter jejuni* was not able to establish itself in the experimental (percolate + food waste) and negative control (percolate only) eudiometer systems. However, the starting inoculum of *C. jejuni* had viable growth (Figure 9) and was able to grow in sterile systems similar to the test systems (data not shown). The lack of growth in the experimental and negative control systems was likely a result to stress (from washing the cells with sterile PBS) and potentially microbial competition. *Campylobacter* spp. could establish in an AD system (Korres et al. 2013). This study indicates both percolate (manure surrogate) and percolate with food waste (manure surrogate with other organics) are both viable options for the reduction of *Campylobacter* spp.

Enterococcus spp. were detected for the longest time period in both experimental and negative control systems. Enterococci are native to the percolate used in this study, therefore extended cultural detection, in comparison to non-native microbes, would be expected. Experimental *Enterococcus* spp. were last detected at day 14 of AD and negative control *Enterococcus* spp. were last detected at day 21 of AD. These data indicate that co-digestion may be better at reducing enterococci than AD of percolate alone. Linear regressions demonstrated that there is a relationship between $\log_{10}$ CFU/mL with time, temperature, pH, and total VFAs for the experimental system but not the negative control system. Co-digestion of a manure surrogate with another organic substrate, over a timer period of at least but not less than fourteen days, appears to a safer method of reducing *Enterococcus* spp. over traditional manure-only AD.

In the experimental systems: *E. coli* was not detectable after two days into AD, *Salmonella enterica* was not detectable after one day of AD, and *Staph. aureus* was not detectable after fifteen minutes of AD (

Figure *15*-17). *Escherichia coli, Salmonella enterica,* and *Staph. aureus* are not native organism to this percolate and therefore were not present in the negative control systems, however, *E. coli* has been isolated from this percolate in the past. These three organisms are capable of being a part of AD and/or have been isolated from manure-based digesters and therefore are a safety concern (Amani et al. 2010; Horan et al. 2004; Kearney et al. 1993; Larsen et al. 1994; Olsen & Laresen 1987; Rabah et al. 2010).

Both objectives in this study were met, with exception to the culturability of *C. jejuni*. The other four organisms were introduced and cultured after introduction into the experimental systems. The second objective (to evaluate, through statistical analyses, if there are relationships between colony forming units per milliliter (CFU/mL) and specific physical-chemical parameters of AD) was only possible with *Enterococcus* spp. as a result of the longevity of enterococci in both experimental and negative control systems. Relationships were calculated for the *Enterococcus* spp. experimental system but not the negative control system.

The hypothesis of this study, if co-fed anaerobic digestion systems results in high VFAs and low pH during acidogenesis, then potentially pathogenic bacteria survival will be reduced by these stressful conditions, could not be fully supported. A statistically significant relationship was not demonstrated between $\log_{10}$ CFU/mL with pH and total VFAs without the consideration of time. Additionally, it was not determined if incomplete acidogenesis occurred. Therefore the hypothesis is rejected and a new hypothesis should be formulated to include time.

In conclusion co-digestion of manure and other organics has the potential to reduce potentially pathogenic bacteria based on these data. Fecal and food related bacteria that could incorporate into AD systems have been reduced in this experimental system within three days of introduction, with the exception of *E. faecalis*.

These data could support the application of the safe reduction of bacteria in large scale co-digestion AD systems. The combination of food waste in manure based digesters could reduce landfill use, further renewable energy through the combustion of methane, while safely reducing the risk of microbial contamination to humans, non-human animals, and the environment.

Future Work

Future work for this study would be to determine:

- Comparative biogas quality and quantity via other benchtop AD systems (e.g. Automated Methane Potential Testing System)
- Closer approximate organismal detection rates via hourly or twice daily sampling periods
- Use of different selective and or differential media for organisms (specifically for *C. jejuni* and *S. aureus*)
- *Enterococcus* species/colony morphology through biochemical assays, molecular sequencing, or comparison through API 20S (identification through bioMérieux).

Biogas from a spiked co-fed system could be compared to a non-spiked system with the same substrates. A sampling port or altered containment system would need to be changed in order to accomplish the biogas capture with measurements, other system measurements, and sampling for microbial detection. Based on these findings, a more accurate final detection time point, as well as different selective and or differential media could be determined for the five microorganisms. Finally, genomic testing could be utilized to determine the reasons behind *Enterococcus* spp. colony morphology and color changes in the system.

APPENDIX A

Culture Colony Counts

Table A-1. *Campylobacter jejuni* cultured concentration in test system during mesophilic AD. The total average CFU/mL is the concentration of *C. jejuni* inoculum in the test system Negative control is not displayed since *Campylobacter* spp. were not found in the negative control system.

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log$_{10}$ CFU/mL	Log$_{10}$ Standard Deviation (for Log$_{10}$ CFU/mL)
Starting Concentration	1.58E+00	2.12E+02	2.87E+00	0.124515323
0.01	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
7	0	0	0	0
14	0	0	0	0
21	0	0	0	0
28	0	0	0	0

Table A-2. *Enterococcus* spp. cultured concentration in test system during mesophilic AD. The total average CFU/mL is the concentration of *Enterococcus* spp. inoculum in the test system experimental eudiometer growth.

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log$_{10}$ CFU/mL	Log$_{10}$ Standard Deviation (for Log$_{10}$ CFU/mL)
Starting Concentration	8.18E+05	707106.7812	5.91E+00	0.000790458
0	1.68E+05	17147.33944	5.23E+00	0.053215088
0.01	8.50E+05	1.16415E-10	5.93E+00	0.082760911
1	1.54E+05	10606.60172	5.19E+00	0.029887056
2	2.68E+04	9545.941546	4.43E+00	0.160211383
3	2.33E+03	459.6194078	3.37E+00	0.099528057
7	3.13E+02	10.60660172	2.49E+00	0.033512025
14	5.50E+02	212.1320344	2.74E+00	0.095051172
21	0.00E+00	0	0.00E+00	0
28	0.00E+00	0	0.00E+00	0

Table A-3. *Enterococcus* spp. negative control (percolate only) eudiometer jars growth

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log_{10} CFU/mL	Log_{10} Standard Deviation (for Log_{10} CFU/mL)
Starting Concentration	N/A	N/A	N/A	N/A
0	1.84E+05	176423.1419	5.27E+00	0.13009944
0.01	1.00E+03	707.1067812	3.00E+00	2.121320344
1	1.50E+02	10.60660172	2.18E+00	0.031882253
2	0.00E+00	0	0.00E+00	0
3	5.67E+02	459.6194078	2.75E+00	0.431988768
7	6.60E+01	12.96362432	1.82E+00	0.188804581
14	4.58E+03	3641.599923	3.66E+00	0.391857179
21	1.35E+02	106.0660172	2.13E+00	0.384714204
28	0.00E+00	0	0.00E+00	0

Table A-4. *Escherichia coli* cultured concentration in test system during mesophilic AD. The total average CFU/mL is the concentration of *E. coli* inoculum in the test system Negative control is not displayed since *E. coli* was not found in the negative control system.

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log_{10} CFU/mL	Log_{10} Standard Deviation (for Log_{10} CFU/mL)
Starting Concentration	2.17E+05	8.49E+06	5.34E+00	0.035818327
0.01	1.58E+05	3.75E+04	5.20E+00	0.161261161
1	5.65E+04	8.49E+03	4.75E+00	0.066120916
2	1.80E+03	3.50E+02	3.26E+00	0.085157783
3	0.00E+00	0	0.00E+00	0
7	0.00E+00	0	0.00E+00	0
14	0.00E+00	0	0.00E+00	0
21	0.00E+00	0	0.00E+00	0
28	0.00E+00	0	0.00E+00	0

Table A-5. *Salmonella enterica* cultured concentration in test system during mesophilic AD. The total average CFU/mL is the concentration of *S. enterica* inoculum in the test system Negative control is not displayed since *S. enterica* was not found in the negative control system.

