ABSTRACT

Title of Document: INCREASING THE SUSTAINABILITY OF PSYCHROPHILIC SMALL-SCALE ANAEROBIC DIGESTERS

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The research was aimed at increasing the energy production efficiency of small-scale anaerobic digesters in temperate climates while quantifying their environmental impacts. Biochemical methane potential tests were used to quantify methane (CH_4) production from separated and unseparated manure during psychrophilic digestion, and compare CH_4 production when pre-incubated alternative inocula (wetland sediment (WS), landfill leachate (LL), mesophilic digestate (MD)) were used. Methanogenic and Archaeal communities were analyzed using T-RFLP and qPCR.

At 24 ºC, unseparated manure produced significantly higher (40%) quantity of CH_4 than separated manure due to higher volatile solids (VS) content, but differences were insignificant at digestion times of ≤16 days. At lower digestion times, farmers could digest liquid, separated manure without sacrificing CH_4 production, but at

longer digestion times, the VS in unseparated manure has the time necessary for CH_4 conversion.

The alternative inocula studies showed that LL inoculum after incubation for 91 days at 25 ºC produced significantly higher quantity (≥20%) of CH_4 than MD and WS during digestion at the same temperature, and was not significantly different in CH_4 quantity than MD that was incubated and digested at 35 ºC (202 ± 4 L/kg VS). *Methanosarcinaceae* was dominant in the LL reactor, while the other reactors were abundant in *Methanosaetaceae,* indicating that inoculum rich in *Methanosarcinaceae* may be beneficial for starting digestion at lower mesophilic temperature ranges. Longer incubation time generally reduced the inoculum amount needed for batch digestion and prevention of volatile fatty acids accumulation. In batch systems with long digestion time (90 days), MD inoculum from well-established digesters, 35% inoculum to substrate ratio, and 35 ºC operation temperature are recommended for highest CH_4 production per unit of digester volume.

Additionally, life cycle assessments (LCA) were conducted to compare the sustainability of an unheated Chinese fixed-dome digester with a heated and insulated small-scale plug-flow digester in the US. The LCA showed that the US plug-flow digester was more sustainable than the Chinese fixed-dome system only in climate change category, but contributed negatively towards 17 impact categories. Digester heating and heating infrastructure were the main contributors towards the negative impacts observed in the US plug-flow digester.

INCREASING THE SUSTAINABILITY OF PSYCHROPHILIC SMALL-SCALE ANAEROBIC DIGESTERS

By

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Foreword

Chapter 2 is a work previously published in *Ecological Engineering, The 13th Annual Conference of the American Ecological Engineering Society: Ecological Engineering and the Dawn of the 21st century* special issue. Inclusion of this Chapter into the dissertation was approved by the dissertation director and has been endorsed by the graduate program's Graduate Director. The student's Dissertation Examining Committee has determined that the student has made substantial contribution towards this work.

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Dedication

To Mom, Dad, and the Witarsa Family. Thank you for being a source of joy!

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1 Introduction

1.1 Anaerobic Digestion Background

Anaerobic digestion is a process in which organic matter is broken down in the absence of oxygen, forming methane (CH_4), carbon dioxide (CO_2), and trace amounts of hydrogen sulfide and ammonia. Initially, hydrolytic bacteria break down and ferment organic polymers to form fatty acids, carbon dioxide, hydrogen, and alcohols (Chynoweth, 1987). Acetogens convert the volatile fatty acids into acetate, hydrogen, and carbon dioxide. Carbon dioxide, hydrogen, and acetate can then be used by methanogens to form methane (Chynoweth, 1987).

The use of anaerobic digestion for treatment of wastewater can provide a number of benefits. The process produces renewable energy in the form of CH_4-enriched biogas that can be used for the production of heat and electricity (Holm-Nielsen et al., 2009: Lansing et al., 2008), reduces odor, allowing farmers to operate in close proximity to communities (Powers et al., 1999), produces fertilizer (Holm-Nielsen et al., 2009), reduces pathogen content within wastewater (Barros et al., 2008; Olsen and Larson, 1987), decreases total solids, volatile solids, and chemical oxygen demand within wastewaters reducing their polluting potential towards water bodies (Barros et al., 2008; Lansing et al., 2008), and capture and mitigate the release of CH_4 associated with manure management (AgSTAR, 2011)

Despite the benefits of this technology, however, the installation of the system is hindered in temperate climates. Anaerobic digestion functions best at mesophilic temperatures ranging from 30-35 °C and at thermophilic temperatures ranging from

50-60 °C (Gerardi, 2003). Energy production from digesters decreases when temperature decreases. Massé et al. (2003) reported a 70% decrease in the quantity of CH_4 produced when digestion temperature was decreased from 20 to 10 °C. Reducing digestion temperature from 30 to 25 and 15 °C also increased the lag-phase from 33 to 66 and 165 days, respectively, before CH_4 production commenced (Zeeman et al., 1988).

As a result, many digesters are heated and operated at mesophilic (25-35 °C) or thermophilic (50-60 °C) temperatures, instead of at ambient or psychrophilic (≤25°C) temperatures. In the US, for instance, the majority of digesters treating livestock waste functions at mesophilic temperatures (AgSTAR, 2006). Heating of digesters could be achieved through the use of waste heat generated from combined heat and power (CHP) generators used for the combustion of biogas, or through the combustion of biogas in boilers. However, the installation of these heating systems increases the capital and operational costs of digesters. Currently, due to economic reason, AgSTAR (2011) only recommends the use of digester in dairy farms with ≥500 cows, making the technology out of reach for >94% of the dairy farms in the US that have less than 500 cows (USNASS, 2014). Reducing heating requirements of the digesters could reduce costs and improve the profitability of the system, creating incentives for small-scale farmers to install this technology.

Energy production also decreases in many unheated digesters in China. China has the highest number of digesters in the world, with 40 million digesters and a growth rate of approximately 1 million units per year (He et al., 2013; Yang et al., 2012b). Digesters in Northern China can only function for 8-9 months of the year

(Chen et al., 2010). There is a need to increase energy production efficiency in temperate climate digesters and research conducted within this dissertation was aimed at accomplishing this goal.

1.2 Research Goal and Background

The goal of the research was to determine operating conditions and inoculum sources that could increase the energy production efficiency and reduce the environmental impacts of small-scale temperate anaerobic digesters.

1.2.1 Manure Separation for Reducing Digester Volume

Manure separation is generally used in farms to separate out coarse particles present in manure. The process can reduce clogging in pipes and pumps, and facilitates the transportation of manure.

Digestion of unseparated manure could produce higher amount of CH_4 than the digestion of separated manure per unit volume (or mass) of manure due to higher volatile solids content within the unseparated manure. For instance, at a hydraulic retention time (HRT) of 16 days, the anaerobic digestion of separated manure produced 10-30% less methane than the digestion of similar amount of unseparated manure (Lo et al., 1983a, b). However, smaller particle size within separated manure could allow faster degradation and conversion of the volatile solids (VS) to CH_4 within separated manure compared to unseparated manure (El-Mashad and Zhang, 2010; Lo et al., 1983a, 1983b; Rico et al., 2007). At lower HRT, separated manure was observed to produce higher amount of CH_4 compared to the same volume of unseparated manure despite the latter having higher volatile solids content. Lo et al.

(1983a, b) found that at 6 and 12 day HRT, separated liquid manure produced almost twice the amount of CH_4 as unseparated manure. The authors concluded that digester volume could be reduced by more than 60% if the coarse fractions are removed and lower HRT is used. Manure separation could thus be a method to reduce digester volume and costs.

Previous studies concerning manure separation were conducted at temperatures ranging from 30-35 °C, with no study at psychrophilic temperatures (≤25 °C). The first study aims to address this research gap by quantifying differences in CH_4 production between digesters treating unseparated and separated dairy manure at two psychrophilic temperatures (14 and 24 °C) over time.

1.2.2 Alternative Inoculum Sources to Increase Methane Production in Digesters

The addition of inoculum is an important part of ensuring successful digestion process. Introducing inoculum helps seed the digester with microorganisms that are ready to reproduce and carry out anaerobic digestion. Previous research has looked at the importance of inoculum in the digestion process.

Compared to uninoculated treatment, Lopes et al. (2004) observed increased organic matter conversion to CH_4 when inoculum was added. Several studies have also investigated the effects of inoculum to substrate ratio (ISR) on digestion processes. Hashimoto (1989), Lopes et al. (2004), and Maya-Altamira et al. (2008) observed increased CH_4 or biogas production when ISR was increased. However, others have observed little to no differences in cumulative CH_4 production when the ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2006). From the literature, it appears that some studies have shown increased

digestion rate when ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2009; Zeng et al., 2010), indicating that increasing ISR could increase the rate of digestion, but not necessarily cumulative CH_4 production.

Other inoculum research involves pre-incubation or acclimatization of the inoculum before utilization in digestion. Zeeman et al. (1988) pre-incubated the inoculum at 18 °C and observed higher CH_4 production rate during low-temperature digestion, compared to treatments that received inoculum from a 35 °C source. Nozhevnikova et al. (1999) observed higher rates of CH_4 production during low-temperature digestion when the inoculum used was pre-acclimated to the same temperature. In a separate study, Collins et al. (2003) observed growth and selection of psychrotolerant microorganisms (microorganisms that could function at psychrophilic temperatures, but still have optimum growth at mesophilic temperatures) in reactors that were running at 15 °C. Thus, the selection and growth of psychrotolerant microorganisms within the inocula during the acclimatization period likely resulted in increased CH_4 production during low temperature digestion.

Some studies have also investigated using alternative inocula from environmental sources to seed anaerobic digesters. All of the above-mentioned inoculum studies, with the exception of Zeeman et al. (1988) who also studied the use of wetland soil as inoculum, utilized conventional inoculum sources: sludge/digestate from anaerobic digesters or bovine rumen fluid. Some researchers studied the use of wetland or river sediments to seed their digesters with the goal of either increasing CH_4 production during low-temperature digestion or to improve the performance of digesters during substrate overload. Bardulet et al. (1990) used river sediment to

inoculate their digesters that were operating at 20 °C and observed stable organic matter removal and biogas production during the experiment. Xing et al. (2010) inoculated a 15 °C digester with lake sediment that was incubated for 225 days at the same temperature. Despite the low digestion temperature, they observed high chemical oxygen demand removal within the reactor. It should be noted that it was not clearly stated why a 225-day incubation period was chosen in the study. On the contrary, Zeeman et al. (1988) observed no reduction in the lag-period in CH_4 production when wetland soil was used as inoculum, compared to uninoculated treatment. Steinberg and Regan (2011) studied the use of acidic bog sediment as inocula for mesophilic digesters and found that the digester inoculated with bog sediment could survive the first, of three, organic shock load better than the reactors that contained inoculum from a municipal sludge or inocula from bog sediment mixed municipal sludge.

Based on literature review, there are currently limited studies that have looked at the use of landfill leachate as an inoculum source for agricultural digesters. In addition, there are also research gaps in terms of the amount of alternative inocula that should be used for digestion (ISR) and the amount of incubation time needed for these inocula before they are used for digestion. The second study was conducted to address this research gap by quantifying CH_4 production using three different inocula: wetland sediment, landfill leachate, and mesophilic digestate, two inoculum incubation periods (91 and 196 days), three ISR (20, 35 and 50% w/w), and three temperatures (15, 25, and 35 ºC) during anaerobic digestion of dairy manure to

determine the effect that inocula type, ISR, and incubation time have on anaerobic digestion of manure at different temperatures.

1.2.3 Understanding Methanogenic Community Shifts During Incubation of Inocula

Molecular techniques refer to a diverse set of tools that can be used to study biochemical components, such as genes and proteins, within a cell. The techniques are powerful methods that can be used to provide information at the cellular level, such as the functions of a protein, or at the ecosystem level, such as the different species of microorganisms present in soils or gut.

Molecular techniques have been used in the digestion field to monitor microbial community shifts during psychrophilic digestion (Collins et al., 2003, McHugh et al., 2004), during start up of a mesophilic batch reactor (Lee et al., 2010), and when organic loading rate was varied (Dollhopf et al, 2001).

The techniques have also been used to provide relationships between the inocula and digestion functionality. Regueiro et al. (2012) found that higher *Bacteroidetes* and Archaea numbers were associated with higher hydrolytic and methanogenic activities, respectively. Using inoculum from lake sediment, Xing et al. (2009, 2010) found that the dominant Archaea present within the 15 °C psychrophilic reactor had 98% similarity to *M. lacustris*, which has a lower optimal temperature than the *Methanosaeta* that dominated a reactor inoculated with mesophilic digestate inoculum. Dollhopf et al. (2001) also found that initial bacterial and Archaeal communities were different for a digester that was inoculated with digestate from anaerobic reactor compared to a reactor inoculated with river sediment, but

convergence of the communities was observed during the digestion process at 34 ºC. Steinberg and Regan (2008) found no overlap in the methanogenic species within a bog sediment and the effluent from a digester treating municipal wastewater. When the two inocula and a mixture of the two were used to inoculate three digesters that received glucose shocks, the reactor inoculated solely with sediment survived an organic shock load better than the other two digesters, and Fen Cluster was observed to dominate the methanogenic community of the bog sediment reactor, indicating that Fen Cluster could play an important role in conferring tolerance to organic shocks in reactors, although it should be mentioned that *Methanosarcina* was more important for resuming CH_4 production (Steinberg and Regan, 2011).

There is still currently a lack of research in terms of how microbial community in alternative inocula changes when they are incubated for different periods of time at different temperatures, and how these changes relate to their effectiveness as inocula. The study in Chapter 4, which was complementary to the study in Chapter 3, was conducted to address this research gap by quantifying changes in methanogenic community during the incubation of alternative inocula and associate these changes to CH_4 production in digesters that received these inocula.

1.2.4 Life Cycle Assessment (LCA) of Small-Scale Digesters

Life Cycle Assessment (LCA) is an environmental accounting technique that is used to quantify the impacts that a system, activity, or product has on the environment during its lifetime, beginning with the extraction of raw materials to construct the system to the disposal of the system after its useful lifetime in a “cradle to grave” analysis (Vigon et al., 1994). A LCA quantifies the inputs and outputs of a

system, process, or the creation of a product and evaluates the environmental impacts of each input and output (Vigon et al., 1994). LCA can be used to determine which process or component of the system has the largest environmental impact in order to target this area for further improvement to minimize the system's negative environmental impacts (Vigon et al., 1994). The results obtained can be used to compare the impacts of different systems, providing a tool for designers, contractors, and policy makers to view the advantages and disadvantages of implementing one system over another (Rehl et al., 2012). The LCA does not take into account social or economic impacts of the process or system, although studies have used hybrid LCA that incorporated economic component, or have separately conducted an economic study, in addition to the LCA, as part of a multi-criteria assessment technique (ISO, 1997; Murray et al., 2008; Nzila et al., 2012).

The LCA process can be broken down into four main components (Özeler et al., 2006; Vigon et al., 1994):

1) Goal and scope: a clear statement of the intended goal and scope of the analysis, including defining the system boundaries, indicating parts of the process that will be included or excluded.

2) Inventory analysis: quantification of all inputs and outputs of the system including energy and raw materials consumed or produced, air and water emissions, and waste products.

3) Impact analysis: quantification of the effects that the inputs and outputs listed in the inventory analysis have on the environment and human well-being.

4) Improvement analysis: evaluation of the focus areas within the system that

could be improved to minimize the system's or process' negative environmental impacts.

A number of LCAs have been conducted for anaerobic digesters. Some studies were focused on comparing different alternatives of waste treatments with scenarios that incorporate anaerobic digesters. Chaya and Gheewala (2007) compared the use of anaerobic digesters with incineration to treat municipal waste in Thailand, and concluded that anaerobic digestion had less global warming potential, acidification potential, photo-oxidant formation, stratospheric ozone depletion, consumption of energy resources, heavy metals impacts, and solid waste discharged to the landfills, but had higher nutrient release into the environment. Özeler et al. (2006) studied various municipal waste treatment options in Ankara, Turkey using LCA and determined that incorporating source reduction within the system resulted in the lowest impacts in terms of non-renewable energy use, hazardous materials, acidification, eutrophication potentials, and human toxicity potential. Incorporating anaerobic digestion resulted in a system that contributed the least towards greenhouse gas emissions.

Some researchers have also used LCA to study the changes in a digestion system's environmental impacts when parameters within the system are changed. Berglund and Börjesson (2006) demonstrated that switching the digestion of biomass waste such as the tops and leaves of sugar beet to the digestion of ley crops, which are planted especially for energy production, increased the energy input/output ratio from approximately 0.27 to 0.40. In addition, the authors found that switching the operation of centralized digestion system to farm-scale anaerobic digestion systems

for the treatment of swine manure increased the energy requirements from 30% to 55% of the produced energy.

Life cycle assessment has also been conducted on small-scale digestion systems. Mezullo et al. (2013) conducted a LCA on a small-scale digester and found that the production of biogas and digestate as fertilizer had beneficial impacts in the categories of climate change and fossil fuel depletion, but had detrimental impacts in the categories of respiratory inorganics emissions and acidification/eutrophication. Life cycle assessment studies have been used to show that the installation of the Chinese household fixed-dome digesters could reduce greenhouse gas emissions by 15.2-25.1 tons of CO_2 eq. (Wang and Zhang, 2012; Zhang et al., 2013). In addition, compared to a farming system without digester, the integration of the Chinese fixed-dome digesters into a farm setting, such as persimmon cultivation, or fish production, and swine rearing, could result in greenhouse gas emission reductions of 3,100-3,400 kg CO_2/year (Chen and Chen, 2013; Yang et al., 2012a), and a net energy production of 3,300 MJ/year (Chen and Chen, 2013).

There has been limited previous LCA research comparing the environmental impacts of small-scale digester with different designs. Nzila et al. (2012) compared the fixed-dome, Taiwanese plug-flow, and the floating drum designs, and found that the Taiwanese plug-flow and the fixed-dome digesters performed better in terms of energy demand, resource depletion, and global warming reduction than the floating drum digester. Pérez et al. (2014) compared the environmental impacts from the construction of fixed-dome design with the Taiwanese plug-flow design in the Andes region. They found that the fixed-dome system had lower abiotic potential,

eutrophication potential, and acidification potential than the plug-flow system, but both systems had similar global warming potential. There are limited studies that have compared the life cycle of small-scale designs, especially for comparison of small-scale designs that are used in a developing nation (designs that are generally are less sophisticated and are unheated) with designs that are used in the developed nations (designs that utilize more heating, insulation, and automation). The fourth study, Chapter 5, aims to address this research gap by comparing the LCA of a small-scale unheated household fixed-dome digester in China with a heated and insulated Taiwanese plug-flow digester in the US.

1.3 Objectives

There were four main objectives for the dissertation research:

1) Quantify the differences in CH_4 production between digesters treating unseparated and separated dairy manure at two psychrophilic temperatures (14 and 24 °C) over time to determine if manure separation could be used to reduce digester volume.

2) Quantify CH_4 production using three different inocula: wetland sediment, landfill leachate, and mesophilic digestate, two inoculum incubation periods (91 and 196 days), three ISR (20, 35 and 50% w/w), and three temperatures (15, 25, and 35 ºC) during anaerobic digestion of dairy manure to determine the effect that inocula type, ISR, and incubation time have on anaerobic digestion of manure at different temperatures.

3) Quantify changes in methanogenic community during the incubation of alternative inocula (from Chapter 3) and compare these shifts to the CH_4 production in digesters that received these inocula sources.

4) Compare the LCA of a small-scale unheated household fixed-dome digester in China with a heated and insulated Taiwanese plug-flow digester in the US to assess the change in sustainability of a small-scale digestion system as it is translated from a developing nation (no heating and less automation) to a developed nation (installed with automation, heating, and insulation) using the same system boundary and assessment methods.

1.4 Research Approach

Both objectives 1 and 2 were conducted at laboratory-scale. In Objective 1 (Chapter 2), biochemical methane potential (BMP) tests, adapted and modified from Moody et al. (2011), were used to monitor differences in CH_4 production between separated and unseparated manure that were inoculated with digestate from a mesophilic digester. In Objective 2 (Chapter 3), wetland sediment, landfill leachate, and digestate from a mesophilic digester were incubated in 4 L reactors at 15, 25, and 35 °C. The reactors were fed with autoclaved manure at regular intervals, and samples were extracted from the reactors on Day 91 and 196 for use as inocula in two BMP tests. In Objective 3 (Chapter 4), terminal restriction fragment length polymorphism (T-RFLP) and quantitative polymerase chain reaction (qPCR) were used to determine changes in Archaeal community and methanogenic number, respectively. In Objective 4 (Chapter 5), LCA for individual digester was conducted using data from field survey and literature using Sima Pro 8 (PRé Consultants, The

Netherlands). Environmental impacts were analyzed using the ReCiPe mid-point hierarchist method integrated within the software (Goedkoop et al., 2013). Together this work seeks to increase energy production efficiency of small-scale temperate anaerobic digesters and minimize their impacts on the environment.

2 Quantifying Methane Production from Psychrophilic Anaerobic Digestion of Separated and Unseparated Dairy Manure

Abstract

In anaerobic digestion, methane (CH_4) production decreases as temperature decreases, resulting in a lower CH_4 production at psychrophilic (≤25 ºC) digestion temperatures. Previous studies at mesophilic temperatures (30-35 ºC) have shown that manure separation and digesting only the liquid fraction could result in the reduction of digester volume without sacrificing CH_4 production. In this research, biochemical methane potential (BMP) tests were used to quantify CH_4 production of unseparated and separated manure at two psychrophilic temperatures: 14 and 24 ºC.

The results showed that CH_4 production decreased by approximately 70% when the temperature was decreased from 24 ºC to 14 ºC. Between Days 20-216 at 24 ºC, higher VS content of the unseparated manure resulted in significantly higher CH_4 production (29-40% more) compared to separated manure, on a volumetric basis, but at digestion times of ≤16 days, faster VS to CH_4 conversion rates in separated manure resulted in no significant differences in CH_4 production between the manure types. Similarly, at 14 ºC, the higher VS content of the unseparated manure resulted in significantly higher CH_4 production (56-147% more) throughout most of 216-day experimental period, when normalized by volume. On a VS basis (mL CH_4/g VS), the separated manure at 24 ºC produced significantly more CH_4 than the other treatments. The study suggests that at 24 ºC, there will be higher CH_4 production, per volume of manure added, from unseparated manure due to the higher VS content, but when operating at a shorter digestion time, the differences could be insignificant.

2.1 Introduction

During anaerobic digestion (AD), both facultative and obligate anaerobic microorganisms work sequentially to extract energy from organic matter fed into the system, with renewable energy in the form of methane (CH_4)-enriched biogas as a product of this metabolism. AD technology can be used to treat wastewater sources, such as dairy manure, resulting in 1) reduction in detrimental impact of manure waste on water bodies by reducing chemical oxygen demand (COD), total solids (TS), and volatile solids (VS) (Lansing et al., 2010); 2) reduction in odor, which can help to improve relationships between farmers and their neighbors (Powers et al., 1999); 3) use of the CH_4-enriched biogas directly as a source of heat or in an electric generator (Lansing et al., 2008); and 4) capture, combustion, and thus, reduction in the quantity of methane (CH_4) released, a greenhouse gas 21 times more powerful than carbon dioxide, compared to traditional open lagoon storage of manure (AgSTAR, 2011; IPCC, 2007).

High costs, however, impede AD installation in temperate regions, such as the United States. ADs function best at mesophilic (30-35 ºC) and thermophilic (50-60 ºC) temperatures (Gerardi, 2003), and CH_4 production decreases when temperature decreases. Massé et al. (2003) found that decreasing the temperature of anaerobic swine manure reactors from 20 °C to 10 °C decreased CH_4 production by 70%. In addition, lower-temperature digesters have a longer lag-phase before CH_4 production commences. One study showed that dairy cow manure digested at less than 15 °C did not produce CH_4 for 165 days, while manure digested at 25 °C and 30 °C experienced shorter lag-phases of 66 and 33 days, respectively (Zeeman et al., 1988).

In order to keep biogas production high during colder months, most agricultural ADs in the US are heated and run at mesophilic temperatures, as opposed to psychrophilic (or ambient) temperatures (≤25 ºC) (AgSTAR, 2006). To keep digesters in the mesophilic range, expensive heating and insulation systems must be installed and maintained. Many heating systems use a portion of the produced biogas to heat the digesters during the colder months, a period when the need for the produced CH_4 is the greatest for other on-farm activities, such as heating barns and buildings. The cost of insulation, installing heat recovery systems from biogas engine generators, and the in-vessel radiant heating mechanisms makes the installation of small-scale ADs in the US largely cost-prohibitive (Klavon et al., 2013), which is one of the reasons AgSTAR (2011) stated that digesters are more economically feasible on dairy farms with more than 500 cows, excluding more than 95% of the US dairy farms (USNASS, 2009). Methods to increase CH_4 production in temperate regions without increasing digester installation costs are thus needed.

This study investigated the effect of manure separation on net CH_4 production from ADs operating at psychrophilic temperature. Manure separation refers to the separation of manure to remove the coarse fractions from the liquid fraction. Separation can reduce clogging in pipes and pumps, and ease manure transportation. While there are several methods for manure separation including the use of mechanical presses, screens, or the addition of flocculants or coagulants to remove the coarse fraction from the liquid filtrate of manure (Pain et al., 1984; Rico et al., 2007), this study concentrated only on the use of a mechanical screw-press separator for solid separation.

Previous research has shown that the VS within separated manure converts to CH_4 more quickly than VS in unseparated manure, likely due to the VS within separated manure having smaller particle sizes leading to quicker degradation (El-Mashad and Zhang, 2010; Lo et al., 1983a, 1983b; Rico et al., 2007). In addition, researchers such as Liao et al. (1984) and Lo et al. (1983a, 1983b) have shown that digester volume could be reduced when digesting separated manure without sacrificing CH_4 production. Therefore, a farmer interested in reducing capital costs for AD construction could build a smaller reactor volume for only the separated manure with shorter retention times and still produce large quantities of CH_4.

Previous experiments that have compared CH_4 production between separated and unseparated manure, however, were conducted at mesophilic conditions (30 ºC-35 ºC), with no published study investigating the effect of manure separation on CH_4 production at psychrophilic temperatures (≤25 ºC). The research objectives for this study were to: 1) quantify differences in CH_4 production at two psychrophilic temperatures, 2) quantify differences in CH_4 production between the separated and unseparated manure fractions at these psychrophilic temperatures, and 3), determine how digestion time affects these differences.

2.2 Methods

Both unseparated manure and separated manure were collected during one sampling trip to the dairy facility at the USDA Beltsville Agricultural Research Center (BARC) in Beltsville, Maryland, USA and stored at 4 ºC before analysis. At the BARC dairy facility, manure and urine, along with some straw bedding from the barn is scraped into a holding pit, which is then pumped into a belt-pressed

mechanical screw-press separator (FAN®) that separates the coarse fractions of the manure from the liquid fraction. The liquid fraction is held at a separate holding pit, before being pumped into a continuous-stirred mesophilic digester. The unseparated manure was collected before the screw-press and the separated manure was collected after the screw press. Approximately 70% of the dry weight solids were removed from the manure during the separation process, resulting in the separated manure having approximately 30% of the dry weight solid mass of the unseparated manure (unpublished data).

Inoculum for the experiment was obtained from the BARC mesophilic digester treating the separated dairy manure. The inoculum contains CH_4-producing microorganisms that speed up the digestion process. It was collected on the same day as the manure and stored at 4 ºC before analysis.

2.2.1 Biochemical Methane Potential (BMP) Testing

The biochemical methane potential (BMP) test used in this study was adapted and modified from the procedures conducted by Moody et al. (2011) and Owen et al. (1979). In this study, the BMP tests were not conducted at the standard 35 ºC, but conducted in two separate chambers operating at 14 ºC and 24 ºC to simulate the average temperatures for Fall (14 ºC) and summer (24 ºC) in Baltimore, Maryland between 1981-2010 (NOAA, 2014).

The BMP test was conducted by monitoring the CH_4 production in 250 mL serum bottles filled with 100 mL of inoculum and 30 mL of either unseparated manure or separated manure. The inoculum volume was kept constant to reduce a potential compounding variable, resulting in an inoculum to substrate VS ratio (ISR)

of 1:1 for the unseparated manure, and 2:1 for the separated manure, which are within the ISR range recommended by Moody et al. (2011) and Raposo et al. (2011). The control bottles consisted of 130 mL of inoculum without manure. No nutrient media was added during the BMP test to simulate field conditions.

To create anaerobic condition within the bottles, each bottle was purged with 70% N_2 and 30% CO_2 before being capped with butyl rubber stoppers. The bottles were incubated in two different chambers with temperatures of 14.0 ± 0.0 ºC and 24.0 ± 0.0 ºC. Triplicate bottles of the different treatments (unseparated manure + inoculum, separated manure + inoculum, and inoculum only) were incubated in each temperature chamber for 216 days. During incubation, no shaking/mixing was conducted to simulate simple, unmixed digestion conditions, such as those for covered lagoon systems.

The quantity of biogas produced in each BMP bottle was measured at least once every week using a graduated, gas-tight, wet-tipped 50 mL glass syringe inserted through the septa and equilibrated to atmospheric pressure. The measured biogas was then vented. After venting, 0.10 mL of biogas was collected from each bottle using a luer-lock, gas tight syringe and injected into an HP 5890 Series II gas chromatograph (GC) to measure percent CH_4. The GC was equipped with a flame ionization detector (FID) and was run with the following parameters: (1) injection temperature of 200 ºC; (2) detector temperature of 250 ºC; and (3) a flow rate of 300 mL/min for helium, the carrier gas.

In order to take into account the CH_4 produced from the inoculum organic matter, the CH_4 production values (mL CH_4/mL inoculum) from the triplicate

inoculum bottles at each temperature were averaged, normalized by inoculum volume, and subtracted from the CH_4 production of each treatment bottle incubated at the same temperature. All CH_4 production volumes were converted using the ideal gas equation to standard temperature and pressure (0 ºC and 1 atm). The CH_4 produced by the manure sources were normalized using two different methods: mL CH_4/g VS, which shows the efficiency of VS conversion to CH_4, and mL CH_4/mL manure to show which type of manure would produce higher CH_4 for a given volume of manure digested.

2.2.2 Substrate Characterization

Total solids, VS, pH, and COD analyses were conducted on the two manure types and the inoculum before the BMP experiment and on each treatment bottle at the end of the BMP test. Total solids and VS were conducted following standard methods (APHA, 2005), and the COD analysis was conducted using the HACH® adapted digestion method (Jirka et al., 1975).

2.2.3 Volatile Solids Destruction and Theoretical Methane Yields

Values for VS and COD destruction for each treatment during the BMP test were calculated by taking the average difference between the pre-digestion values (calculated from the individual VS and COD concentrations of the manure and inoculum) and the post-digestion values for each treatment. It should be noted that the VS and COD destruction values do not distinguish between destruction of the manure and inoculum components. Difference between the CH_4 yields of each treatment and the theoretical CH_4 yield were also calculated. The theoretical CH_4 yield (350 mL

CH_4/g COD destroyed) is based on calculated conversion of COD to CH_4 during the digestion process (Angelidaki and Ellegaard, 2003).

