## EXPLORING THE IMPACT OF BIOGAS QUANTITY AND QUALITY IN DIFFERENT DIGESTER TYPES WITH VARIATIONS IN TEMPERATURE

By

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#### ABSTRACT

## EXPLORING THE IMPACT OF BIOGAS QUANTITY AND QUALITY IN DIFFERENT DIGESTER TYPES WITH VARIATIONS IN TEMPERATURE

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The energy sector in the U.S. has been pushing for policies such as the Renewable Portfolio Standard (RPS) to mitigate the impacts of GHG emissions. Biogas from anaerobic digesters is a viable form of renewable energy, due to its CH<sub>4</sub> composition, it can be used as a replacement for power and heat generation or upgraded and sold as biomethane. This study analyzed the effects of temperature in biogas quality and quantity of dairy cow manure in order to compare two main systems, a CSTR and a covered lagoon. A biochemical methane potential (BMP) test was performed to determine material biodegradability of dairy cow manure with respect to temperature. The results show that all samples are anaerobically biodegradable with samples yielding 86, 168, 440, 475 and 448 L biogas per kg initial VS for 15°C, non-mixed; 20°C, non-mixed; 30°C, nonmixed; 39°C, non-mixed; and 39°C, mixed, respectively. The BMP results demonstrated so significant difference between 30°C, non-mixed; 39°C, non-mixed; and 39°C, mixed, respectively. In addition, the effects of psychrophilic, unregulated, and mesophilic conditions were tested in small scale lab pilot digesters. Results show that mesophilic condition yielded the highest cumulative biogas production, while the psychrophilic and unregulated conditions presented higher methane yield. A life cycle analysis was performed to compare two popular anaerobic digestion systems, a CTSR and a covered lagoon, versus current manure management systems for dairy cow manure. The LCA revealed that both systems have less environmental burdens when compared to current waste management systems and a CSTR has less environmental burdens than a covered lagoon.

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# **KEYS TO ABBREVIATIONS**

AAP	Air Acidification Potential
AD	Anaerobic Digester
ADREC	Anaerobic Digestion Research and Education Center
BMP	Biological Methane Potential
CH <sub>4</sub>	Methane
$CO_2$	Carbon Dioxide
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DQI	Data Quality Inventory
EPA	Environmental Protection Agency
FU	Functional Unit
GWP	Global Warming Potential
$H_2S$	Hydrogen Sulfide
HRT	Hydraulic Retention Time
LCA	Life Cycle Analysis
LCFS	Low Carbon Fuel Standard
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
MSU	Michigan State University
MSUSCAD	Michigan State University South Campus Anaerobic Digester
N <sub>2</sub> O	Nitrous Oxide

OLR	Organic Loading Rate
REC	Renewable Energy Credit
RFS	Renewable Fuel Standard
RNG	Renewable Natural Gas
RPS	Renewable Portfolio Standards
TS	Total Solids
VS	Volatile Solids
WCP	Water Consumption Potential
WEP	Water Eutrophication Potential

## **1. INTRODUCTION**

### **1.1Problem statement**

Temperature is one of the main factors that affects digester performance. Microbial communities inside the digester are highly sensitive to temperature changes. It is well known in literature that digesters cannot handle more than a 2°C change within 24 hours, or the microbial communities will be highly disturbed (Schnaars, 2012; Meegoda et al., 2018). The analysis of different temperature profiles provides an opportunity to evaluate impacts on not only biogas production is affected, but its quality. Increased data on temperature impacts on anaerobic digestion will aid the technology selection and operational strategy for manure-based systems across the country. Different regions of the country face varied ambient temperatures seasonal; information generated during this research will improve management of energy inputs necessary for the system to properly function. With a better understanding of how temperature profiles can affect anaerobic digestion, it can allow for easier implementation of different digester types and its variables that might be affected. Better understanding of temperature impacts in anaerobic digestion performance will allow for system to be better optimized for system cost, performance, and energy output.

The capital costs for anaerobic digester projects are broken down into equipment costs and associated markup factors. The initial equipment cost is more expensive for CSTR when compared to a covered lagoon due to additional investments such as a mixing system, heating system and safety features. Additionally, a CSTR has controlled operations and higher maintenance requirements. Another comparison is the main structural unit of a CSTR and a covered lagoon. A CSTR consists of a tank in which depending on sizing can range from \$0.40 to \$1.00 per gallon. In the AD market, the smaller the tank, the higher the cost. For a covered lagoon, the cover price

will be roughly \$5 per square foot of lagoon surface. An additional 10% of square foot is accounted for in order to anchor the cover to the ground. Covered lagoons are considered passive systems due that it takes the advantage of using existing systems such as aerobic lagoons and placing an impermeable cover through which biogas is collected. This type of system works as a two for one due that it provides storage as well as treatment of waste.

## 1.2 Goal and objectives

The goal of my research is to explore the impact of biogas quantity and quality from covered lagoon anaerobic digesters over a range of temperatures and temperature changes. The focus is based on understanding temperature impacts on dairy cow manure digestion, in addition to achieving the three following objectives:

- (1) Analyze the biodegradability of dairy cow manure with variations in temperature.
- (2) Compare the biogas production from cow manure while trying to represent lagoon conditions with variations in temperature and the lack of supplemental mixing.
- (3) Compare a life cycle analysis for a covered lagoon system and a complete mix digester.

## 2. LITERATURE REVIEW

# 2.1 Energy Systems

An energy system is defined as a system composed of various technologies and infrastructures utilized to deliver energy services to end users. Throughout the years, energy systems have been highly affected by factors such as resource availability, environmental impacts, and technological innovation (Saundry, 2019). According to MIT Professor Richard Schmalensee, the innovation in energy systems is derived from an economic standpoint where it is not based on the idea of running out of a fossil fuels such as petroleum, but it is about the price increase with the decrease in source availability. In the past, energy systems have focused only on the supply aspect of energy systems but without considered the energy demand. The U.S. is one of the highest energy demand countries in the world. With an estimated 2.1% increase in demand per year and availability to expand the energy sector with new forms of energy are now being implemented in order to supply the increasing demand in energy.