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log_{10} CFU/mL	Log_{10} Standard Deviation (for Log_{10} CFU/mL)
Starting Concentration	2.30E+04	5.98E+06	4.4E+00	0.248025433
0.01	1.00E+03	7.10E+02	3.0E+00	2.121320344
1	2.17E+02	2.50E+02	2.3E+00	0.237440872
2	0	0	0.0E+00	0
3	0	0	0.0E+00	0
8	0	0	0.0E+00	0
14	0	0	0.0E+00	0
21	0	0	0.0E+00	0
28	0	0	0.0E+00	0

Table A-6. *Staphylococcus aureus* cultured concentration in test system during mesophilic AD. The total average CFU/mL is the concentration of *S. aureus* inoculum in the test system Negative control is not displayed since *S. aureus* was not found in the negative control system.

Sample Time (Days)	Total Average CFU/mL	Standard Deviation (for CFU/mL)	Log_{10} CFU/mL	Log_{10} Standard Deviation (for Log_{10} CFU/mL)
Starting Concentration	1.80E+05	37299882.71	5.26E+00	0.194660422
0.01	1.51E+05	140714.2495	5.18E+00	0.875794619
1	0	0	0.00E+00	0
2	0	0	0.00E+00	0
3	0	0	0.00E+00	0
8	0	0	0.00E+00	0
14	0	0	0.00E+00	0
21	0	0	0.00E+00	0
28	0	0	0.00E+00	0

APPENDIX B

Statistical Analyses Tables for pH, Temperature, and Total VFAs

Table B-1. Experimental *C. jejuni* sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’.

Sample Time (Days)	pH	x̄ pH	Std. dev. pH	Temperature (°C)	x̄ Temperature (°C)	Std dev Temperature (°C)
0	7.65	7.65	0	36.1	36.4	0.424264
	7.65			36.7		
0.01	7.44	7.46	0.021213	37.0	37.0	0
	7.47			37.0		
1	7.60	7.70	0.141421	36.9	36.9	0.070711
	7.80			36.8		
2	7.67	7.68	0.014142	37.1	37.2	0.070711
	7.69			37.2		
3	7.72	7.72	0.007071	37.3	37.3	0
	7.71			37.3		
7	7.60	7.60	0	37.4	37.5	0.070711
	7.60			37.5		
14	7.84	7.82	0.035355	37.7	37.4	0.494975
	7.79			37.0		
21	7.89	7.90	0.014142	37.4	37.5	0.070711
	7.91			37.5		
28	7.93	7.94	0.007071	37.4	37.5	0.141421
	7.94			37.6		

Table B-2. Experimental *C. jejuni* sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	0	0	4	14	17	0
	0	0	0	0	0	0
0.01	0	0	8	14	16	0
	593	23	6	15	11	0
1	282	16	8	15	5	0
	30	0	0	7	0	0
2	39	0	0	0	0	0
	18	0	0	0	0	0
3	0	0	0	0	0	0
	20	0	0	0	0	0
7	19	0	0	0	0	0
	19	0	0	0	0	0
14	24	0	0	0	0	0
	22	0	0	0	0	0
21	0	0	0	0	0	0
	0	0	0	3	0	0
28	19	0	0	0	0	0
	16	0	0	0	0	0

Table B3. Experimental *C. jejuni* sample time and AD parameter statistics for VFAs.

Sample Time (Days)	x̄ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	x̄ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	x̄ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	x̄ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	x̄ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	0.0	0	0	0	2	2.828427	7	9.899495	0	0
0.01	297	419.314321	12	16.26346	7	1.414214	15	0.707107	0	0
1	156	178.190909	8	11.31371	4	5.656854	11	5.656854	0	0
2	29	14.8492424	0	0	0	0	0	0	0	0
3	10	14.1421356	0	0	0	0	0	0	0	0
7	19	0	0	0	0	0	0	0	0	0
14	23	1.41421356	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	2	2.12132	0	0
28	18	2.12132034	18	2.12132	0	0	0	0	0	0

Table B-4. Experimental *E. coli* sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’.

Sample Time (Days)	pH	x̄ pH	Std. dev. pH	Temperature (°C)	x̄ Temperature (°C)	Std dev Temperature (°C)
0	7.68	7.67	0.021213203	36.8	36.9	0.141421356
	7.65			37		
0.01	7.46	7.46	0	36.5	36.2	0.424264069
	7.46			35.9		
1	7.60	7.59	0.014142136	36.9	36.9	0
	7.58			36.9		
2	7.67	7.67	0	37.1	37.1	0
	7.67			37.1		
3	7.70	7.70	0	37.3	37.4	0.070710678
	7.70			37.4		
7	7.60	7.60	0.007071068	37.2	37.2	0.070710678
	7.59			37.1		
14	7.79	7.79	0.007071068	37.8	37.6	0.353553391
	7.78			37.3		
21	7.87	7.88	0.007071068	37.4	37.6	0.212132034
	7.88			37.7		
28	7.93	7.92	0.021213203	37.4	37.7	0.353553391
	7.90			37.9		

Table B-5. Experimental *E. coli* sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	29	0	14	17	21	0
	0	0	13	17	25	0
0.01	504	24	6	13	12	0
	22	0	9	15	20	0
1	37	0	0	0	0	0
	26	0	0	0	0	0
2	20	0	0	0	0	0
	19	0	0	0	0	0
3	29	0	0	0	0	0
	0	0	0	0	0	0
7	23	0	0	0	0	0
	20	0	0	0	0	0
14	27	0	0	0	0	0
	29	0	0	0	0	0
21	0	0	0	0	0	0
	19	0	0	0	0	0
28	0	0	0	0	0	0
	0	0	0	0	0	0

Table B-6. Experimental *E. coli* sample time and AD parameter statistics for VFAs.

Sample Time (Days)	x̄ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	x̄ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	x̄ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	x̄ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	x̄ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	14.5	20.50609665	0	0	13.5	0.707106781	17	0	0	0
0.01	263.0	340.8254685	12	16.97056275	0	0	14	1.414213562	0	0
1	31.5	7.778174593	0	0	0	0	0	0	0	0
2	19.5	0.707106781	0	0	0	0	0	0	0	0
3	14.5	20.50609665	0	0	0	0	0	0	0	0
7	21.5	2.121320344	0	0	0	0	0	0	0	0
14	28.0	1.414213562	0	0	0	0	0	0	0	0
21	9.5	13.43502884	0	0	0	0	0	0	0	0
28	0.0	0	0	0	0	0	0	0	0	0

Table B-7. Experimental *Enterococcus* spp. sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’.

Sample Time (Days)	pH	x̄ pH	Std dev pH	Temperature (°C)	x̄ Temperature	Std dev Temperature (°C)
0	7.63	7.65	0.021213	37.2	37.3	0.070711
	7.66			37.3		
0.01	7.44	7.45	0.007071	37.1	37.0	0.212132
	7.45			36.8		
1	7.58	7.58	0	37.2	37.0	0.282843
	7.58			36.8		
2	7.67	7.67	0	36.6	36.8	0.282843
	7.67			37.0		
3	7.68	7.70	0.021213	37.3	37.4	0.070711
	7.71			37.4		
7	7.60	7.61	0.007071	37.3	37.4	0.141421
	7.61			37.5		
14	7.84	7.83	0.021213	37.8	37.7	0.212132
	7.81			37.5		
21	7.89	7.95	0.077782	37.3	37.3	0
	8.00			37.3		
28	7.93	7.94	0.007071	37.9	37.9	0
	7.94			37.9		

Table B-8. Experimental *Enterococcus* spp. sample time and VFA concentrations.