2.2.4 Statistical Analyses

Tukey-Kramer analyses were performed based on temperature and manure type (separated and unseparated) to compare differences in treatments during the 216-day experiment for percent CH_4, and CH_4 production based on the final CH_4 values using two normalization procedures (volumetric and VS normalization). It should be noted that the Tukey-Kramer analyses were conducted on the sub-replicates in each temperature chamber. Two-tailed t-tests were also performed to compare CH_4 production from separated and unseparated manure at approximately 5-day intervals at each temperature. Statistical analyses were conducted using the Proc Mixed (with repeated/group statement to account for any variance heterogeneity) and Proc ttest procedures in SAS® 9.3. An *alpha* value of 0.05 was used in each analysis.

2.3 Results

2.3.1 Wastewater Characterization

The TS and VS concentrations of the unseparated manure were approximately twice the concentration of the separated manure (Table 2.1). After 216 days of digestion, VS reduction was 2-9% higher at 24 ºC compared to 14 ºC for both manure types (Table 2.2). The pH of the unseparated manure, separated manure, and inoculum before digestion and of the post-digested mixtures was within the ideal pH-range (6.5-8) for digestion (Seadi et al., 2008) (Tables 2.1 and 2.2).

Table 2.1: Wastewater characterization, including total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and pH.

Substrates	TS (g/L)	VS (g/L)	COD (g/L)	pH
Unseparated Manure	73.6 ± 2.0	64.8 ±1.9	55.9 ± 2.5	6.93
Separated Manure	41.1 ± 0.06	32.4 ± 0.1	52.1 ± 0.4	6.82
Inoculum	28.3 ± 0.2	19.5 ± 0.3	31.5 ± 0.5	7.57

Values are averages ± standard errors.

2.3.2 Methane Production

The unseparated manure digested at 24 ºC produced significantly more CH_4 than the other treatments on a volumetric basis ($p < 0.001$ for all comparisons), and was 40% greater than the separated manure at 24 ºC (Table 2.2, Figure 2.1). At 14 ºC, there was no significant difference between the quantities of CH_4 produced by the two types of manure ($p = 0.094$). There was 68 to 71% less CH_4 production at 14 ºC for the unseparated and separated manure, respectively, compared to 24 ºC. An additional BMP test conducted at 2.5 ºC ± 3.5 ºC had CH_4 production values <0.3 mL CH_4/mL manure for both manure types (results not shown).

Table 2.2: Biochemical methane potential (BMP) results, with CH_4 production normalized by volatile solids (VS) and volume of manure added, percent CH_4, percent decrease in VS, and final pH.

Treatment	Temperature (ºC)	Decrease in VS (%)	Final pH	Cumulative CH_4 (mL/g VS)	Cumulative CH_4 (mL/mL manure)	% CH_4
Unseparated Manure	24	29	7.42 ± 0.01	335 ± 11[a]	21.7 ± 0.7[a]	60.3 ± 0.2 [b]
Separated Manure	24	32	7.46 ± 0.01	479 ± 9[b]	15.5 ± 0.3[b]	63.7 ± 0.2 [a]
Unseparated Manure	14	27	7.36 ± 0.01	107 ± 13[c]	7.0 ± 0.8[c]	49.5 ± 1.5 [c]
Separated Manure	14	23	7.41 ± < 0.01	137 ± 12[c]	4.5 ± 0.4[c]	51.0 ± 2.5 [c]

Values are averages ± standard errors. Lower-case letters indicate significant differences from Tukey-Kramer analysis between treatments within each column.

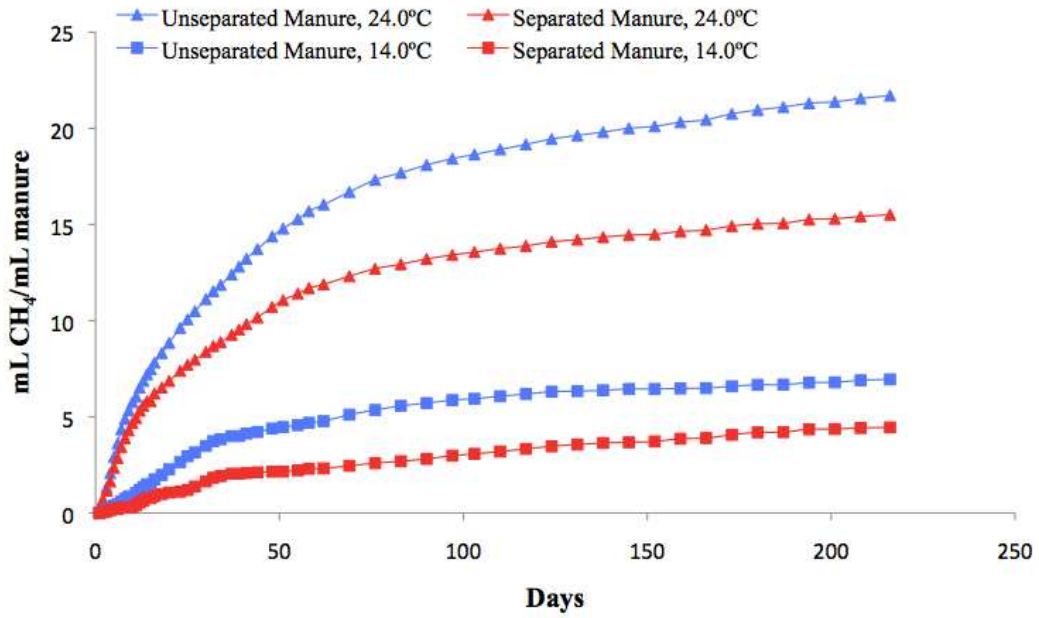

Figure 2.1: Cumulative CH_4 production for the biochemical methane potential (BMP) testing normalized by volume (mL CH_4/mL manure) for separated and unseparated manure at two psychrophilic temperatures.

On a VS basis (mL CH_4/g VS), the separated manure at 24 ºC produced significantly more CH_4 than the other treatments ($p < 0.001$ for all comparisons), with 43% more CH_4 production than the unseparated manure (Table 2.2, Figure 2.2). The effect of temperature on CH_4 production was similar when normalized by VS basis, with a 68% and 71% reduction in CH_4 production for the unseparated and separated manure, respectively, when the temperature fell from 24 ºC to 14 ºC and no significant difference between the separated and unseparated manure at 14 ºC (p = 0.36).

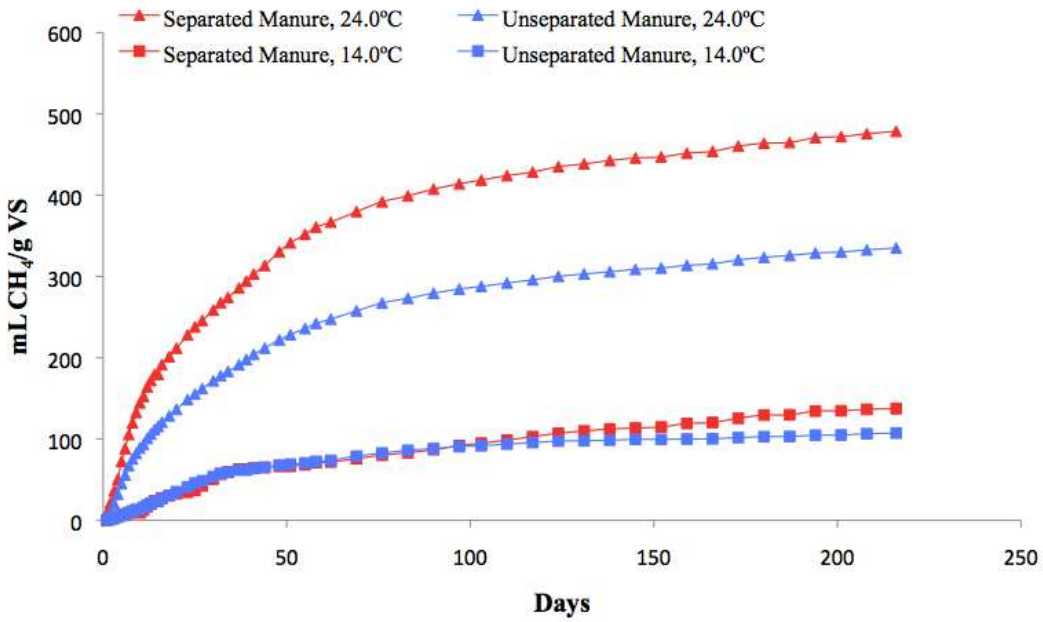

Figure 2.2: Cumulative CH_4 production for the biochemical methane potential (BMP) testing normalized by volatile solids (mL CH_4/g VS) for separated and unseparated manure at two psychrophilic temperatures.

Biogas quality, as measured by percent CH_4, was significantly higher in the separated manure at 24 ºC than in other treatments ($p < 0.005$ for all comparisons) (Table 2.2, Figure 2.3). Decreasing the temperature from 24 ºC to 14 ºC decreased the CH_4 content within the biogas by more than 10% for both types of manure. At 14 ºC, there was no significant difference in biogas quality between the separated and unseparated manure ($p = 0.96$).

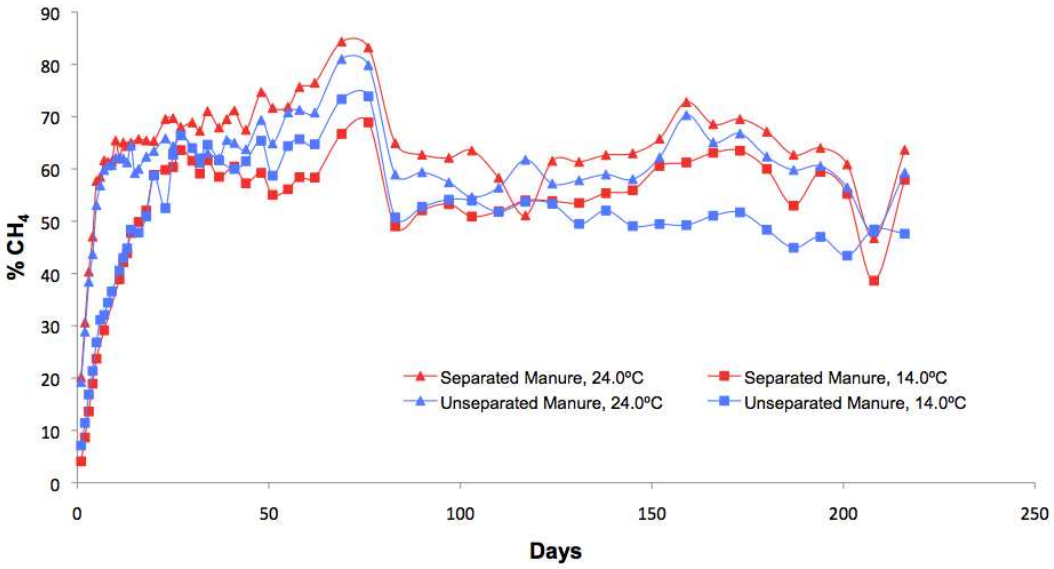

Figure 2.3: Percent CH_4 in biogas during the biochemical methane potential (BMP) testing of separated and unseparated manure at two psychrophilic temperatures.

2.3.3 Comparison of Actual and Theoretical Methane Yields

The CH_4 yields based on COD destroyed for unseparated manure were 54% to 117% higher than the CH_4 yields for the separated manure (Table 2.3). Methane production values were closer to the theoretical methane yield as the temperature increased and when digesting unseparated manure.

Table 2.3: Comparison of actual CH_4 and theoretical CH_4 yields

Treatment	Temperature (ºC)	Theoretical CH_4 Yield (mL/g COD)	Cumulative CH_4 Produced (mL)	COD Decrease (g)	Actual CH_4 Yield (mL/g COD)	% Yield
Unseparated Manure	24	350	621 ± 21	1.49 ± 0.11	424 ± 44	121
Separated Manure	24		465 ± 8	1.70 ± 0.09	275 ± 12	78.6
Unseparated Manure	14		201 ± 23	0.80 ± 0.08	273 ± 65	77.9
Separated Manure	14		134 ± 11	1.09 ± 0.09	126 ± 19	36.1

Values are averages ± standard errors.

Table 2.4: Cumulative CH_4 from the biochemical methane potential (BMP) testing normalized by volatile solids (VS) and the percent of the total CH_4 produced at various digestion time periods for separated and unseparated manure at 14 ºC and 24 ºC.

Treatment	Temperature (ºC)	Cumulative CH_4 (mL CH_4/g VS) and % of total CH_4 produced							
		16 Days	41 Days	62 Days	90 Days	124 Days	152 Days	194 Days	216 Days
Unseparated Manure	24	121 (36%)	204 (61%)	248 (74%)	279 (83%)	300 (90%)	310 (93%)	329 (98%)	335 (100%)
Separated Manure	24	192 (40%)	303 (63%)	367 (77%)	407 (85%)	435 (91%)	447 (93%)	471 (98%)	479 (100%)
Unseparated Manure	14	27.0 (25%)	63.8 (59%)	73.7 (69%)	88.2 (82%)	97.4 (91%)	99.5 (93%)	105 (98%)	107 (100%)
Separated Manure	14	27.3 (20%)	64.2 (47%)	71.6 (52%)	86.9 (63%)	107 (78%)	115 (84%)	134 (98%)	137 (100%)

2.3.4 Effect of Digestion Time on Methane Production

At 14 ºC and 24 ºC, the quantity of CH_4 produced (normalized by VS) at various digestion periods were calculated (Table 2.4). At 24 ºC, the highest rate of CH_4 production occurred during the first 16 days of digestion. As temperature decreased, the percent of the total CH_4 produced in the first 16 days also decreased for both manure types. Both the separated and unseparated manure at 24 ºC and the unseparated manure at 14 ºC had produced 80% of the total CH_4 of the 216-day test by Day 90.

The statistical analyses conducted at approximately 5-day intervals showed no significant differences at 24 ºC between the quantities of CH_4 produced in the separated and unseparated manure for the first 16 days of digestion (by volume) (*p*-values ranging from 0.054 to 0.16). From Day 20 to Day 216 (the end of the experiment), the unseparated manure produced significantly more CH_4 (29-40% more) than the separated manure (*p*-values ranging from 0.001 to 0.037), on a volumetric basis. On a VS basis, the separated manure produced significantly more CH_4 (43 to 63% more) than unseparated manure throughout the 216-day experimental period (*p*-values ranging from 0.0004 to 0.009).

At 14 ºC, the unseparated manure produced significantly more CH_4 than the separated manure (by volume) during the first 201 days, ranging from 56-147% more CH_4 (*p*-values ranging from 0.0008 to 0.049), but no significant difference was observed after Day 201 (*p*-values ranging from 0.0505 to 0.0512). On a VS basis, there were no statistical differences in the quantity of CH_4 produced between the two

manure types throughout the 216-day experimental period (*p*-values ranging from 0.097 to 0.99).

By Day 5 at 24 ºC, both manure substrates had over 50% CH_4 in the produced biogas, and the percent CH_4 remained above 50% throughout most of the experimental period (Figure 2.3). At 14 ºC, the substrates took longer (16 days) to reach 50% CH_4 and generally remained above this value throughout the experimental period (Figure 2.3).

2.4 Discussion

2.4.1 Methane Production

With no mixing, psychrophilic operating temperatures, and a long digestion period, the conditions of this study were similar to conditions found in simple digestion systems, such as those for covered lagoon digesters. Safley and Westerman (1992) found an average CH_4 production of 390 mL/g VS from a covered lagoon that treated separated dairy manure at temperatures ranging from approximately 10 ºC to 30 ºC, which is similar to this study when the 24 ºC and 14 ºC separated manure values were averaged (308 mL/g VS). Interestingly, however, the CH_4 values found at 24 ºC in this study were higher than values found for mixed, mesophilic (30-35 ºC) dairy manure digesters (137-264 mL/g VS) (Moody et al., 2011; Pain et al., 1984). Higher CH_4 production values in this study could be due to longer digestion time (216 days) compared to digestion times of 20-60 days used in other studies.

At 24 ºC, the average percent CH_4 was higher for the separated manure than the unseparated manure, with similar observations seen in previous studies at mesophilic (32-35 ºC) temperatures (El-Mashad and Zhang, 2010; González

Fernández et al., 2008), likely due to the higher portion of more readily available substrates for CH_4 production in separated manure. At 24 ºC, higher CH_4 production by separated manure compared to unseparated manure, when normalized by VS, indicates higher VS conversion efficiency to CH_4 for the separated manure due to the more biodegradable VS present within the separated manure. El-Mashad and Zhang (2010) and Rico et al. (2007) also observed a higher production of CH_4 on a VS basis from separated manure compared to unseparated manure when digested at 35 ºC, although the difference in CH_4 production observed by these authors (21-25%) were lower than that observed in this study (43%). Lower temperature in this study could have increased the use of more readily degradable dissolved substrates compared to the coarse fractions in the unseparated manure, resulting in a higher difference for the CH_4/g VS produced by separated manure compared to unseparated manure.

It should be stipulated, however, that the organic loading rate (OLR) could influence the conversion efficiency of VS. In this study's BMP experiment and that of Rico et al. (2007), equivalent amount of separated and unseparated manure were used in a batch system with equal volumes of inoculum additions, and since unseparated manure contains higher VS content, higher OLRs were utilized for the unseparated manure. Given similar OLR (g VS/L/day) and sufficient time, Lo et al. (1983a, 1983b) showed that reactors fed with unseparated manure and separated manure could produce equivalent CH_4 on a VS basis when digested at 30 ºC. Hence, the degradation of the unseparated manure in our study and other studies is likely affected by both VS conversion efficiency of complex solids in the unseparated manure and the effect of OLR in the experiment.

At 24 ºC, this study found a similar increase in CH_4 production (approximately 40%) with unseparated manure compared to separated manure on a volumetric basis as the Rico et al. (2007) digestion study conducted at 35 ºC. Higher VS concentration in the unseparated manure resulted in higher CH_4 production by volume even when the efficiency in converting VS to CH_4 was lower compared to separated manure.

The decrease in CH_4 production observed in this study when temperature was decreased from 24 ºC to 14 ºC was similar to values observed by Massé et al. (2003), who saw a 70% decrease in CH_4 production when the temperature for the digestion of swine manure was decreased from 20 ºC to 10 ºC. Since the final pH of all treatments were within the ideal pH-range for AD process, acidification was not a confounding factor in any treatment, illustrating that the lack of methanogenic activity at 14 ºC was likely due more to the temperature conditions than the pH conditions within the digesters. In addition, lowering the temperature also reduced the biogas quality to the lower end (50%) of typical CH_4 concentration in biogas from anaerobic digesters (Seadi et al., 2008). This observation, however, was in contrast to results by Massé et al. (2003), who observed an increase in CH_4 content when digestion temperature was reduced from 20 ºC to 10 ºC. Massé et al. (2003) postulated that an increase in homoacetogenic activity at lower temperatures could have converted H_2/CO_2 to acetate, which could be converted to CH_4. Indeed, previous researchers have discussed the increasing importance of homoacetogenic activities when temperature of methanogenic system decreases (Fey and Conrad, 2000; Kotsyurbenko et al., 1993; Kotsyurbenko et al., 2001; Kotsyurbenko, 2005). However, besides H_2/CO_2,

acetogens can utilize other substrates, such as sugars, which could release CO_2 during the process (Chidthaisong et al., 1999; Conrad, 1999). Furthermore, the homoacetogenic degradation of sugars could be an important degradation pathway at lower temperatures (Chin and Conrad, 1995). If the CO_2/CH_4 ratio increases as temperature decreases, then CH_4 content within biogas could decrease with decreasing temperature. In this study, further indication that a lower fraction of the degraded organic matter was transformed to CH_4 at lower temperatures was provided by the greater deviation of the actual CH_4 yield from the theoretical yield as temperature decreased.

2.4.2 Effect of Digestion Time and Digester Volume

Both Lo et al. (1983a, 1983b) and Liao et al. (1984) found that at shorter retention times, separated manure produced higher CH_4 (on a volume basis) than unseparated manure at 30-35 ºC. However, at 24 ºC in our batch study, the lower rates of CH_4 production during the initial 16 days resulted in the separated manure to not outperform the unseparated manure at shorter digestion times (on a volume basis). Nevertheless, the results suggest that given a smaller digester volume with a digestion time of ≤16 days and operational temperature of 24 ºC, farmers could digest separated manure without sacrificing CH_4 production. At the 16-day digestion time, approximately 40% of the CH_4 production (on a VS basis) for separated manure was obtained, representing a relatively high CH_4 production rate compared to longer digestion time. Given a larger digester size with a longer digestion time, however, more CH_4 production would be obtained at 24 ºC using unseparated manure.

In contrast to the findings at 24 ºC, separated manure at 14 ºC produced less CH_4 per volume of manure than unseparated manure at digestion times ≤201 days due to the insignificant difference in the efficiency of VS conversion to CH_4 between the two manure types and higher VS concentrations within the unseparated manure. Hence, for a given digester volume at 14 ºC, digesting unseparated manure would produce more CH_4 than separated manure, but the overall CH_4 produced would be approximately 70% less than at 24 ºC.

2.5 Conclusion

In this research, CH_4 production in AD treating dairy manure decreased by approximately 70% when the digestion incubation temperature was lowered from 24 ºC to 14 ºC. At 24 ºC, farmers will get the highest CH_4 production, per volume of manure added, from unseparated manure due to the higher VS content, but if operating at a shorter digestion time (less than 16 days), the difference in CH_4 production between the two manure types might be insignificant. While there was much less CH_4 production in general at 14 ºC, the unseparated manure would likely produce more CH_4 than separated manure, per volume of manure loaded.

3. Alternative Inoculum Sources for Psychrophilic and Mesophilic Anaerobic Digestion

Abstract

The main objective of the research was to compare methane (CH_4) production potential when wetland sediment (WS), landfill leachate (LL), and mesophilic digestate (MD) were used as inoculum sources for anaerobic digestion of dairy manure at 15, 25, and 35 ºC. All three inoculum sources (WS, LL, and MD) were initially incubated (acclimated) at 15, 25, and 35 ºC and subsequently used as inocula in 90-day biochemical methane potential (BMP) tests at the same temperatures. Two BMP tests were conducted for two inoculum incubation periods: 91 (BMP1) and 196 days (BMP2) using three inoculum to substrate ratios (ISR) (20, 35, 50% w/w).

The results showed that all inocula were viable for digestion at 25 ºC and 35 ºC, but none of the inoculum sources were productive at 15 ºC. In BMP1, digesters with LL at 25 ºC had CH_4 yield (194 ± 7 L/kg VS) that was not significantly different than digesters at 35 ºC with MD (202 ± 4 L/kg VS). Compared to BMP1, there were less differences in CH_4 yield between 35 and 50% ISRs at 25 and 35 ºC in BMP2, indicating that longer incubation time could allow less inoculum to be used. Furthermore, 35% ISR was sufficient in preventing VFA accumulation in most treatments at 25 ºC and 35 ºC in BMP2, compared to the required 50% ISR in BMP1. Overall, in batch systems with long digestion time (90 days), MD inoculum from well-established digesters (i.e. long incubation time), 35% ISR, and 35 ºC operation temperature are recommended for highest CH_4 production per unit of digester volume. Research represents the first study that compares the effectiveness of wetland

sediment, landfill leachate, and mesophilic digestate as inoculum sources for anaerobic digestion at three temperatures using three ISRs and two inoculum incubation periods.

3.1 Introduction

Anaerobic digestion (AD) is a microbial-based technology that converts organic matter (OM) in manure into methane (CH_4)-enriched biogas, a renewable energy source that can be used to supply heat or electricity. The use of AD can also reduce odors and CH_4 emissions from manure management and create an organic fertilizer that can be used for crop production (AgSTAR, 2011; Holm-Nielsen et al., 2009; Powers et al., 1999).

Despite these benefits, transfer of AD technology to smaller-scale farms in temperate climates, such as the U.S., has been hampered by decreased CH_4 production in the winter. Previous laboratory research has found a 70% decrease in CH_4 production when temperature decreased from 24 ºC to 14 ºC, as well as a longer lag-phase (33 and 132 days at 25 ºC and 15 ºC, respectively) compared to 30 ºC (Witarsa and Lansing, 2015; Zeeman et al., 1988). In temperate digesters, heating mechanisms are installed that use a portion of the produced biogas or waste heat from combined heat and power systems for digester heating, greatly increasing initial capital costs. As a result, most US-based AD systems are installed on large to medium-scale farms (≥500 dairy cows) and are operated at mesophilic temperatures (25-35 ºC) rather than ambient or psychrophilic temperatures (≤25 ºC). High cost is recognized as a major factor for the low installation rate of AD systems in the US, especially for the >94% of US dairy farms that are classified as small and medium-

scale (<500 dairy cows) (AgSTAR, 2011; USNASS, 2014). Finding a method to increase year-round CH_4 production in temperate AD systems could reduce costs and create greater incentives for small-scale farmers to install AD technology.

The addition of inoculum has been shown to increase rates of OM conversion to CH_4 (Lopes et al., 2004). Hashimoto (1989), Lopes et al. (2004), and Maya-Altamira et al. (2008) observed increased CH_4 or biogas production when inoculum to substrate ratio (ISR) was increased, although others observed little to no differences in cumulative CH_4 production when ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2006). Some studies have also shown increases in digestion rate when ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2009; Zeng et al., 2010), indicating that increased ISR could increase digestion rate but not necessarily cumulative CH_4 production. In an attempt to increase CH_4 production during low-temperature digestion, some studies have investigated the use of inocula that were pre-incubated or acclimated to lower temperatures. Zeeman et al. (1988), for instance, observed increased CH_4 production rate during low-temperature digestion when the inoculum was acclimated to a lower temperature. With the exception of Zeeman et al. (1988) who used wetland soil as inoculum, all previous research results stated above have used sludge/digestate from anaerobic digesters or bovine rumen fluid as their inocula source.

In this study, the use of alternative inocula, specifically wetland sediment and landfill leachate, to seed digester and increase CH_4 production at 15, 25, and 35 ºC was evaluated. These methanogenic systems are exposed to fluctuating temperatures and could potentially contain more psychrotolerant methanogens, making them better

inoculum sources for psychrophilic digesters. Studies on the use of landfill leachate as an inoculum source for agricultural AD systems are limited. Sediments have been used as a psychrophilic AD inoculum source but results have varied. Bardulet et al. (1990) and Xing et al. (2010) observed stable OM removal or biogas production when lake or river sediments were used as inocula for psychrophilic AD. On the other hand, Zeeman et al. (1988) did not observe a reduction in the start-up time of AD inoculated with wetland soil compared to the un-inoculated treatment. In addition, the effects of ISR and incubation time on CH_4 production when wetland sediments or landfill leachate are used as inocula have not been studied.

The objective of this study was to quantify CH_4 production using three different inocula: wetland sediment (WS), landfill leachate (LL), and mesophilic digestate (MD), two inoculum incubation periods (91 and 196 days), three ISR (20, 35 and 50% w/w), and three temperatures (15, 25, and 35 ºC) during anaerobic digestion of dairy manure using biochemical CH_4 potential (BMP) testing. The results can be used to better understand the benefits of using alternative inoculum sources for increasing CH_4 production in temperate AD systems and the effect of incubation and ISR when operating at various temperatures.

3.2 Methods

3.2.1 Inoculum Collection

A preliminary analysis utilizing specific methanogenic assays, adapted and modified from Sørensen and Ahring (1993), was used to determine CH_4 production potential of WS samples from five different wetlands and LL samples from five landfills. The two WS and LL sites with the highest CH_4 production from this

preliminary research were carried through to the incubation phase, and the one WS and one LL site with the highest CH_4 production in the incubation phase were used in the BMP tests (preliminary results not shown).

The WS used in the BMP analyses was collected from the Jugbay Wetland Sanctuary (38.78º N, 76.71º W) located adjacent to the Patuxent River, Anne Arundel County, Maryland, USA. The WS was collected from a depth of 5-10 cm and was degassed within 2 hours with an N_2:CO_2 (70:30) mixture. The LL sample used in the BMP analyses was collected from Stafford County Landfill, Virginia (38.38º N, 77.42º W), which was opened in 1987, holds 715,000 tons of waste and produces an average 46 L of biogas/ton of waste/day (USEPA, 2011). The MD sample was collected from a complete mixed digester (540 m^3) located at the US Department of Agriculture (USDA) Beltsville Agriculture Research Center (BARC) dairy facility (39.03º N, 76.89º W). All samples were stored at 4 ºC and were used within 25 days.

3.2.2 Reactor Configurations and Inocula Incubation

Nine incubation reactors were constructed using 10.2 cm diameter (4-in) PVC pipe with a height of 0.5 m. Each reactor was sealed with a total knockout closet flange on the bottom and a mechanical test plug on top. Manure feeding and sample extraction were conducted using two 1.27 cm diameter (0.5-in) ball valves and one 2.54 cm diameter (1-in) ball valve. Biogas was collected using Tygon® tubing connected to a 5 L multi-foil bag (Figure 3.1). There were initially two reactors from two different WS and LL sites used in incubation, and one MD reactor in each temperature controlled chamber (15, 25, and 35 ºC), resulting in a total of 15 incubation reactors. In addition to the MD reactor from each temperature chamber,

the highest performing WS and LL reactors (based on CH_4 production across all three temperatures) during incubation were selected from each temperature chamber for subsequent BMP tests, resulting in a total of 3 reactors (one MS, one WS and one LL) chosen from each of the three temperature chambers.