The sustainability of energy supplies is highly dependent on three factors: society, environment, and economy. There is two form of sustainability related to energy systems: "energy sustainability" and "sustainable energy". Energy sustainability refers to the ability of a population to obtain or acquire energy sources without causing an unbalance in the three factors mentioned above; while sustainable energy refers to the energy producing system which has achieved optimum impacts in all the three factors mentioned above. According to Sikdar (2018), "The sustainability of an energy systems depends on its availability to reduce the adverse environmental, societal and economical aspects associated with such systems.". In recent years, the sourcing of materials and production of energy from fossil fuels have provided a bigger picture on the environmental, economic, and societal needs to find sustainable energy sources. There is a need to

find either forms of reducing the emissions from fossil fuels by investing in technologies to trap GHG or invest in forms of renewable energy (Dunlap, 2015; Kreith, 2015; Tester et al., 2005).

New forms of renewable energy such as wind, solar and biogas have been gaining popularity in recent years. The 1970's energy crisis provided the development of programs to develop renewable energy and utilize energy conservation measures in building, homes and vehicles (Turner, 1999). Renewable energy systems in the U.S. alone avoid the release of 70 million metric tons of carbon dioxide  $(CO_2)$  if the same amount of electricity was generated by conventional methods (Pena, 1997). In recent years, policies have been implemented to not only reduce emissions in the energy sector, but also in the transportation sector. In 2018, Elon Musk in an interview with Joe Regan presented that a key aspect to change to a greener world is the reformation of the transportation sector and transitioning from gasoline to electric and renewable natural gas (RNG) vehicles. In California, 37% of greenhouse gases (GHG) emissions are correspondent to the transportation sector, and passenger vehicles such as car and buses account for a quarter of these emissions. Significant reduction of GHG emissions in the transportation sector can be accomplished by the substitution of fleets utilizing conventional fuel into fleets utilizing low carbon fuels. Low carbon fuels, such as RNG, can be obtained from anaerobic digesters. According to Marianne Mintz of Argonne National Laboratory's Energy System Division Renewable, RNG can "achieve the greatest GHG reductions of any transportation fuel today—70% more as compared to gasoline or diesel.".

### 2.1.1 Fossil Fuels

Fossil fuels, such as natural gas, coal, and oil, are non-renewable resources that formed through millions of years due to the decay of organic matter that was buried under sedimentation. Under changes in temperature and pressure, the organic matter transformed into complex hydrocarbon chains which are used as fossil fuels today. According to the U.S. Department of Energy (DOE), 80% of the domestic energy production per year originated from fossil fuels over the past decade. Fossil fuel production in the U.S. includes natural gas, oil, and coal.

Fossil fuels release some of the highest concentrations of GHG when converted to electricity. These GHG are factors that affect the atmosphere and contributing to climate change. The United States is the second highest emitter of GHG) from the conversion and utilization of fossil fuels. In 2017, the United States was the largest country per capita of GHG emissions. According to the EPA, 65% of CO<sub>2</sub> emission is observed from burning fossil fuels and industrial processes. Before the industrial revolution, the CO<sub>2</sub> concentration in the atmosphere was approximately 280 ppm. Today, the CO<sub>2</sub> concentration in the atmosphere is approximately 47% times higher than before the industrial age. Since 2000, the concentration of CO<sub>2</sub>has increased from approximately 370 parts per million (ppm) to 413 ppm, an 11% change in only two decades (NASA, 2021).

Electricity is defined as the flow of electrical power or charge. The daily human routine consists of utilizing electricity to run common household items such as microwaves and ovens to running massive operations such as factories, hospitals, and airport terminals. Electricity has become crucial in the development of a thriving economy. In a study conducted by Ferguson et al. (2000), wealthy countries have a higher correlation between economic development and electricity consumption in comparison to underdeveloped or monetary unstable countries. In 2019, the total electricity consumption in the United States alone was 3.9 trillion kilowatt-hours (kWh), from which, approximately 65% was obtained from burning fossil fuels such as coal, natural gas, and petroleum, while the remainder was obtained from nuclear energy and renewables,

respectively. Natural gas is the largest electricity production with roughly 38% of all electricity was obtained from the processing of this.

Natural gas is predominantly methane (CH<sub>4</sub>), composed of four hydrocarbon atoms and one carbon atom. In the U.S., natural gas is obtained by the process of hydraulic fracturing, also known as fracking. This process consists of drilling into the rock formation where the natural gas is located. The whole process of drilling the well takes roughly 3 to 5 months, but natural gas and oil can be extracted from a well for roughly 20 to 40 years after drilling. Approximately, 60% of all the oil and natural gas in the U.S. is obtained through this process. However, natural gas wells also result in GHG emissions, approximately 29% of CH<sub>4</sub> emissions in 2018 were from natural gas wells (U.S. Energy Information Administration (EIA), 2020).

### 2.1.2 Renewable Fuels

According to the Environmental Protection Agency (EPA), only a small percentage of the world's energy resources, 0.8%, are obtained from renewable energy such as solar, wind or geothermal (2019). Although sustainability goals have been implemented globally, there is an inadequate technological development towards the renewable energy field. In the U.S. in 2019, only 11% of electricity generation was from renewable sources.

### 2.1.2.1 Wind, Solar, and Biogas for Electricity

Renewable energy forms such as windfarms or solar panels are highly used worldwide as renewable energy systems; but they have negative environmental impacts. Wind power, as the name implies, refers to the process of obtaining electricity from air flow. Wind energy is perceived as one of the cleanest renewable energy production systems due that there are no pollutants or GHG during their operation, but the issues arise during the installation and disposal of the mechanisms. Wind farms are created by installing wind turbines on open land. These farms are highly dependent on wind; thus, the optimal locations are limited by average wind speed. The optimal locations are often in unpopulated regions or offshore, resulting in transmission challenges to populated regions.

Moreover, other concerns with wind energy include disturbance to nearby populations due to noise and the effects on animal populations. In the U.S., it is estimated that half a million birds are killed with turbine collisions each year (U.S. Fish and Wildlife Service). Once the useful life of 20 to 25 years is reached, there are issues with blade disposal. A typical wind turbine single blade is 120 feet, which is approximately the size of a commercial plane. All other components of a wind turbine can be reused or recycled while the main issue consists in the disposal of the blades. The blades are often buried in landfills or burned through pyrolysis (EIA, 2020). Although the popularity of wind power has increased with time, the negative side effects seem to counteract the benefits (Covert et al., 2016).