Sample Time	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	41	0	15	18	25	0
	20	0	9	15	16	0
0.01	22	0	9	15	20	0
	742	24	6	17	8	0
1	202	0	0	6	0	0
	25	0	0	0	0	0
2	37	0	0	0	6	0
	78	0	0	0	0	0
3	0	0	0	0	0	0
	0	0	0	0	0	0
7	22	0	0	0	0	0
	24	0	0	0	0	0
14	21	0	0	0	0	0
	24	0	0	0	0	0
21	22	0	0	0	0	0
	18	0	0	0	0	0
28	21	0	0	0	0	0
	20	0	0	0	0	0

Table B-9. Experimental *Enterococcus* spp. sample time and AD parameter statistics for VFAs.

Sample Time	Average Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	Average Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	Average Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	Average Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	Average Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	30.5	14.84924	0	0	12	4.242641	16.5	2.12132	0	0
0.01	382.0	509.1169	12	16.97056	7.5	2.12132	16	1.414214	0	0
1	113.5	125.1579	0	0	0	0	3	4.242641	0	0
2	57.5	28.99138	0	0	0	0	0	0	0	0
3	0.0	0	0	0	0	0	0	0	0	0
7	23.0	1.414214	0	0	0	0	0	0	0	0
14	22.5	2.12132	0	0	0	0	0	0	0	0
21	20.0	2.828427	0	0	0	0	0	0	0	0
28	20.5	0.707107	0	0	0	0	0	0	0	0

Table B-10. Negative control for *C. jejuni*, *E. coli*, and *Enterococcus* spp. sample time and AD parameter statistics – average pH and temperature. Where “Std dev” represents ‘standard deviation’.

Sample Time	pH	Average pH	Stand. Dev. pH	pH Temperature (C)	Average pH Temperature (C)	Std dev pH Temperature (C)
0	7.72	7.74	0.0212132	37.4	37.1	0.494975
0	7.75			36.7		
0.01	7.78	7.74	0.06363961	36.7	36.8	0.141421
0.01	7.69			36.9		
1	7.89	7.94	0.06363961	37.2	37.0	0.282843
1	7.98			36.8		
2	7.90	7.90	0.00707107	36.8	37.1	0.424264
2	7.89			37.4		
3	7.91	7.92	0.01414214	37.4	37.1	0.424264
3	7.93			36.8		
7	7.81	7.80	0.01414214	37.4	37.4	0.070711
7	7.79			37.3		
14	7.93	7.96	0.04242641	37.6	37.3	0.494975
14	7.99			36.9		
21	8.01	8.02	0.01414214	37.0	37.0	0.070711
21	8.03			36.9		
28	8.00	8.04	0.04949747	37.4	37.4	0
28	8.07			37.4		

Table B-11. Negative control for *C. jejuni*, *E. coli*, and *Enterococcus* spp. sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	0	0	0	0	0	0
	0	0	0	0	0	0
0.01	21	0	0	0	0	0
	15	0	0	0	0	0
1	0	0	0	0	0	0
	0	0	0	0	0	0
2	0	0	0	0	0	0
	19	0	0	0	0	0
3	0	0	0	0	0	0
	17	0	0	0	0	0
7	20	0	0	0	0	0
	0	0	0	0	0	0
14	0	0	0	0	0	1
	0	0	0	0	0	0
21	20	0	0	0	0	0
	29	0	0	0	0	0
28	26	0	0	0	0	0
	20	0	0	0	0	0

Table B-12. Negative control for *C. jejuni*, *E. coli*, and *Enterococcus* spp. sample time and AD parameter statistics for VFAs.

Sample Time (Days)	x̄ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	x̄ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	x̄ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	x̄ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	x̄ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	0	0	0	0	0	0	0	0	0	0
0.01	18.0	4.242640687	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	9.5	13.43502884	0	0	0	0	0	0	0	0
3	8.5	12.02081528	0	0	0	0	0	0	0	0
7	10.0	14.14213562	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0.5	0.707106781
21	24.5	6.363961031	0	0	0	0	0	0	0	0
28	23.0	4.242640687	0	0	0	0	0	0	0	0

Table B-13. Experimental *S. enterica* sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’

Sample Time (Days)	pH	x̄ pH	Std. dev. pH	Temperature (°C)	x̄ Temperature (°C)	Std dev Temperature (°C)
0	7.88	7.89	0.01414214	27.9	27.0	1.272792206
	7.90			26.1		
0.01	7.92	7.93	0.01414214	26.2	26.0	0.282842712
	7.94			25.8		
1	7.86	7.91	0.06363961	33.5	34.2	0.989949494
	7.95			34.9		
2	7.96	7.99	0.03535534	34.1	34.6	0.707106781
	8.01			35.1		
3	8.06	8.07	0.00707107	35.2	35.4	0.282842712
	8.07			35.6		
8	8.35	8.37	0.0212132	37.9	37.9	0
	8.38			37.9		
14	8.05	8.06	0.01414214	37.7	37.7	0
	8.07			37.7		
21	7.82	7.81	0.0212132	35.9	36.2	0.353553391
	7.79			36.4		
28	7.90	7.92	0.0212132	36.3	36.3	0
	7.93			36.3		

Table B-14. Experimental *S. enterica* sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	145	0	0	0	0	0
	134	0	0	0	0	0
0.01	186	0	0	0	0	0
	183	0	0	0	0	0
1	868	142	82	115	86	0
	667	0	0	91	0	0
2	422	122	83	88	82	0
	477	120	84	87	82	0
3	324	130	83	81	83	0
	328	122	81	79	80	0
8	69	0	0	0	0	0
	72	0	0	0	0	0
14	66	0	0	0	0	0
	62	0	0	0	0	0
21	82	0	0	0	0	0
	82	0	0	0	0	0
28	61	0	0	0	0	0
	0	0	0	0	0	0

Table B-15. Experimental *S. enterica* sample time and AD parameter statistics for VFAs.

Sample Time (Days)	$\bar{x}$ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	$\bar{x}$ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	$\bar{x}$ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	$\bar{x}$ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	$\bar{x}$ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	140	7.778174593	0	0	0	0	0	0	0	0
0.01	185	2.121320344	0	0	0	0	0	0	0	0
1	768	142.128463	71	100.4091629	41	57.98275606	103	16.97056275	0	0
2	450	38.89087297	121	1.414213562	84	0.707106781	88	0.707106781	0	0
3	326	2.828427125	126	5.656854249	82	1.414213562	80	1.414213562	0	0
8	71	2.121320344	0	0	0	0	0	0	0	0
14	64	2.828427125	0	0	0	0	0	0	0	0
21	82	0	0	0	0	0	0	0	0	0
28	31	43.13351365	0	0	0	0	0	0	0	0

Table B-16. Experimental *S. aureus* sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’.