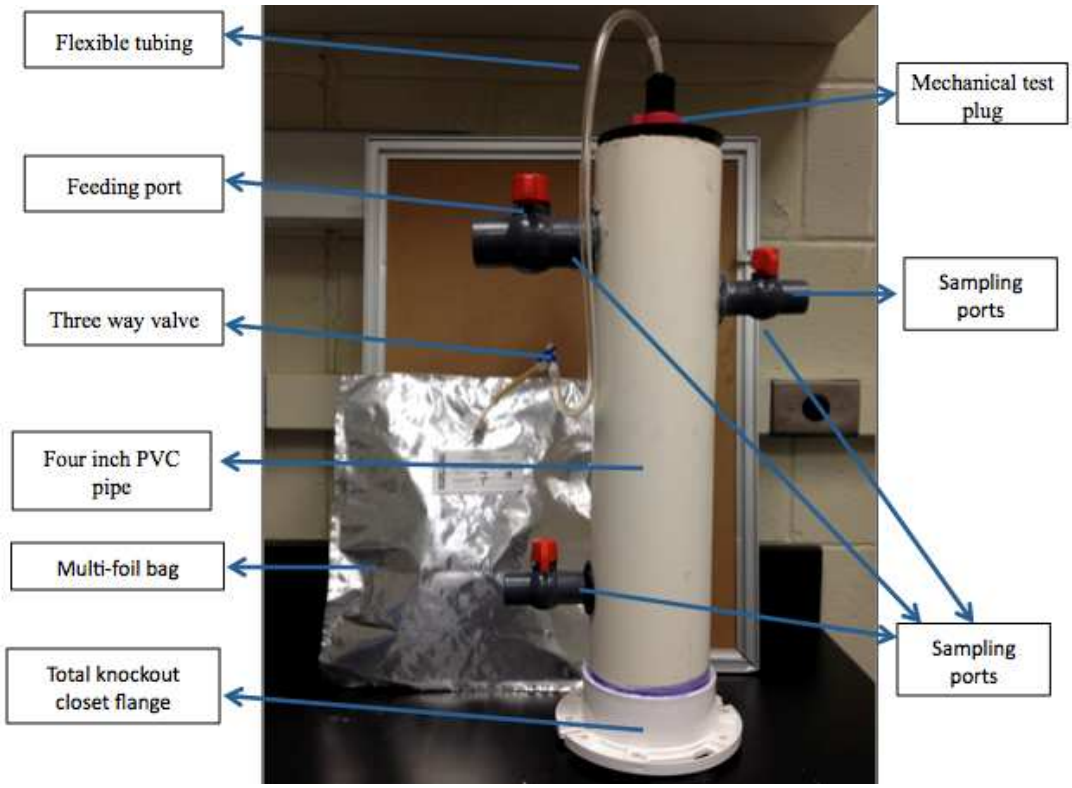

Figure 3.1: Inoculum incubation reactor

An equal amount of the source inoculum and nutrient media (≈ 1.25 L) was added into each incubation reactor. The nutrient media, detailed in Speece (1996), contains micro and macronutrients to encourage the growth of methanogens. Following the inoculum and media addition, the headspace of each reactor was

purged with N_2:CO_2 (70:30) mixture for 10 min. to remove O_2. The reactors were then sealed and incubated at the target temperature.

The reactors were fed at approximately 4-day intervals with unseparated, scraped manure from the USDA BARC facility. The collected manure was autoclaved and stored at 4 ºC to minimize the introduction of methanogens within the manure source to the inoculum incubation reactors. A step-wise loading regime, adapted from Bardulet et al. (1990) and Bull et al. (1983), of autoclaved manure and methanol was used, with the methanol gradually phased out over a 34-day period (Table 3.1). Due to the viscous nature of the unseparated manure, a ratio of 1.09 g COD/g VS for manure was used to determine the manure/methanol loading (USEPA, 2002). The reactors were mixed by hand after feeding.

Table 3.1: Feeding regime of the inoculum incubation reactors.

Days	Autoclaved manure loading (g VS/L digester content/day)	Methanol loading (g methanol/L digester content/day)
0-9	0.046	0.033
10-19	0.12	0.083
20-24	0.23	0.17
25-29	0.35	0.083
30-34	0.52	0.13
35-39	0.69	0
40-49	0.92	0
50-196	1.2	0

Biogas and percent CH_4 analyses were conducted before each feeding. Biogas collected in 5 L multi-foil bag was measured using 140 and 60 mL syringes, while

percent CH_4 was analyzed by injecting 0.10 mL sample, using a luer-lock, gas tight syringe, into an Agilent HP 7890A GC equipped with a thermal conductivity detector (TCD) with the following parameters: 1) injection temperature of 250 ºC; 2) detector temperature of 250 ºC; 3) oven temperature of 60 ºC; and 4) a carrier gas flow rate of 8.6 mL He/min.

The reactors functioned as semi-batch reactors until Day 150 when semi-continuous feeding of the reactors was achieved, with the wasting of the digester content equal to the feedstock addition. Inoculum was extracted from each reactor on the 91st day for the 1st BMP test (1.75 L for the 33 BMP bottles utilized) and on the 196th or 197th day for the 2nd BMP tests. Day 178 was the last day when biogas and percent CH_4 measurements were conducted on the incubation reactors.

3.2.3 Biochemical Methane Potential (BMP) Tests

The BMP test methods were adapted from Moody et al. (2011). The BMP test is a laboratory batch study used to characterize CH_4 production potentials where substrate, inoculum, and nutrient media are added into 250 mL serum bottles, purged with N_2:CO_2 gas, capped, and incubated at 35 ºC. Biogas and CH_4 concentration are monitored at regular intervals for 30-40 days, or until biogas production has largely ceased.

In this study, two BMP tests were performed using the three inoculum sources after incubation of the inoculum sources for 91 days (BMP1) and 196 days (BMP2), with the BMP testing conducted at the same temperature at which the inocula were incubated (i.e. inocula incubated at 15, 25, and 35 ºC were used for BMP testing at 15, 25, and 35 ºC, respectively). The substrate for the BMP tests was unseparated

manure obtained from the BARC dairy facility and stored at 4 ºC before use. For BMP1, inocula obtained from reactors were stored at 4 ºC for a maximum of 7 days before use, while inocula for BMP2 were obtained fresh from the reactors and loaded on Day 196 or Day 197 into the BMP bottles. Nutrient media and mixing were not used in either BMP test to simulate covered lagoon digestion.

For BMP1, a 120 g mixture of inoculum and manure were added to 250 mL serum bottles at different inoculum to substrate ratios (ISR) (w/w): (0%, which equated to 120 g of manure and 0 g of inoculum), 20% (24 g of inoculum and 96 g manure), 35% (42 g of inoculum and 78 g manure), and 50% (60 g of inoculum and 60 g manure). While researchers commonly use VS ratio for ISR, inoculum loading rate in this study was conducted using wet weight in order to keep the volume of inoculum consistent between the different treatments. For reference purposes, the ISR based on VS (for both BMPs) could be calculated using Equation 1:

$$\text{ISR (based on VS)} = (M_{inoc} * VS_{inoc}) / (M_{manure} * VS_{manure} + M_{inoc} * VS_{inoc}) * 100 \quad (1)$$

Where M_{inoc} is mass of inoculum added, VS_{inoc} is concentration of volatile solids in inoculum, M_{manure} is mass of manure added, and VS_{manure} is concentration of volatile solids in manure. Values for the VS of each inoculum source are listed in Table 3.2 for the two BMP tests. The manure VS concentration in BMP1 and BMP2 were 133 ± 2 g/kg manure and 141 ± 3 g/kg manure, respectively.

Inoculum-only bottles containing 120 g of the each inoculum type were used as controls. All treatments were conducted in triplicate except for inoculum-only and manure-only bottles (0% ISR), which were conducted in duplicate, resulting in 105

total BMP bottles for BMP1. To create anaerobic conditions, each bottle was purged with N_2:CO_2 (70:30) before being capped with butyl rubber stoppers. The BMP test was carried out for approximately 90 days.

The frequency of the biogas and CH_4 measurements was conducted in accordance with the quantity of biogas produced in the bottles in each temperature chamber. The higher the biogas production, the more frequent the measurements were made. Measurements for bottles in the 25 and 35 ºC chambers were conducted daily for the first five days and gradually decreased to approximately once a week during the 90-day BMP test. Biogas measurements for bottles in the 15 ºC chamber were conducted approximately every 4 days during the first 14 days and then decreased to weekly or bi-weekly. Biogas measurements were made using a graduated, gas-tight, wet-tipped 50 mL glass syringe inserted through the septa and equilibrated to atmospheric pressure. The measured biogas was then vented. After venting, to measure percent CH_4, 0.10 mL of biogas was collected from each bottle using a luer-lock, gas tight syringe and injected into an Agilent HP 7890A gas chromatograph (GC), with the parameters listed in *Section 3.2.2.*

The average CH_4 (L/kg inoculum) produced from the duplicate inoculum-only bottles at each temperature was adjusted to the volume of inoculum used in the treatment bottles and subtracted from the CH_4 production of the treatment bottles to account for residual CH_4 production from the inoculum source. All CH_4 production volumes were converted to standard temperature and pressure (0 ºC and 1 atm) using the ideal gas equation and normalized to the volatile solids (VS) of manure added to each bottle (L CH_4/kg VS).

The procedures for BMP2 were similar to those in BMP1, with the following modifications: 1) the 20% ISR treatments were not included due to the low quantity of CH_4 produced during BMP1, and 2) at 15 ºC, only the inocula with the highest CH_4 production (MD and WS) at 50% ISR were used in addition to manure and inocula-only controls, resulting in 64 total BMP bottles in BMP2. BMP2 was run for approximately 100 days, 10 days longer than BMP1, but all CH_4 values presented for BMP2 were based on the cumulative CH_4 production values on approximately Day 90 to allow comparisons between the two BMP tests.

3.2.4 Wastewater Characterization

Total solids (TS), volatile solids (VS), and volatile fatty acids (VFA) analyses were conducted on the manure and incubated inocula before each BMP test, and on each treatment bottle at the end of each BMP test. pH analyses were conducted on individual treatment bottle before and after the BMP tests. Total solids and VS were measured by heating samples at 103-105 ºC to constant mass, followed by heating samples at 550 ºC to constant mass (APHA, 2005). For measurement of VFAs (acetic, propionic, n-butyric, and n-valeric acids), samples were acidified with concentrated sulfuric acid to pH below 2 (diluted by ≤10%) and filtered to 0.22 µm before injection into a HP 7890A GC equipped with a flame ionization detector (FID) with the following parameters: 1) injection temperature of 250 ºC; 2) detector temperature of 300 ºC; 3) oven temperature of 100 ºC for 2 minutes and increased by 10 ºC/min for a total run time of 10 min; and 4) a carrier gas flow rate of 1.80 mL He/min. Total volatile fatty acids (TVFA) content was calculated by adding the concentrations of acetic, propionic, butyric, and valeric acids, expressed as mg/L

acetic. Extrapolations of individual acid standard curves below 1 mM were conducted to determine values within the range of 0.1-1 mM. Values <0.1 mM (6.01 mg/L) were assumed to be zero, as these concentrations were considered negligible compared to TVFA values associated with unstable digestion: >2,000 mg/L (as acetic) (Varel et al., 1977), and had no discernable differences with DI blanks verifications.

3.2.5 Statistical Analyses

Two Tukey-Kramer analyses using Proc Mixed procedure in SAS® 9.3 (Cary, NC) were conducted to compare treatments within BMP1 and within BMP2. Equal variances were used for the Tukey-Kramer analyses in BMP1. For BMP2, variances within the treatments were grouped into three categories: 1) WS at 15 ºC and 50% ISR, and manure-only treatments at 15 and 25 ºC; 2) LL at 25 ºC at 50% and 35% ISR, and MD at 35 ºC at 50% and 35% ISR; and 3) all remaining treatments. A t-test was also conducted using the Proc ttest procedure in SAS® 9.3 (Cary, NC) to compare CH_4 production at approximately Day 20 of two treatments (50% ISR LL at 25 ºC and 50% ISR MD at 35 ºC) within BMP1. An α value of 0.05 was used in each analysis. All Tukey-Kramer and t-test analyses were conducted on sub-replicates of each reactor and temperature chamber.

Correlation between the final TVFA and L CH_4/kg VS were analyzed for each BMP results using Proc NLIN in SAS® 9.3 (Cary, NC). Two models, a linear and a logarithmic model, were used to fit the curves, with the logarithmic models yielding a better fit with lower sum of squares error.

3.3 Results and Discussion

3.3.1 Methane Production and Effluent Characteristics of Incubation Reactors

Cumulative CH_4 from the first 91 days of the 196-day incubation period (mid-point of the incubation period) was 22-36% of the total cumulative CH_4 for all reactors (Table 3.2). The MD reactors had the highest cumulative CH_4 production at all three temperatures on Days 91 and 178. The LL and WS reactors at 15 ºC had TVFA values above the optimum range <2,000 mg/L (Varel et al., 1977) and cumulative CH_4 production values that were 2% and 22%, respectively, of the MD incubation reactors at 15 ºC. Even with a 10 ºC decrease in temperature, the cumulative CH_4 production of the reactors at 25 ºC was only 2-14% lower than 35 ºC. The pH of the inocula used in both BMP1 and BMP2 were within the ideal range of 6.5-8 (Seadi et al., 2008), except for LL at 15 ºC (6.18).

3.3.2 BMP1 (91-day inocula incubation)

After the 91-day inoculum incubation, the three inoculum sources (WS, LL and MD) were shown to be effective in enhancing CH_4 production during AD (Figures 3.2, 3.3; Table 3.3). Compared to the manure-only treatments, adding incubated inoculum increased CH_4 production by 1,900-2,800% at 25 ºC, and 2,400-3,000% at 35 ºC at 50% ISR ($p < 0.001$). BMP bottles inoculated with LL at 50% ISR at 25 ºC and 35 ºC produced significantly higher volume of CH_4 than all other treatments ($p < 0.007$), except MD inoculum incubated at 35 ºC (50% ISR), which was not significantly different ($p = 1.000$). Methane production by LL (50% ISR) at 25 ºC (194 L/kg VS) was in the range observed by Møller et al. (2004) (148 L/kg VS)

and Moody et al. (2011) (252 L/kg VS) for batch digestion of manure at 35 ºC, and was significantly higher than MD at 25 ºC. The results support the idea that the incubated LL can be used to seed 25 ºC digesters to produce similar quantity of CH_4 as digesters operating at 35 ºC using traditional inoculum sources.

Table 3.2: Cumulative CH_4 production and effluent characteristics from the inoculum incubation reactors for landfill leachate (LL), wetland sediment (WS), and mesophilic digestate (MD).

	Day 91				Day 196			
	Cumulative CH_4 (L/L reactor)	**pH***	**TVFA+ (mg/L as acetic)**	**VS* (g/kg)**	**Cumulative CH_4#(L/L reactor)**	**pH***	**TVFA+ (mg/L as acetic)**	**VS* (g/kg)**
		15 ºC				**15 ºC**		
LL	0.07	6.18 ± 0.01	7700	38.8 ± 1.4	0.23	**	11500	78.5 ± 1.7
WS	1.01	6.96 ± 0.01	2510	53.0 ± 1.0	3.15	6.96 ± 0.06	5980	56.1 ± 2.0
MD	3.84	7.33 ± 0.03	185	37.4 ± 0.2	14.3	7.38 ± 0.10	200	51.9 ± 0.9
		25 ºC				**25 ºC**		
LL	3.81	7.31 ± 0.03	257	31.3 ± 0.6	17.3	7.58 ± 0.04	111	54.4 ± 1.7
WS	6.35	7.20 ± 0.04	113	38.7 ± 0.2	19.2	7.46 ± 0.02	181	35.9 ± 0.7
MD	7.40	7.27 ± 0.01	71.1	33.9 ± 0.7	21.7	7.38 ± 0.02	49.4	51.7 ± 1.2
		35 ºC				**35 ºC**		
LL	5.80	7.39 ± 0.02	103	24.8 ± 0.4	20.1	7.99 ± 0.02	61.2	51.3 ± 3.0
WS	4.91	7.41 ± 0.01	116	36.0 ± 1.0	19.7	7.62 ± 0.04	46.7	32.8 ± 1.1
MD	8.11	7.44 ± 0.03	62.0	26.1 ± 1.3	22.2	7.64 ± 0.02	37.0	56.1 ± 1.5

Note: Reactor effluents were used as inocula for BMP1 (91 days of incubation) and BMP2 (196 days of incubation).
* Average pH and volatile solids (VS) ± standard error. ** pH was not measured since inoculum was not used in BMP test.
+ Total volatile fatty acids (TVFA) were calculated as the sum of acetic, propionic, butyric, and valeric acids.
# Final CH_4 measurements for reactors were collected on Day 178.

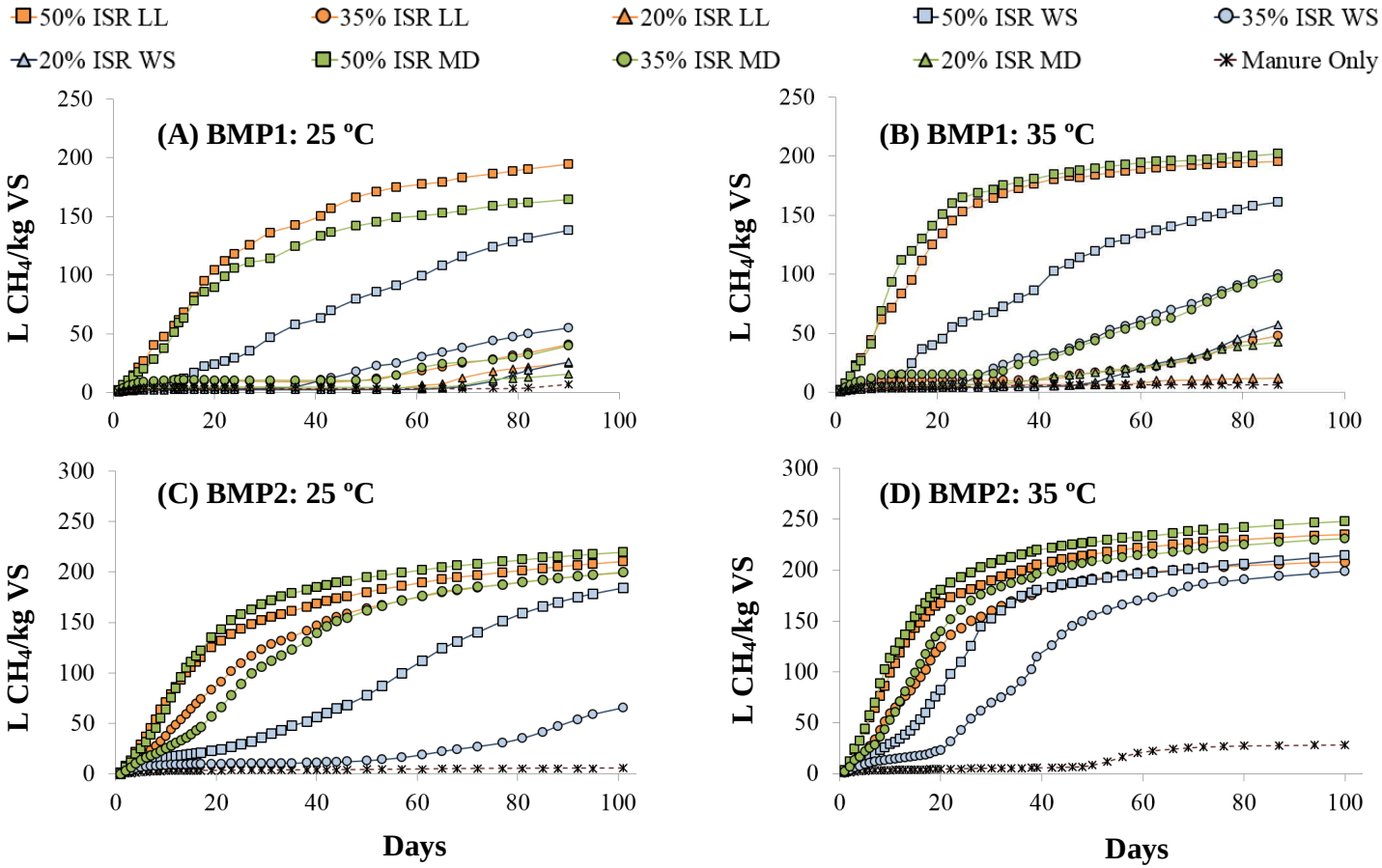

Figure 3.2: Cumulative CH_4 for BMP1 and BMP2 over time using wetland sediment (WS), landfill leachate (LL) and mesophilic digestate (MD) as inocula at three inoculum to substrate ratios (ISR).

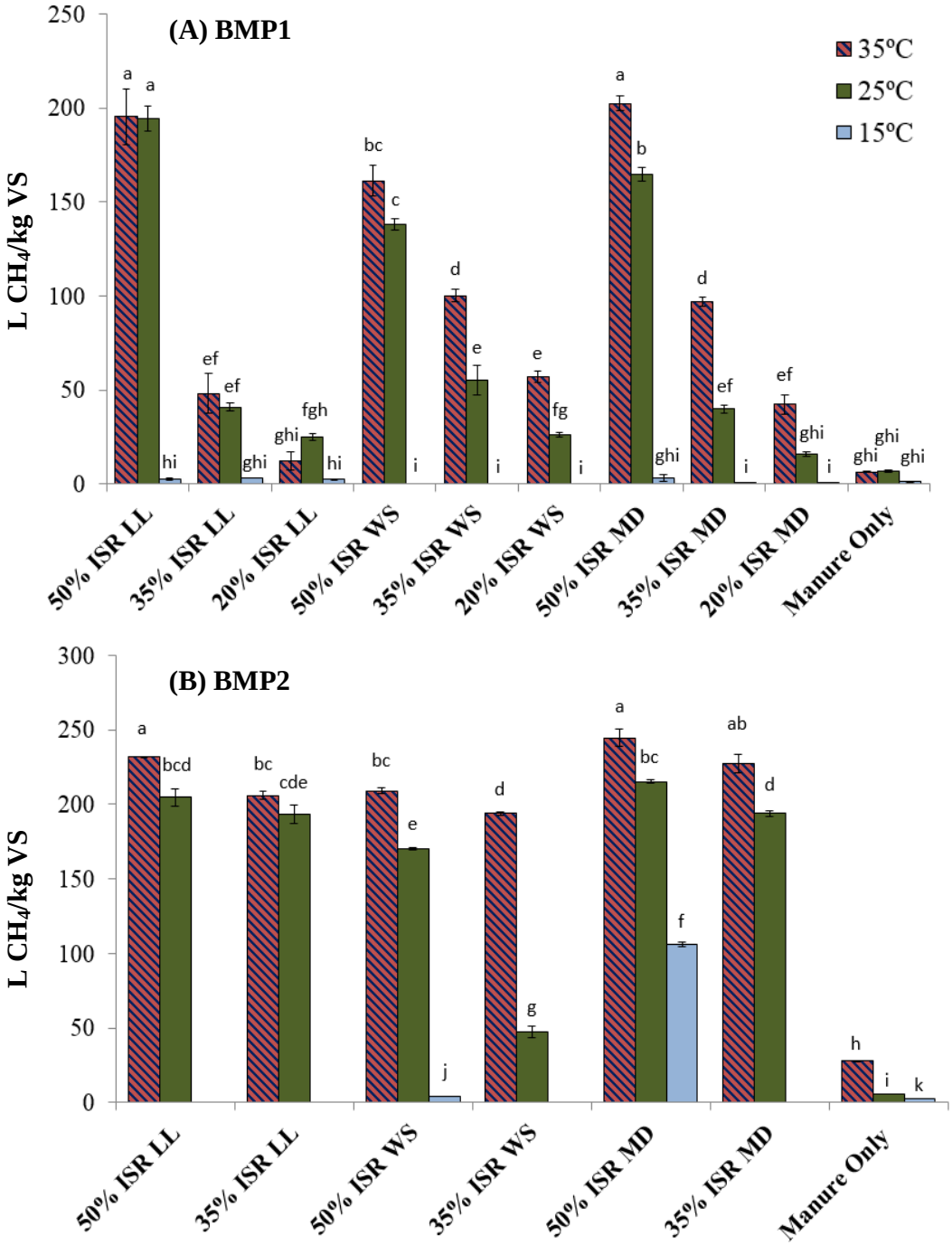

Figure 3.3: Cumulative CH_4 for BMP1 and BMP2 using wetland sediment (WS), landfill leachate (LL) and mesophilic digestate (MD) as inocula at three inoculum to substrate ratios (ISR). Letters indicate significant differences ($p < 0.05$).

Table 3.3: Cumulative CH_4 production at Day 20 and Day 90 in BMP1 using landfill leachate (LL), wetland sediment (WS), and mesophilic digestate (MD) inoculum sources at three inoculum to substrate ratios (ISR).

	Day 20*				Day 90*			
	50% ISR	**35% ISR**	**20% ISR**	**0% ISR**	**50% ISR**	**35% ISR**	**20% ISR**	**0% ISR**
			15 ºC				**15 ºC**	
LL	1.60 ± 0.21	1.83 ± 0.15	1.53 ± 0.12		2.64 ± 0.55	3.16 ± 0.17	2.27 ± 0.11	
WS	1.71 ± 0.51	1.40 ± 0.20	1.06 ± 0.08		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
MD	6.57 ± 1.21	2.73 ± 0.98	1.18 ± 0.41		2.99 ± 1.96	0.06 ± 0.06	0.18 ± 0.18	
Manure-only				0.75 ± 0.10				1.11 ± 0.15
			25 ºC				**25 ºC**	
LL	104 ± 4	9.87 ± 0.36	3.74 ± 0.14		194 ± 7	40.7 ± 2.1	24.9 ± 1.9	
WS	23.9 ± 4.1	3.02 ± 0.19	2.33 ± 0.06		138 ± 3	55.0 ± 7.8	26.2 ± 1.3	
MD	89.5 ± 7.3	10.5 ± 0.49	4.73 ± 0.41		165 ± 4	39.8 ± 2.2	15.8 ± 1.4	
Manure-only				3.03 ± 0.20				6.78 ± 0.54
			35 ºC				**35 ºC**	
LL	135 ± 6	10.2 ± 0.3	6.26 ± 0.17		195 ± 15	48.1 ± 10.4	12.2 ± 4.9	
WS	45.0 ± 0.5	5.64 ± 0.34	4.10 ± 0.11		161 ± 8	100 ± 3	57.1 ± 2.9	
MD	151 ± 4	15.3 ± 0.4	6.81 ± 0.14		202 ± 4	96.7 ± 2.4	42.3 ± 5.1	
Manure-only				4.20 ± 0.09				6.42 ± 0.12

*All values are averages ± standard error in L CH_4/kg VS.

Other studies have also found that biodegradability and CH_4 production potential did not increase significantly when temperature was increased within the range of 25 ºC and 35 ºC (Mahmoud et al, 2004; Torres-Castillo et al., 1995). Torres-Castillo et al. (1995) found that CH_4 yield was higher at 25 ºC than at 35 ºC when sufficient time was given for the complete digestion of waste. Temperature increase from 25 ºC to 35 ºC has been shown to reduce the lag-phase and increase digestion rate, but not necessarily overall CH_4 production or biodegradability (Mahmoud et al., 2004; Torres-Castillo et al., 1995). At Day 20, a common AD retention time, MD at 35 ºC (50% ISR) produced significantly more CH_4 than LL at 25 ºC (50% ISR) (p = 0.002) (Table 3.3). When digestion time was long (90-days), LL at 25 ºC was not significantly different than MD at 35 ºC, but with a lower digestion time (20 days), MD inoculum at 35 ºC produced significantly more CH_4.

At 25 and 35 ºC, increasing ISR from 20 to 35% significantly increased CH_4 production by 63-295% ($p \leq 0.038$), except for LL at 25 ºC (p = 0.655). Further increasing the ISR from 35 to 50% significantly increased CH_4 production by 61-377% ($p < 0.001$) (Figure 3.3; Table 3.3). Increasing the inoculum volume in a batch digester with a fixed volume does decrease the total volume available for substrate addition; for instance, increasing the ISR from 35% to 50% will result in a 15% decrease in the substrate content within the digester. However, overall CH_4/digester will still be 24-267% higher when the higher ISR is used. Therefore, based on the large increases seen in BMP1 with increasing ISR, the highest ISR tested (50%) would be recommended given space availability within a batch digestion reactor. Increasing the ISR above 50% (w/w) was not tested. Previous studies have also

shown that increasing the ISR in a batch AD system increased CH_4 (or biogas) (Hashimoto, 1989; Lopes et al., 2004; Maya-Altamira et al., 2008), although it should be reiterated that these previous studies used digestate/sludge from anaerobic digesters or bovine rumen fluid, and not wetland or landfill leachate, as inocula.

At 15 ºC, the final cumulative CH_4 in all BMP bottles was <3.2 L/kg VS, and the values from the three inoculum sources were not significantly different from each other (p = 1.000) (Figure 3.3; Table 3.3). Methane production at 15 ºC was ≥98% lower than CH_4 production observed for MD (35 ºC, 50% ISR) and LL (25 ºC, 50% ISR) (p < 0.001). Regardless of the inoculum source, the incubated inocula did not significantly increase CH_4 production at 15 ºC, with no significant differences between the manure-only treatment and the inoculum+substrate bottles (p = 1.000). The pH values at 15 ºC indicated instability during the digestion process. At the start of the BMP, the LL inoculum-only control and LL at 50% ISR had pH values below the optimum range of 6.5-8 (Seadi et al., 2008) due to the low pH values in the incubation reactors. By the end of BMP1, the pH of all treatments, except WS and MD inoculum-only treatments, were below 6.5. The higher pH values in WS and MD inoculum-only bottles resulted in higher CH_4 production in the control compared to their corresponding manure+inoculum treatments over time.

3.3.3 BMP2 (196-day inocula incubation)

In BMP2, the addition of inocula (50% ISR) at 25 ºC and 35 ºC significantly increased CH_4 production compared to manure-only treatments (Figures 3.2, 3.3; Table 3.4), with LL producing 3,700% (25 ºC) and 740% (35 ºC) more CH_4 than the manure control, WS producing 3,100% (25 ºC) and 660% (35 ºC) more CH_4, and MD

producing 3,900% (25 ºC) and 780% (35 ºC) more CH_4 than the manure control ($p < 0.001$). The highest cumulative CH_4 production in BMP2 was MD (50% ISR) at 35 ºC ($p \leq 0.006$), but this value was not statistically different than LL (50% ISR) and MD (35% ISR) at 35 ºC (Figure 3.3) ($p \geq 0.821$). At 25 ºC, MD (50% ISR) produced the highest cumulative CH_4 volume but was not significantly different than LL (50% and 35% ISR) ($p > 0.105$).

When the inoculum incubation period was increased from 91 days (BMP1) to 196 days (BMP2), there was less discernable differences in CH_4 production between 35 and 50% ISR (Figure 3.3). In BMP1 at 25 and 35 ºC, increasing ISR from 35 to 50% resulted in ≥110% increase in CH_4 production in all but one treatment (WS at 35 ºC). In BMP2, the 50% ISR bottles produced only 6-12% more CH_4 than 35% ISR (except WS at 25 ºC with 261% more CH_4). This could be due to higher concentration of methanogens in the inocula with a longer incubation time. Molecular analyses were conducted to verify this claim, with the results presented in Chapter 4.

Table 3.4: Cumulative methane CH_4 production for Day 20 and Day 90 in BMP2 using landfill leachate (LL), wetland sediment (WS), and mesophilic digestate (MD) as inoculum sources at two inoculum to substrate ratios (ISR).

	Day 20*			**Day 90***		
	50% ISR	**35% ISR**	**0% ISR**	**50% ISR**	**35% ISR**	**0% ISR**
		15 ºC			**15 ºC**	
WS	4.24 ± 0.04	-		4.26 ± 0.03	-	
MD	19.1 ± 1.9	-		106 ± 1	-	
Manure-only			0.78 ± 0.01			2.19 ± 0.04
		25 ºC			**25 ºC**	
LL	132 ± 4	90.8 ± 4.0		205 ± 6	194 ± 6	
WS	24.1 ± 0.6	9.99 ± 0.14		170 ± 1	47.1 ± 3.8	
MD	143 ± 1	66.0 ± 1.2		215± 1	194 ± 2	
Manure-only			3.43 ± 0.03			5.35 ± 0.08
		35 ºC			**35 ºC**	
LL	167 ± 1	125 ± 2		232 ± < 1	206 ± 2	
WS	82.2 ± 0.7	23.2 ± 0.5		209 ± 2	194 ± 1	
MD	180 ± 3	140 ± 4		245 ± 6	227 ± 7	
Manure-only			4.52 ± 0.02			27.7 ± 0.6

*All values are averages ± standard error in L CH_4/kg VS.