Solar power, as the name infers, is the energy obtained from the sun. Solar panels collect sunlight into a photovoltaic cell. The energy is then passed through an inverter and either stored in batteries or used immediately. Solar power has become tremendously popular since installation costs have almost halved between 2007 to 2019. By 2018, companies such as Apple, Target, Amazon, and Walmart have summed up 1.1 gigawatts of total installed solar capacity (Solar Energy Industry Association). It is estimated that solar power accounts for roughly 72 billion kWh in electricity generation in the U.S. (EIA).

Even though solar panels are considered environmentally friendly given the lack of GHG emissions during the energy collection process in compared to coal and natural gas. The harshest environmental impacts rely during the manufacturing and disposal process. Solar panels are made of materials such as silicon and plexiglass. During the manufacturing process, various chemicals

are utilized in order to build the semiconductors and maintain them. The entire life cycle of a solar panel from material sourcing to end-of-life disposal require vast amounts of energy for material sourcing, transformations, installation, and recycling at the end of lifetime. All this process requires intensive labor and heavy machinery (National Renewable Laboratory, 2012). Although taking the full lifetime of the cell provides negative insights, it is an improvement in comparison to fossil fuels when it comes to GHG reductions.

An upcoming technology, that has gained popularity as a renewable energy source, is biogas. Biogas is one of the major products of anaerobic digestion. Anaerobic digestion (AD) is a natural occurring process in which microorganisms, such as bacteria, breakdown organic material and transform into biogas and digestate. Biogas has two major components: CH<sub>4</sub> and CO<sub>2</sub> (Weiland, 2010). Biogas is a viable form of renewable energy, due to its CH<sub>4</sub> composition, it can be used as a replacement for power and heat generation or upgraded and sold as biomethane. The U.S. currently has approximately 2,000 operating biogas systems (Tanigawa, 2017). Wastewater treatment plants, landfills and livestock farms have adapted biogas systems due to the high efficiency to convert the organic material into a usable byproduct while also treating organic waste and controlling emissions. The key aspect of AD is that CH<sub>4</sub> is a form of renewable energy with the reduction of GHG. According to Fehrendbach et al., (2008), biogas production through anaerobic digestion can be classified as "one of the most energy-efficient and environmentally beneficial technology for bioenergy production".

### 2.1.2.2 Renewable Natural Gas

Renewable natural gas (RNG), also known as biomethane, is the term used to describe biogas that has been purified into CH<sub>4</sub> concentration of 90% or above. Raw biogas can have a CH<sub>4</sub> concentration of 50 to 70% CH<sub>4</sub>, depending on the process and feedstock. Biogas can be obtained from landfills, wastewater treatment plants, livestock farms and waste management operations. RNG can be distributed by a natural gas pipeline and converted to electricity, thermal applications, or vehicle fuel. Due to the purification process and molecular form, RNG and fossil natural gas are identical (Wiley, 2018). RNG vehicles in compared to diesel or gasoline vehicles are able to reduce the GHG emissions by approximately 75% (AFLEET Tool). The RNG trend is capable of creating negative carbon footprints due to the avoidance of CH<sub>4</sub> emissions from current waste management operations in places such as livestock farms (CARB & California Environmental Protection Agency, 2014).

## **2.2 Policy Drivers**

#### 2.2.1 Renewable Portfolio Standards

Renewable portfolio standards (RPS), also referred as renewable electricity standards (RES), are regulatory mandates intended to increase energy production from renewable sources such as wind, solar, biomass, etc. and reduce the energy consumption from other sources such as fossil fuels and nuclear energy. These standards encourage energy suppliers to diversify the grid by introducing a certain value or percentage of energy obtained from renewable sources. The purpose of these policies is to promote the diversification of energy generation towards renewable energy sources and encourage a new market beneficial for the owners of the renewable energy source (AgStar, 2019). These policies have been implemented in states such as Michigan, Arizona, and California. RPS policies have been developed across the states individually but there is no electricity policy current in place at the federal level. Each state has various definitions and goals for their policies which brings to variations in the definitions of terms such as "carbon-free", "carbon neutral" or "clean energy".

A common feature between state to state for this policy is the renewable energy credit (REC) trading system. Renewable energy credits (RECs) are payments that a utility company or other businesses will provide to a renewable energy provider for the exchange of their energy production being placed into the grid. One REC is equivalent to 1 megawatt-hour of electricity obtained from a renewable energy source (Binkley et al., 2011). Under RFS, utility companies are required and/or expected to obtain a number of RECs during a certain time period. RECs work as a trading mechanism due that can be sold or bought within energy companies in the state in order to meet the standard.

Nationally, the RPS requirements have played a key role in increasing the renewable energy drive within the country and results can be seen not only at state level but federally. It is estimated that more than 50% increase in renewable energy usage in the last decade can be attributed to the implementation of RPS policies in the majority of Northeast and Mid-Atlantic states (SEIA).

## 2.2.2 Renewable Fuels Standard

Renewable Fuel Standards (RFS) is a federal program that creates incentives to promote the integration of vehicle fuels obtained from renewable resources. According to the Renewable Fuel Association, this policy has been categorized as "the single most successful clean fuels policy in the United States". This federal policy was amended to the Energy Policy Act in 2005 by Congress. This policy was later renewed, and it is now known as the Clean Energy Act (CAA). The Environmental Protection Agency (EPA) is the designated entity to establish targets and implements the policy at federal level.

The target volume is designated from investigating projected trendlines of gasoline and diesel consumption and reviewing the compliance to reach previous years goals. The renewable

fuel in question has to be utilized for sectors such as transportation fuel or jet fuel to be considered. The biofuel needs to have a reduction in GHG when compared to a 2005 petroleum baseline. In previous years, various factors such as government grants and technology limitations have proven to rise challenges when it comes to reaching target volumes in the categories mentioned.