Sample Time (Days)	pH	x̄ pH	Std. dev. pH	Temperature (°C)	x̄ Temperature (°C)	Std dev Temperature (°C)
0	7.85	7.86	0.014142136	29.3	30.2	1.272792206
	7.87			31.1		
0.01	7.86	7.86	0.007071068	32.3	31.7	0.919238816
	7.85			31.0		
1	7.91	7.89	0.035355339	35.2	35.7	0.636396103
	7.86			36.1		
2	8.02	8.00	0.028284271	35.1	35.2	0.070710678
	7.98			35.2		
3	8.06	8.11	0.070710678	35.7	35.5	0.353553391
	8.16			35.2		
8	8.34	8.37	0.042426407	37.9	37.9	0.070710678
	8.40			37.8		
14	8.02	8.02	0.007071068	37.9	37.9	0.070710678
	8.01			37.8		
21	7.77	7.79	0.021213203	37.1	37.2	0.141421356
	7.80			37.3		
28	7.91	7.93	0.021213203	36.9	36.9	0
	7.94			36.9		

Table B-17. Experimental *S. aureus* sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	145	0	0	0	0	0
	65	0	0	0	0	0
0.01	173	0	0	0	0	0
	159	0	0	0	0	0
1	834	141	82	113	85	0
	884	176	83	113	85	0
2	600	154	94	101	88	0
	351	107	80	83	80	0
3	351	172	85	83	83	0
	313	110	82	79	82	0
8	70	0	0	0	0	0
	68	0	0	0	0	0
14	70	0	0	0	0	0
	63	0	0	0	0	0
21	82	0	0	0	0	0
	82	0	0	0	0	0
28	62	0	0	0	0	0
	70	0	0	0	0	0

Table B-18. Experimental *S. aureus* sample time and AD parameter statistics for VFAs.

Sample Time (Days)	x̄ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	x̄ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	x̄ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	x̄ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	x̄ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	105	56.56854249	0	0	0	0	0	0	0	0
0.01	166	9.899494937	0	0	0	0	0	0	0	0
1	859	35.35533906	159	24.74873734	83	0.707106781	113	0	0	0
2	476	176.0695885	131	33.23401872	87	9.899494937	92	12.72792206	0	0
3	332	26.87005769	141	43.84062043	84	2.121320344	81	2.828427125	0	0
8	69	1.414213562	0	0	0	0	0	0	0	0
14	67	4.949747468	0	0	0	0	0	0	0	0
21	82	0	0	0	0	0	0	0	0	0
28	66	5.656854249	0	0	0	0	0	0	0	0

Table B-19. Negative control for *S. enterica* and *S. aureus* sample time and AD parameter statistics – average pH and temperature where “Std dev” represents ‘standard deviation’ and “x̄” represents ‘average’.

Sample Time (Days)	pH	x̄ pH	Std. dev. pH	Temperature (°C)	x̄ Temperature (°C)	Std dev Temperature (°C)
0	7.86	7.89	0.035355339	34.3	34.4	0.070710678
	7.91			34.4		
0.01	7.86	7.93	0.091923882	34.4	34.5	0.141421356
	7.99			34.6		
1	8.13	8.15	0.028284271	35.4	35.6	0.282842712
	8.17			35.8		
2	8.23	8.28	0.06363961	24.8	30.0	7.283199846
	8.32			35.1		
3	8.22	8.26	0.056568542	35.8	36.3	0.707106781
	8.30			36.8		
8	8.46	8.46	0.007071068	37.8	37.8	0.070710678
	8.45			37.7		
14	8.15	8.01	0.197989899	37.7	39.2	2.121320344
	7.87			40.7		
21	7.88	7.91	0.035355339	37.3	37.2	0.141421356
	7.93			37.1		
28	8.02	8.04	0.028284271	36.8	36.8	0
	8.06			36.8		

Table B-20. Negative control for *S. enterica* and *S. aureus* sample time and VFA concentrations.

Sample Time (Days)	Acetic Acid (mg/Kg WWB)	Propanoic Acid (mg/Kg WWB)	Isobutanoic Acid (mg/Kg WWB)	Butanoic Acid (mg/Kg WWB)	Isovaleric Acid (mg/Kg WWB)	Valeric Acid (mg/Kg WWB)
0	65	0	0	0	0	0
	63	0	0	0	0	0
0.01	64	0	0	0	0	0
	66	0	0	0	0	0
1	64	0	0	0	0	0
	71	0	0	0	0	0
2	63	0	0	0	0	0
	63	0	0	0	0	0
3	65	0	0	0	0	0
	67	0	0	0	0	0
7	69	0	0	0	0	0
	70	0	0	0	0	0
14	63	0	0	0	0	0
	67	0	0	0	0	0
21	67	0	0	0	0	0
	67	0	0	0	0	0
28	70	0	0	0	0	0
	69	0	0	0	0	0

Table B-21. Negative control for *S. enterica* and *S. aureus* sample time and AD parameter statistics for VFAs.

Sample Time (Days)	x̄ Acetic Acid (mg/Kg WWB)	Std Dev Acetic Acid	x̄ Propanoic Acid (mg/Kg WWB)	Std Dev Propanoic Acid	x̄ Isobutanoic Acid (mg/Kg WWB)	Std Dev Isobutanoic Acid	x̄ Butanoic Acid (mg/Kg WWB)	Std Dev Butanoic Acid	x̄ Valeric Acid (mg/Kg WWB)	Std Dev Valeric Acid
0	64	1.414213562	0	0	0	0	0	0	0	0
0.01	65	1.414213562	0	0	0	0	0	0	0	0
1	68	4.949747468	0	0	0	0	0	0	0	0
2	63	0	0	0	0	0	0	0	0	0
3	66	1.414213562	0	0	0	0	0	0	0	0
7	70	0.707106781	0	0	0	0	0	0	0	0
14	65	2.828427125	0	0	0	0	0	0	0	0
21	67	0	0	0	0	0	0	0	0	0
28	70	0.707106781	0	0	0	0	0	0	0	0

APPENDIX C

Statistical Analyses for *Enterococcus* spp

Table C-1. Linear regression: $\log_{10}$ CFU/mL and time

	Experimental	Negative Control
p value (α = 0.05)	**1.028E-08**	0.2444
R^2 value	0.8779	0.08366
F statistic actual	115	1.461
F statistic calculated	4.49	4.49
degrees of freedom	1, 16	1, 16
n	18	18
Standard Error	0.01799	0.03626
Estimated Relationship	-0.19299 (Negative)	-0.04382 (Negative)

Table C-2. Linear Regression: $\log_{10}$ CFU/mL and temperature

	Experimental	Negative Control
p value (α = 0.05)	**0.0008687**	0.4304
R^2 value	0.5102	0.03929
F statistic actual	16.66	0.6544
F statistic calculated	4.49	4.49
Degrees of freedom	1, 16	1, 16
n	18	18
Standard Error	0.9977	1.2207
Estimated Relationship	-4.0725 (Negative)	-0.9874 (Negative)

Table C-3. Linear Regression: $\log_{10}$ CFU/mL and pH

	Experimental	Negative Control
p value (α = 0.05)	**2.232E-07**	0.3676
R^2 value	0.8213	0.05098
F statistic actual	73.51	0.8595
F statistic calculated	4.49	4.49
Degrees of freedom	1, 16	1, 16
n	18	18
Standard Error	1.327	3.249
Estimated Relationship	-11.373 (Negative)	-3.013 (Negative)

Table C-4. Linear Regression: pH and Temperature

	Experimental	Negative Control
p value (α = 0.05)	**0.003852**	0.7067
R^2 value	**0.416**	0.009087
F statistic actual	11.4	0.1467
F statistic calculated	4.49	4.49
Degrees of freedom	1, 16	1, 16
n	18	18
Standard Error	0.4205	0.09292
Estimated Relationship	0.2930 (positive)	0.03559 (positive)

Table C-5. Linear regression models for the average Enterococcus spp. $\log_{10}$ CFU/mL as a function of Time with Total VFAs as an additional variable in Experimental and Negative Control systems.