Previous research has also observed little to no differences in cumulative CH_4 production when the ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2006), but some studies have shown increases in digestion rate when ISR was increased (González-Fernández and García-Encina, 2009; Raposo et al., 2009; Zeng et al., 2010), indicating that increased ISR could increase digestion rate, but not necessarily cumulative CH_4 production. At Day 20, the 50% ISR treatments in BMP2 produced 29-255% more CH_4 than the 35% ISR treatments (Table 3.4), but the difference in CH_4 production decreased as the digestion period increased, implying that with longer inoculum incubation and digestion times, less inoculum and more manure could be added to a digester, allowing higher VS loading and higher absolute CH_4 production by the digester. Based on results from BMP2, at 35 ºC, using 35% ISR from MD incubated for 196 days could allow a higher proportion of manure (65%, by mass) to be added to the digester, resulting in 21% more CH_4/digester. It should be noted, however, that while a comparison of BMP1 and BMP2 was made, there were differences in storage of inocula before use, and the manure source (manure for BMP1 was collected in September and manure for BMP2 was collected in December), which could affect CH_4 production results. One noticeable difference was the pH of the manure for BMP1 and BMP2: 6.76 ± 0.04 and 7.58 ± 0.03, respectively.

At 15 ºC, MD (50% ISR) produced significantly more CH_4 than the other treatments ($p < 0.001$) (Figure 3.3; Table 3.4). While this volume was 4,700% more than the manure-only treatment, it was still ≥50% lower than the highest quantities of CH_4 observed at 25 ºC and 35 ºC ($p < 0.001$) in BMP2. Hence, unless the energy

savings from reducing the digester temperature from 25 ºC to 15 ºC exceeds the expected 50% decrease in CH_4 production, operating the AD systems at 15 ºC would not be recommended.

3.3.4 Volatile Fatty Acids

There were negative correlations between CH_4 production and final TVFA values in inoculum+substrate bottles in BMP1 and BMP2 (Figure 3.4). In BMP1, all bottles that had TVFA values ≤530 mg/L produced ≥130 L CH_4/kg VS (except for the triplicate bottles from WS at 35% ISR, 35 ºC), while all bottles with ≥2,600 mg/L TVFA produced ≤100 L CH_4/kg VS. In BMP2, all bottles with ≤110 mg/L TVFA produced ≥170 L CH_4/kg VS (except for a replicate bottle from MD at 50% ISR, 15 ºC) while bottles with ≥3,200 mg/L TVFA produced ≤50 L CH_4/kg VS. These results agree with the observations made by Varel et al. (1977) that digestion becomes unstable at TVFA concentrations >2,000 mg/L. Logarithmic curves were fitted for the correlation between TVFA and CH_4 production values for both BMPs (Figure 3.4). Both curves had approximately the same factors associated with the parameters. Based on the logarithmic nature of the correlation curves, there is a threshold ISR level for preventing VFA accumulation and digestion failure. Specifically, at 25 and 35 ºC in BMP1, a 50% ISR resulted in all the VFA values to be lower (≤530 mg/L), which did not occur with a 35 or 20% ISR. In BMP2, a 35% ISR was sufficient to keep TVFA concentration ≤110 mg/L at 25 and 35 ºC (with the exception of the triplicate WS bottles at 35% ISR and 25 ºC). At lower ISR ranges, Hashimoto (1989) observed large increases in CH_4 production as the ISR (on VS basis) was increased from ≤16% to 20%, with lower increases in CH_4 production as the ISR increased

further from 20% to 92%. Raposo et al. (2008) observed significant increases in CH_4 production when the ISR (on a VS basis) was increased from ≤60 to 75%, but not when ISR was increased from 44 to 60%; VFA accumulation occurred at ≤60% ISR. A subsequent study by Raposo et al. (2009) showed large increases in potential CH_4 yield when ISR was increased to 44% ISR and more gradual increases at ≥ 44% ISR, with accumulation of VFAs at 33 and 44% ISR but not at higher ISRs (50-75%).

3.3.5 Inoculum Use Recommendations and Farm-Scale Analysis

The results show that for batch digestion lasting 90 days, inoculum that has been incubated for a longer period is preferable because it allows for a lower ISR (35% ISR vs. 50%) to be used with minimal VFA accumulation and higher overall CH_4 production per digester. Under these conditions, MD as inoculum and an operational temperature of 35 ºC is recommended for highest CH_4 yield. Using MD as inoculum is also a better option because of other factors that need to be considered when WD and LL are used: wetlands are protected under the Clean Water Act in the US and obtaining sediments from wetlands could disturb this habitat. LL is readily available, but since digestate from agricultural digesters is mainly used for fertilizing plant crops, digestate from digesters inoculated with LL may need to undergo testing to ensure that it can safely be used as fertilizer. Currently, there is no conclusive study that has evaluated the safety of using anaerobically digested landfill leachate as fertilizer. Furthermore, MD as inoculum sources are more readily available, whereas incubation will be needed should one want to use wetland sediments or landfill leachate as inocula.

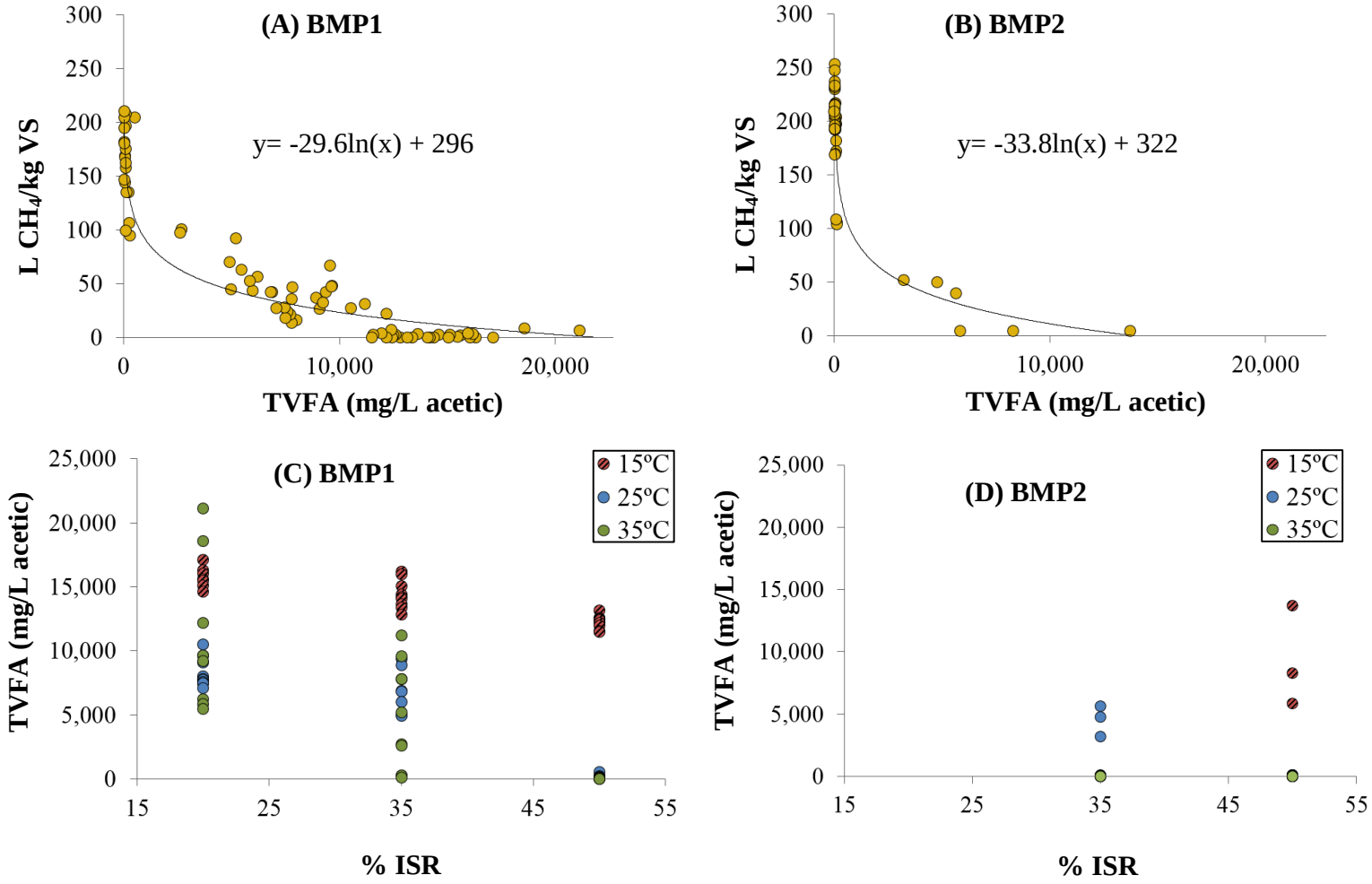

Figure 3.4: Correlation between cumulative CH_4 and total volatile fatty acids (TVFA) and between TVFA and inoculum to substrate ratio (ISR) for BMP1 and BMP2.

Results from this study were extrapolated for an energy-yield analysis of a covered lagoon treating dairy manure from a 100-cow dairy farm. A covered lagoon was selected because it is generally unheated, unmixed, and has a longer digestion times as lagoons are used for storing manure during times when spreading of manure in the field is not allowed (i.e. winter and fall, depending on the local regulations). For the analysis, manure was assumed to be stored in the covered lagoon for 90 days since manure field application is not allowed during certain times of the year. In Maryland (USA), for instance, farmers are not allowed to spread manure between November to March (Maryland Department of Agriculture, 2012). The covered lagoon was assumed to be operating at 25 °C or 35 °C, and at 35% or 50% ISR.

Based on the farm-scale exploration, the highest energy yield over a 90-day digestion period would be obtained when operating the covered lagoon digester at 35 ºC and inoculated at 50% ISR ($5.28x10^8$ BTU) (Table 3.5). This amount was 8%, 14%, and 26% higher than the respective CH_4 quantity and energy yield for the covered lagoon at 35 ºC with 35% ISR, 25 ºC with 50% ISR, and 25 ºC with 35% ISR. However, reducing the ISR from 50% to 35% could reduce the digester volume by 261 m^3, which could reduce capital and operation costs. Methane production per unit of digester volume was calculated to be highest when the covered lagoon was operated at 35 ºC with 35% ISR ($5.66x10^5$ BTU/m^3), with an energy yield that was 21% higher than at 35 ºC with 50% ISR.

Table 3.5: Energy yield of a covered lagoon treating manure from 100 cows at 90-day digestion time.

Operational Temperature	25 ℃		35 ℃	
ISR[b]	35%	50%	35%	50%
Manure VS added (kg)[a,c]	$6.72x10^4$	$6.72x10^4$	$6.72x10^4$	$6.72x10^4$
Digester Volume (m^3)[d]	869	1130	869	1130
CH_4 Yield (m^3)	$1.30x10^4$	$1.45x10^4$	$1.53x10^4$	$1.64x10^4$
Energy Yield (BTU)	$4.19x10^8$	$4.65x10^8$	$4.91x10^8$	$5.28x10^8$
Energy Yield per Digester Volume (BTU/m^3)	$4.82x10^5$	$4.12x10^5$	$5.66x10^5$	$4.68x10^5$

[a]Based on 54.4 kg of manure/cow-day (ASAE, 2003) and manure density of 1.00 kg/L.
[b] Inoculum to Substrate Ratio (ISR).
[c]VS: volatile solids value from study: 0.137 kg VS/kg manure.
[d]Assumes that headspace is 15% of digester volume.

3.4 Conclusion

All inocula were viable sources of methanogens for batch anaerobic digestion at 25 ºC and 35 ºC, but none of the inoculum sources were productive at 15 ºC. Compared to BMP1 (91-day inoculum incubation time), there were less differences in CH_4 yield between 35 and 50% ISRs at 25 ºC and 35 ºC in BMP2 (196-day inoculum incubation time), indicating that longer incubation time could allow less inoculum to be used. Furthermore, 35% ISR was sufficient in preventing VFA accumulation in most treatments at 25 ºC and 35 ºC in BMP2, compared to the required 50% ISR in BMP1. In batch systems with long digestion time (90 days), inoculum from well-established mesophilic digesters (i.e. long incubation time), 35% ISR, and a digester operation temperature of 35 ºC are recommended for highest CH_4 yield per unit of digester volume and low VFA accumulation. Research represents the first study that compares the effectiveness of wetland sediment, landfill leachate, and mesophilic digestate as inocula for anaerobic digestion at three temperatures using three ISRs and two inoculum incubation periods.

4. Incubation of Innovative Methanogenic Communities to Seed Anaerobic Digesters

Abstract

The objective of this study was to determine changes in the methanogenic and Archaeal community in three digester inocula, wetland sediment (WS), landfill leachate (LL), and mesophilic digestate (MD), during incubation at 15, 25, and 35 ºC for two incubation periods (91 and 196 days). After incubation, the inocula were used in biochemical methane potential (BMP) tests at the same three temperatures. Differences in the methanogenic and Archaeal community were then related to the CH_4 production from the BMP experiments. Terminal restriction fragment length polymorphism (T-RFLP) and quantitative polymerase chain reaction (qPCR) were used to study the changes in the communities within the inocula during the two incubation periods. The results showed that the *mcrA* numbers only increased significantly in the LL samples after incubation at 25 and 35 ºC for 196 days, and there was no significant correlation between the inoculum *mcrA* gene copy numbers and CH_4 production observed in the BMP tests. Samples from MD reactors at 25 and 35 ºC did not experience major shifts in Archaeal community. After 196 days of incubation at 25 and 35 ºC, the LL and WS Archaeal community generally converged with the MD samples at the same temperature. This cluster was associated with high relative abundance of terminal restriction fragment (TRF) putatively identified as *Methanosaetaceae*, and incubation reactors within this cluster were associated with low acetic acid concentrations (0.62-2.56 mM). After long incubation (196 days) at 15 ºC, the samples generally clustered in an area of dominance of TRF putatively

identified *Methanosarcinaceae* and were associated with high acetic acid concentrations (3.20-133.6 mM). Thus, during inocula incubation, low acetic acid accumulation appeared to be important in promoting the growth of *Methanosaetaceae*, while accumulation of acetic acid, which occurred at most reactors after 15 ºC incubation, appeared to be important in promoting the growth of *Methanosarcinaceae*. After incubation at 25 ºC for 91 days, the LL reactor had higher relative abundance of TRF identified as *Methanosarcinaceae* and produced significantly higher quantity of CH_4 (≥18%) than the WS and MD samples, indicating that inoculum rich in *Methanosarcinaceae* may be more beneficial than inoculum rich in *Methanosaetaceae*, when starting a digester at the lower mesophilic temperature range (25 ºC).

4.1 Introduction

The production of renewable energy in the form of methane (CH_4)-enriched biogas through anaerobic digestion is a microbial-based process largely affected by temperature. As much as 70% decrease in methane production has been observed with a 10 °C decrease in temperature: from 24 to 14 °C (Witarsa and Lansing, 2014) and from 20 to 10 °C (Massé et al., 2003). As a result, digesters in temperate climates are heated to maintain digestion temperature, which increases capital and operational costs.

Inoculum has been recognized as an important component to increase organic matter conversion to CH_4 in digesters (Lopes et al., 2004). Previous researchers have studied the effects of inoculum to substrate ratios (ISRs) on CH_4 production and digestion rate (Hashimoto, 1989; González-Fernández and García-Encina, 2009;

Lopes et al., 2004; Maya-Altamira et al., 2008; Raposo et al., 2006; Zeeman et al., 1988), and others, such as Nozhevnikova et al., (1999) and Zeeman et al. (1988), have studied the effects of pre-incubating the inocula for use in psychrophilic digestion. However all these studies, with the exception of Zeeman et al. (1988) who used wetland soil as inoculum, performed the studies using bovine rumen fluid, and sludge or digestate from anaerobic digesters as inocula.

The use of alternative inocula, specifically wetland sediment (WS) and landfill leachate (LL), could potentially introduce new microbial communities that are more adapted to low-temperature digestion. Steinberg and Regan (2008) illustrated that there was no overlap in the methanogenic species between a fen and an anaerobic digester. The organisms in WS and LL are exposed to seasonal temperature fluctuations, and hence it was expected that these fluctuations could increase their potential to harbor cold-adapted, psychrophilic (≤25 ºC) methanogens. Ferroni and Kaminski (1980), for instance, found that psychrophilic and psychrotrophic heterotrophic microorganisms were higher in numbers than mesophilic bacteria in a lake experiencing seasonal temperature fluctuations.

Currently, there are limited studies that have looked at the use of LL as inocula for agricultural digesters. A couple of previous studies have looked at the use of WS as digester inoculum. Steinberg and Regan (2011) studied the use of acidic bog sediment as inocula for mesophilic digesters and found that the digester inoculated with the bog sediment could survive the first of three organic shock loads better than the reactors that contained inoculum from a municipal sludge or inocula from bog sediment mixed with municipal sludge. Results on the use of WS for

psychrophilic digestion have varied. Bardulet et al. (1990) and Xing et al. (2010) observed stable organic matter removal or biogas production when wetland or river sediment was used as inocula for psychrophilic digesters, but Zeeman et al. (1988) did not find a decrease of lag-time when wetland sediment was used as inocula for psychrophilic digestion when compared to the un-inoculated treatment. In addition, there are still research gaps in terms of the amount of ISR for these alternative inocula or the pre-incubation time needed to acclimate the inocula for digestion.

Molecular techniques have been used to monitor microbial community shifts in digesters at psychrophilic temperature (Collins et al., 2003, McHugh et al., 2004), during start up of a mesophilic batch reactor (Lee et al., 2010), and at varied organic loading rate (Dollhopf et al, 2001). McMahon et al. (2004) reported digesters with higher quantities of Archaea and more *Methanosaeta concilii* compared to *Methanosarcina* sp. had a more successful start-up period. Regueiro et al. (2012) found that higher *Bacteroidetes* and Archaea numbers were associated with higher hydrolytic and methanogenic activities, respectively.

Using inoculum from lake sediment, Xing et al. (2009, 2010) found that the dominant Archaea present within the 15 °C psychrophilic reactor had 98% similarity to *M. lacustris*, which has a lower optimal temperature than the *Methanosaeta* that dominated a reactor inoculated with mesophilic digestate inoculum, though the two studies were conducted using different reactor designs and at different times. Dollhopf et al. (2001) also found that initial bacterial and Archaeal communities were different between a digester that was inoculated with digestate from anaerobic reactor and a reactor inoculated with river sediment, but the convergence of the communities

was observed during the digestion process when operated at 34 ºC. Steinberg and Regan (2011) studied three mesophilic (30 ºC) digesters that were inoculated with acidic bog sediment, municipal sludge, and a mixture of the two. The digester inoculated solely with sediment survived an organic shock load better than the other two digesters, with Fen Cluster observed to dominate the methanogenic community of the bog sediment reactor, indicating that Fen Cluster could play an important role in conferring tolerance to organic shocks in reactors. It should be mentioned, however, that *Methanosarcina* was more important for resuming CH_4 production (Steinberg and Regan, 2011).

There is still a lack of research in terms of how the microbial community in alternative inocula changes when incubated for different periods of time at different temperatures, and how these changes relate to their effectiveness as an inoculum source for enhanced CH_4 production. This research compared three inocula (mesophilic digestate (MD) obtained from a dairy manure anaerobic digester, wetland sediment (WS), and landfill leachate (LL) inocula) incubated at 15, 25, and 35 °C and used in dairy manure digestion at the same three temperatures using two incubation time. Specifically, the research focused on determining changes in methanogenic number and Archaeal community structure during inocula incubation and relating these differences to the CH_4 production from biochemical methane potential (BMP) experiments.

4.2 Methods

4.2.1 Inoculum Incubation and Biochemical Methane Potential Tests

The complete methods for inocula incubation and biochemical methane potential (BMP) testing can be found in Chapter 3. The inocula used were WS obtained from the Jugbay Wetland Sanctuary (38.78º N, 76.71º W) located adjacent to the Patuxent River, Anne Arundel County, Maryland, USA, LL sample collected from Stafford County Landfill, Virginia (38.38º N, 77.42º W), and MD sample collected from a complete mixed digester (540 m^3) located at the US Department of Agriculture (USDA) Beltsville Agriculture Research Center (BARC) dairy facility (39.03º N, 76.89º W). The WS, LL, and MD inocula were mixed separately with nutrient media from Speece (1996) in nine 4 L anaerobic reactors made of 10.2-cm diameter (4") PVC pipes with a height of 0.5 m and purged with N_2:CO_2 (70:30) mix for 10 minutes before being sealed and incubated in three temperature chambers (15, 25, or 35 °C). The reactors were fed at approximately four-day intervals with autoclaved unseparated scraped manure and methanol according to a feeding regimen adapted from Bardulet et al. (1990) and Bull et al. (1983) (Table 3.1). The reactors were shaken by hand after each feeding session. Samples were taken out from each incubation reactor to be used as inocula on Day 91 for BMP1 and Day 196 (or 197) for BMP2 and digested at the same temperature as incubation.

The procedures for BMP tests were adapted from Moody et al. (2011). Generally, the BMP tests in this study consisted of 250 mL serum bottles that contain inoculum and substrate that were purged with N_2:CO_2 (70:30), sealed with butyl rubber septa, and incubated in triplicates without shaking at 15, 25, 35 ºC for a period

of 90-100 days. In addition, duplicates of control bottles containing 120 g of the only inoculum from each individual reactor were set up in each temperature chamber to quantify CH_4 production from the inocula. Average cumulative CH_4 volumes (L CH_4/kg inoculum) from the duplicate control bottles were adjusted to the volume of inoculum used in the treatment bottles and subtracted from the CH_4 production of the treatment bottles to account for residual CH_4 production from the inoculum source.

The substrate used was unseparated scraped manure from the USDA BARC facility using three ISRs. Only 50% ISR (w/w) (60 g of manure + 60 g of inoculum), however, was considered in this microbial study since this treatment generally produced the highest amount of CH_4 in both BMPs. In BMP2, no LL treatment was used at 15 ºC. It should also be noted that in BMP1, the inocula were stored for a maximum of seven days at 4 °C before use in the BMP test, while in BMP2, the inocula were introduced directly from the reactors into BMP bottles.

Biogas was measured by inserting a graduated wet-tipped glass syringe into the rubber septum that capped each serum bottle, with the measured amount of biogas vented. A 0.1 mL sample was then obtained from the bottle and injected into an Agilent HP 7890A gas chromatograph (GC) to measure the CH_4 content. The following parameters were used for the GC: 1) injection temperature of 250 ºC; 2) detector temperature of 250 ºC; 3) oven temperature of 60 ºC; and 4) a carrier gas flow rate of 8.6 mL He/min.

4.2.2 Effluent Characteristics of Inoculum Incubation Reactors

Volatile solids (VS), acetic acid analyses, and pH were conducted on the incubated inocula before each BMP test. Total solids and VS were measured by

heating samples at 103-105 ºC to constant mass, followed by heating samples at 550 ºC to constant mass (APHA, 2005). For measurement of acetic acid concentrations, samples were acidified with concentrated sulfuric acid to a pH below 2 (diluted by ≤10%) and filtered to 0.22 µm before injection into a HP 7890A GC equipped with a flame ionization detector (FID) with the following parameters: 1) injection temperature of 250 ºC; 2) detector temperature of 300 ºC; 3) oven temperature of 100 ºC for 2 minutes and increased by 10 ºC/min for a total run time of 10 min; and 4) a carrier gas flow rate of 1.80 mL He/min. Extrapolations of acetic acid standard curves below 1 mM were conducted to determine values within the range of 0.1-1 mM. Values <0.1 mM were assumed to be zero, as these concentrations had no discernable differences with DI blanks verifications.

4.2.3 DNA Extraction

Duplicate samples were taken from each incubation reactor for microbial analyses on Day 91 and Day 196 when samples were extracted from the reactors for the BMP tests. Microbial analyses were also conducted on the original samples (WS, LL, and MD) before they were placed in the incubation reactors (referred to as seeds henceforth).

Samples were kept in sterile 15 mL centrifuge tubes and stored at -20 °C (<10 days) before being transferred on ice into a -80°C freezer. DNA samples were extracted using PowerFecal® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA), during which cells were lysed and DNA was separated from non-DNA particles, such as polysaccharides and proteins. The amount of DNA was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY).

4.2.4 Quantitative Polymerase Chain Reaction (qPCR)

Quantitative polymerase chain reaction (qPCR) was used to quantify the total number of methanogens present within the seeds and reactor samples on Day 91 and Day 196. The gene sequence targeted for amplification was the alpha subunit of the methyl coenzyme M reductase (*mcrA*), an enzyme used by methanogens in the last step of CH_4 formation (Ferry, 1992), and has been used by many previous researchers for studying the methanogenic community (Alvarado et al., 2014).

Briefly, in this procedure, the *mcrA* in the extracted DNA from each sample was replicated in a qPCR machine. A fluoresecent dye that could bind to the replicated gene was added and the number of cycles it took for fluorescence to be detected for each sample was compared to samples in which the *mcrA* quantities were known. This allowed the quantification of *mcrA* gene present within each sample.

Extracted DNA samples were diluted to 1.25 ng/µL DNA concentration to prevent process inhibition, except for duplicate samples of the seed LL that already had DNA concentration <1.0 ng/ µL. The qPCR was conducted using the following conditions: heat activation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 56 °C for 45 seconds, primer elongation at 72 °C for 60 seconds, and acquisition at 80 °C for 10 seconds. The forward and reverse primers used for the amplification process were mcrA_1035F (5'- GGTGGTGTMGGATTCACACARTAYGCWACAGC-3') and mcrA_1530R (5'- TTCATTGCRTAGTTWGGRTAGTT-3') (Pereyra et al., 2010).

The plasmid standard for standard curves was prepared according to Prasse et al. (2015). Each 20 µL qPCR reaction mixture contained 10.0 µL of KICQSTART

SYBR Green qPCR Ready Mix with ROX (Sigma Aldrich, St. Louis, MO), 0.5 µM of forward and reverse primers, 6 µL of qPCR grade water, and 2 µL of template DNA. The qPCR were conducted in triplicates for each sample and each standard using a StepOne Plus Real-Time PCR instrument (Applied Biosystems, Foster City, CA). At least three of the diluted plasmid standards were used for creating the standard curves. All standard curves had R^2 >0.96 and efficiencies of 99.6-102%.

4.2.5 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Briefly, in this procedure, Archaeal 16S rRNA gene within each sample was replicated in a PCR machine to increase its abundance for analysis. After PCR, the replicated 16S rRNA gene of interest was separated from unwanted materials such as excess nucleic acids and buffers (i.e. cleaned). The cleaned samples were digested with a restriction enzyme that cut the gene of interest at different points, producing different gene fragments that could then be separated and viewed in a capillary gel electrophoresis. Because Archaeal 16S rRNA genes in different species or family could have different sequences of nucleic acids, the different fragment lengths present within the sample represents different types of Archaea within the sample.

Archaeal gene sequences were first amplified using PCR in an Eppendorf MasterCycler Pro S (Eppendorf, New Hamburger, Germany). Q-PCR grade water was added to samples that had more than 25 ng/ µL of DNA to obtain DNA concentration of 25 ng/µL, except for the original WS samples that were diluted to 2.5 ng/µL to overcome inhibition during the PCR process. The forward primer used was AR109F (5'-ACKGCTCAGTAACACGT-3'), while the reverse primer was AR915r (5'-GTGCTCCCCCGCCAATTCCT-3') (Sigma-Aldrich®, St. Louis, MO).

The reverse primer was fluorescently labeled. PCR amplification process was conducted with the following conditions: 5 minutes of heat activation, followed by 30 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds, primer elongation at 72 °C for 1 min, and a final extension at 72 °C for 7 minutes (Chin et al., 1999).

Go Taq® PCR Core System I kit (Promega Corporation, Madison, WI) was used for the PCR runs, which was conducted using 50 µL of reaction mixtures that contained 19.47 µL PCR-grade water, 8.13 µL of 0.4% BSA, 10.67 µL of 5x buffer, 1.87 mM of $MgCl_2$, 0.0535 mM of dNTP's, 0.266 µM of reverse primer, 0.266 µM of forward primer, 0.27 µL of TAQ polymerase, and 4 µL of extracted DNA samples. 0.5 µM of forward and reverse primers were used for a replicate sample from the MD seed, the duplicate WS seed, and a replicate of the WS reactor after 91 days of incubation at 15 °C and 25 °C. In addition, 2 µL of the extracted DNA sample were used in the duplicate WS seed and a replicate of the WS reactor after 91 days of incubation at 15 °C and 25 °C to prevent inhibition.

Samples obtained from the PCR process were cleaned using UltraClean® PCR Clean-Up kit (MO BIO Laboratories, Inc., Carlsbad, CA). The samples were then digested at 37 °C for 120 minutes, followed by 80 °C for 20 minutes, in 20 µL of the reaction mixture consisting of 17.5 µL of cleaned DNA samples, 2 µL of 10x buffer (Promega Corporation, Madison, WI), and 0.5 µL of *TaqI* restriction enzyme (Promega Corporation, Madison, WI). Two T-RFLP runs were conducted to ensure peaks were in the measurement range. The first T-RFLP plate contained 9 µL of Hi-Di™ Formamide (Life Technologies, Carlsbad, CA) and GeneScan™-1000 Rox™

mixture, and 2 µL of digested DNA samples in each well, while the second T-RFLP plate contained 9 µL mixture of Hi-Di™ Formamide (Life Technologies, Carlsbad, CA) and reduced quantity of GeneScan™-1000 Rox™, 0.2 µL of digested DNA samples, and 1.8 µL of water to obtain better peak resolution. Both T-RFLP runs underwent a denaturing process for 3 minutes at 95 °C and cooled before they were inserted into a 3730xl DNA analyzer (Life Technologies, Carlsbad, CA).

Relative abundance of detected peaks within each sample was calculated, and peaks that had less than 1% relative abundance were removed from each sample. Remaining peaks for all samples that were ≤1.5 base pairs apart were considered a single peak, and the fragment length of this peak was obtained by averaging all the fragment lengths considered for this peak. The relative abundances were then recalculated using the remaining peaks present within each sample.

4.2.6 Statistical Analysis

All statistical analyses were conducted on the sub-replicates of each reactor and temperature chamber.

4.2.6.1 BMP tests

Two Tukey-Kramer analyses using Proc Mixed procedure in SAS® 9.3 (Cary, NC) were conducted to compare treatments within BMP1 and within BMP2 (included all ISR treatments; Chapter 3). Equal variances were used for the Tukey-Kramer analyses in BMP1. For BMP2, variances within the treatments were grouped into three categories: 1) WS at 15 ºC and 50% ISR, and manure-only treatments at 15 and 25 ºC; 2) LL at 25 ºC at 50% and 35% ISR, and MD at 35 ºC at 50% and 35% ISR;

and 3) all remaining treatments. An *alpha* of 0.05 was used to indicate significant difference.