EPA is in charge of setting the targets of fuel volume required from the following biofuel categories: total renewable fuel, total advanced biofuel, cellulosic biofuel, and biomass-based diesel. The total renewable fuel requirement is composed of two subcategories: conventional biofuel and advanced biofuel. Conventional biofuel is any fuel obtained from feedstocks such as grain and corn. Conventional biofuel has to demonstrate a reduction of approximately 20% in lifecycle GHG emissions. Moreover, advance biofuels are composed of two subcategories: cellulose biofuels and biomass-based diesel. Advanced biofuels are derived from cellulosic or advanced feedstocks such as sugarcane, sugar beet, vegetable oil and others. Cellulosic biofuels are any diesel fuel substitute obtained from lignin, hemicellulose, or cellulose. These biofuels most demonstrate an approximate reduction of at least 60% in lifecycle GHG emissions. Biomass based diesel is created from renewable feedstocks and must demonstrate approximately 50% reduction in lifecycle GHG emissions.

Similar to the RECs, utilized for electricity production at the state level, for RFS we can encounter a similar trading mechanism identified as Renewable Identification Numbers (RIN). RIN is a credit obtained by a company when they produce one gallon of renewable fuels. At the end of a time period, the company must demonstrate enough RIN to be compliant with the RFS policy. In similar manner to RECs, RIN can be sold or bought within renewable fuel production companies (Brackmort, 2020, EPA, DOE).

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### 2.2.3 Carbon Cap and Trade

Carbon cap and trade, as the name implies, is the combination of two components when it comes to carbon emissions into the environment. The cap represents the maximum number of GHG emissions set by the government; while the trade is where the government sells, or issues permits to businesses or entities that are emitters of GHG. Any company that emits GHG as part of their day-to-day operations needs one permit for every ton of GHG emitted. These permits are allowed to be traded between companies, as necessary. Due to associating a monetary incentive, companies have made efforts to reduce their GHG emissions. If a company has more permits than what it needs, it is allowed to sell to other companies, therefore providing a value market associated with environmental impacts (Center for Climate and Energy Solutions).

### 2.2.3.1 Carbon Intensity

Carbon intensity is defined as the measurement of GHG emissions associated with transportation fuel. This metric is usually presented in grams of  $CO_2$  per megajoule of energy. When calculating carbon intensity, it is composed of considering the following processes within its scope: extraction, refinement, distribution, storage, and combustion. In other words, these scores take into consideration a complete life cycle analysis of a specific fuel.

### 2.2.3.1.1 Model

The current model to calculate a CI score is the CA-GREET 3.0 Model and Tier 1 Simplified Carbon Intensity calculators. GREET is the abbreviation for Greenhouse Gases, Regulated Emissions and Energy Use in Transportation. The GREET model is a tool utilized to input data and perform a life cycle analysis about the environmental impacts of vehicle technologies, fuels, and energy systems. The GREET model can be used to calculate total energy consumption, emission of GHG and air pollutants and water consumption. Department of Energy and Argonne labs partnered in order to develop this model and continue to introduce improvements with advancements in industry and technology.

#### 2.2.3.2 California LCFS process

In recent years, states have adopted a vast majority of standards and policies in order to reduce GHG emissions into the environment by providing various forms of incentives. A major topic within policies drivers has been vehicle fuel consumption and usage. Companies such as Amazon and Target have adopted the usage of electric vehicles for local and nationwide deliveries. Even though a single electric truck can be priced around \$70,000, states such as California have adopted a policy that is changing the transportation game for companies (Electrek, 2020). This policy is the Low Carbon Fuel Standards (LCFS). This is an action established in the state of California by the California Air and Resources Board in 2009. This policy has been adopted by states in a similar realm such as Oregon and Washington. LCFS has even been implemented in international countries such as Canada and Brazil. This standard is implemented to decrease the carbon intensity of transportation fuel by providing benefits and income to low carbon and renewable fuel providers. In other words, any industrial vehicle from which CO<sub>2</sub> emissions fall below the standards implemented by the government, will receive a LCFS credit. One LCFS credit represent 1 metric ton of CO<sub>2</sub> prevented from being released into the environment. LCFS credits are generates by low carbon or renewable fuel producers and purchased by gasoline and diesel production companies. The main contradictions for this process are the high pricing and resources to maintain records for the generation and retail of these (U.C. Davis Institute of Transportation Studies, 2020).

#### 2.2.3.3 How Dairy Manure Fits?

Biogas derived from dairy manure fits the majority of the state and federally level programs focused on reduction of GHG emissions and renewable energy and/or fuels. Cow manure contains some of the highest concentrations of CH<sub>4</sub>, but also has significant GHG emissions during longterm storage. Dairy manure, as an AD feedstock, can not only reducing GHG emissions, but it is also producing a renewable energy source.

## **2.3 Dairy Manure Management Systems**

#### 2.3.1 Overview of Standard Practice

A single dairy cow can produce a rough estimate of 68 kilograms of manure per day (ASABE Standards, 2005). Proper manure management results in the collection and eventual land application as a fertilizer without significant impacts on air, soil, or water quality. Manure is collected from dairy barns or loading pens and either directly land applied or stored in anaerobic ponds. Some farms use solid liquid separation prior to storage to improve management and generate bedding or solid fertilizer products. The liquid portion of the manure can be irrigated as a form of fertilizer and crop water source.

### 2.3.2 Environmental Impacts

Current, waste treatment solutions for manure and slurries systems are unfavorable for natural environments due to possible soil pollution and negative environmental impacts, such as contaminating nearby water streams and food crops with pathological entities (US EPA, 2018). When poorly managed, animal handling sectors such as farms account for around 18% of GHG emissions in the world, without including other materials such as nitrous oxide and ammonia (Esfandiari, Khosrokhavar & Sekhavat, 2011). In the United States alone, livestock manure management contributes roughly 14% to ammonia emissions which cause acid rain (Eckert et al. 2018). Anaerobic digestion solves these problems through storage, control, and reduction of waste.

#### 2.3.2.1 Emissions generation during lagoon storage

Dairy liquid manure storage is one of the major sources of GHG emissions in the agricultural sector. The major GHG produced is CH<sub>4</sub>. In a study conducted by Leytem et al. (2017), six manure storage lagoons in the Western United States were analyzed for GHG emissions. The average CH<sub>4</sub> emissions from the lagoons were 22 to 517 kg per day. The variation was due to the monitoring occurring over a full year span and therefore providing variations due to temperature changes with respect to seasonal changes. Western United States due to low humidity there is a high evaporation rate from liquid waste storages, therefore reflecting higher GHG emissions in comparison to other locations around the country (Grant & Boehm, 2020).