	Experimental	Negative Control
p value for model (α = 0.05)	**1.702E-08**	0.1871
p value for Time (α = 0.05)	**2.08E-08**	0.769
p value for Total VFAs (α = 0.05)	**0.0429**	0.160
R^2 value for model	**0.9079**	0.2003
Estimated Relationship of $\log_{10}$ CFU/mL with Time	-0.1813549 (Negative)	-0.01225 (Negative)
Estimated Relationship of $\log_{10}$ CFU/mL with Total VFAs	0.0020313 (Neutral/Positive)	-0.05466 (Negative)
F statistic actual	73.96	1.878
F statistic calculated	3.68	3.68
Degrees of freedom	2, 15	2, 15
n	18	18
Standard Error Time	0.0169741	0.04098
Standard Error Total VFAs	0.0009183	0.03696

APPENDIX D

Biogas Data

Table D-1. Positive control eudiometer biogas data (part 1) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-1	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
1/3/2017	16:11	20	1007	x	0	0	0
1/4/2017	9:04	20	1012		30	30	30
1/5/2017	8:45	19	1015		60	80	70
1/6/2017	8:14	19	1021		170	180	175
1/7/2017	11:30	19	1029	x	510	510	510
1/8/2017	15:20	19	1033	x	800	800	800
1/9/2017	8:23	20	1022	x	690	720	705
1/10/2017	8:25	20	996	x	>800	260	260
1/11/2017	9:33	21	1007	x	690	520	605
1/12/2017	15:00	21	1021		270	60	165
1/13/2017	12:25	21	1042	x	420	60	240
1/15/2017	14:37	20	1026		340	320	330
1/17/2017	8:12	21	1009	x	520	510	515
1/21/2017	15:00	20	995		260	60	160
1/24/2017	8:08	20	1009	x	370	60	215
1/31/2017	9:19	21	1002	x	280	290	285

Table D-2. Positive control eudiometer biogas data (part 2) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
1/3/2017	0.0	0.0	0.00	0.0	0.0	0.00
1/4/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/5/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/7/2017	36.9	42.8	39.85	35.1	36.9	36.00
1/8/2017	39.6	39.4	39.50	60.3	60.5	60.40
1/9/2017	29.5	28.8	29.15	70.4	71.1	70.75
1/10/2017	32.8	31.9	32.35	67.1	68.0	67.55
1/11/2017	44.0	43.1	43.55	55.9	56.8	56.35
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	55.0	*	55.00	44.9	*	44.90
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	61.2	59.3	60.25	38.7	40.6	39.65
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	62.1	*	62.10	37.8	*	37.80
1/31/2017	62.4	58.1	60.25	37.5	40.1	38.80

Table D-3. Positive control eudiometer biogas data (part 3) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
1/3/2017	0	0	0.00			
1/4/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/5/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/7/2017	140	190	165.00	0	0	0
1/8/2017	370	360	365.00	0	0	0
1/9/2017	295	375	335.00	0	0	0
1/10/2017	235	300	267.50	0	0	0
1/11/2017	215	285	250.00	0	0	0
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	180	*	180.00	0		0
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	120	165	142.50	0	0	0
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	120	*	120.00	0		0
1/31/2017	100	135	117.50	0	0	0

Table D-4. *Campylobacter jejuni* eudiometer biogas data (part 1). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-1	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
1/3/2017	16:11	20	1007	x	0	0	0
1/4/2017	9:04	20	1012	x	540	450	495
1/5/2017	8:45	19	1015	x	470	520	495
1/6/2017	8:14	19	1021	x	380	340	360
1/7/2017	11:30	19	1029	x	500	460	480
1/8/2017	15:20	19	1033	x	335	340	337.5
1/9/2017	8:23	20	1022		170	150	160
1/10/2017	8:25	20	996	x	380	340	360
1/11/2017	9:33	21	1007		220	190	205
1/12/2017	15:00	21	1021		300	270	285
1/13/2017	12:25	21	1042	x	370	330	350
1/15/2017	14:37	20	1026		200	190	195
1/17/2017	8:12	21	1009	x	330	320	325
1/21/2017	15:00	20	995		270	270	270
1/24/2017	8:08	20	1009	x	350	350	350
1/31/2017	9:19	21	1002	x	260	270	265

Table D-5. *Campylobacter jejuni* eudiometer biogas data (part 2). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
1/3/2017	0.0	0.0	0.00	0.0	0.0	0.00
1/4/2017	23.6	21.4	22.50	41.2	40.4	40.80
1/5/2017	22.6	24.7	23.65	32.3	34.9	33.60
1/6/2017	21.0	21.7	21.35	27.4	26.5	26.95
1/7/2017	16.4	16.0	16.20	21.1	20.4	20.75
1/8/2017	39.1	35.8	37.45	28.3	27.5	27.90
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	49.7	48.2	48.95	30.1	29.3	29.70
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	17.6	15.3	16.45	20.4	20.0	20.20
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	35.4	33.7	34.55	25.1	24.8	24.95
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	15.6	15.2	15.40	20.9	21.5	21.20
1/31/2017	11.9	10.8	11.35	19.4	19.2	19.30

Table D-6. *Campylobacter jejuni* eudiometer biogas data (part 3). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
1/3/2017	0	0	0.00			
1/4/2017	80	70	75.00	6	7	6.5
1/5/2017	115	150	132.50	4.9	3.4	4.15
1/6/2017	80	115	97.50	5.6	5.8	5.7
1/7/2017	140	95	117.50	8.1	8.4	8.25
1/8/2017	270	170	220.00	0	0	0
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	235	205	220.00	0	0	0
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	135	105	120.00	6.7	7	6.85
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	150	145	147.50	0	0	0
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	105	80	92.50	2.9	3.1	3
1/31/2017	100	70	85.00	2.1	3.5	2.8

Table D-7. *Enterococcus faecalis* eudiometer biogas data (part 1). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-1	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
1/3/2017	16:11	20	1007	x	0	0	0
1/4/2017	9:04	20	1012	x	375	590	482.5
1/5/2017	8:45	19	1015	x	480	510	495
1/6/2017	8:14	19	1021	x	390	680	535
1/7/2017	11:30	19	1029	x	490	465	477.5
1/8/2017	15:20	19	1033	x	310	300	305
1/9/2017	8:23	20	1022		160	150	155
1/10/2017	8:25	20	996	x	370	340	355
1/11/2017	9:33	21	1007		180	170	175
1/12/2017	15:00	21	1021		270	240	255
1/13/2017	12:25	21	1042	x	340	310	325
1/15/2017	14:37	20	1026		210	190	200
1/17/2017	8:12	21	1009	x	360	330	345
1/21/2017	15:00	20	995		260	230	245
1/24/2017	8:08	20	1009	x	350	320	335
1/31/2017	9:19	21	1002	x	260	230	245

Table D-8. *Enterococcus faecalis* eudiometer biogas data (part 2). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
1/3/2017	0.0	0.0	0.00	0.0	0.0	0.00
1/4/2017	26.9	27.0	26.95	49.4	50.1	49.75
1/5/2017	25.0	25.8	25.40	36.1	37.4	36.75
1/6/2017	25.5	25.5	25.50	30.6	29.9	30.25
1/7/2017	17.6	16.5	17.05	21.4	20.9	21.15
1/8/2017	37.7	37.6	37.65	28.1	27.4	27.75
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	50.2	49.1	49.65	29.8	29.0	29.40
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	18.7	16.2	17.45	21.9	20.8	21.35
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	35.9	33.7	34.80	26.1	25.2	25.65
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	16.6	15.7	16.15	22.7	21.9	22.30
1/31/2017	13.0	12.8	12.90	20.8	20.9	20.85

Table D-9. *Enterococcus faecalis* eudiometer biogas data (part 3). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-2	Average O_2%
1/3/2017	0	0	0.00			
1/4/2017	80	95	87.50	5	4.4	4.7
1/5/2017	130	140	135.00	3.4	2.4	2.9
1/6/2017	140	155	147.50	3.2	3.8	3.5
1/7/2017	95	65	80.00	8.1	8.1	8.1
1/8/2017	160	135	147.50	0	0	0
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	200	170	185.00	0	0	0
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	105	45	75.00	5.8	6.1	5.95
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	155	110	132.50	0	0	0
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	100	45	72.50	2.3	1.7	2
1/31/2017	80	40	60.00	1.8	0.3	1.05