4.2.6.2 qPCR Statistical Analysis

The *mcrA* gene copy numbers were expressed in logarithmic form for statistical analyses.

Analysis of variance (ANOVA) tests were performed for all the different treatments, except for the WS reactor that was incubated for 90 days at 15 ºC since only a replicate of the duplicate samples was amplified during qPCR.

Correlations between the log *mcrA* numbers and CH_4 values from BMP tests with 50% inoculum to substrate ratio (ISR) at each incubation time (BMP1 or BMP2) and both incubation times (BMP1 and 2 combined) were analyzed. Since low temperatures could suppress CH_4 production, the same correlation analyses without incorporating 15 ºC (i.e. only 25 and 35 ºC) were also conducted. The WS reactor that was incubated at 15 ºC for 90 days was not included in all correlation analyses since only a replicate of the duplicate samples was amplified during qPCR.

An *alpha* of 0.05 was used to indicate significant differences or correlations, and SAS® 9.3 (Cary, NC) was used for correlations and ANOVA analyses.

4.2.6.3 T-RFLP Statistical Analysis

The T-RFLP data was analyzed by non-metric dimensional scaling (NMS) ordination using PC-ORD version 6 (MjM Software Design, Gleneden Beach, OR).

4.3 Results

4.3.1 Wastewater Characteristics and BMP Results

The pH of the inocula used for the BMP tests were within the ideal pH range (6.5-8) (Seadi et al., 2008), with the exception of LL reactor incubated at 15 ºC on Day 91 (Table 3.2). Acetic acid concentrations were generally lower for reactors incubated at 25 and 35 ºC (0.62-4.13 mM) than reactors incubated at 15 ºC (1.61-133.6 mM) (Table 4.1).

Table 4.1: Acetic acid concentrations of contents from inoculum incubation reactors for landfill leachate (LL), wetland sediment (WS), and mesophilic digestate (MD). Reactor contents were used as inocula for BMP1 (91 days of incubation) and BMP2 (196 days of incubation).

	Day 91	**Day 196**
Incubation Reactors	**Acetic Acid Concentrations (mM)**	**Acetic Acid Concentrations (mM)**
	15 ºC	
LL	99.2	133.6
WS	15.4	46.3
MD	1.61	3.20
	25 ºC	
LL	4.13	1.73
WS	1.69	2.56
MD	1.03	0.82
	35 ºC	
LL	1.58	1.02
WS	1.65	0.62
MD	0.99	0.62

The LL inoculum at 25 ºC after 91 days of incubation resulted in significantly higher CH_4 production (18% higher) than the WS and MD samples ($p \leq 0.002$) at the same temperature (Table 3.3, Figure 3.3). In addition, LL inoculum at 25 ºC also did not produce significantly different amount of CH_4 when compared to the highest CH_4

producer in BMP1, the MD inoculum at 35 ºC. In BMP2 (after 196 days of inocula incubation), the LL inoculum source at 25 ºC still produced significantly higher quantity of CH_4 (21% more; $p < 0.001$) compared to WS at 25 ºC, but did not produce significantly different amount of CH_4 than MD at 25 ºC (Table 3.4; Figure 3.3). In addition, LL at 25 ºC produced 12 to 16% less CH_4 than MD and LL at 35 ºC ($p \leq 0.016$).

4.3.2 qPCR

All inoculum samples contained between 10^8 to 10^{10} copies of *mcrA* genes/g of sample (Figure 4.1). Original inoculum samples from MD contained significantly higher amount of gene copies (2,050% more) than the LL inoculum source ($p \leq 0.021$), but no significant difference was observed between LL and WS inoculum sources ($p = 0.167$), and between MD and WS inoculum sources ($p = 1.000$).

Gene copy numbers for LL samples increased significantly (≥2,140%) after 196 days of incubation at 25 and 35 ºC compared to the inoculum seed source ($p \leq 0.014$). In addition, after 196 days of incubation, the LL samples from the 25 ºC incubation had significantly higher number of gene copy numbers (4,430% more) than the LL samples incubated at 15 ºC ($p = 0.0173$).

No significant correlation was observed between the gene copy numbers and CH_4 produced from the BMP tests when all the temperatures (15, 25, and 35 ºC) were analyzed (Figure 4.2). Removal of 15 ºC data and analysis with only CH_4 values from 25 and 35 ºC also did not yield significant correlation.

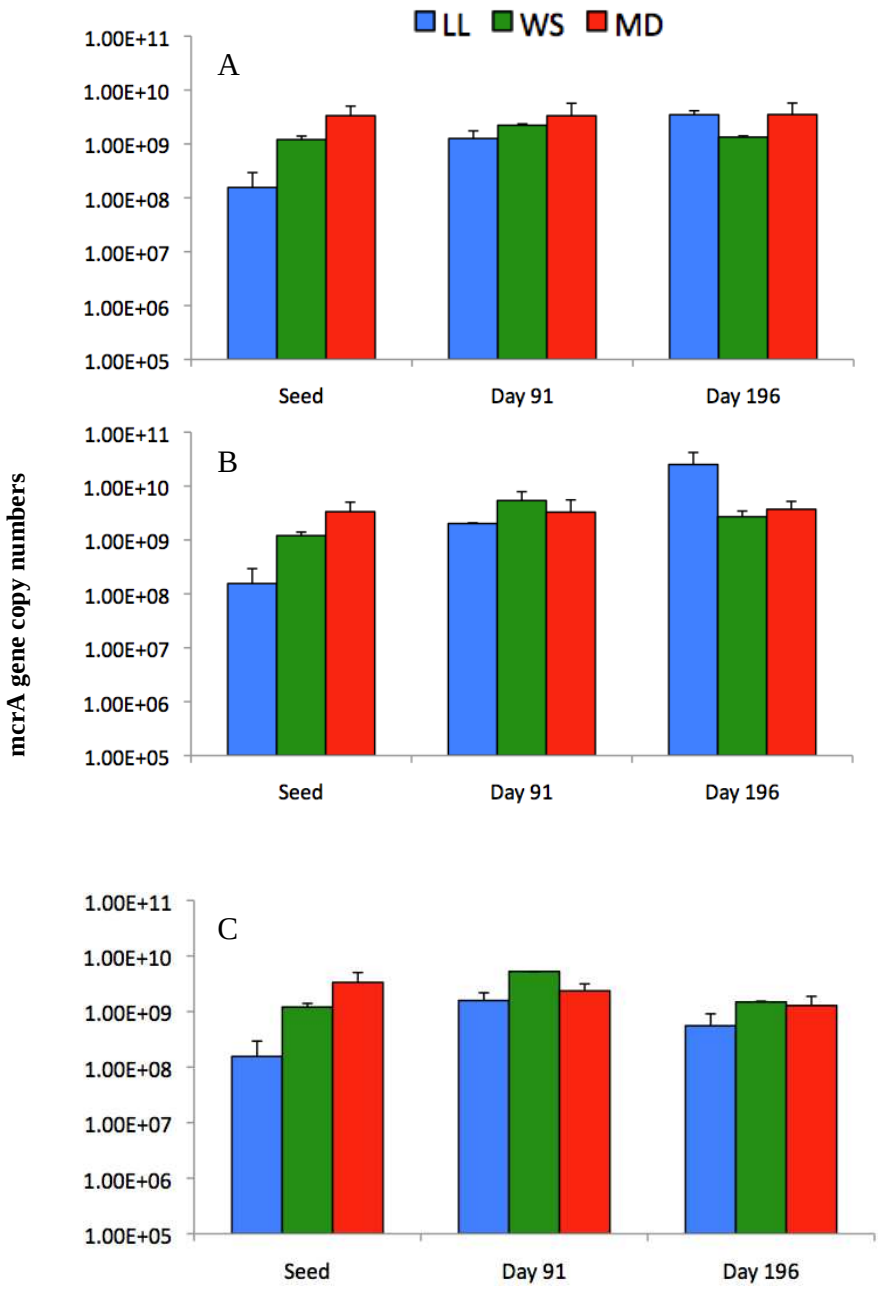

Figure 4.1: *mcrA* gene copy numbers for seed inocula and incubation reactors for the inocula, which were incubated at 35 ºC (A), 25 ºC (B), and 15 ºC (C) for 91 and 196 days. WS, LL, and MD refer to wetland sediment, landfill leachate, and mesophilic digestate, respectively.

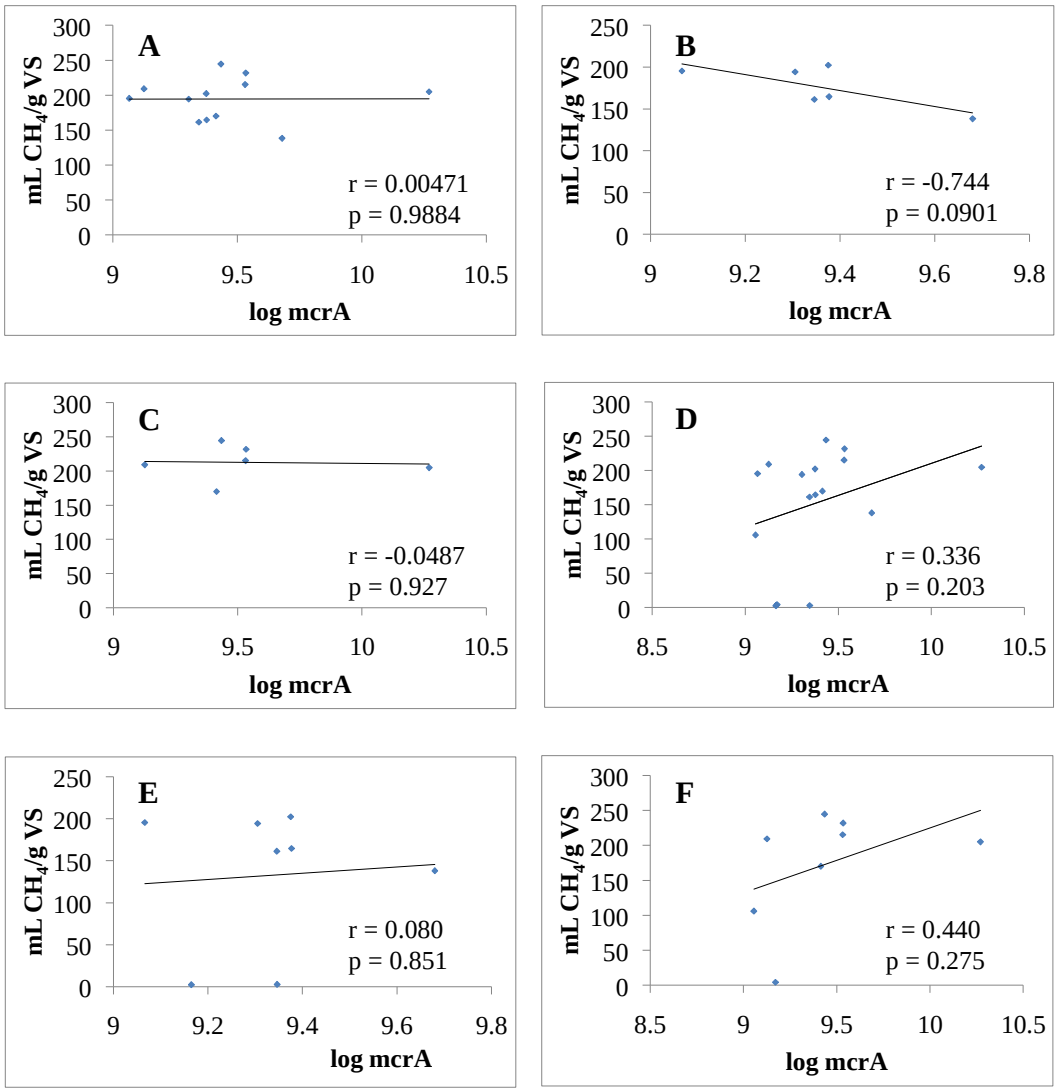

Figure 4.2: Correlations between inocula log *mcrA* numbers and CH_4 produced from: A) BMP1 and 2 at 25 and 35 °C; B) BMP1 at 25 and 35 °C; C) BMP2 at 25 and 35 °C; D) BMP1 and 2, at 15, 25, and 35 °C; E) BMP1, at 15, 25, and 35 °C; and F) BMP2, at 15, 25, and 35 °C.

4.3.3 T-RFLP Analysis for Seed and Reactor Samples after Incubation

A total of 27 terminal restriction fragments (TRF) with >1% relative abundance was observed for all samples. Terminal restriction fragments 199 and 302 were the two most common TRF in all the samples, appearing in at least 98 and 88% of samples analyzed, respectively. TRF 413 was the next most common fragment, appearing in 76% of samples analyzed.

Seed samples from the duplicate MD samples showed a total of 8 peaks, with TRF 302 constituting at least 85% of the relative abundance. Duplicate WS samples had a total of 10 TRFs, with a more evenly distributed fragments, with each TRF constituting ≤25% of the relative abundance. The LL samples had a total of 18 peaks, and similar to the WS samples, the TRFs were more evenly distributed with an average relative abundance of ≤24% for each TRF.

Two important clusters were recognized within the NMS ordination of the T-RFLP results (Figure 4.3). Cluster 1 consisted of the seed MD inoculum and the following reactors: 1) all MD reactors except for MD reactor after incubation for 196 days at 15 ºC; 2) WS reactors after incubation for 91 and 196 days at both 25 and 35 ºC; and 3) LL reactors after incubation for 196 days at 25 and 35 ºC. It should be noted that not all of the duplicate samples from these reactors fell within Cluster 1 due to heterogeneity in sampling procedures. Samples within Cluster 1 were characterized by high relative abundance of TRF 302 and low acetic acid concentrations (0.62-2.56 mM) (Table 4.1).

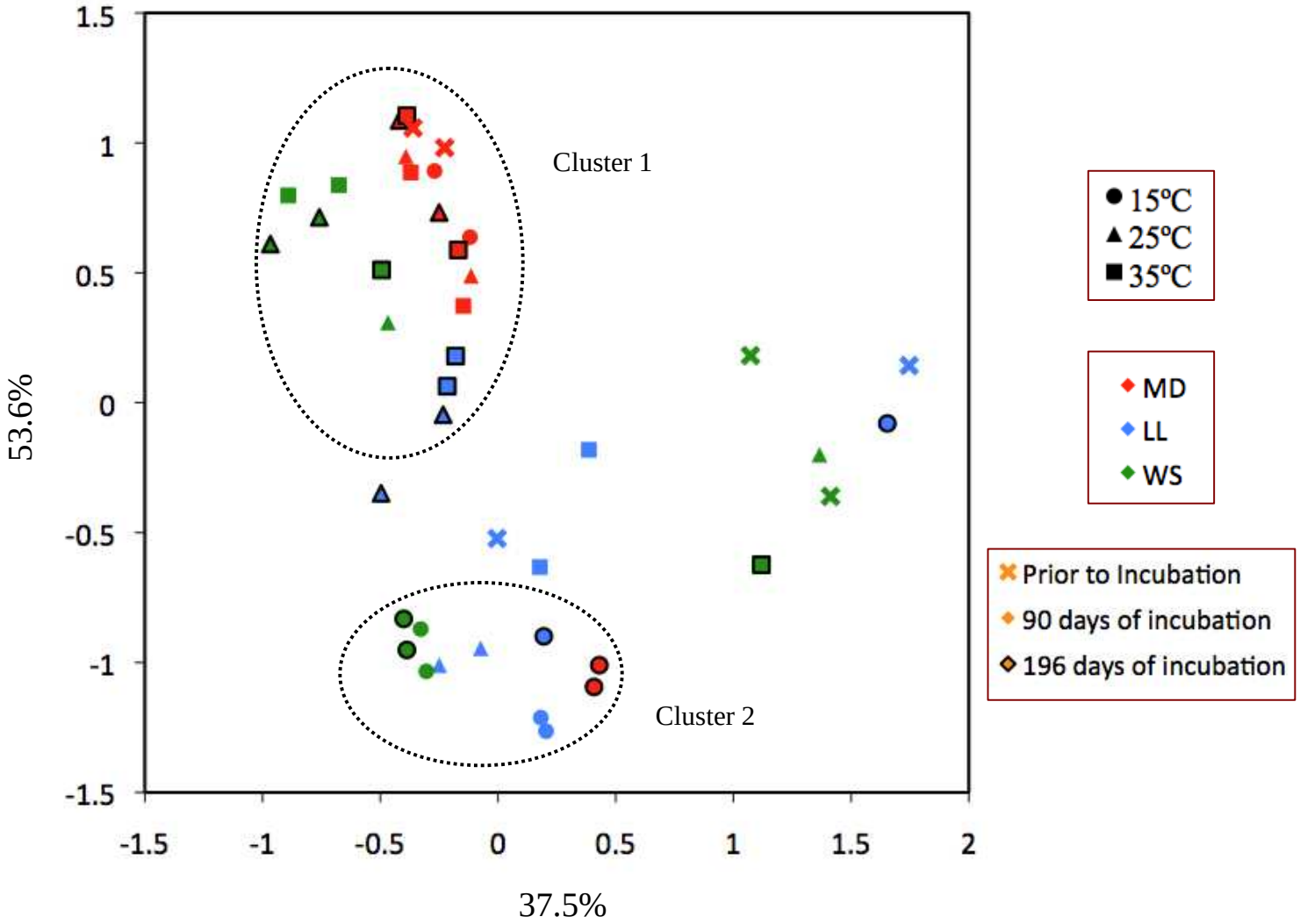

Figure 4.3: Non-metric multidimensional scaling (NMS) ordination of T-RFLP peaks of samples from seed inocula and incubation reactors. WS, MD, and LL refer to wetland sediment, mesophilic digestate, and landfill leachate, respectively.

The Archaeal community in the MD reactors did not experience major shifts after incubation and remained closely clustered with the seed MD inoculum in Cluster 1, with the exception of MD reactor incubated for 196 days at 15 ºC that migrated to Cluster 2 (Figure 4.3). The WS and LL samples generally shifted towards the MD cluster (Cluster 1) after incubation at 25 and 35 ºC, although it took the LL samples 196 days before the samples approached the MD cluster (Cluster 1).

After incubation at 25 and 35 ºC, TRF 302 continued to dominate the Archaeal community in the MD samples in Cluster 1, with ≥60% relative abundance in each replicate sample analyzed. TRF 302 was the most abundant peak in the WS samples that clustered together with the MD cluster (≥44% relative abundance) in Cluster 1, with the exception of a replicate sample of the WS reactor after incubation at 25 ºC for 196 days that had high abundance of TRF 302 (relative abundance of 39%), but was dominated by TRF 413 (relative abundance of 56%). Within the LL samples that were in the Cluster 1, TRF 302 was the most abundant peak (≥47% relative abundance), with the exception of a replicate sample with an incubation of 196 days at 25 ºC, which had high abundance of TRF 302 (36%), but was dominated by TRF 199 (43%).

Cluster 2 contained all reactor samples that were incubated at 15 ºC, with the exception of MD samples that were incubated for 91 days and a replicate sample of LL reactor that was incubated for 196 days (Figure 4.3). In addition, Cluster 2 also contained LL samples that were incubated at 25 ºC for 91 days. TRF 199 was dominant in all samples within Cluster 2, with relative abundances ranging from 34 to 85% in all the replicate samples. The reactors ordinated within Cluster 2 contained

higher concentrations of acetic acids (3.20-133.6 mM) compared to reactors within Cluster 1 (0.62-2.56 mM).

4.4 Discussion

4.4.1 Methanogenic Numbers

Significant increase in *mcrA* gene copy numbers was only observed in the LL reactors after incubation at 25 and 35 ºC for 196 days, with higher numbers at the end of the incubation at 25 ºC compared to 15 ºC, likely due to the higher temperature that stimulated higher growth of methanogens. The results showed that there were no significant correlations between the inoculum *mcrA* gene copy numbers and CH_4 production (Figure 4.2). Traversi et al. (2012) reported significant correlations between biogas production and *mcrA* gene numbers within their bioreactor, but did not study the correlations between *mcrA* numbers in the inoculum and biogas production. Morris et al. (2014) did determine that there was a significant correlation between the *mcrA* gene copy numbers from inoculum obtained from incubation reactors and CH_4 production values from a SMA that used H_2/CO_2 as substrate, but no significant correlations were observed between the SMA results and inoculum gene copy numbers when acetate or propionate were used as the substrate. The lack of significant correlations when liquid substrates were used confirms the lack of correlation observed in our study. The MD, WS, LL study used a mixed, complicated substrate (manure) that likely affected the observed lack of correlation. Alvarado et al. (2014) and Freitag and Prosser (2009) discussed the ineffectiveness of *mcrA* gene copy numbers in predicting CH_4 production activity due to the possible presence of dormant cells. The presence of inactive or dormant cells within our inoculum could

affect the correlation between the CH_4 production rate and *mcrA* gene copy numbers. Additionally, the gene copy numbers are only indicative of the total amount of methanogens and not necessarily the community of methanogens, which shifted during incubation according to the NMS ordination from the T-RFLP results.

4.4.2 Archaeal Community within Incubation Reactors

The T-RFLP results showed that the terminal restriction fragments (TRF) at 199 and 302 were the most common TRFs. Aceticlastic methanogens are generally the dominant methanogens within a balanced anaerobic digester, contributing up to 70% of CH_4 produced (Alvarado et al., 2014; Gerardi, 2003). Additionally, Chin et al. (1999) used the same T-RFLP methodology as our study and showed that the TRFs at 199 and 302 were aceticlastic methanogens belonging to the *Methanosarcinaceae* and *Methanosaetaceae* family, respectively. Our study had a difference of 14 and 16 base pairs, respectively, when compared to results from the Chin et al. (1999) study.

Organisms within the *Methanosarcinaceae* family (TRF 199) are involved in the dismutation of methyl compounds and use acetate and CO_2/H_2 to produce CH_4 (Kendall and Boone, 2006). However, only *Methanosarcina* sp. within the *Methanosarcinaceae* family uses acetate as substrate for CH_4 production, in addition to methanol, methylamines, CO, and CO_2/H_2 (Kendall and Boone, 2006). There is only one genus within the *Methanosaetaceae* family (TRF 302), *Methanosaeta,* which only uses acetate as the energy source (Kendall and Boone, 2006). Additionally, *Methanosarcina* sp. have been found to have a higher minimum acetate threshold (0.2-1.2 mM) and growth rate than *Methanosaeta* (7-70 µM acetate

threshold) (Jetten et al., 1992). As a result, *Methanosaeta* tends to dominate in environments with low acetate concentrations (Smith and Ingram-Smith, 2007).

High abundance of TRF 302 within the MD seed indicated high numbers of *Methanosaeta* sp. within the inoculum. In previous studies, *Methanosaeta* was the most commonly observed Archaea in 44 digesters, which included the fixed biofilm, continuously stirred, fluidized bed, sequential batch, and upflow anaerobic sludge blanket digester designs. However, within the continuously stirred reactors (CSTR), *Methanosarcina*, along with an unidentified Archaea, was found to be dominant (Leclerc et al., 2004). In survey of 15 digesters, Karakashev et al. (2005) found that *Methanosarcinaceae* dominated most of the manure digesters, and the manure-based digesters had higher VFA values (>5 mM as acetic acid equivalent), while *Methanosaetaceae* dominated all digesters fed exclusively with sludge, with these digesters having a lower VFA values (0.2-1.17 mM as acetic acid equivalent). Thus, VFA values were shown to have an important role in determining the dominant methanogens. A separate analysis of samples from the digester in which the MD inoculum was taken from revealed that the acetic acid levels were ≤0.95 mM (unpublished results), which is considered a sufficient concentration for *Methanosaetaceae* to thrive, but too low for *Methanosarcinaceae* to thrive. The low levels of acetate of this manure-based digester is likely due to the use of liquid manure (after solid separation with a screw press) used as the influent for the digester. In addition, while the digester was built as a CSTR, it was noted that the mixing system had not been working for many years, which together with the lower VS input

of the liquid manure could have resulted in the non-dominance of *Methanosarcinaceae*.

Two important clusters were recognized in the NMS ordination of the T-RFLP results (Figure 4.3). Cluster 1 was driven mainly by the presence of abundant TRF 302, putatively identified as *Methanosaetaceae.* Cluster 1 contained the MD inoculum incubated at 25 and 35 ºC, which experienced no major shift in Archaeal community. This was expected considering there was no major change in substrate and conditions for these reactors. Acetic acid concentrations within these reactors were ≤1.0 mM, which is more likely to promote the growth of *Methanosaeataceae.* At 25 and 35 ºC, Archaeal community within the LL and WS samples generally shifted and became more tightly clustered with the MD samples in Cluster 1, especially at the higher incubation time (196 days).

Dollhopf et al. (2001) also observed convergence of both Archaeal and bacterial communities in two glucose-based reactors with one reactor inoculated with wetland sediment and the other reactor inoculated with sewage sludge. The small shift in the MD Archaeal community and the larger shift in the WS and LL samples observed in our study were similar to the results of Pagaling et al. (2014), where pre-conditioned inocula experienced smaller shifts when placed in a new microcosm compared to inocula sources that were not pre-conditioned.

After incubation at 15 ºC, samples from all three inocula sources generally clustered together in Cluster 2. The results showed that TRF 199, putatively identified as *Methanosarcinaceae,* was dominant in the reactors within Cluster 2, in contrast to Cluster 1, which was driven by high abundance of *Methanosaetaceae*. Additionally,

acetic acid concentration was also observed to be important drivers of the two clusters, with higher concentrations in Cluster 2 (3.20-133.6 mM) than in Cluster 1 (0.62-2.56 mM). Homoacetogens become an important tropic group when temperature decreases (Fey and Conrad, 2000; Kotsyurbenko et al., 1993; Kotsyurbenko et al., 2001; Kotsyurbenko, 2005), which could increase acetate concentration and allow *Methanosarcinaceae*, with its higher growth rate and higher acetate threshold, to outcompete *Methanosaetaceae.* Thus, it appears that at 15 ºC, the reactors accumulated higher acetic acid values, which promoted the growth of *Methanosarcinaceae* and caused the samples to be ordinated in Cluster 2. If the acetic acid levels remained low, *Methanosaetaceae* would likely have dominated and samples could have shifted to Cluster 1. This was clearly illustrated by the MD inoculum that was incubated at 15 ºC for 90 days. Despite incubation at 15 ºC, acetic acid levels remained low (1.61 mM) and thus *Methanosaetaceae* was dominant and samples were ordinated within Cluster 1.

While TRFs 199 and 302 were putatively identified as *Methanosarcinaceae* and *Methanosaetaceae*, it should be noted that Chin et al. (1999) also assigned TRF 199 to *Crenarchaeota* Rice Cluster VI, and TRF 302 to a novel *Euryarchaeota* Rice Cluster V and *Crenarchaeota* Rice Cluster IV. To our knowledge, Rice cluster V and VI have not been reported to be in anaerobic digesters. Sekiguchi (2006) also reported that Rice Cluster VI has not been found in anaerobic digesters, but reported that Rice Cluster IV was observed in anaerobic digesters. Unfortunately, with the lack of specificity with the T-RFLP analysis, it was not possible to conclusively distinguish between Rice Cluster IV and *Methanosaetaceae* in the samples analyzed. However,

the relationships between the VFA levels and the abundance and/or dominance of TRFs 302 and 199 did agree well with the assumptions of these two TRF peaks as *Methanosaetaceae* and *Methanosarcinaceae.*

4.4.3 Inoculum Archaeal Communities and CH_4 Production in BMP Tests

After incubation at 25 ºC for 91 days, LL inoculum at 50% ISR produced significantly higher quantity of CH_4 (194 L CH_4/kg VS) than the MD (165 L CH_4/kg VS) or WS (138 L CH_4/kg VS) at 50% ISR and 25 ºC (Table 3.3). A higher rate of CH_4 production was also observed for LL compared to MD and WS inoculum sources within the first twenty days of digestion: 5.2, 4.5, and 1.2 L CH_4/kg VS/day for LL, MD, and WS, respectively. The pH and VS content of the three inocula sources were similar: 7.27-7.31 and 31.3-38.7 g/kg VS, respectively (Table 3.2). With insignificant differences between the *mcrA* gene copy numbers of the LL, WS, and MD inoculum sources after incubation at 25 ºC for 91 days, the differences in CH_4 production were likely due to the fact that the incubated LL Archaeal community was dominated by *Methanosarcinaceae* (Cluster 2), which resulted in higher CH_4 production compared to the incubated MD and WS inocula sources that were generally dominated by *Methanosaetaceae* (Cluster 1). *Methanosarcinaceae* has previously been reported as a “heavy duty” methanogen that could, among others, withstand overloading shock and temperature changes better than other methanogens (De Vrieze et al., 2012). De Vrieze et al. (2012) discussed the possibility of adding inoculum rich in *Methanosarcina* during perturbation to promote digester recovery from organic loading shock. Results from the current study showed that inoculum

dominated by *Methanosarcinaceae* may indeed enhance the performance of a digestion system.

4.5 Conclusion

The research showed that low acetic acid accumulation likely promoted the growth of *Methanosaetaceae*, while accumulation of acetic acid, which occurred in most samples after 15 ºC incubation, likely promoted the growth of *Methanosarcinaceae*. The inoculum sources rich in *Methanosarcinaceae* (LL) may be more beneficial than inoculum rich in *Methanosaetaceae*, when starting a digester at the lower mesophilic temperature range (25 ºC). The *mcrA* numbers only increased in the LL samples after incubation at 25 and 35 ºC for 196 days, and as *mcrA* numbers do not discern between viability and community groups present, there was no significant correlation between the inoculum *mcrA* gene copy numbers and the CH_4 production observed in the BMP tests. While the MD-inoculated reactors at 25 and 35 ºC did not experience major shifts in Archaeal community, the Archaeal community in the LL and WS inoculated reactors generally converged with the MD samples at 25 and 35 ºC, forming a cluster that was associated with high abundance of *Methanosaetaceae* and low acetic acid concentrations. After long incubation (196 days) at 15 ºC, the inoculated reactors generally clustered together in a second cluster associated with high acetic acid concentrations and dominance of *Methanosarcinaceae.* Future work involving sequencing could help identify unidentified peaks in the T-RFLP results and shed insights into methanogenic and Archaeal species within inocula that are important for digester functionality.