## **2.4 Anaerobic Digestion**

#### 2.4.1 Process of Anaerobic Digestion

The process of anaerobic digestion consists of four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the hydrolysis process, organic materials such as cellulose and hemicellulose are hydrolyzed to simple monomers and oligomers such as soluble sugars or alcohols. Hydrolysis products will be transformed during the acidogenesis process to yield volatile fatty acids that mainly consist of formic, acetic, propionic, and butyric acid. During the acetogenesis and methanogenesis stages, methanogenes will utilize formic and acetic acid, while propionic and butyric acid will be converted into acetic acid by acetogenes (Shen et al., 2018).

The anaerobic digestion process is driven by the various microbial communities that carry out the four stages mentioned. Most of these communities are highly dependent various factors such as temperature and material availability. There are vast number of bacterial species involved in the process and each specie will require individual parameters to thrive within the AD process. Different bacterial communities breakdown complex organic molecules by the process of hydrolysis and fermentation. Once simple molecules, organic acids, are available, a specific bacterial community, methanogens, utilize volatile solids and produce the main component of biogas: CH<sub>4</sub> and CO<sub>2</sub>. This natural occurring process is found in different environments such as soil and lakes (Meegod et al., 2018).

Anaerobic digestion has been used by human population since the early starts of civilization. In the 17th century, Jan Baptita Van Helmont discovered that flammable gases could evolve from the decay of organic matter. In 1808, Humphrey Davy determines that CH<sub>4</sub> is naturally produced by cattle manure. Approximately, 51 years later, the first anaerobic digestion plant was built in 1859 in India (Zullo, 2016).

#### 2.4.1.1 Digesters 101

An anaerobic digester is a controlled system that executes this natural process to collect the biogas for future use in energy production. AD systems produce valuable products such as biogas and digestate. Biogas can be combusted onsite to create electricity and heat or refined and utilized as biomethane or RNG. Digestate provides a substitution for inorganic fertilizer introduced into the environment as it includes stable forms of nitrogen and phosphorus readily accessible for soil absorption (Tambone et al., 2010; Weiland, 2010).

## 2.4.2 Factors influencing Anaerobic Digestion

#### 2.4.2.1 Temperature

Anaerobic digesters operate in one of three temperature regimes: psychrophilic (15–23°C), mesophilic (35–41°C), and thermophilic (52–58°C). Psychrophilic temperatures have been presented in unheated digester systems where the temperatures are below 23°C. According to Chen

and Neibling (2014), microbial degradation of feedstocks within this temperature range stops therefore reducing both biogas quality and quantity. The most common types of digester studied in the United States are mesophilic digesters. Mesophilic digesters have been well studied with the use of cow, swine, or chicken manure. Due that mesophilic digesters have been well studied in digestion of manures, the shift in research has been in the analysis of co-digestion processes for this temperature profile. Thermophilic temperatures have been well studied in literature as part of municipal waste management systems. Co-digestion is the mixing of microbial rich, low energy feedstocks like manures with, energy rich feedstocks like food waste and raw carbon wastes.

In various studied presented in academics, temperature in the mesophilic range is considered the ideal temperature to promote bacterial activity within the digester. Thermophilic digester is often seen in literature in order to treat wastewaters or highly pathogenic sludges. Heating of a complete mix digester will usually occur through a heated water loop which is heated by the waste heat from the electricity production of the digester. The thermal energy demand by the system is derived by four main factors: ambient temperature, feed rates, degree of insulation and digester cover type.

According to Song et al. (2004), heating provides an ideal system that promotes the bacteria to be continuously active and promoting solids destruction. Acetogens and methanogens are crucial bacterial communities that depend in these heating conditions in order to convert the organic material presented as feed into biogas and digestate.

Covered lagoon systems are highly understudied due to the difficulty of evaluating them at pilot scale and a lack of commercial system data that is publicly available. The systems are somewhat unpredictability due to the influence of ambient temperatures. Although covered lagoons may not thrive in colder climates or psychrophilic as a biogas producing system, it could
possibly thrive in different states across the United States where the ground and air temperatures are higher. For example, shown in Figure 2.1, the mean earth temperature in States such as Florida, Texas or Arizona is above 19°C. As part of my research is to identify a transition between psychrophilic temperatures and mesophilic temperatures in order to create a possible model for biogas quantity and quality.



Figure 2.1. Mean annual earth temperature observations at individual stations, superimposed on well-water temperature contours.

In 2011, a study was performed a 100 head dairy farm in South Dakota. The farm utilizes a covered lagoon as a flush system for barn output. The lagoon in this study had a synthetic cover that was weighted down by concrete tubes that also serve as the gas collection system. The study presented preliminary results for variations between ambient temperature and effluent temperature at the side and bottom of the lagoon. As seen in Figure 2.2, and presented as a preliminary discussion point, even though, there was a variation in temperature recording, the lagoon sludge seemed to maintain a psychrophilic state during the winter season (Darrington & Cortus, 2011).



Figure 2.2. Measured temperature at the side and bottom of sludge line and ambient temperature at a 100 head farm in South Dakota (Darrington & Cortus, 2011)

## 2.4.2.2 Mixing

Although mixing will not be taken into consideration due to time constraints, this specific parameter will be discussed for further information and recommendations. Mixing the active sludge within the digester allows for all the diverse microbial communities within the digester to destroy complex polymers into monomers and other bacteria such as methanogens and acetogens that destroy this food and convert into biogas which is formed by CH<sub>4</sub> and CO<sub>2</sub>. There are diverse goals and benefits described in literature; but a lack of comparison between non-mixing scenarios and how specifically mixing does affect biogas quantity and quality. Some of the goals of mixing are:

- Exposing the microorganisms to the maximum amount of food available
- Reducing the volume occupied by material settling
- Preventing the formation of a floating crust layer that will reduce the percolation of biogas
- Eliminating stratification of the system

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Although these are the goals of mixing, there is a vast of literature resources that due provide the pros and cons of mixing. One well defined benefit of mixing provided in literature is that it does provide faster volatile solid reduction; due that mixing will provide a homogeneous environment for microbial degradation and reducing the potential for upsets within digester due to matter separation. One con with regards to mixing is the high cost of purchasing and maintaining the equipment as well as the energy consumption required for mixing.