Table D-10. *Escherichia coli* eudiometer biogas data (part 1). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-1	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
1/3/2017	16:11	20	1007	x	0	0	0
1/4/2017	9:04	20	1012	x	390	440	415
1/5/2017	8:45	19	1015	x	500	380	440
1/6/2017	8:14	19	1021	x	690	410	550
1/7/2017	11:30	19	1029	x	500	480	490
1/8/2017	15:20	19	1033	x	320	300	310
1/9/2017	8:23	20	1022		160	150	155
1/10/2017	8:25	20	996	x	370	340	355
1/11/2017	9:33	21	1007		200	170	185
1/12/2017	15:00	21	1021		270	270	270
1/13/2017	12:25	21	1042	x	340	340	340
1/15/2017	14:37	20	1026		190	200	195
1/17/2017	8:12	21	1009	x	320	350	335
1/21/2017	15:00	20	995		240	260	250
1/24/2017	8:08	20	1009	x	320	350	335
1/31/2017	9:19	21	1002	x	240	240	240

Table D-11. *Escherichia coli* eudiometer biogas data (part 2). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
1/3/2017	0.0	0.0	0.00	0.0	0.0	0.00
1/4/2017	20.9	27.6	24.25	39.3	48.9	44.10
1/5/2017	25.0	24.7	24.85	35.6	35.1	35.35
1/6/2017	23.4	25.0	24.20	28.9	29.3	29.10
1/7/2017	15.9	18.3	17.10	20.7	21.8	21.25
1/8/2017	36.8	40.5	38.65	28.0	28.4	28.20
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	49.9	52.4	51.15	29.8	30.1	29.95
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	17.9	18.4	18.15	21.6	22.1	21.85
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	33.9	37.9	35.90	25.3	26.2	25.75
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	15.4	18.4	16.90	22.8	23.9	23.35
1/31/2017	11.1	13.2	12.15	20.2	21.5	20.85

Table D-12. *Escherichia coli* eudiometer biogas data (part 3). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
1/3/2017	0	0	0.00			
1/4/2017	55	70	62.50	8	4.7	6.35
1/5/2017	155	150	152.50	3.3	4.7	4
1/6/2017	150	150	150.00	4.1	4.8	4.45
1/7/2017	90	100	95.00	8.3	7.4	7.85
1/8/2017	170	170	170.00	0	0	0
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	210	210	210.00	0	0	0
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	105	110	107.50	5.8	6	5.9
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	145	155	150.00	0	0	0
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	85	105	95.00	2	1.9	1.95
1/31/2017	70	80	75.00	1.4	1.3	1.35

Table D-13. Negative control eudiometer biogas data (part 1) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-1	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
1/3/2017	16:11	20	1007	x	0	0	0
1/4/2017	9:04	20	1012		130	125	127.5
1/5/2017	8:45	19	1015	x	80	50	65
1/6/2017	8:14	19	1021		30	30	30
1/7/2017	11:30	19	1029		50	50	50
1/8/2017	15:20	19	1033		60	60	60
1/9/2017	8:23	20	1022		110	110	110
1/10/2017	8:25	20	996	x	200	200	200
1/11/2017	9:33	21	1007		60	60	60
1/12/2017	15:00	21	1021		40	40	40
1/13/2017	12:25	21	1042		60	50	55
1/15/2017	14:37	20	1026		180	160	170
1/17/2017	8:12	21	1009	x	270	250	260
1/21/2017	15:00	20	995		100	100	100
1/24/2017	8:08	20	1009		180	190	185
1/31/2017	9:19	21	1002	x	140	140	140

Table D-14. Negative control eudiometer biogas data (part 2) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
1/3/2017	0.0	0.0	0.00	0.0	0.0	0.00
1/4/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/5/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/7/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/8/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	18.5	18.2	18.35	15.9	14.2	15.05
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/31/2017	11.4	11.3	11.35	17.1	14.9	16.00

Table D-15. Negative control eudiometer biogas data (part 3) for *C. jejuni*, *E. coli*, and *E. faecalis*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-2	Average O_2%
1/3/2017	0	0	0.00			
1/4/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/5/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/7/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/8/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/9/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/10/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/11/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/12/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/13/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/15/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/17/2017	60	45	52.50	0	0	0
1/21/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/24/2017	N/A	N/A	N/A	N/A	N/A	N/A
1/31/2017	35	50	42.50	0.4	1.9	1.15

Table D-16. Positive control eudiometer biogas data (part 1) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-11	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
8/29/2016	12:53	22	1023	x	0	0	0
8/30/2016	8:13	22	1018	x	70	70	70
8/31/2016	8:26	22	1019		0	0	0
9/1/2016	8:33	21	1023		50	60	55
9/2/2016	14:53	22	1024	x	330	400	365
9/3/2016	9:08	22	1024	x	480	560	520
9/5/2016	9:20	21	1015	x	450	470	460
9/6/2017	8:53	22	1013	x	500	510	505
9/7/2016	11:44	21	1013	x	510	510	510
9/8/2016	18:27	21	1009	x	600	585	592.5
9/10/2016	15:26	22	1010	X	720	30	375
9/12/2016	13:13	21	1015	x	190	210	200
9/18/2017	13:00	22	1012		360	380	370
9/19/2016	13:14	23	1013	x	420	430	425
9/26/2016	13:18	22	1008		420	410	415

Table D-17. Positive control eudiometer biogas data (part 2) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
8/29/2016	0.0	0.0	0.00	0.0	0.0	0.00
8/30/2016	N/A	N/A	N/A	N/A	N/A	N/A
8/31/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/1/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/2/2016	32.0	35.6	33.80	23.8	27.8	25.80
9/3/2016	35.5	37.0	36.25	46.2	50.8	48.50
9/5/2016	42.4	42.2	42.30	57.5	57.7	57.60
9/6/2017	48.5	48.8	48.65	51.4	51.1	51.25
9/7/2016	55.8	55.0	55.40	44.1	44.9	44.50
9/8/2016	60.7	60.0	60.35	39.2	39.9	39.55
9/10/2016	64.2	*	64.20	35.7	*	35.70
9/12/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	69.2	68.4	68.80	30.7	31.5	31.10
9/26/2016	68.6	68.6	68.60	31.3	31.5	31.40

Table D-18. Positive control eudiometer biogas data (part 3) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
8/29/2016	0	0	0.00			
8/30/2016	N/A	N/A	N/A	N/A	N/A	N/A
8/31/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/1/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/2/2016	120	160	140.00	0	0	0
9/3/2016	260	290	275.00	0	0	0
9/5/2016	265	360	312.50	0	0	0
9/6/2017	275	420	347.50	0	0	0
9/7/2016	235	345	290.00	0	0	0
9/8/2016	160	285	222.50	0	0	0
9/10/2016	170	*	170.00	0	*	0
9/12/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	80	115	97.50	0	0	0
9/26/2016	80	110	95.00	0	0	0

Table D-19. *Salmonella enterica* eudiometer biogas data (part 1). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-11	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
8/29/2016	12:53	22	1023	x	0	0	0
8/30/2016	8:13	22	1018	x	460	425	442.5
8/31/2016	8:26	22	1019	x	270	525	397.5
9/1/2016	8:33	21	1023	x	640	610	625
9/2/2016	14:53	22	1024	x	670	590	630
9/3/2016	9:08	22	1024		360	290	325
9/5/2016	9:20	21	1015	x	370	620	495
9/6/2017	8:53	22	1013	x	140	130	135
9/7/2016	11:44	21	1013		130	130	130
9/8/2016	18:27	21	1009		200	190	195
9/10/2016	15:26	22	1010		340	310	325
9/12/2016	13:13	21	1015	x	360	400	380
9/18/2017	13:00	22	1012		280	300	290
9/19/2016	13:14	23	1013	x	330	350	340
9/26/2016	13:18	22	1008		290	260	275