5. Comparative Life Cycle Assessment (LCA) of a Chinese Fixed-Dome Digester and a US Plug-Flow Digester

Abstract

A comparative LCA was conducted between an insulated, heated and automated Taiwanese plug-flow digester in the US and a Chinese fixed-dome digester that was not heated, buried underground, and produced lower quantity of biogas energy during the year. The goal of the LCA was to compare the environmental impacts of the construction and operation of the two types of digestion systems used to produce energy to replace alternative fuel use. Eighteen LCA impact categories were assessed in this study, including climate change, fossil fuel depletion, metal depletion, and water depletion. The results showed that the US plug-flow digester was more sustainable than the Chinese fixed-dome system only in the climate change category, but performed worse in all the other categories. Heating and heating equipment were the main contributor towards the more adverse impacts observed in the plug-flow digester. The LCA highlighted the need to reduce heating requirement, specifically the need to increase the insulation of the US plug-flow system. The LCA of the Chinese fixed-dome system revealed that the type of fuel displaced by the digestion system was important in determining the sustainability of the system. The US small-scale plug-flow digester with its use of more sophisticated heating and insulation to maintain biogas production throughout the year resulted in higher greenhouse gas emission reductions but was not overall more sustainable than the Chinese fixed-dome digester constructed with minimal amount of materials and producing lower biogas quantities during the winter.

5.1 Introduction

The installation of anaerobic digesters provides numerous benefits, including the production of renewable energy and fertilizer from wastewater and the reduction of odor, pathogens, and greenhouse gas emissions during wastewater treatment (AgSTAR, 2011; Barros et al., 2008; Holm-Nielsen et al., 2009; Olsen and Larson, 1987; Powers et al., 1999). Currently, most of world's digesters are household systems built in developing countries, with approximately 40 million digesters in China and 4.5 million digesters in India (He et al., 2013; Indian Ministry of New and Renewable Energy, 2013). Different designs exist within this setting, with majority of systems built as Chinese fixed-dome digesters, and others built as Taiwanese plug-flow or floating drum digesters. Many of these systems are buried underground and unheated, or heated using simple techniques, such as the use of a greenhouse cover.

Because anaerobic digestion is dependent on microbial reactions, the efficiency of biogas production decreases when temperature decreases. The optimal digestion temperature is in the mesophilic range (25-35 ºC), which means that the reaction can proceed largely without heating in tropical climates. In temperate climates, however, the efficiency of biogas production decreases or ceases during the winter. Without any heating source, digesters in the Shandong province in northern China, for instance, produced biogas for only 230 days of the year (Qi et al., 2005). As the installation of digesters increased in developed nations, more complex digester designs were implemented to overcome the decrease in energy production during the winter. In the US, the general trend is to install digesters that are heated, insulated,

and operated at mesophilic temperatures, which could decrease the overall sustainability of the digestion systems.

As implementation of anaerobic digestion technology increases, it is important to ensure that designs are environmentally sustainable. Life cycle assessment (LCA) represents a tool that quantifies the impacts that a system or product has on the environment during its lifetime, from the extraction of raw materials to the construction and operation of the system and finally the disposal of the system after its lifetime in a "cradle to grave" analysis (Vigon et al., 1994). In a LCA, the inputs and outputs of a system or process are quantified and its environmental impacts are calculated (Vigon et al., 1994). LCA results can be used to determine which process or component of the system has the largest environmental impact in order to target this area for further improvement to minimize a system's negative environmental impacts (Vigon et al., 1994). The results obtained can also be used to compare the impacts of different systems, providing a tool for designers, contractors, and policy makers to understand the advantages and disadvantages of implementing one system over another (Rehl et al., 2012).

A number of LCAs have been conducted for AD systems. Researchers have used LCA to determine which process within an AD system releases the most greenhouse gases (Ishikawa et al., 2006), the change in environmental impacts when AD is incorporated into waste-management systems (Chaya and Gheewala, 2007; Özeler et al., 2006), and the change in energy production when parameters within AD systems are modified (Berglund and Börjesson, 2006; Poeschl et al., 2012). Life cycle assessment has also been used to analyze the sustainability of small-scale AD systems

in countries such as the UK, China, Kenya, and Vietnam (Chen and Chen, 2013; Chen et al., 2012; Mezzulo et al., 2013; Nzila et al., 2012; Pérez et al., 2014; Vu et al., 2015; Wang and Zhang, 2012), but many of these studies only focused on greenhouse gas emissions, resource depletion, and energy demand or production (Chen and Chen, 2013; Nzila et al., 2012; Wang and Zhang, 2012). Furthermore, there are limited comparative LCA studies focusing on different small-scale designs. Pérez et al. (2014) compared the abiotic depletion, global warming potential, acidification potential, and eutrophication potential of a fixed-dome system and a Taiwanese plug-flow system built in the Andes region. They found that the fixed-dome system had lower abiotic potential, eutrophication potential, and acidification potential than the plug-flow system, but both systems had similar global warming potential. Nzila et al. (2012) compared the fixed-dome, Taiwanese plug-flow, and floating drum designs, and found that the fixed-dome and plug-flow designs performed better in terms of energy demand, resource depletion, and global warming reduction than the floating drum digester.

The objective of this study was to conduct a comparative life cycle assessment of a small-scale unheated household fixed-dome digester in China and a heated and insulated plug-flow digester in the US to assess the change in sustainability of a small-scale digestion system as it is translated from a developing nation (no heating and less automation) to a developed nation (installed with automation, heating, and insulation) using the same system boundary and assessment methods.

5.2 Methods

The following recommendations from the ISO 14040 and ISO 14041 standards (ISO, 1997, 1998) were used in the study: 1) a clearly defined goal stating the purpose, potential application, and audience for the study; 2) a clearly defined scope with a description of systems, function of systems, functional unit, system boundary, data description, and impact assessment methods and impacts; 3) a clearly defined inventory analysis of the data collection and calculations, relating the data to the functional unit; 4) modified sensitivity analyses through the use of different scenarios; 5) clearly defined impact assessment methods and types of impacts analyzed; and 6) a discussion of limitations. The LCA was conducted in SimaPro 8 (PRé Consultants, The Netherlands).

5.2.1 US Plug-Flow Digester Description

The US digester analysis was based on a digester system located at the United States Department of Agriculture (USDA) Beltsville Agricultural Research Center (BARC) in Beltsville, MD (39.03° N, 76.89° W). Full description of the system can be found in Lansing et al. (2015). The system consisted of six field-scale, plug-flow digesters that each had a digester volume of 3 m^3 with a liquid capacity of 2 m^3. Each digester was made up of a PVC bag with an approximate diameter of 0.9 m and length of 5.2 m. The bottom half of each digester was surrounded by polystyrene foam that had a thickness of 76.2 mm, while the top half was covered by a radiant heating barrier made up of polyethylene sheet sandwiched between two aluminum foil layers. The PVC bag, radiant heating barrier, and polystyrene foam were surrounded by a black 1.07 m diameter corrugated drain pipe, which was surrounded

by a 0.1 mm plastic sheet, followed by 50.8 mm polystyrene insulation boards and another 0.1 mm plastic sheet. Spray foam was used to fill the spaces between the HDPE corrugated pipe and the insulation board.

Dairy manure was scraped from the barn and separated using a screw press to remove approximately 70% of the solid material. Each day, approximately 144 L of the separated manure (liquid portion with 30% of the solids from the manure source) was pumped into the heating kettle and then flowed via gravity into the digesters. The heating kettle consisted of an outer and inner chamber, which received the manure, but during data collection stage, manure was not heated before entering into the digesters. Water and ethylene glycol solution were heated in the outer chamber and distributed via a network of polyethylene tubing (PEX) to ethylene propylene diene monomer (EPDM) pipes that ran underneath the black corrugated pipes.

The retention time for each digester was approximately 17 days and effluent flowed by gravity into a holding tank before being pumped into an existing lagoon that stored effluent from an existing mesophilic continuously stirred 540 m^3 anaerobic digester treating the same separated manure source. The existing lagoon and 540 m^3 digester were not included in the LCA analysis.

Water traps made of polycarbonate cylinders (1 L) filled with silica gel were installed to remove moisture from the biogas. One of the six digesters also had a polycarbonate cylinder (1.9 L) filled 0.08 L of pea gravel, 3.2 kg of iron-based adsorbent, and a biomat filter to remove H_2S from the biogas. Manure flow and the flow of heat exchanger fluid (water and propylene glycol mix) were controlled via automatic valves. Biogas quantity was measured before the biogas was burned.

5.2.2 Chinese Household Fixed-Dome Digester Description

The Chinese digester analyzed was a fixed dome design with a volume of 8 m^3 that was buried and assumed to be made of concrete per Compilation of Rural Energy Standards (Ministry of Agriculture Science and Education Division and Ministry of Agriculture Chinese Rural Energy and Environment Agency, 2013).

Operational parameters of the digester were based on field survey data collected from two household digesters in Yangling, Shaanxi, China (34.30° N, 108.07° E). The field survey was conducted to determine: 1) the type of waste that entered the digester; 2) the amount of energy produced from the digesters; 3) the type of fuels that the biogas was replacing (more details in *Section 5.2.6*); and 4) the alternative waste management system. All quantitative data from the field survey was averaged before use in the LCA. One household functioned as a small restaurant and the digester within this household received food waste from the restaurant and crop straw. The amount of food waste entering the digester increased during the summer due to increased number of customers. The other household digester received both kitchen food waste and human manure. An iron oxide scrubber system (0.6 L) was installed to remove H_2S in both household digesters, but the iron oxide was not changed periodically. Both digesters were buried underground and unheated.

The produced biogas was used for cooking. When biogas production was low, one household would use liquefied petroleum gas (LPG), while the other household would use coal, firewood, and straw for cooking. Pressure of both digesters was ≥9 kPa when we visited the systems, indicating that not all the biogas produced was being used.

The climate for the two digestion system types (in US and China) are similar: annual mean temperature for Maryland, USA and Yangling, China are 15.4 and 12.8 °C, respectively, with highest temperature occurring in July (27.7 °C for Yangling and 25 °C in Maryland), and lowest temperature occurring in January (1.3 °C for Yangling and 0.5 °C in Maryland) (NOAA, 2015a,b). Temperature data for Yangling was based on 10 years of average temperature data between 1999-2008 for Xian, which is located approximately 97 km east of Yangling.

5.2.3 Goal and Scope

The goal of the LCA was to assess and compare the sustainability of an unheated small-scale fixed-dome digester in China and a heated and insulated small-scale Taiwanese plug-flow digester in the US, with the end goal of recommending changes to the systems to improve their sustainability. The study will be useful for policy makers and those interested in implementing Chinese fixed-dome or Taiwanese model digesters that are heated or unheated by increasing their understanding of the sustainability of the systems and identifying the materials and operational methods that can minimize negative environmental impacts of these systems.

The main purpose of both digestion systems was to produce energy from wastes and replace the use of alternative fuels that would be used in the absence of the digesters. The functional unit for the analysis was the production of 1 J of heat energy from the combustion of biogas. All inputs and outputs within the system were normalized to the functional unit to allow easy comparison.

The system boundaries for the analysis of both systems included the extraction of raw materials to the production of heat energy, with a functional unit of 1 J of heat energy from biogas (Figure 5.1). The following details the main processes included in the LCA: 1) construction materials and assembly of digesters; 2) electricity use for the plug-flow digester; 3) heating for the plug-flow-digester; 4) scrubbing and combustion of biogas; 5) avoided impacts from the displacement of alternative fuels; 6) insulation for the plug-flow digester; 7) methane (CH_4) leakage from the digesters; and 8) avoided greenhouse gas emissions from alternative waste management systems.

The following processes were not included in the LCA study: 1) disposal of the systems due to the uncertainty of how the materials would be disposed, especially in China, where digesters are often left non-functioning underground; 2) digester effluent (digestate) handling, use, and avoided (or resulting) emissions from the use of digestate, as the main goal of this study was the displacement of alternative fuels; 3) upstream processes for the production of manure or waste were not included since the waste products would be produced regardless of whether or not the digesters were built; and 4) transportation of wastes since the wastes in both systems were produced on-site and only required the use of pumps in the plug-flow system.

Tables 5.1 and 5.2 detail the different components entered into the LCA (termed as the original LCA) for the fixed-dome and plug-flow digesters. Detailed calculations can be found in Appendix A and B.

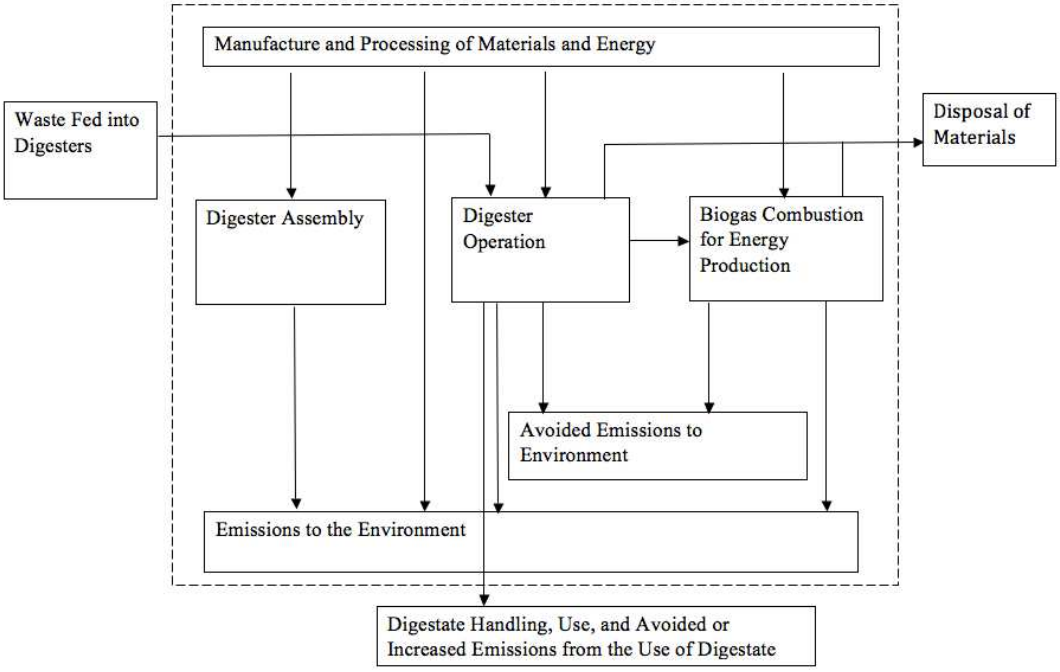

Figure 5.1: System boundary for life cycle assessment. Components within the dashed lines were included in the LCA.

Table 5.1: Components used in the LCA of US plug-flow digester

	Full System	**Per J of biogas energy***
Construction Materials and Energy		
Mass of PVC bags	5.69E+00 kg/year	1.04E-10 kg
Mass of cast iron	4.78E+00 kg/year	8.70E-11 kg
Mass of copper	1.04E+00 kg/year	1.89E-11 kg
HDPE culvert	5.91E+01 kg/year	1.08E-09 kg
Diesel	1.31E+01 L/year	2.38E-10 L
Mass of PVC pipes	1.43E+01 kg/year	2.60E-10 kg
Mass of polyethylene (holding tank)	2.15E+00 kg/year	3.91E-11 kg
Electricity		
Electricity used to run pumps	2.65E+07 J/year	4.82E-04 J
Heating Infrastructure and Energy		
Propane	3.05E+03 kg/year	5.54E-08 kg
PEX mass	2.78E-01 kg/year	5.05E-12 kg
EPDM mass	1.07E+01 kg/year	1.94E-10 kg
Stainless steel	2.38E+01 kg/year	4.34E-10 kg
Water	9.98E+01 kg/year	1.82E-09 kg
Propylene glycol	4.13E+01 kg/year	7.51E-10 kg
Insulation		
Polystyrene foam mass	1.62E+01 kg/year	2.94E-10 kg
Aluminum mass	1.36E+00 kg/year	2.48E-11 kg
Polyethylene core	2.37E-01 kg/year	4.31E-12 kg
Plastic (polyethylene)	9.93E-01 kg/year	1.81E-11 kg
Scrubbing and Combustion of Biogas		
Fe needed to scrub H_2S	1.75E+01 kg/year	3.19E-10 kg
HDPE container	2.47E-03 kg/year	4.50E-14 kg
CH_4 released during combustion (biogenic)	5.49E-02 kg/year	9.99E-13 kg
CH_4 Emission from Alternative System		
CH_4 emission from lagoons (emission reduction) (biogenic)	-1.64E+03 kg/year	-2.98E-08 kg
CH_4 Leakage from Digester		
CH_4 leakage (biogenic)	1.19E+02 kg/year	2.16E-09 kg
Boilers and associated needs and emissions for combusting biogas and propane, and displacement of fuels	(See Appendix A)	

* Based on an energy yield of 5.50E+10 J/year

Table 5.2: Components used in the LCA of Chinese fixed-dome digester.

Construction Materials	Full System	Per J of Biogas Energy*
Cement	5.21E+01 kg/year	2.31E-08 kg
Sand	1.09E+02 kg/year	4.85E-08 kg
Gravel	1.65E+02 kg/year	7.31E-08 kg
Polyethylene pipe	1.79E-01 kg/year	7.94E-11 kg
Scrubbing and Combustion of Biogas		
High Density Polyethylene	2.47E-03 kg/year	1.10E-12 kg
Fe needed to scrub H_2S	9.36E-01 kg/year	4.16E-10 kg
Copper (stove)	2.26E-02 kg/year	1.00E-11 kg
Cast iron (stove)	1.55E-01 kg/year	6.87E-11 kg
Stainless steel (stove)	7.42E-02 kg/year	3.29E-11 kg
CH_4 leaked during combustion (biogenic)	2.25E-03 kg/year	9.99E-13 kg
CH_4 Emission from Alternative System		
CH_4 emission from septic system (emission reduction) (biogenic)	-6.57E+00 kg/year	-2.92E-09 kg
CH_4 Leakage from Digester		
CH_4 leaked from digester (biogenic)	4.87E+00 kg/year	2.16E-09 kg
Displacement of fuels	(See Appendix B)	

* Based on an energy yield of 2.25E+09 J/year

5.2.4 Construction of Digesters

Materials used for the construction of the plug-flow digester were obtained from Klavon (2011), Lansing et al. (2015), and Moss et al. (2014). Diesel for operating the excavation machine was included within the construction of the digesters. Materials needed for the construction of the fixed-dome digester was based on the Compilation of Rural Energy Standards (Ministry of Agriculture Science and Education Division and Ministry of Agriculture Chinese Rural Energy and Environment Agency, 2013). The fixed-dome digester was assumed to be made of concrete and constructed using only human labor, without the use of machines or equipment. Both digesters were assumed to have a lifespan of 20 years.

5.2.5 Heating, Insulation, and Electricity Use of Digesters

Data for heating and insulation systems for the plug-flow digesters were obtained from a previous study that ran the digestion system from May to September 2013 (Lansing et al., 2015). Average propane to heat the digester was approximately 4.5 kg/day or 2.26E+08 J to overcome a daily loss of 1 ºC (average digestion temperature of 27.5 ºC and average ambient temperature of 21.7 ºC (NOAA, 2015a)). It was thus calculated that for every 5.8 ºC difference between the digester and ambient temperature, there was a need to input 2.26E+08 J of heat per day. Considering that the average temperature from October to April is 6.5 ºC, a total of 8.17E+08 J/day would be needed to overcome a 3.6 ºC drop in daily digester temperature. The total heat energy needed to heat the digesters throughout the year was calculated to be 2.08E+11 J/year. It should be mentioned that the heat transfer efficiency in Lansing et al. (2015) was calculated to be 27% (2.26E+08 J/day needed instead of theoretical value of 6.10E+07 J/day needed to overcome a 1 ºC drop in temperature). In the actual study, propane was used as the heat source mainly for research purposes. However, biogas would be the preferred heat source over propane. Therefore, for the LCA, it was assumed that all heat energy for heating the digesters was obtained from the biogas, but if biogas energy was insufficient for heating the digesters, propane was used to provide the extra energy needed.

Water and the propylene glycol solution for heating were included within the LCA. The solution contained 30% propylene glycol (Lansing et al., 2015) to fill the spaces within the PEX and EPDM tubing (0.07 m^3), and was replaced twice per year.

Electricity use for the plug-flow digester was for running the pumps, and was calculated based on the power rating of the pumps, an assumed flow rate for the pumps, and the amount of manure that needed to be pumped daily.

No electricity use or heating was included in the LCA of the fixed-dome digester due to the absence of machineries, pumps, or heating systems.

5.2.6 Biogas Scrubbing and Combustion, and Displacement of Alternative Fuels

The average yearly biogas, CH_4, and hydrogen sulfide (H_2S) production for the US plug-flow system was based on the experimental run that occurred from May to September of 2013 (Lansing et al., 2015). The CH_4 and H_2S concentrations in the biogas were 67.2% and 0.43%, respectively, with a gross annual biogas production of 2,680 m^3. The system was assumed to contain scrubbing systems that could reduce the H_2S content to 0 ppm. The scrubbing system was assumed to be made of 0.6 L HDPE containers containing iron oxide. Stoichiometric calculations were performed to determine the amount of iron oxide needed to remove all produced H_2S. No separate water trap was used in the system. Biogas was burned in a natural gas boiler and gas product from the combustion of the biogas was assumed to be only biogenic CO_2. Methane emitted (i.e. leaked) during biogas combustion was calculated based on recommendations by Eastern Research Group Inc. (2011), with correction by Klavon (2011). Biogas produced from the plug-flow digestion system was assumed to replace energy needed from the combustion of natural gas.

Biogas for the Chinese fixed-dome system was assumed to contain 60% CH_4 and 0.5% H_2S (Seadi et al., 2008), with the remainder being biogenic CO_2. A field survey conducted in December 2014 was used to collect the biogas data for the fixed-

dome LCA. Two household digesters were studied, with the biogas quantities averaged, resulting a gross biogas yield of 123 m^3 per year. Specifically, biogas used for cooking over a ten-day period was determined in two households in Yangling, Shaanxi, China through user documentations. In addition, either at the end or the beginning of the ten-day period, the temperature of water and the time needed for 500 mL of water to boil were measured. Specific heat capacity was then used to determine the amount of energy flow per minute. It should be mentioned that one of the digesters (restaurant digester) had a leak in the system (H_2S detector detected H_2S even though the system was off). However, the value for the digester was included to simulate a real-life scenario where the digester may not be maintained. Estimated time of cooking during the summer was obtained for the first digester (restaurant digester) (personal communication), and the ratio of summer to winter values was used in the second digester (note that summer in this study refers to a period of six months ranging from May to October, while winter refers to the other six months). Methane emitted (i.e. leaked) during biogas combustion was calculated for the two digesters based on recommendations by Eastern Research Group Inc. (2011), with correction by Klavon (2011) and averaged before use in the LCA. Due to the large uncertainty of the biogas data, a scenario was run in which a literature value for biogas from a fixed-dome digester in northern China was used (38 m^3/m^3/year from Qi et al. (2005) compared to 15.4 m^3/m^3/year in the original scenario, which was the average biogas produced from the two households) (more details in *Section 5.2.10*).

Biogas scrubbing system for both household digesters was made of a 0.6 L HDPE container that contained iron oxide. In the LCA, the scrubbing system was

assumed to remove H_2S concentration to 0 ppm, with the iron oxide replaced periodically. Stoichiometric calculations were performed for both digesters to determine the amount of iron oxide needed for removing all H_2S produced, with the quantity of iron oxide averaged for the two digesters. Combustion of biogas in the fixed-dome system was assumed to be conducted in a Mei Jia Si® (Rong Gui Mei Jia, Guangdong, China) cooking stove. Biogas produced from the fixed-dome digestion system was assumed to replace energy needed from the combustion of 50% liquefied petroleum gas (LPG) and 16.7% of coal, 16.7% of straw, and 16.7% of wood, which was the average mix of fuels based on field survey that determined a dominance of LPG use in one household, and a mix of coal, straw, and wood in the second household.

5.2.7 Greenhouse Gas Leakage and Emission Reductions from Alternative Systems

Incorporation of biogas leakage has been shown to be important in creating a complete account of the greenhouse gas emissions from a digestion system (Börjesson and Berglund, 2006). Previous studies have estimated leakage rate to be as low as 3.1% to as high as 40% (Bruun et al., 2014; Flesch et al., 2011). In this study, a leakage rate of 10% was used for both systems, based on recommendations by the Eastern Research Group Inc. (2011).

In the absence of a digester, manure in the Maryland system would be stored in a lagoon until the manure could be applied to the field. The lagoon emits greenhouse gases due to the anaerobic nature and the use of a plug-flow anaerobic digester to treat manure results in the capture and combustion of CH_4 that would

otherwise be emitted. The amount of CH_4 emission reduction was calculated based on the recommendation detailed in the Eastern Research Group Inc. (2011) report.

For the fixed-dome system, data from the two household digesters collected during the field survey were averaged to determine the greenhouse gas emission reductions from alternative waste management system. The two households were connected to the wastewater treatment system. Digester 1 received food waste and straw and these waste products were assumed to be disposed aerobically in the absence of a digester, yielding no CH_4 emissions. In the absence of digester, the food waste in digester 2 was similarly assumed to be disposed aerobically, while the human waste would enter the wastewater treatment system. However, considering that digestion is only a form of primary treatment, emissions from the treatment of the human waste in septic system was used as the reduced methane emissions from digester installation. The CH_4 emission from septic system was calculated based on Doorn et al. (2006).

5.2.8 LCA Calculations

Where available, emissions for the different components in Tables 5.1 and 5.2 were obtained from the Ecoinvent, USLCI, and ELCD databases (European Commission Joint Research Center, 2013; NREL, 2012; Weidema et al., 2013) in the Sima Pro software (PRé Consultants, The Netherlands). Items chosen from the database were selected to represent the LCA components as closely as possible. Methane emissions (from leakage, emissions from combustion, and avoided emissions from alternative waste management systems) were entered directly. In some instances, modifications were made to the inventories within the database to

make the values more appropriate for the LCA, including: 1) a small-scale natural gas boiler in SimaPro was used with the emissions and natural gas input eliminated to calculate emissions from the construction and operation of propane boiler in the plug-flow system; 2) only the emissions component from the combustion of propane in an industrial boiler was used to calculate the emissions from the combustion of propane in both the plug-flow and the fixed-dome systems, but the construction of the boiler was not included; and 3) a small-scale natural gas boiler without the air emissions from combustion and natural gas input was used to calculate emissions for the construction and operation of biogas combustion system for the US plug-flow system. The avoided impacts from the displacement of LPG in the Chinese fixed-dome digesters required creating a propane burner within the system, which was assumed to have the same specifications as the stove used for burning biogas.

Most of the inventories used for the two LCA were inventories meant for global use or for countries outside Europe. Full details on the different inventories used for LCA calculations can be found in Appendix A and B.

5.2.9 Impact Analysis

The ReCiPe mid-point world hierarchist methodology in the SimaPro 8 software (PRé Consultants, The Netherlands) was used to assess the impacts of the different inputs and emissions for both systems (Goedkoop et al., 2013). The hierarchist method incorporates a mean adaptation ability (by the human population), in contrast to the individualist method that assumes full ability to adapt, or egalitarian approach that assumes no adaptation ability (Goedkoop et al., 2013). Eighteen impact categories were analyzed in the ReCiPe mid-point method: ozone depletion, human

toxicity, ionizing radiation, photochemical oxidant formation, particulate matter formation, terrestrial acidification, climate change, terrestrial ecotoxicity, agricultural land occupation, urban land occupation, natural land transformation, marine ecotoxicity, marine eutrophication, freshwater eutrophication, freshwater ecotoxicity, fossil fuel depletion, metal depletion, and water depletion (Goedkoop et al., 2013). The use of the same impact assessment method for digesters in both countries was important due to the comparative nature of this study. Thus other methods, such as TRACI, which would have been more applicable for US systems, were not used, as the application to China would not have been as accurate.

5.2.10 Different Scenario Analysis

Based on results from the original LCA, the following different scenarios were run for the two digestion systems:

1) Plug-flow system:

i) Heat transfer efficiency was observed to be 27% efficient in Lansing et al. (2015) mainly due to low efficiency of the heating kettle. A scenario was conducted to determine the LCA of the system if the heat transfer efficiency was 100%. Therefore, instead of using 2.08E+11 J of heat energy per year to heat the digesters, only 5.61E+10 J/year were needed to heat the digester (or only 27% of the energy needed in the original scenario).

ii) A scenario in which digester was shut down in the winter and the heat transfer efficiency was 100% was run. Considering that there is a higher expenditure of heat energy for heating the digesters during winter, this scenario was conducted to determine if the sustainability of the digester could

improve if it was not operated during the winter. Within this analysis, the amount of energy produced, electricity use for pump operation, greenhouse gas emission reductions from using biogas, water and propylene glycol, and heat energy needed were adjusted to nine months of operation, with additional adjustments in the amount of CH_4 produced, the amount of CH_4 leaked from digester and emitted during combustion of biogas, and H_2S produced, accordingly. Since energy produced from biogas was higher than the quantity of energy needed for heating, the displacement of natural gas was also accounted for in this scenario.

iii) A scenario was run to determine the sustainability of the system if the system was located in a tropical climate that did not need heating input, equipment, and insulation. In this scenario, heating requirement and equipment were eliminated, but biogas production was assumed to be constant. Displacement of natural gas was accounted for in this scenario.

iv) A scenario was run to determine the sustainability of the system that required only insulation but was not heated to maintain its temperature. This could be a scenario in which the system is located in a region that experiences large temperature drops during the night. Displacement of natural gas was accounted for in this scenario.

2) Fixed-dome system:

i) Due to the uncertainty of the yearly biogas production values obtained from the field survey, a scenario was run in which gross biogas volume before leakage was increased to 38 m^3/m^3 digester/year (totaling 302 m^3 per year).

This quantity was derived from the 272 m^3 biogas production observed by Qi et al. (2005), which was assumed to be the net biogas production volume post leakage. The 302 m^3 per year value was assumed to be after leakage (gross biogas volume: 336 m^3 per year). Increasing the amount of biogas changed the volume of H_2S produced and the volume of CH_4 leaked and emitted during biogas combustion.

ii) A scenario was run assuming that all the biogas was used to displace only LPG. For each joule of biogas energy, the production of one joule of LPG energy was avoided.

iii) A scenario was run assuming that the biogas was used to displace 50% straw and 50% wood. For each joule of biogas energy, the production of half a joule of energy from wood and half a joule of energy from straw was avoided.

iv) A scenario was run assuming that the biogas was used to displace only coal. For each joule of biogas energy, the production of one joule of coal energy was avoided.

5.3 Results

5.3.1 US Plug-Flow Digester

The installation of the plug-flow digester resulted in a net benefit in terms of climate change, which was driven by CH_4 emission reductions from the storage lagoons (Table 5.3; Figure 5.2). However, in all other impact categories the US system had adverse impacts, with the majority of the impacts caused by the heating component, which contributed ≥81% of negative environmental impacts. Even with 100% heat transfer efficiency (reduction of heat requirement to 27% of the amount

needed in the original scenario), the system still had negative impacts in 16 of the 18 impact categories (Table 5.3), with the heating component contributing ≥68% of the negative impacts within these 16 categories. Calculation of energy produced from CH_4 and propane energy needed for the system with 100% heat transfer efficiency revealed that more energy was needed to heat the system (5.61E+10 J/year) than the total energy produced (5.50E+10 J/year). In this study, the break-even point where the quantity of energy produced from biogas equaled the amount of energy required for heating was determined to be 26.4% of heat energy required in the original scenario.