#### 2.4.2.3 Hydraulic Retention Time

Hydraulic Retention Time (HRT) is defined as the theoretical average time the substrate remain inside the reactor. This parameter is often represented in literature as  $\theta$  and is calculated as a ratio between reactor volume and the reactor feed rate. The HRT of the reactor presents a crucial parameter for proper functioning. The HRT in other ways is correlated to the loading rate. A smaller HRT represents a higher volumetric loading rate, while a larger HRT, smaller loading rate (Kim et al, 2013). There have been various studies in research proposing variations depending on feedstock and digester size. A key conclusion presented is that, at low HRTs, methanogens can be washed out from the reactor therefore producing an unfavorable environment for CH<sub>4</sub> production (Velvizhi, 2019). Longer HRTs although have proven efficient for higher biogas yields, it provides additional costs and decreasing the process efficiency to handle larger volumes of wastes (Zhang et al., 2006)

## 2.4.2.4 Organic Loading Rate

The organic loading rate (OLR) refers to the amount of organic material fed to the digester per day. The OLR is a ratio of the mass of volatile solids (VS) or chemical oxygen demand (COD) in the feedstock and the reactor volume. The OLR is an indicator of the overall performance of the digester and represents how much reactor space is actually being utilized. The OLR range will be dependent not only on digester type but also on the type of feedstocks introduced into the digester (Ferguson et al., 2016).

Total solids (TS) indicate the total mass of the material that is being added daily to the reactor, while VS is the material available for biogas conversion. TS will impact the volatile fatty acids to alkalinity ratio, OLR and the volume of gas production. A high or low feeding rate can lead to poor performance. High feeding rates can cause an accumulation of volatile fatty acids which will highly impacts the process of methanogenesis (MSU Anaerobic Digester Operator Training, 2019). In recent studies, it has been deducted that the microbial community within the digester after an overloading event might be adapting and able to increase the loading rate with succession in loading events (Ferguson et al., 2016). Under feeding a system can starve the microbial community of needed energy, resulting in lower activity and biogas production.

#### 2.4.2.5 Feedstocks

Feedstocks is defined as any organic material that can be introduced into a digester and converted to biogas by the microbial community. There is a great range of feedstock available such as animal manure, municipal waste, lignocellulosic material, and food waste. The end goal is feeding the digester substrates that will provide the maximum CH<sub>4</sub> yield possible. Cow manure and swine manure by themselves will provide a reasonable amount of biogas. Cattle manure can provide a range of 0.13-0.24 m<sup>3</sup> CH<sub>4</sub> per kg of VS; while swine and poultry manure can produce a range of 0.29-0.45 and 0.02-0.39, respectively (Díaz-Vázquez et al., 2020). Co-digestion is the anaerobic digestion of multiple feedstocks within a single AD system. The effects on biogas potential of a specific feedstock will vary highly on its individual chemical composition. Lipids, carbohydrates, and proteins have biogas potentials of 1.42, 0.83 and 0.95 L biogas per g VS (Alves et al., 2009). The addition of 20 to 30% materials such as food waste and crop residues are ale to

increase methane production by at least 15% when compared to the digestion of manure by itself (Lehtomäki et al., 2007). Some biogas potentials for mixtures with cow manure and slaughterhouse blood, out of date beverages and grease trap waste are 0.40, 0.82 and 0.83 mL CH<sub>4</sub> per mg of initial TS (Ma et al., 2017). Although we compare feedstocks against one another, pretreatments, chemical addition, and other processes can highly vary a single feedstocks biogas production.

## 2.4.2.6 Digester Types

Many large-scale farms have adopted anaerobic digestion systems as waste management systems within recent years. In the past two decades, as show in Figure 2.3, anaerobic digesters have doubled within the U.S. In 2019, there were 245 operational digesters, 9 newly operational, 1 shut down and 32 under construction. From these trends, 204 are dairy manure digesters. (EPA, 2019)



# Figure 2.3. Anaerobic Digesters Operating in the United States from 2000 to 2019 (EPA , 2019)

Across the United States, as shown in Figure 2.4, there are 3 major types of anaerobic digesters used: plug flow, complete mix, and covered lagoons. Plug flow and complete mix (e.g. completely stirred tank rectors) digesters are the two most popular systems due to their high

biogas productivity. Both systems have supplemental mixing or heating and are usually controlled by an operator (Klinghoffer & Castaldi, 2013).



**Figure 2.4. Designs for Operating Anaerobic Digesters in the United States (EPA, 2019)** 2.4.2.6.1 Complete Mix Stirred Reactors

Complete mix stirred reactors, also known as CSTR, are mainly used in the bioenergy industry due to their vast availability of data. This system can accept high variety in feedstock ranges and can be widely implemented in various weathers. The CSTR has cylindrical tanks that are constructed from steel or concrete. This system requires supplemental mixing and heating. The main feature of this system includes the tanks, mixers, covers, and heating systems. It typically has a 5 to 20 days hydraulic retention time (HRT). CSTR systems can handle a wide range of influent total solids and agricultural flush. This digester type provides uniformity of temperature, mixing, and substrate concentration (Usack et al., 2012). CSTR systems can function in a variety of climates due to the closed heating system that accompanies this digester type.

#### 2.4.2.6.2 Covered Lagoon

Another popular type of AD system in the United States are covered lagoons. Covered lagoons are underground systems covered by a turf or lining for biogas collection. Typically, these systems are designed to store diluted wastewaters of sludges with less than 5% TS. Covered lagoon systems do not require supplemental mixing or heating. These lagoons are more common in

warmer climates since they are not normally heated and will be inefficient in temperate or cold climates. These systems are still used in colder climates for digestate storage and odor control. It typically seen within the United States in states such as Arizona or Texas or outside of the United States in countries such as Ecuador and Chile. Moreover, this type of digester can produce enough quantity and quality of biogas in climates that have elevated year-round temperatures (Penn State Extension, 2013). Table 2.1 represents a comparison of this system with respect to a CSTR.