Table D-20. *Salmonella enterica* eudiometer biogas data (part 2). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
8/29/2016	0.0	0.0	0.00	0.0	0.0	0.00
8/30/2016	32.6	26.5	29.55	30.3	28.6	29.45
8/31/2016	26.2	27.0	26.60	29.0	28.5	28.75
9/1/2016	28.1	24.0	26.05	25.0	23.7	24.35
9/2/2016	29.8	24.4	27.10	24.1	22.0	23.05
9/3/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/5/2016	59.0	54.3	56.65	26.7	24.6	25.65
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	29.0	26.0	27.50	22.0	21.3	21.65
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	21.5	18.6	20.05	21.5	20.4	20.95
9/26/2016	19.1	16.4	17.75	20.9	19.7	20.30

Table D-21. *Salmonella enterica* eudiometer biogas data (part 3). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
8/29/2016	0	0	0.00			
8/30/2016	70	80	75.00	5.9	7.1	6.5
8/31/2016	140	245	192.50	4.5	3.5	4
9/1/2016	215	245	230.00	4.3	5.4	4.85
9/2/2016	215	230	222.50	3.5	5	4.25
9/3/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/5/2016	255	195	225.00	0	0	0
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	35	90	62.50	1.7	1.7	1.7
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	65	75	70.00	0.9	0.9	0.9
9/26/2016	60	45	52.50	0.9	0.7	0.8

Table D-22. *Staphylococcus aureus* eudiometer biogas data (part 1). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-11	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
8/29/2016	12:53	22	1023	x	0	0	0
8/30/2016	8:13	22	1018	x	430	460	445
8/31/2016	8:26	22	1019	x	395	325	360
9/1/2016	8:33	21	1023	x	330	630	480
9/2/2016	14:53	22	1024	x	630	620	625
9/3/2016	9:08	22	1024	x	320	300	310
9/5/2016	9:20	21	1015	x	390	375	382.5
9/6/2017	8:53	22	1013	x	140	130	135
9/7/2016	11:44	21	1013		130	120	125
9/8/2016	18:27	21	1009		220	205	212.5
9/10/2016	15:26	22	1010		350	310	330
9/12/2016	13:13	21	1015	x	430	380	405
9/18/2017	13:00	22	1012		280	250	265
9/19/2016	13:14	23	1013	x	330	300	315
9/26/2016	13:18	22	1008		300	280	290

Table D-23. *Staphylococcus aureus* eudiometer biogas data (part 2). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
8/29/2016	0.0	0.0	0.00	0.0	0.0	0.00
8/30/2016	26.8	24.5	25.65	28.9	28.2	28.55
8/31/2016	29.1	25.9	27.50	29.4	28.0	28.70
9/1/2016	25.5	25.0	25.25	24.4	23.3	23.85
9/2/2016	28.7	25.0	26.85	23.7	21.8	22.75
9/3/2016	50.4	45.7	48.05	27.2	25.0	26.10
9/5/2016	61.9	58.8	60.35	25.0	24.5	24.75
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	29.4	26.4	27.90	22.6	21.7	22.15
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	20.4	17.8	19.10	20.8	19.9	20.35
9/26/2016	18.1	16.2	17.15	19.9	19.3	19.60

Table D-24. *Staphylococcus aureus* eudiometer biogas data (part 3). Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
8/29/2016	0	0	0.00			
8/30/2016	80	125	102.50	7.3	7.3	7.3
8/31/2016	255	275	265.00	3.5	4.2	3.85
9/1/2016	225	300	262.50	4.7	5.6	5.15
9/2/2016	235	325	280.00	3.8	5.1	4.45
9/3/2016	245	355	300.00	0	0	0
9/5/2016	165	260	212.50	0	0	0
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	75	190	132.50	1.6	1.8	1.7
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	55	95	75.00	1.1	1.1	1.1
9/26/2016	35	70	52.50	0.8	0.7	0.75

Table D-25. Negative control eudiometer biogas data (part 1) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	Time	Air Temp (°C)	Pressure (mbar)	Zeroed	Gas Volume (mL) Sample E-11	Gas Volume (mL) Sample E-2	Average Gas Volume (mL)
8/29/2016	12:53	22	1023	x	0	0	0
8/30/2016	8:13	22	1018	x	100	130	115
8/31/2016	8:26	22	1019	x	90	105	97.5
9/1/2016	8:33	21	1023	x	10	50	30
9/2/2016	14:53	22	1024		20	20	20
9/3/2016	9:08	22	1024		50	40	45
9/5/2016	9:20	21	1015		150	130	140
9/6/2017	8:53	22	1013	x	200	180	190
9/7/2016	11:44	21	1013		80	100	90
9/8/2016	18:27	21	1009		75	70	72.5
9/10/2016	15:26	22	1010		150	150	150
9/12/2016	13:13	21	1015	x	210	200	205
9/18/2017	13:00	22	1012		150	130	140
9/19/2016	13:14	23	1013	x	180	150	165
9/26/2016	13:18	22	1008		150	110	130

Table D-26. Negative control eudiometer biogas data (part 2) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	% Methane Sample E-1	% Methane Sample E-2	Average % Methane	% CO_2 Sample E-1	% CO_2 Sample E-2	Average % CO_2
8/29/2016	0.0	0.0	0.00	0.0	0.0	0.00
8/30/2016	N/A	N/A	N/A	N/A	N/A	N/A
8/31/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/1/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/2/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/3/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/5/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/26/2016	13.2	11.3	12.25	16.2	15.6	15.90

Table D-27. Negative control eudiometer biogas data (part 3) for *S. enterica* and *S. aureus*. Where E-1 = eudiometer duplicate 1 and E-2 = eudiometer duplicate 2. * = unable to obtain gas quality. N/A = not applicable/no sampling.

Date	H_2S (ppm) Sample E-1	H_2S (ppm) Sample E-2	H_2S (ppm) Average	O_2% E-1	O_2% E-% E-	Average O_2%
8/29/2016	0	0	0			
8/30/2016	N/A	N/A	N/A	N/A	N/A	N/A
8/31/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/1/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/2/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/3/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/5/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/6/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/7/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/8/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/10/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/12/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/18/2017	N/A	N/A	N/A	N/A	N/A	N/A
9/19/2016	N/A	N/A	N/A	N/A	N/A	N/A
9/26/2016	60	40	50.00	0.2	0	0.1

References

Abdul P, D Lloyd (1985) Pathogen survival during anaerobic digestion: fatty acids inhibit anaerobic growth of *Escherichia coli*. Biotechnology Letters 7(2): 125-128.

Al Seadi T, Rutz D, Prassl H, Köttner M, Finsterwalder T, Volk S, Janssen R (2008) Biogas handbook. Esbjerg, Denmark.

Amani T, Nosrati M, Sreekrishnan TR (2010) Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects — a review. Environmental Reviews 18(1): 255-278.

American Biogas Council (2017) https://www.americanbiogascouncil.org/biogas_maps.asp Accessed 1 Sept 2016

BD (2013) Campylobacter Agar with 5 Antimicrobics and 10% Sheep Blood (Campy BAP). PA-254001.06

The Board of Regents of the University of Wisconsin system (2013) Wisconsin geological and natural history survey. https://wgnhs.uwex.edu/water-environment/karst-sinkholes/ Accessed 15 Jun 2017

Byappanahalli MN, Nevers MD, Korajkic A, Staley ZR, Harwood VJ (2012) Enterococci in the environment. Microbiology and Molecular Biology Reviews (76)4: 685-706.