Table 5.3: LCA of the plug-flow digester in different scenario.

Impact category	Unit	Original Scenario*	100% Heat Transfer Efficiency*	Winter Shut Down; 100% Heat Transfer Efficiency*	Absence of Heating and Insulation*	Absence of Heating*
Climate change	kg CO_2 eq	-3.62E-07	-5.37E-07	-6.19E-07	-6.87E-07	-6.85E-07
Ozone depletion	kg CFC-11 eq	1.59E-14	5.40E-15	-6.14E-16	-9.44E-15	-9.40E-15
Terrestrial acidification	kg SO_2 eq	5.21E-10	1.98E-10	6.44E-11	-7.48E-11	-7.00E-11
Freshwater eutrophication	kg P eq	1.03E-11	5.66E-12	3.28E-12	-2.95E-12	-2.83E-12
Marine eutrophication	kg N eq	2.17E-11	8.05E-12	2.90E-12	-1.11E-12	-9.66E-13
Human toxicity	kg 1,4-DB eq	1.49E-08	8.53E-09	5.87E-09	-2.00E-09	-1.90E-09
Photochemical oxidant formation	kg NMVOC	3.72E-10	-5.09E-11	-2.15E-10	-3.36E-10	-3.29E-10
Particulate matter formation	kg PM10 eq	1.99E-10	8.03E-11	3.47E-11	-2.21E-11	-2.05E-11
Terrestrial ecotoxicity	kg 1,4-DB eq	4.38E-12	2.43E-12	1.67E-12	-6.00E-13	-5.64E-13
Freshwater ecotoxicity	kg 1,4-DB eq	4.60E-10	3.16E-10	2.97E-10	-6.50E-11	-6.12E-11
Marine ecotoxicity	kg 1,4-DB eq	5.60E-10	3.49E-10	3.06E-10	-6.78E-11	-6.42E-11
Ionising radiation	kBq U235 eq	1.44E-08	4.98E-09	1.15E-09	-1.53E-09	-1.47E-09
Agricultural land occupation	m^2a	6.37E-10	3.97E-10	3.30E-10	-9.39E-11	-8.35E-11
Urban land occupation	m^2a	4.26E-10	2.04E-10	1.26E-10	-4.05E-11	-3.58E-11
Natural land transformation	m^2	6.25E-11	1.78E-11	-1.78E-12	-1.38E-11	-1.38E-11
Water depletion	m^3	1.38E-07	9.32E-08	8.27E-08	-3.57E-08	-3.49E-08
Metal depletion	kg Fe eq	9.17E-09	7.65E-09	8.86E-09	-1.52E-10	-1.40E-10
Fossil depletion	kg oil eq	7.88E-08	2.58E-08	1.31E-09	-2.39E-08	-2.32E-08

*Note that positive values indicate detrimental impacts and negative values indicate beneficial impacts.

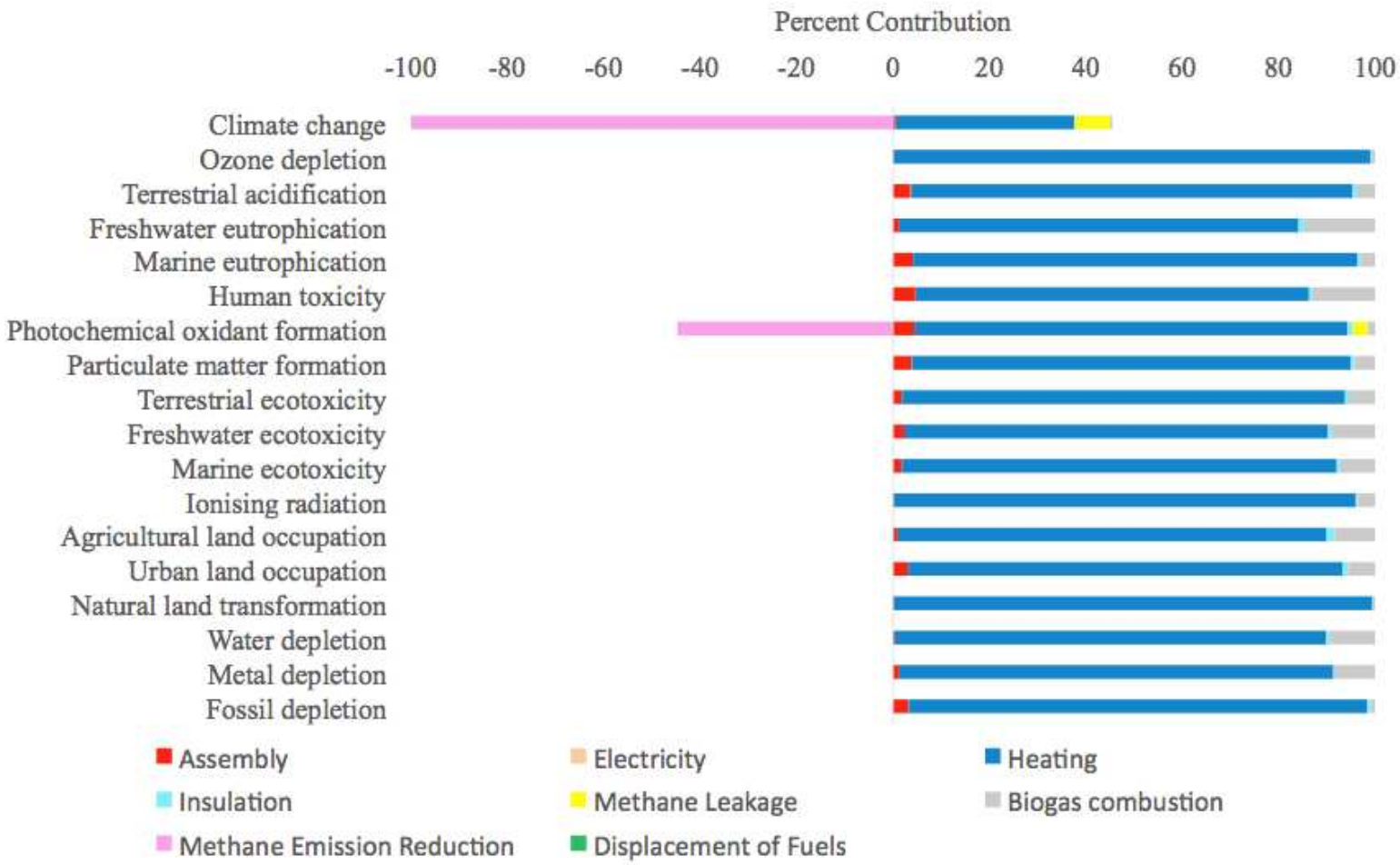

Figure 5.2: Component contributions towards LCA of the US plug-flow digester in the original scenario. Note that positive values indicate detrimental impacts and negative values indicate beneficial impacts.

A scenario was run where the plug-flow digester was shut down during the winter (December, January, and February) to cut down on heat energy usage and with the assumption that the heat transfer efficiency was 100%. The results showed that the amount of energy produced by the biogas (4.12E+10 J/year) was higher than the amount of energy needed for heating the system (3.18E+10 J/year). Operation of the digester using this method resulted in beneficial impacts in four categories: climate change, ozone depletion, photochemical oxidant formation, and natural land transformation, while the other impacts were still negative (Table 5.3).

Assuming the digester could function and produce the same amount of biogas without the need for heating and insulation system, the digester became sustainable in all impact categories studied, although the net benefit in metal depletion was marginal. Adding insulation back into the scenario resulted in minimal change (≤13%) in net impacts from all categories (Table 5.3).

5.3.2 Chinese Fixed-Dome Digester

In contrast to the plug-flow digester, the Chinese fixed-dome digester had beneficial impacts in 16 of the 18 impacts studied, with negative impacts in water depletion, in which the assembly contributed the most impact, and metal depletion, in which biogas combustion contributed the most towards the negative impact (Table 5.4; Figure 5.3). Cement was the highest contributor in the assembly stage for all categories, contributing ≥53% of the impact. Combustion of biogas had high impact on metal depletion mainly due to the use of copper and steel in the biogas stove.

Table 5.4: LCA of the fixed-dome digester in different scenario.

Impact category	Unit	Original Scenario*	Increased Biogas Production*	Displacement of LPG*	Displacement of Coal*	Displacement of Straw and Wood*
Climate change	kg CO_2 eq	-5.41E-08	-3.42E-08	-6.89E-08	-1.41E-07	1.14E-08
Ozone depletion	kg CFC-11 eq	-2.14E-15	-2.48E-15	-4.52E-15	-4.18E-16	5.71E-16
Terrestrial acidification	kg SO_2 eq	-1.37E-10	-1.97E-10	-4.19E-11	-7.04E-10	3.35E-12
Freshwater eutrophication	kg P eq	-4.14E-12	-6.15E-12	2.09E-12	-3.51E-11	2.00E-12
Marine eutrophication	kg N eq	-3.50E-12	-5.83E-12	-2.30E-12	-9.15E-12	-2.46E-12
Human toxicity	kg 1,4-DB eq	-5.97E-09	-8.63E-09	2.04E-09	-4.46E-08	1.34E-09
Photochemical oxidant formation	kg NMVOC	-2.25E-10	-2.63E-10	-1.14E-10	-5.67E-10	-2.21E-10
Particulate matter formation	kg PM10 eq	-9.00E-11	-1.13E-10	-1.10E-11	-2.79E-10	-1.14E-10
Terrestrial ecotoxicity	kg 1,4-DB eq	-2.39E-12	-2.85E-12	-5.11E-14	-1.06E-11	-1.78E-12
Freshwater ecotoxicity	kg 1,4-DB eq	-3.86E-11	-9.42E-11	4.05E-11	-5.17E-10	8.17E-11
Marine ecotoxicity	kg 1,4-DB eq	-6.25E-11	-1.23E-10	1.23E-11	-5.68E-10	7.83E-11
Ionising radiation	kBq U235 eq	-1.26E-09	-1.90E-09	-3.08E-09	-3.68E-10	1.02E-09
Agricultural land occupation	m^2a	-2.64E-10	-3.81E-10	1.26E-10	-2.28E-09	1.59E-10
Urban land occupation	m^2a	-2.26E-11	-1.72E-10	1.62E-10	-9.69E-10	1.74E-10
Natural land transformation	m^2	-9.42E-12	-1.17E-11	-1.79E-11	-7.79E-12	2.45E-12
Water depletion	m^3	3.05E-08	7.56E-09	2.77E-08	2.46E-08	3.77E-08
Metal depletion	kg Fe eq	9.29E-10	3.14E-10	6.30E-10	1.07E-09	1.31E-09
Fossil depletion	kg oil eq	-1.43E-08	-1.65E-08	-2.17E-08	-2.78E-08	3.66E-09

* Note that positive values indicate detrimental impacts and negative values indicate beneficial impacts.

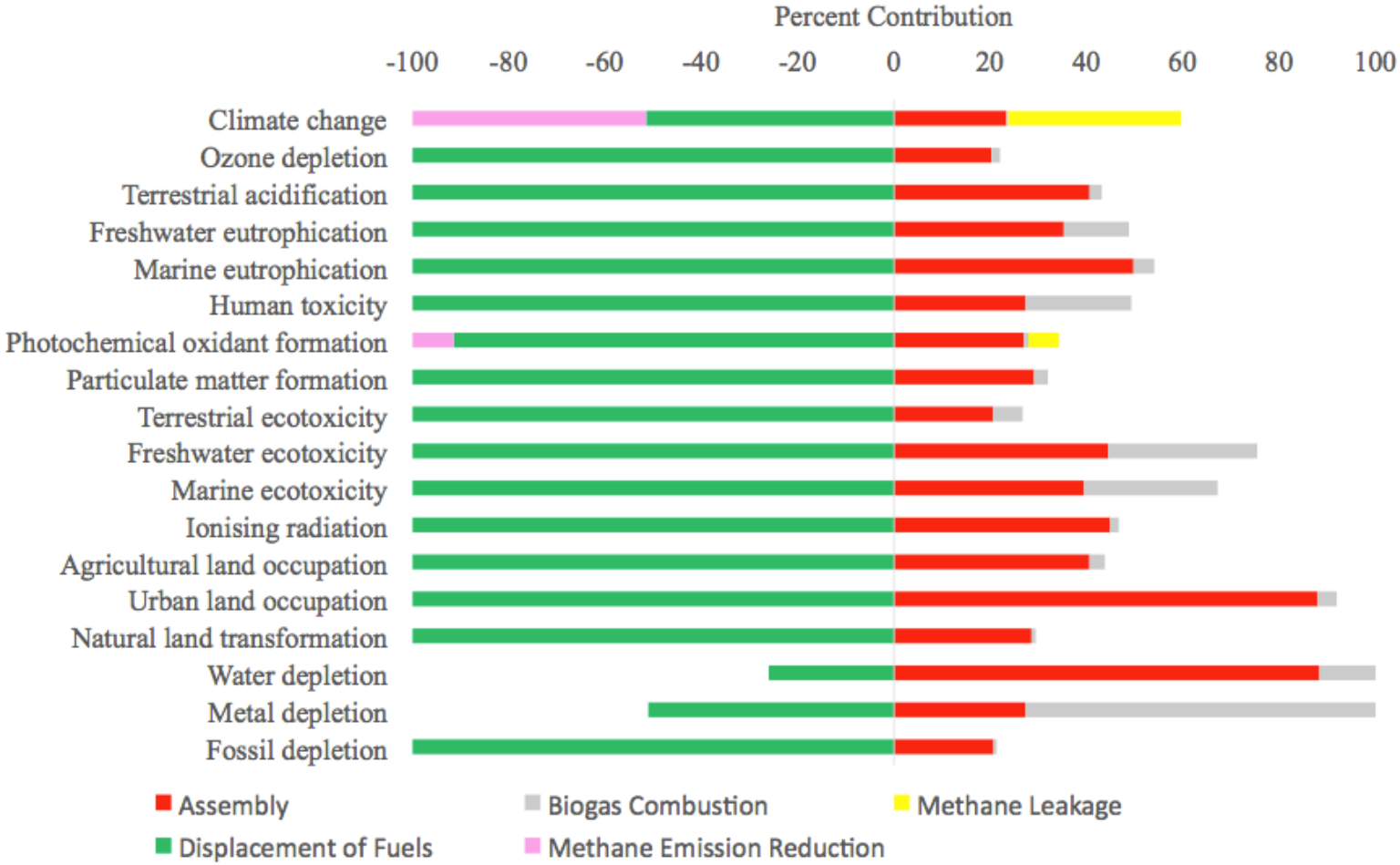

Figure 5.3: Component contributions towards LCA of the Chinese fixed-dome digester in the original scenario. Note that positive values indicate detrimental impacts and negative values indicate beneficial impacts.

Increasing the biogas production from 123 m^3/year to the literature value of 302 m^3/year resulted in improvements in all categories, with the exception of climate change, as the increased biogas production resulted in increased leakage, but the overall impact was still beneficial (Table 5.4). Metal depletion and water depletion were still had negative impacts in this scenario.

There were large differences in impacts from changing the fuels that the biogas displaced. Compared to the original scenario where biogas displaced coal, LPG, and straw and wood, when biogas displaced only LPG, there were improvements in seven categories: climate change, ozone depletion, ionizing radiation, natural land transformation, water depletion, and metal depletion, and fossil depletion, but had increased environmental burden or less beneficial impacts in other categories, with six additional categories having negative impacts: freshwater eutrophication, human toxicity, freshwater ecotoxicity, marine ecotoxicity, agricultural land occupation, and urban land occupation (Table 5.4). Switching from the original scenario to displacement of solely coal resulted in improvements in 14 of the impact categories (Table 5.4). Impacts for four categories (ozone depletion, ionizing radiation, natural land transformation, and metal depletion) became less beneficial or had higher environmental burden when biogas only displaced coal. Compared to the original scenario, displacement of only straw and wood resulted in improvement only in the particulate matter formation category, reduced benefits in three impact categories (terrestrial ecotoxicity, photochemical oxidant formation, marine eutrophication), and 12 additional categories having negative impacts: climate change, ozone depletion, terrestrial acidification, freshwater eutrophication, human

toxicity, freshwater ecotoxicity, marine ecotoxicity, ionizing radiation, agricultural land occupation, urban land occupation, natural land transformation, and fossil depletion (Table 5.4).

5.4 Discussion

The comparative LCA of the two digestion systems showed that even with 96% less energy production in the fixed-dome system (2.25E+09 J/year) compared to the plug-flow system (5.50E+10 J/year), the fixed dome system was more sustainable in all categories except for climate change. The installation of the fixed-dome digestion resulted in beneficial impacts in 16 categories, whereas the installation of the plug-flow digestion resulted in beneficial impact only in climate change category. The fixed-dome system was found to perform sustainably even with a biogas value (123 m^3/year) that was lower than previously observed (302 m^3) in Qi et al., 2005, or 300 m^3 used in other LCA studies (Wang and Zhang, 2012; Zhang et al., 2013).

Foley et al. (2010) mentioned the importance of viewing their LCA study within the limits of assumptions and methods used within the study. Thus, even though comparisons between results from current study and other studies were made, caution needs to be taken due to differences in the study contexts. Results from the fixed-dome LCA were comparable to results in previous studies. In Pérez et al. (2014), cement was also found to be a major contributor towards acidification, eutrophication, and global warming potential during the construction of a fixed-dome digester in the Andes. In addition, climate change, terrestrial acidification, and freshwater eutrophication values for the construction of the fixed-dome digester was comparable to results observed by Pérez et al. (2014), Wang and Zhang (2012), and

Zhang et al. (2013) (Table 5.5). Carbon dioxide reduction from the displacement of alternative fuels in our study were also comparable to results found in Wang and Zhang (2012) and Zhang et al. (2013), with differences likely due to the differences in alternative fuel allocation and different accounting methods for biogenic CO_2 (no biogenic CO_2 was accounted in our study). The scenario with displacement of only coal in our study, for instance, resulted in a CO_2 emission reduction value that was closer to literature values (2.84 kg CO_2 eq/m^3 biogas).

The LCA of the fixed-dome system showed that the type of fuel that the system displaced was important in determining the sustainability of the system. In terms of climate change impact, the installation of digesters in communities that use solely coal will be more beneficial than communities that solely use straw and wood as fuel. Bruun et al. (2014) showed that the type of fuel that the digesters displaced could affect the amount of leakage that digesters could tolerate before digesters become a contributor towards climate change. Coal was described as one of the most polluting fuel and emitted higher amount of greenhouse gases than wood or LPG. Our study also showed that the substitution of coal using biogas could provide more benefits in terms of climate change compared to replacing other alternative fuels, such as straw and wood.

Table 5.5: Comparison of Chinese fixed-dome LCA values with previous research values.

Studies	Climate Change for Construction of Digesters (kg CO_2 eq/m^3 biogas)	Terrestrial Acidification for Construction of Digesters (kg SO_2/ m^3 biogas)	Freshwater Eutrophication for Construction of Digesters (kg P eq/m3 biogas)	CO_2 Reduction from Displacement of Alternative Fuels (kg CO_2 eq/m^3 biogas)
This Study	5.7E-01	1.8E-03	5.2E-05	1.3E+00
Pérez et al. (2014)*	2.5E-01	1E-03	2.3E-05	-
Wang and Zhang (2012)	3.9E-01	-	-	3.6E+00
Zhang et al. (2013)	3.3E-01	-	-	3.8E+00

*Approximate values calculated based on figures within the study.

The plug-flow digester was shown to contribute positively towards reducing climate change, mainly due to the alternative manure management system of storing manure in a lagoon that emits CH_4 into the atmosphere. The lagoon is not covered, and therefore, the CH_4 is not captured and combusted. In the other categories, however, the system was found to be less sustainable mainly due to heating and the heating equipment installed. Lansing et al. (2015) showed that the digestion system could produce more energy than the energy needed to heat the system given an efficient heating system, but the study was conducted during the May to September, and the higher requirement projected in the current study for heating during the winter increased the total additional heat energy needed beyond the total biogas produced by the system. Due to these projected heating requirements, shutting down the digester during the winter increased the net energy produced by the system to a positive value and allowed for biogas to be used as the sole heat source. However, the system still had negative impacts with respect to 14 impact categories.

Berglund and Börjesson (2006) used a lower biogas energy production value (5.60E+08 J/ton manure) than the US plug-flow system in our study (1.32E+09 J/ton manure; manure loading rate of 114 L/day; density of 1 kg/L; energy production of 5.50E+10 J/year), yet they found that energy requirement for heating farm-scale digester was only 44% of the energy produced by the digester. This difference was mainly due to the high energy required for heating the US plug-flow digester (5.0E+03 MJ/ton manure), compared to 250 MJ/ton manure used in the Berglund and Börjesson (2006) study.

There is a need to optimize energy production from the plug-flow digester or reduce the amount of heating. As shown in one of the scenarios, removing the heating component resulted in a sustainable system with respect to all impact categories. Insulation contributed minimal negative impacts towards the sustainability of the system, and thus should be prioritized as a method for reducing heating requirement. The use of sustainable insulation, such as soil, could also become an important method to reduce the temperature drop in digesters. It should be noted that the current study did not investigate the use of biogas for electric generation. Waste heat from a combined heat and power generator can be used to heat the digester, but the impacts of the infrastructure of a generator system was not evaluated in this study.

Other limitations within the study include the omissions of the system disposal, digestate handling, and avoided impacts (positive or negative) from the use of digestate from digesters compared to alternative systems. Disposal of system was not included due to uncertainties in disposal methods for both systems, while the use of digestate was not included as the main focus was on the displacement of alternative fuels. Inclusion of these processes could affect the sustainability of the systems. Precision in terms of the amount of heat needed for the plug-flow system could also be improved. Within the Lansing et al. (2015) study used for the LCA, instead of heating manure directly within the kettle system, cold manure was injected into the digestion system and the digestion was then heated via the PEX and EPDM heat exchanger system that carried the heated glycol mixture. This could represent a big loss of heat energy from the system. Finally, most of the inventories used for the LCA were based on a global context or based on general data for countries outside Europe,

and may not be specific to either the US or China. This could affect the sustainability assessment results. Since LCA is an iterative process, it is important that the uncertain processes were omitted from the analysis, with more precise data collected from further research included in future iterations.

Nevertheless, within the context of the study, the results showed that the use of more sophisticated and complicated system with heating and insulation in a small-scale digester to maintain biogas production throughout the year (plug-flow system) did not result in a more sustainable system than one that was constructed with minimal amount of materials, manual labor, and allowed to produce lower amount of biogas during the winter (fixed-dome system). AgSTAR, the government body that promotes the dissemination of anaerobic digesters in the US, does not recommend the installation of digesters in farms with less than 500 cows due to economic reasons (AgSTAR, 2011). The current study shows that in addition to economic profitability, there is also a need to consider the sustainability of digester systems to ensure that digester installation does not create more environmental harm than benefits.

5.5 Conclusion

A comparative LCA was conducted of an insulated, heated and automated Taiwanese plug-flow digester in the US and a Chinese fixed-dome digester that was not heated, buried underground, and produced lower quantity of energy during the year. The results showed that the US plug-flow digester was more sustainable than the Chinese fixed-dome system only in the climate change category, but contributed negatively towards the other categories. Heating and heating equipment were the main contributors towards the detrimental impacts observed in the plug-flow digester.

A need to reduce heating requirements, especially by increasing the insulation of the system in the plug-flow system was recognized. The LCA of the fixed-dome system also showed that the type of fuel displaced by the digestion system was important in determining the sustainability of the system.

6. Conclusion

6.1 Intellectual Merit

Chapter 2 of this dissertation represents the first study that compared the effect of manure separation on CH_4 production during digestion under psychrophilic temperatures. The results showed that in digestion conducted at 24 ºC, which is in the high range of psychrophilic bacteria and low range of mesophilic bacteria, with long digestion times (≥20 days), the unseparated manure produced 29-40% more CH_4 than separated manure due to higher volatile solids content of the unseparated manure broken down over time. At lower digestion times (≤16 days), the CH_4 production from the separated manure was not significantly different than unseparated manure due to faster VS conversion rates in liquid, separated manure. This indicated that given a smaller digestion volume and shorter digestion times, farmers operating digesters at ~24 ºC could digest separated manure without sacrificing CH_4 production.

Chapter 3 and 4 represent the first study comparing the effectiveness of wetland sediment, landfill leachate, and mesophilic digestate as inocula for agricultural anaerobic digesters at three temperatures using three inoculum to substrate ratios and two inoculum incubation periods, with quantification of the methanogenic community and of the ordinal changes in the Archaeal community during inocula incubation. The alternative inocula tested, wetland sediment (WS) and landfill leachate (LL), after incubation 25 or 35 ºC were shown to be viable inocula for batch digestion at the same temperature. Inocula incubated and digested at 15 ºC

did not produce viable quantities of CH_4. Longer incubation time generally reduced the amount of inoculum needed for batch digestion and reduced volatile fatty acids accumulation. Overall, MD inoculum from well-established digesters at a 35% inoculum to substrate ratio (w/w) and 35 ºC operational temperature is recommended for highest CH_4 production per unit of digester volume.

Molecular techniques provided insights into the methanogenic and Archaeal community shifts during the incubation of alternative inocula. High acetic acid levels appeared to drive the dominance of *Methanosarcinaceae,* while low acetic acid levels drove the abundance of *Methanosaetaceae. Methanosaetaceae* abundance increased in wetland sediment and landfill leachate reactors incubated at 25 and 35 ºC, and caused samples from these reactors to generally converge with the MD reactors that were incubated at the same temperature and had high abundance of *Methanosaetaceae. Methanosarcinaceae* generally dominated the reactors incubated at 15 ºC, resulting in clustering in the T-RFLP for the reactors incubated at 15 ºC, regardless of inoculum source. The study also indicated that the use of inoculum rich in *Methanosarcinaceae* could be beneficial in starting digesters at the lower mesophilic temperature range (25 ºC).

Finally, Chapter 5 of the dissertation was the first study to assess the change in sustainability of a small-scale digestion system as it is translated from a developing nation (no heating and less automation) to a developed nation (installed with automation, heating, and insulation) using the same system boundary and assessment methods. The LCA showed that the heated and insulated US plug-flow digester contributed beneficially towards greenhouse gas reductions as the alternative manure

management system of storing manure in lagoon results in CH_4 emissions from the manure storage lagoon. However, the Chinese fixed-dome digester was more sustainable than the US plug-flow digester in 17 of 18 environmental impact categories and contributed beneficially to 16 out of the 18 impacts studied, despite producing less biogas per year and operating inefficiently during the winter months. Heating and heating equipment were the main contributors towards the detrimental impacts observed in the plug-flow digester. There is a need to reduce heating needs or provide more efficient digester heating methods. The LCA of the fixed-dome digester showed that the type of fuels that the digester displaced should be taken into account before the system is built, as coal replacement was shown to be more sustainable than replacing straw and wood.

6.2 Broader Impacts

Results from the research indicated that at low mesophilic operational temperature, farmers with small digestion volumes or low digestion times could choose to digest separated or unseparated manure. Additionally, results from the inoculum study points to the possibility of using inoculum rich in *Methanosarcinaceae* to promote higher CH_4 production for digesters functioning at lower mesophilic temperatures. Future research should concentrate on applying this knowledge to formulate an inoculum seed and apply it to lab, pilot, or field-scale. Finally, the LCA study further reinforces the importance of continuing to reduce the heating needs of digesters. Insulation, with its minimal impacts, could prove to be useful in minimizing temperature drop in digesters and reduce heating. AgSTAR promotes the adoption of anaerobic digesters in farms with more than 500 cows due

to economic reasons. While cost is an important factor in the installation of digesters, one should also consider the environmental sustainability of digesters before a system is built.

Appendices

Appendix A: Detailed Calculations and Information for Plug-Flow Digester LCA

A1. Data and Database for US plug-flow digester

Table A1: Data and database used in SimaPro for US plug-flow digester LCA

Components in LCA	Specific Data Used in SimaPro	Database[a,b]
PVC bags	Polyvinylchloride, bulk polymerised {GLO}| market for | Alloc Def, U	Ecoinvent 3
Cast iron	Cast iron {GLO}| market for | Alloc Def, U	Ecoinvent 3
Copper	Copper wire, technology mix, consumption mix, at plant, cross section 1 mm² EU-15 S	ELCD
HDPE culvert	Polyethylene, high density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Diesel	Diesel, combusted in industrial equipment/US	USLCI
PVC pipes	Polyvinylchloride, bulk polymerised {GLO}| market for | Alloc Def, U	Ecoinvent 3
Polyethylene (holding tank)	Polyethylene, low density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Electricity used to run pumps	Electricity, at grid, US/US	USLCI
Propane	Liquefied petroleum gas {RoW}| market for | Alloc Def, U	Ecoinvent 3
Boiler for propane	Heat, central or small-scale, natural gas {RoW}| heat production, natural gas, at boiler modulating <100kW | Alloc Def, U minus Emissions to air and natural gas input	Ecoinvent 3
Emissions from combustion of propane	Only emissions to air from Liquefied petroleum gas, combusted in industrial boiler/US	USLCI
PEX mass	Polyethylene, high density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
EPDM mass	Synthetic rubber {GLO}| market for | Alloc Def, U	Ecoinvent 3
Stainless steel	Steel, chromium steel 18/8 {GLO}| market for | Alloc Def, U	Ecoinvent 3
Water	Tap water, at user {RoW}| market for | Alloc Def, U	Ecoinvent 3

Propylene glycol	Propylene glycol, liquid {GLO}| market for | Alloc Def, U	Ecoinvent 3
Polystyrene foam mass	Polystyrene foam slab {GLO}| market for | Alloc Def, U	Ecoinvent 3
Aluminum mass	Aluminium sheet, primary prod., prod. mix, aluminium semi-finished sheet product RER S	ELCD
Polyethylene core	Polyethylene, low density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Plastic (polyethylene)	Polyethylene, low density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Fe needed to scrub H_2S	Iron pellet {GLO}| market for | Alloc Def, U	Ecoinvent 3
HDPE container	Polyethylene, high density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Gas Boiler and associated electrical needs for combusting biogas	Heat, central or small-scale, natural gas {RoW}| heat production, natural gas, at boiler modulating <100kW | Alloc Def, U minus Emissions to air and natural gas input	Ecoinvent 3
Displacement of Natural Gas	Heat, central or small-scale, natural gas {RoW}| heat production, natural gas, at boiler modulating <100kW | Alloc Def, U	Ecoinvent 3

[a] From Sima Pro database.
[b] European Commission Joint Research Center (2013), NREL (2012), and Weidema et al. (2013).