	CSTR	Covered Lagoon
Typical HRT	5 to 20 days	40 to 60 days
TS Range	Variable	<5%
OLR Range	1 to 10 kg COD/m <sup>3</sup> /day	0 to 0.2 kg $COD/m^3/day$
Insulation	System dependent	Not typical
Supplemental Heating	System dependent	Not typical
Supplemental Mixing	Yes (pump or motor)	Not typical

 Table 2.1. Overall Comparison of a Complete Mix Digester versus a Covered Lagoon

## 2.4.4.2.3 Costs

As presented in Table 2.2, there is a case study that analyzed different digester types and compared the capital cost and payback period for each one. Presented are the results for the calculated payback periods of each case study. The paper failed to present if the economics reviewed the monetary benefits within its analysis (AgSTAR Project Profiles).

 Table 2.2. Case Studies regarding Capital Costs and Payback Period for Different Digester

 Types

Case Study	Digester Type	Capital Cost	Payback Period
Tollenaar Holsteins Dairy	Complete Mix (CSTR)	\$1.7 million	10 years
Butler Farms	Covered Lagoon	\$650,000	8 to 10 years
Lloyd Ray Farms	Covered Lagoon	\$1.2 million	N/A
Quasar Energy Group	Complete Mix (CSTR)	\$6 million	4 to 6 years

The economics regarding an anaerobic digestion facility are not only based on costs, operation and management, and revenue. As seen in Table 2.2, the payback period changes vastly across different digester types and sizes. The capital cost will have a wide range due that it is dependent on digester type, sizing, and feedstock available. The operation and management expenses will include all consumables, workers, and maintenance procedures. Equipment maintenance should be taking into consideration in the initial stages of project proposal and rather facilitating preventative maintenance to keep the proper functioning of pumps, heaters, valves, and others. All though digester costs are high possible forms of revenue should be taking into consideration as part of the planning. Different forms of revenue will be discussed in the following section (Sheffler, 2018).

#### 2.4.3 Environmental Benefits

There are five major benefits to anaerobic digestion which are the following: emission control, pathogen reduction, odor control, waste stabilization and nutrient availability. Through AD systems, there is a reduction in GHG emissions from livestock manure into the atmosphere. Instead of CH<sub>4</sub> being released, it is used for either electricity or fuel (EPA). Odor control and waste stabilization are achieved by inserting an AD system prior to releasing the manure in the storage lagoon. Through AD, the effluent released into the storage lagoon contains more stable organic material than manure itself and less volatile odorants. Nutrients can be retrieved from nature through the AD process. These nutrients are then available in the digestate utilized for land application. Animal wastes and municipal wastes can contain several forms of pathogen and contaminants. The competition with other microbes within the digester can cause pathogen reduction, in contribution, with the presence of organic acids to inhibit pathogen growth (Wilkie, 2005).

## 2.4.4 Revenue

Anaerobic digestion is an emerging form of renewable energy across the United States and even though digesters cost is high, there are many forms of revenue throughout the byproducts of anaerobic digestion. There is the existence of different markets for an anerobic digester to receive some form of revenue such as digestate, energy or fuel credits and feeding fees.

The average pricing for these revenue streams will vary from digester to digester due to location and operating procedures. One key example of these is feeding fees. Feeding fees are rates charged to a company disposing of waste materials as digester influent. The feeding rates vary on the water content of the materials. FOG (Fats, Oils and Grease) has high amounts of water content therefore it has a low monetary value of \$0.10 per gallon; but forms of dry material with low water concentration for about \$12.00 per gallon. Additionally, digestate can be sold to nearby farms or composting facilities for approximately \$7.00 per ton (Dr. Dana Kirk, 2020).

Some of the markets mentioned previously are RINs and RECs related to the current policy drives at state or federal level. According to the EPA (2020), the price range for a RIN is between \$0.01 and \$3.50. This value price will be dependent on the source of the biofuel. RECs prices due to be highly varying between state to state, the prices are affected by changes in policy and the availability of RECs within the state. Between 2014 and 2017, drops in all the states regarding to REC prices could be noted with regards to new introductions of renewable energy policies within those years. Most of the current RECs are met through technologies such as wind and solar therefore creating a deviation in the market for the prices.

# **3. MATERIALS AND METHODS**

# **3.1 Waste Collection and Handling**

Samples were collected and analyzes by the MSU Anaerobic Digestion Research and Education Center (ADREC).

# 3.1.1 Anaerobic Filtrate

Seed material, also known as filtrate, was collected from MSU South Campus Anaerobic Digester (MSU SCAD). MSU SCAD is a complete mix digester. The feedstock of the digester consists of a 50/50 mix of cow manure from MSU Dairy Farms and food waste. Effluent from the MSU SCAD is processed through a screw press to separated solids and liquids (filtrate). Filtrate was collected prior to every BMP round and prior to initial pilot seeding.

## 3.1.2 Liquid Cow Manure

Liquid manure was collected from a dairy farm near Webberville, Michigan. The cow manure was collected from a storage pit post to the manure passing through the sand separation system and rotary solid liquid separator. This manure was chosen due to the low number of total solids (approximately 4% TS), making it suitable to compare to manures utilized in lagoon operations. Fresh manure was collected prior to every BMP round and every two weeks for pilot feeding.

#### 3.1.3 Sample Storage

All samples collected were stored in a refrigerator at 4°C.

# **3.2 Waste Characterization**

The raw samples obtained were characterized for pH, conductivity (EC), total solids (TS), and volatile solids (VS). TS and VS analysis performed during this research utilized the EPA accepted Hach methods 8271 and 8276, respectively. For TS, the procedure was modified from a 6-hour oven holding time to 24 hours in order to ensure complete drying. The time was also increased from 1 hour to 6 hours for the VS procedure in order to ensure complete sample combustion. pH and EC measurements were tested using an Accumet Excel XL60 meter by Fisher Scientific.