Composting Council (2011) USCC position statement: keeping organics out of landfills. Ronkonkoma, NY

Composting Council. 2014. http://compostingcouncil.org/landfill-ban-map-10-2014/

DeBruyn J, Hilbron D (2007) Anaerobic digestions basics: fact sheet. Ontario. Ministry of Agriculture, Food and Rural Affairs 07-057: 1-8.

Deutsches Institut für Normung (1985) DIN 38414, part 8, German standard method for the examination of water, waste water, and sludge; sludge and sediments (group S) – determination of the amenability to anaerobic digestion.

Deutsches Institut für Normung (1998) DIN EN 12176, Characterization of sludge – determination of pH value.

Deutsches Institut für Normung (2001) DIN EN 12880, Characterization of sludges – determination of dry residue and water content.

Deutsches Institut für Normung (2004) DIN EN 12879, Chemical analyses – determination of loss on ignition in sediment, sludge, soil, and waste.

Deutsches Institut für Normung (2006) DIN 38414-18/ VDI 4630. ICS 13.030.30; 27-190. Fermentation of organic materials characterization of the substrate, sampling, collection of material data, fermentation tests.

Difco™ & BBL™ Manual, 2nd Edition. mTEC Agar, Modified (2009). Available from: https://bd.com/europe/regulatory/Assets/IFU/Difco_BBL/215044.pdf

Environmental Research and Innovation Center (ERIC) Laboratory (2012). Standard Operating Procedure ERIC-VFA-02.

Environmental Research and Innovation Center (ERIC) Laboratory (2017a) Allen Farms Digester – General Overview.

Environmental Research and Innovation Center (ERIC) Laboratory (2017b) Rosendale Digester – General Overview.

Gunaseelan VN (1997) Anaerobic digestion of biomass for methane production: a review. Biomass and Bioenergy 13(1-2): 83-114. http://dx.doi.org/10.1016/S0961-9534(97)00020-2

Horan NJ, Fletcher L, Betmal SM, Wilks SA, Keevil CW (2004) Die-off of enteric bacterial pathogens during mesophilic anaerobic digestions. Water Research 38: 1113-1120.

IDEXX Enterolert ™ instructions via American Society for Testing and Materials (ASTM) D6503-99 Standard Test Method for Enterococci in Water Using Enterolert™. (1999). DOI:10.1520/D6503-14

Jones PW (1979) Health hazards associated with the handling of animal wastes. The Veterinary Record 106: 4-7.

Jones PW, Matthews PRJ (1975). Examination of slurry from cattle for pathogenic bacteria. The Journal of Hygiene 74: 57-64.

Kearney TE, Larkin MJ, Levett PN (1993) The effect of slurry storage and anaerobic digestion on survival of pathogenic bacteria. Journal of Applied Bacteriology 74: 86-93.

Kleinheinz G (2017) Personal Communication.

Korres NE, O'Kiely P, Benzie JAH, West JS (2013) Bioenergy production by anaerobic digestion using agricultural biomass and organic wastes. New York, NY.

Kusch S, Schäfer W, Kranert M (2011) Dry digestion of organic residues, integrated waste management - Volume I, Mr. Sunil Kumar (Ed.), ISBN: 978-953-307-469-6, InTech, Available via: https://www.intechopen.com/books/integrated-waste-management-volume-i Accessed 11 April 2017.

Larsen HE, Munch B, Schlundt J (1994) Use of indicators for monitoring the reduction of pathogens in animal waste treated in biogas plants. International Journal of Hygiene and Environmental Medicine (Zentralblatt f*ü*r Hygiene und Umweltmedizin) 195(5-6): 544-555.

Mitscherlich E, Marth EH (1984). Microbial survival in the environment: bacteria and rickettsiae important in human and animal health. Berlin, Germany

National Center for Biotechnology Information, U.S. National Library of Medicine (NCBI) (2017) *Enterococcus faecalis* Complete Genome. Available from: https://www.ncbi.nlm.nih.gov/genome/?term=enterococcus%20faecalis Accessed 6 October 2016.

Olsen JE, Larsen HE (1987) Bacterial decimnation times in anaerobic digestion of animal slurries. Biological Wastes 21:153-168.

Rabah AB, Baki AS, Hassan LG, Musa M, Ibrahim AD (2010) Production of biogas using abattoir waste at different retention time. Science World Journal 5(4):23-26.

Ramsey M, Hartke A, Huycke M (2014) The physiology and metabolism of enterococci. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, (eds) Enterococci: from commensals to leading causes of drug resistant infection Boston: Massachusetts, pp. 1-43.

Sobsey MD, Khatib LA, Hill VR, Alocilja E, Pillai S (2006) Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate. In: JM Rice, FD Caldwell, FJ Humenik, (eds) Animal Agriculture and the Environment: National Center for Manure and Animal Waste Management White Papers. ASABE, St. Joseph, Michigan pp 609-666.

Sluiter A, Hames B, Hyman D, Payne C, Ruiz R, Scarlata C, Sluiter J, Templeton D, Wolfe J (2008) Biomass and Total Dissolved Solids in Liquid Process Samples Laboratory Analytical Procedure (LAP) Technical Report. National Renewable Energy Laboratory. Available via: NREL/TP-510-42621. Accessed 5 May 2017.

Termorshuizen AJ, Volker D, Blok WJ, ten Brummeler E, Hartog BJ, Janse JD, Knol W, and Wenneker M (2003) Survival of human and plant pathogens during anaerobic mesophilic digestion of vegetable, fruit, and garden waste. European Journal of Soil Biology 39: 165–171.

Topp E, Scott A, Lapen DR, Lyautey E, Duriez P (2009) Livestock waste treatment systems for reducing environmental exposure to hazardous enteric pathogens: some considerations. Bioresource Technology 100: 5395-5398.

United States Department of Agriculture (1995) Animal manure management. Available via: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/null/?cid=nrcs143_014211 Accessed 6 October 2017.

United States Environmental Protection Agency (2002) Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using modified membrane- thermotolerant *Escherichia coli* agar (modified mTEC) EPA-821-R-02-023.

United States Environmental Protection Agency (2013) Literature review of livestock and poultry manure EPA 820-R-13-002 pp. 1-137

United States Environmental Protection Agency (2015) Advancing sustainable materials management: 2013 fact sheet assessing trends in material generation, recycling and disposal in the United States. Available via: https://www.epa.gov/sites/production/files/2015-09/documents/2013_advncng_smm_fs.pdf Accessed 6 October 2017.

United States Environmental Protection Agency (2016) America's food waste problem. Available via: https://www.epa.gov/sciencematters/americas-food-waste-problem Accessed 6 October 2017.

Wagner AO, Gstraunthaler G, Illmer P (2008) Survival of bacterial pathogen during the thermophilic anaerobic digestion of biowaste: laboratory experiments and *in situ* validation. Anaerobe 14: 181-183.

Wu QL, Guo WQ, Zheng HS, Lao HC, Feng XC, Yin RL, Ren NQ (2016) Enhancement of volatile fatty acid production by co-fermentation of food waste and excess sludge without pH control: the mechanism and microbial community analyses. Bioresource Technology 216:653-660.

Willey JM, Sherwood LM, Woolverton CJ (2014) Prescott's microbiology. New York, NY.

Zar JH (1999) Biostatistical analysis in Appendix B, 4th edn. Prentice Hall, Upper Saddle River, New Jersey, pp. App21-App22