A2. Assembly of Plug-Flow Digester

PVC Bags

Length of each PVC bag	5.2 m	Lansing et al. (2015)
Width of each PVC bag	2.83 m	Lansing et al. (2015)
Thickness of each PVC bag	0.001 m	Lansing et al. (2015)
Density of Flexible PVC	1290 kg/m^3	StelRay Plastic Products Inc. (2014)
Assumed life span	20 years	
Number of PVC bags	6	

Mass of PVC per year:

Length * height * width * density * number of bags / lifespan

= (5.2*2.83*.001)*1290*6/20 = 5.70 kg/year

Cast-Iron Pumps

Mass of cast-iron pumps (two Zoeller pumps 382 and one Zoeller pump 267)	95.6 kg	Klavon (2011), Zoeller (2015)
Mass of cast iron	95.6 kg	
Life span	20 years	

Mass of cast-iron pumps per year:

mass of cast iron pump / life span

= 95.6/20 = 4.78 kg/year

Copper Wiring

Mass of copper (assumed to be scaled up linearly from Moss et al. (2014); volume of digester in current study is 1.15 times the amount in Moss et al. (2014))	20.8 kg
Life span	20 years

Mass of copper wiring per year:

mass of copper / life span

= 20.8/20 = 4.78 kg/year = 1.04 kg/year

PVC Pipe

Pit to holding tank (3")	120 ft	Klavon (2011)
holding tank to kettle (2")	65 ft	Klavon (2011)
kettle to digeters (2")	60 ft	Estimate
digester to septic tank (2")	56 ft	Klavon (2011)
septic tank to lagoon (3")	240 ft	Klavon (2011)
Weight (2")	0.308 kg/ft	The Engineering Toolbox (2015)
Weight (3")	0.64 kg/ft	The Engineering Toolbox (2015)
Life span	20	

Mass of PVC pipe per year:

(total length of 2” pipe * weight of 2” pipe + total length of 3” pipe * weight of 3” pipe) / life span

= ((65+60+56)*0.308+(120+240)*0.64)/20 = 14.3 kg/year

Holding Tank

Volume	500 gallon	
Mass (assumed to be low density polyethylene)	43 kg	estimated
Life span	20 years	

Mass of polyethylene:

Mass of tank / life span

= 43/20 = 2.15 kg/year

HDPE Culvert Pipe

Length of each pipe	5 m	Lansing et al. (2015)
Mass (N-12, 42" pipe)	39.4 kg/m	ADS, Inc. (1999)
Number of digesters	6	
Life span year	20 years	

Mass of HDPE culvert per year:

mass of HDPE culvert per meter * length * number of digesters / life span

39.4*5*6/20 = 59.1 kg/year

Diesel for Excavation

Diesel (scaled up linearly from Moss et al. (2014); volume of digester in current study is 1.15 times the amount in Moss et al. (2014))	262 L
Life span year	20 years

Volume of diesel per year:

Volume of diesel / life span

= 262/20 = 13.1 L/year

A3. Electricity Use for Plug-Flow Digester

Electricity for Running Pumps

Assumed flow rate for influent from septic tank to lagoon	133 L/min	Klavon et al. (2011)
Volume needed to be pumped per day	114 L/day	Lansing et al. (2015)
Assumed flow rate for influent from holding tank to kettle	56.8 L/min	Klavon et al. (2011)
Assumed flow rate for effluent pit to holding tank	303 L/min	Klavon et al. (2011)
Horsepower of pump	0.5 hp	Zoeller (2015)
Conversion rate	746	W/hp

Electricity needed per year:

(Time needed for pump 1 to run per day + time needed for pump 2 to run per day + time needed for pump 3 to run per day)* days in one year * power

= (114/133+114/56.8+114/303)/60*365*0.5*746 = 7350 Wh/year = 2.65E+07 J/year

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A4. Methane and Biogas Production

Volume of CH_4 produced (after leakage)	1620 m^3/year	Lansing et al. (2015)
Percent leakage rate	10%	Eastern Research Group Inc. (2011)
Percent retained	90%	
% CH_4 in biogas	67.2%	Lansing et al. (2015)
Density of CH_4	0.66 kg/m^3	
CH_4 emitted during combustion	$1.00*10^{-9}$ kg/BTU	Eastern Research Group Inc. (2011), with correction by Klavon (2011)
Conversion factor	33898 BTU/m^3	
CH_4 energy content	3.39E+07 J/m^3	The Engineering Toolbox (2015)

Mass of CH_4 leaked:

Volume of CH_4 after leakage * leakage rate (%) / percent retained * density of CH_4

= 1620*10/90*0.66 = 119 kg/year

Volume of biogas produced:

Volume of CH_4 after leakage / percent retained *100 / % CH_4 in biogas * 100

= 1620/90*100/67.2*100 = 2680 m^3/year

Mass of CH_4 released during combustion (Eastern Research Group Inc. (2011)):

Volume of CH_4 (after leakage) * conversion factor * CH_4 emitted during combustion

= 1620 * 33898 * $1.00*10^{-9}$ = 0.0549 kg/year

Volume of net CH_4:

Volume of CH_4 after leakage – (CH_4 released during combustion/density of CH_4)

= 1620-(0.0549/0.66) = 1620 m^3/year

Energy value from CH_4:

Volume of net CH_4* CH_4 energy content

=1620*3.39E+07 = 5.50E+10 J/year

A5. Heating

PEX Tubing (Cross-Linked HDPE 2.5cm)

Length (estimate)	20.8 m	
Wall Thickness (estimate)	0.00318 m	
Diameter of tube	0.025 m	
Density of PEX	948 kg/m^3	Lyons and AHR Architects (2015)
Life span	20 years	

Mass of PEX tubing per year:

(Volume of PEX tubing – volume of inner space) * density / life span

= $((\pi*((0.00318*2+0.025)/2)^2*20.8)-(\pi*(0.025/2)^2*20.8)) * 948 /20$

= 0.278 kg/year

Ethylene Propylene Diene Monomer (EPDM)

Surface area covered per digester	17 m^2	Lansing et al. (2015)
Inner Diameter	0.00476 m	Lansing et al. (2015)
Length of culvert	5 m	
OD of each tube based on 3/16"ID	0.00953 m	
Density of EPDM	1170 kg/m^3	WRS (2015)
Life span year	20	
Number of digester	6	

Mass of EPDM per year:

Surface area covered per digester / surface area of each tube * (volume of tube – volume of tube inner space) * number of digesters * density / life span

$= 17/(\pi*0.00953*5)*(\pi*(0.00953/2)^2*5- \pi*(0.00476/2)^2*5)*6*1170/20$

= <u>10.7 kg/year</u>

Stainless Steel:

Stainless Steel (assumed to be scaled up linearly from Moss et al. (2014); volume of digester in current study is 1.15 times the amount in Moss et al. (2014))	477 kg
Life span	20 years

Mass of stainless steel per year:

mass of stainless steel / life span

= 477/20 = <u>23.8 kg/year</u>

Tap Water and Propylene Glycol for Heating

Number of times water is replaced per year (assumed)	2	
Percent water	70%	Lansing et al. (2015)
Percent propylene glycol	30%	Lansing et al. (2015)
Density of water	1000 kg/m^3	
Density of propylene glycol	965 kg/m^3	

Mass of water needed:

Volume of inner space within EPDM and PEX tubing (see above) * number of times solution is changed per year (assumed to be 2) * percent water * density of water

$= (17/(\pi*0.00953*5)*(\pi*(0.00476/2)^2*5)*6 + \pi*(0.025/2)^2*20.8) *2*70/100 *1000 =$ <u>99.8 kg/year</u>

Volume of propylene glycol needed:

Volume of inner space within EPDM and PEX tubing (see above) * number of times solution is changed per year (assumed to be 2) * percent propylene glycol * density of propylene glycol

$= (17/(\pi*0.00953*5)*(\pi*(0.00476/2)^2*5)*6 + \pi*(0.025/2)^2*20.8)*2*30/100 *965 =$ <u>41.3 kg/year</u>

Mass and Energy Content of Propane Gas needed for Heating

Propane use between May and September	4.5 kg/day	Lansing et al., 2015
Temperature drop in digesters between May and September	1 °C/day	Lansing et al., 2015
Average temperature between May and September	21.7 °C	NOAA (2015a)
Average temperature of digesters between May and September	27.5 °C	Lansing et al., 2015
Average temperature between October and April	6.5 °C	NOAA (2015a)
Energy Content of propane	5.02E+07 J/kg	The Engineering Toolbox (2015)
Propane density	0.508 kg/L	The Engineering Toolbox (2015)

Mass of propane needed for the year:

amount of propane needed per day * number of days between May and September + estimated temperature drop in digester between October and April * amount of propane needed between May and September /daily temperature drop between May and September * number of days between October and April

= 4.5*153+ (27.5-6.5)/(27.5-21.7)*4.5/1*212

= 4,143 kg/year

Amount of energy needed from propane for heating:

Mass of propane needed per year * energy content of propane

= 4,143* 5.02E+07 = 2.08E+11 J/year

Biogas was used to heat the system, but propane was used if biogas energy was not sufficient. In the original scenario, energy needed for heating was 2.08E+11 J/year, but only 5.50E+10 J/year was produced from the biogas. Therefore, 2.08E+11 - 5.50E+10 = 1.53E+11 J/year is needed for heating in the original scenario.

Mass of propane needed:

Amount of energy needed / energy content of propane

= 1.53E+11/5.02E+07 = 3.05E+03 kg/year

Inputs	Specific Data Used in SimaPro	Database	Amount of input
Boiler for propane	Heat, central or small-scale, natural gas {RoW}| heat production, natural gas, at boiler modulating <100kW | Alloc Def, U minus Emissions to air and natural gas input	Ecoinvent 3	2.78 J
Emissions from combustion of propane	Only emissions to air from Liquefied petroleum gas, combusted in industrial boiler/US	USLCI	1.09E-07 L

Amount of boiler for propane:

Amount of energy needed per year/joules of energy from biogas per year

= 1.53E+11/5.50E+10

= 2.78 J

Amount of propane used to calculate emissions from combustion of propane:

Mass of propane needed / joules of energy from biogas per year / density of propane

= 3.05E+03 / 5.50E+10 / 0.508

= 1.09E-07 L

A6. Insulation

Polystyrene foam at the bottom of Bags

Length	5 m	Lansing et al. (2015)
Diameter of each foam (based on culvert)	1.07 m	Lansing et al. (2015)
Density of expanded polystyrene	22.5 kg/m^3	The Engineering Toolbox (2015)
Number of foams	6	Lansing et al. (2015)
Volume of each PVC bag	3 m^3	Lansing et al. (2015)
Assumed life span	20 years	

Mass of polystyrene foam per year:

(volume of culvert – volume of PVC bag) * number of bags * density of polystyrene / life span:

$(\pi*(1.07/2)^2*5-3)*6*22.5/20$

= 10.1 kg/year

Polystyrene foam around culvert and spray foam

Length of culvert	5 m	Lansing et al. (2015)
Diameter of foam + culvert (assumed to circular)	1.17 m	Lansing et al. (2015)
Density of expanded polystyrene	22.5 kg/m^3	The Engineering Toolbox (2015)
Assume life span	20 years	
Number of PVC bag	6	

Polystyrene foam mass per year:

(volume of (culvert+outer foam) – volume of culvert)*density of polystyrene*number of digesters/life span

$= ((\pi*(1.17/2)^2*5)-(\pi*(1.07/2)^2*5))*22.5*6/20$ = 6.06 kg/year

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Aluminum Foil

length	5 m	Lansing et al. (2015)
Width	1.68 m	Lansing et al. (2015)
Thickness	0.0001 m	Estimated
Density of aluminum	2700 kg/m^3	
Number of aluminum sheets per digester	2	Lansing et al. (2015)
Number of digesters	6	
Life span year	20 years	

Mass of aluminum foils per year:

Volume of aluminum * density of aluminum * number of aluminum sheets per digester * number of digesters / life span

= 5*1.68*0.0001*2700*2*6/20

= 1.36 kg/year

Polyethylene Core within Radiant Heating Barrier

length	5 m	Lansing et al. (2015)
Width	1.68 m	Lansing et al. (2015)
Thickness	0.0001 m	Estimate
Density of polyethylene	940 kg/m^3	The Engineering Toolbox (2015)
Number of digesters	6	
Life span year	20 years	

Mass of polyethylene core per year:

Volume of polyethylene * density of polyethylene * number of digesters / life span

= 5*1.68*0.0001*940*6/20 =0.237 kg/year

Plastic Sheets Covering Outer Foam

Length	5 m	Lansing et al. (2015)
Circumference for culvert/width 1 (based on 1.07 m diameter)	3.36 m	Lansing et al. (2015)
Circumference for outer foam/width 2 (Assumed to be circular and based on 1.17 m diameter)	3.68 m	
Thickness	0.0001 m	Lansing et al. (2015)
Density of plastic (assumed to be polyethylene)	940 kg/m^3	The Engineering Toolbox (2015)
Number of digesters	6	
Life span year	20 years	

Mass of polyethylene sheets per year:

Volume of plastic sheet 1 covering culvert + volume of plastic sheet 2 covering outer foam * density * number of digesters /life span

= ((5*3.36*0.0001)+(5*3.68*0.0001))*940*6/20

= 0.993 kg/year

A7. Scrubbing and Combustion of Biogas

HDPE Container for Iron Pellets

HDPE container mass	0.0494 kg	Estimated based on fixed-dome system
Life span	20 years	

Mass of HDPE per year:

HDPE container mass / life span

= 0.0494/20 = 0.00247 kg/year

Mass of Iron Oxide (Assumed to be Iron) Needed for Scrubbing

Quantity of biogas	2679 m^3/year	
% H_2S in biogas	0.43%	Lansing et al. (2015)
Assumed temperature	25 °C	
Conversion factor	40.9 moles/m^3	PV=nRT
Mole ratio for iron to H_2S	2/3	
Molecular weight for iron	0.0558 kg/mole	

$Fe_2O_3(s)+3H_2S(g) \rightarrow 2FeS(s)+3H_2O(l)$

Mass of iron needed:

$\%H_2S$ * biogas volume * conversion factor * mole ratios * molecular weight for iron

= 0.43/100*2679*40.9*2/3*0.0558 = <u>17.7 kg/year</u>

Combustion of biogas

Components in LCA	Specific Data Used in SimaPro	Database	Input
Gas Boiler and associated electrical needs for combusting biogas	Heat, central or small-scale, natural gas {RoW}| heat production, natural gas, at boiler modulating <100kW | Alloc Def, U minus Emissions to air and natural gas input	Ecoinvent 3	1 J

Methane emitted during combustion of biogas:

Calculations can be found in *Appendix Section A4*.

A8. Greenhouse Gas Emissions from Lagoons (Emission Reduction)

VS production per cow	6.4 kg VS/day/cow	ASAE, 2003
Number of cows	12	Estimate
Days in year	365	
CH_4 production rate	0.335 m^3/kg VS	Witarsa and Lansing, 2014
Conversion factor	0.67 kg CH_4/m^3 CH_4	
MCF (liquid/slurry with crust cover)	26%	Eastern Research Group Inc. (2011)

Mass of CH_4 emission reduction from lagoons (Eastern Research Group Inc. (2011)):

VS production per cow * number of cows * number of days in year * CH_4 production rate * MCF * conversion factor

= 6.4*12*365*0.335*26/100*0.67 = <u>1636 kg CH_4/year</u>

Appendix B: Detailed Calculations and Information for Chinese Fixed-Dome Digester LCA

B1. Data and Database for fixed-dome digester

Table B1: Data and database used in SimaPro for Chinese fixed-dome digester LCA

Components in LCA	**Specific Data Used in SimaPro**	**Database[a,b]**
Cement	Cement, Portland {RoW}| market for | Alloc Def, U	Ecoinvent 3
Sand	Sand {GLO}| market for | Alloc Def, U	Ecoinvent 3
Gravel	Gravel, crushed {GLO}| market for | Alloc Def, U	Ecoinvent 3
Polyethylene pipe	Polyethylene, high density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
HDPE Container	Polyethylene, high density, granulate {GLO}| market for | Alloc Def, U	Ecoinvent 3
Fe needed to scrub H_2S	Iron pellet {GLO}| market for | Alloc Def, U	Ecoinvent 3
Copper (stove)	Copper {GLO}| market for | Alloc Def, U	Ecoinvent 3
Cast iron (stove)	Cast iron {GLO}| market for | Alloc Def, U	Ecoinvent 3
Stainless steel (stove)	Steel, chromium steel 18/8 {GLO}| market for | Alloc Def, U	Ecoinvent 3
Heat Energy from wood	Log, energy wood, split, measured as solid wood under bark {CH}| heat production, mixed logs, at wood heater 6kW | Alloc Def, U	Ecoinvent 3
Heat Energy from straw	Stalk {GLO}| treatment of, in wood heater 6kW | Alloc Def, U	Ecoinvent 3
Heat Energy from coal	Heat, central or small-scale, other than natural gas {RoW}| heat production, hard coal briquette, stove 5-15kW | Alloc Def, U	Ecoinvent 3
Emissions from combustion of LPG and LPG use	Only emissions to air from Liquefied petroleum gas, combusted in industrial boiler/US, and Liquefied petroleum gas {RoW}| market for | Alloc Def, U	USLCI, Ecoinvent 3
Stove for LPG	Assumed to be the same stove used for burning biogas (Copper {GLO}| market for | Alloc Def, U; Cast iron {GLO}| market for | Alloc Def, U; Steel, chromium steel 18/8 {GLO}| market for | Alloc Def, U)	Ecoinvent 3

[a] From Sima Pro database.
[b] NREL (2012), and Weidema et al. (2013).

B2. Assembly of Fixed-Dome Digester

Cement

Mass of cement	1042 kg	Ministry of Agriculture Science and Education Division (MOASED) and Ministry of Agriculture Chinese Rural Energy and Environment Agency (MOACREEA) (2013)
Assumed life span	20 years	

Mass of cement needed:

mass of cement / life span

= 1042/20 = 52.1 kg/year

Medium Sand

Volume of sand	1.51 m^3	MOASED and MOACREEA (2013)
Density of sand	1440 kg/m^3	The Engineering Toolbox (2015)
Assumed life span	20 years	

Mass of sand needed:

Volume of sand * density / life span

= 1.51*1440/20 = 109 kg/year

Gravel

Volume of gravel	2.17 m^3	MOASED and MOACREEA (2013)
Density of sand	1520 kg/m^3	The Engineering Toolbox (2015)
Assumed life span	20 years	

Mass of gravel needed:

Volume of gravel * density / life span

= 2.17*1520/20 = 165 kg/year

Polyethylene (PE) Pipe (Assumed to be HDPE)

Length of pipe (16mm diameter)	30 m	Estimate
Weight of PE pipe	0.119 kg/m	The Engineering Toolbox (2015)
Assumed life span	20 years	

Mass of PE pipe needed:

Length of pipe * weight / life span

= 30*0.119/20 = 0.179 kg/year

B3. Biogas and Methane Production

Digester 1		
Time used for cooking over 10 days (assumed to be winter period)	220 minutes	Field Survey
Time data was collected	December 2014	
Volume of water for energy flow test	500 mL	
Density of water	1 g/mL	
Initial temperature of water for energy flow test	8.8 °C	
Final water temperature for energy flow test	100 °C	

Time for water to boil	4.55 minutes	
Specific heat capacity of water	4.184 J/g/°C	
Mass of stainless steel pot	188 g	
Specific heat capacity of stainless steel	0.510 J/g/°C	Dirac Delta Consultants Ltd (2015)
Assumed Percent CH_4	60%	Seadi et al. (2008)
Energy content of CH_4	3.39E+07 J/m^3	
Estimated summer values for cooking	105 minutes/day	Field Survey
Estimated number of days for summer	182.5 days	
Estimated number of days for winter	182.5 days	

Energy flow for digester:

((Volume of water * density * specific heat capacity * temperature change) + (mass of stainless steel * specific heat capacity * temperature change)) / time for water to boil

= ((500*1*4.184*(100-8.8))+(188*0.510*(100-8.8)))/4.55 = 4.39E+04 J/min

Energy produced by biogas per day during winter:

total minutes from survey / time range for survey *energy flow

= 220/10*4.39E+04 = 9.66E+05 J/day

Volume of CH_4 for cooking per day (after leakage and after emission during combustion) in winter:

Energy flow produced by biogas per day / energy content of CH_4

= 9.66E+05/3.39E+07= 0.0285 m^3/day

Energy produced by biogas per day during Summer:

= total minutes per day *energy flow

= 105*4.39E+04 = 4.61E+06 J/day

Volume of CH_4 for cooking per day in Summer (after leakage and after emission during combustion):

Energy flow produced by biogas per day / energy content of CH_4

= 4.61E+06/3.39E+07= $\underline{0.136\ m^3/day}$

Total CH_4 produced per year:

Number of days in winter * CH_4 produced in winter + number of days in summer * CH_4 produced in summer

= 182.5* 0.0285 + 182.5*0.136 = $\underline{30.0\ m^3/year}$

Digester 2		
Time used for cooking over 4 days	266 minutes	Field Survey; data was only filled out only over a period of four days
Time data was collected	December 2014	
Volume of water for energy flow test	500 mL	
Density of water	1g/mL	
Initial temperature of water for energy flow test	7.1 °C	
Final water temperature for energy flow test	100 °C	
Time for water to boil	4.08 minutes	
Specific heat capacity of water	4.184 J/g/°C	
Mass of stainless steel pot	188	
Specific heat capacity of stainless steel	0.510 J/g/°C	Dirac Delta Consultants Ltd (2015)
Assumed Percent CH_4	60%	Seadi et al. (2008)
Energy content of CH_4	3.39E+07 J/m^3	
Factor to estimate summer CH_4 values for cooking	4.77	Based on the same factor in Digester 1

Energy flow for digester:

((Volume of water * density * specific heat capacity * temperature) + (mass of stainless steel * specific heat capacity * temperature change)) / time for water to boil

= ((500*1*4.184*(100-7.1)) + (188*0.510*(100-7.1))) /4.08

= 4.98E+04 J/min

Energy produced by biogas per day in winter:

total minutes for survey / time range for survey *energy flow of water

= 266/4*4.98E+04 = 3.31E+06 J/day

Volume of CH_4 for cooking per day (after leakage and after emission during combustion) in winter:

Energy flow produced by biogas per day / energy content of CH4

= 3.31E+06/3.39E+07= 0.0977 m^3/day

Energy produced by biogas per day during summer:

factor to estimate summer values * total minutes per day in winter*energy flow

= 4.77*266/4*4.98E+04 = 1.58E+07 J/day

Volume of CH_4 for cooking per day (after leakage and after emission during combustion) in summer:

Energy flow produced by biogas per day / energy content of CH_4

= 1.58E+07/3.39E+07= 0.466 m^3/day

Total CH_4 produced per year:

Number of days in winter * CH_4 produced in winter + number of days in summer * CH_4 produced in summer

= 182.5* 0.0977 + 182.5*0.466 = 103 m3/year

Average CH_4 for cooking per year from Digesters 1 and 2:

(Volume of CH_4 for cooking per year from Digester 1 + Volume of CH_4 for cooking per year from Digester 1)/2

= (30.0+103)/2 = 66.4 m^3/year

Average CH_4 energy for cooking per year from Digesters 1 and 2:

Volume of CH_4 produced * energy content of CH_4

= 66.4*3.39E+07= 2.25E+09 J/year

Volume of CH_4 for cooking	66.4 m^3/year	See above
Conversion Factor	33,898 BTU/m^3	
CH_4 emitted during combustion	$1.00*10^{-9}$ kg/BTU	Eastern Research Group Inc. (2011), with correction by Klavon (2011)
Density of CH_4	0.66 kg/m^3	
Assumed leakage rate	10%	Eastern Research Group Inc. (2011)
Percent retained	90%	
Assumed percent CH_4 content in biogas	60%	Seadi et al. (2008)

Volume of CH_4 emitted from combustion (Eastern Research Group Inc. (2011), with corrections by Klavon (2011)):

Volume of CH_4 for cooking per year/(1/conversion factor/CH_4 emitted during combustion - 1) / Density of CH_4

= 66.4 / (1/(33898)/($1.00*10^{-9}$)-1)/0.66= $\underline{0.00341\ m^3/day}$

Mass of CH_4 emitted from combustion:

Volume of CH_4 emitted from combustion * Density of CH_4

= 0.00341*0.66 = $\underline{0.00225\ kg/year}$

Methane utilized for combustion (methane after leakage):

Volume Methane used for energy production + methane released from combustion

= 66.4+0.00341 = $\underline{66.4\ m^3/year}$

Volume of CH_4 Leaked:

Volume of methane after leakage / percent retained * percent leaked

= 66.4/(90/100)*10/100 = $\underline{7.38\ m^3/year}$

Mass of CH_4 Leaked:

Volume of CH_4 leaked * density of CH_4

= 7.38*0.66 = $\underline{4.87\ kg/year}$

Volume of biogas produced:

(CH_4 leaked + CH_4 utilized for combustion) / percent CH_4 in biogas

= (7.38+66.4)/60*100 = $\underline{123\ m^3/year}$

B4. Biogas Scrubbing, Combustion, and Displacement of Alternative Fuels

HDPE Container for Iron Pellets

HDPE container mass	0.0494 kg	Measured
Life span	20 years	

Mass of HDPE needed per year:

HDPE container mass / life span

= 0.0494/20 = <u>0.00247 kg/year</u>

Mass of Iron Oxide (Assumed to be Iron) Needed for Scrubbing

Quantity of biogas	123 m^3/year	
% H_2S in biogas	0.5%	Seadi et al. (2015)
Assumed temperature	25 °C	
Conversion factor	40.9 moles/m^3	PV=nRT
Mole ratio for iron to H_2S	2/3	
Molecular weight for iron	0.0558 kg/mole	

$Fe_2O_3(s)+3H_2S(g) \rightarrow 2FeS(s)+3H_2O(l)$

Mass of iron needed:

%H_2S * biogas volume * conversion factor * mole ratios * molecular weight for iron

= 0.5/100*123*40.9*2/3*0.0558 = <u>0.936 kg/year</u>

Copper Mass for Stove

Mass of copper	0.451 kg	Estimated from Mei Jia Si® stove (Rong Gui Mei Jia, Guangdong, China)
Life span	20 years	

Mass of copper needed per year:

Copper mass / life span

= 0.451/20 = 0.0226 kg/year

Cast Iron Mass for Stove

Mass of cast iron	3.09 kg	Estimated from Mei Jia Si® stove (Rong Gui Mei Jia, Guangdong, China)
Life span	20 years	

Mass of cast iron needed per year:

Cast iron mass / life span

= 3.09/20 = 0.155 kg/year

Stainless Steel Mass for Stove

Mass of stainless steel	1.48 kg	Estimated from Mei Jia Si® stove (Rong Gui Mei Jia, Guangdong, China)
Life span	20 years	

Mass of stainless steel needed per year:

Stainless steel mass / life span

= 1.48/20 = 0.0742 kg/year

Methane emitted during combustion of biogas:

Calculations can be found under *Appendix Section B3*.

Displacement of Alternative Fuels

Components in LCA	Specific Data Used in SimaPro	Database	Amount of Input
Heat Energy from wood	Log, energy wood, split, measured as solid wood under bark {CH}| heat production, mixed logs, at wood heater 6kW | Alloc Def, U	Ecoinvent 3	-1.77E-11 m^3
Heat Energy from straw	Stalk {GLO}| treatment of, in wood heater 6kW | Alloc Def, U	Ecoinvent 3	-1.21E-08 kg
Heat Energy from coal	Heat, central or small-scale, other than natural gas {RoW}| heat production, hard coal briquette, stove 5-15kW | Alloc Def, U	Ecoinvent 3	0.167 J
Emissions from combustion of LPG and LPG use	Only emissions to air from Liquefied petroleum gas, combusted in industrial boiler/US, and Liquefied petroleum gas {RoW}| market for | Alloc Def, U	USLCI, Ecoinvent 3	1.962E-08 L
Stove for LPG	Assumed to be the same stove used for burning biogas (Copper {GLO}| market for | Alloc Def, U; Cast iron {GLO}| market for | Alloc Def, U; Steel, chromium steel 18/8 {GLO}| market for | Alloc Def, U)	Ecoinvent 3	0.5 J

Each joule of energy from the biogas was used to displace the following fuels:

0.5 J of LPG, 0.167 J of firewood, 0.167 J of straw, and 0.167 J of LPG

Amount of straw input:

Amount of energy to be displaced / energy content from stalk

= 0.167/1.38E+07 = <u>1.21E-08 kg</u>

Where 1.38E+07 J/kg is energy content of stalk from the Ecoinvent database. Note that value has to be entered into the system as negative due to the negative nature of the specified data.

Amount of wood input:

Amount of energy to be displaced / energy content from wood

= 0.167/9.43E+09 = 1.77E-11 m^3

Where 9.43E+09 J/m^3 is energy content of stalk from the Ecoinvent database. Note that value has to be entered into the system as negative due to the negative nature of the specified data.

Volume of LPG displaced:

Energy from LPG/energy content of propane / density of propane

= 0.5/5.02E+07/0.508

= 1.962E-08 L

Where 5.02E+07 J/kg is the energy content of propane, and 0.508 kg/L is the density of propane.

The stove for LPG was assumed to be made of the same materials as the stove for biogas (0.0226 kg/year copper, 0.155 kg/year cast iron, and 0.0742 kg/year stainless steel).

B5. Greenhouse Gas Emissions from Septic Tank (Greenhouse Gas Emissions Reduction)

Default maximum CH_4 for wastewater	0.6 kg/kg BOD	Doorn et al. (2006)
MCF (methane correction factor)	0.5	Doorn et al. (2006)
Number of persons for Digester 1	0	
Number of persons for Digester 2	3	
BOD estimate	0.040 kg/person/day	Doorn et al. (2006)
Number of days per year	365	
CH_4 emission per year	13.14	kg CH_4/year

Greenhouse gas emission reductions from Digester 1 only (Doorn et al., 2006):

Default maximum CH_4 for wastewater * MCF * Number of persons * BOD estimate * number of days

= 0.6*0.5*3*0.040*365 = <u>13.1 kg/year</u>

Average Greenhouse gas emission reductions from both digesters:

= (greenhouse gas emission reductions from Digester 1 + Digester 2)/2

= (13.1+0)/2= <u>6.57 kg/year</u>

Appendix C: Endorsement Letter for Inclusion of Published Work in Dissertation

UNIVERSITY OF MARYLAND
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July 10, 2015

Charles Caramello
Dean of the Graduate School
University of Maryland
2123 Lee building,
College Park, MD 20742

Dear Dean Caramello,

This letter is written to certify and inform you that the Dissertation Examining Committee, Dissertation Director, and the program's Graduate Director for Freddy Witarsa has approved and endorsed the inclusion of a previously published work into Freddy Witarsa's dissertation. The work is in Chapter 2 of the dissertation and was previously published in *Ecological Engineering, The 13th Annual Conference of the American Ecological Engineering Society: Ecological Engineering and the Dawn of the 21st century* special issue. Freddy Witarsa was the first author in the previously published work and his Dissertation Examining Committee has determined that Freddy Witarsa has made substantial contribution to the work. Thank you for your kind attention.

Sincerely,

Stephanie Lansing, Ph.D.
Ph.D.
Assistant Professor of Ecological Engineering
Dissertation Director

(Acting DGS)
Martin Rabenhorst, 7/13/15
Brian Needelman
Professor
ENST Graduate Director

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