The pre- and post-BMP digestion and pre-and post-pilot analysis performed included pH, EC, TS, VS, and soluble chemical oxygen demand (SCOD). The SCOD testing consists of obtaining the soluble portion of the sample and the using EPA accepted Hach Method 8000. The filters utilized to obtain the soluble portion of the sample are presented in table 3.1.

Order	Diameter	Characteristic
1	47 mm	Ashless, 41, Whatman
2	47 mm	Qualitative, Whatman
3	42.5 mm	Qualitative, Whatman
4	42.5 mm	Qualitative, VWR
5	42.5 mm	Hardened, Whatman
6	47 mm	Glass Microfiber Filters, GF/A, Whatman
7	47 mm	Glass Microfiber Filters, GF/F, Whatman
8	0.45 µm	Microporous membrane, Whatman

Table 3.1. Filters utilized during the SCOD process

# **3.3** Biochemical methane potential Test (BMP)

Three round of BMP assays were performed at ADREC during January 2020 to July 2020. For each round, new filtrate and cow manure samples were collected. Samples were collected throughout 2020 and maintained in refrigeration at 4°C. BMPs were performed to evaluate performance differences related to mixing and temperature with respect to material biodegradability. The assay consisted of 5 different BMPs which had the conditions presented in Table 3.2. All the bottles were maintained at their respective temperatures and mixing conditions during the duration of the experiment.

Categories	Temperature	Mixing
8	(°C)	(Y/N)
1	15	Ν
2	20	N
3	30	N
4	39	N
5	39	Y

Table 3.2. Summary of BMP assays with variations in temperature and mixing

#### 3.3.1 Set-up

The BMPs were set up utilizing the procedure and all analyses are obtained from the paper presented by Faivor and Kirk (2011). After performing raw characterization on both filtrate and cow manure, blends were created to have an initial VS:VS ratio of 2:1 in terms of filtrate: cow manure. All blends are set up in triplicates including three bottles only containing seed material, which serve as a control group. For purposes of this experiment, a positive control group was set up. Cellulose microcrystalline was utilized as a positive control group. The filtrate bottles allow us to calculate the biogas production by subtracting the biogas production of inoculum and materials minus inoculum itself. However, a control group such as filtrate does provide enough information to verify inoculum performance; therefore, a positive control must be prepared. The positive control allows to verify the fitness of the inoculum for testing.

A total amount of 300 mL per bottle is prepared. From that 300 mL blend, 150 mL was sealed in a bottle with a septum and an aluminum crimp, while remaining 150 mL was retained for pre digestion analysis. The pre digestion samples were preserved in a refrigerator at 4°C. All blends were prepared on a mass basis rather than a volume basis. The bottles are flushed with nitrogen at a flowrate of 750 mL per minute for 10 minutes and placed into their respective temperature

profiles. After two hours, gas was released from the bottles and time was recorded as starting time. The BMP bottles were sealed and monitored for 30 days.

## 3.3.2 Operation and Monitoring

Gas production was measured either daily or every other day using a 10-, 30-, 50- or 100mL glass syringe. Syringe volume selection was based on prior day reading or estimated guess between time elapsed between previous reading. Gas composition was analyzed using a HayeSep D column in an SRI 8610 Gas Chromatograph with a flame ionization detector (FID) and thermal conductivity detector (TCD). Gas chromatography was performed weekly, and the following parameters were measured: CH<sub>4</sub>, CO<sub>2</sub>, N<sub>2</sub> and H<sub>2</sub>S. The sample was taken from each individual bottle's headspace after daily gas measurement had been performed. After 30 days, the bottles were uncapped, and post digestion analysis were performed in the digested sample.

# 3.3.3 BMP Calculations

Raw gas is measured in a lab maintained at  $22^{\circ}$ C and is assumed saturated. Gas is normalized for standard temperature (0°C) and pressure (1 atm) (STP) using the Equation 1.

$G_{STP} = G_R \times$	< 0.897	(1)
Gstp	gas normalized for standard temperature and pressure, mL	
G <sub>R</sub>	raw gas production, mL	
0.897	STP conversion factor for conditions in East Lansing, MI	

Each bottle's biogas production is normalized to the control bottles that contain only filtrate and DI water using Equation 2.

$$G_N = G_{STP} - \frac{Control_1 + Control_2 + Control_3}{3}$$
(2)

G<sub>N</sub> normalized gas production, mL

Control <sub>1</sub>	biogas production from control 1, mL
Control <sub>2</sub>	biogas production from control 2, mL
Control <sub>3</sub>	biogas production from control 3, mL

The VS content is calculated for the bottles based on the VS of the raw sample using

# Equation 3.

$$VS_N = VS_R \times S \times \frac{1}{1000}$$
(3) $VS_N$ volatile solids content in the bottle, mg $VS_R$ volatile solids content of the raw sample, mg/kgSmass of sample in the bottle, g $1/1000$ conversion factor, kg/g

The biogas content of the respective bottles (BMP<sub>i</sub>) was found by using Equation 4.

$$BMP_i = \frac{G_N}{VS_N} \tag{4}$$

i bottle number

The triplicate bottles are then averaged using Equation 5.

$$BMP = \frac{BMP_1 + BMP_2 + BMP_3}{3} \times \frac{1}{1000} \times 10^6$$
(5)

BMP biochemical methane potential, L biogas/kg initial VS

- 1/1000 conversion factor, L/mL
- 10<sup>6</sup> conversion factor, mg/kg

# 3.4 Pilot systems design, operation, and analysis.

Three temperature profiles were explored during the pilot systems. As seen in Table 3.3, three temperature profiles were evaluated in duplicate. The pilot systems were not mixed. Pilots 1,2, 5 and 6 were maintained in control temperature rooms while pilots 3 and 4 were left in an uncontrolled temperature area. The data was collected for three, 45-day HRT's.

Pilots	Temperature
1	10°C
2	19 C
3	$0^{\circ}$ C to $28^{\circ}$ C
4	9°C to 28°C
5	30°C
6	39 C

 Table 3.3. Pilots Temperature Profiles

#### 3.4.1 Pilot Vessel/Structure

The pilot systems are cylindrical shaped made from polyvinyl chloride (PVC) piping with dimensions of 6-inch diameter and 7.5-inch height. A PVC flange socket was fixed as the top of the digester. A gasket was placed on the flange socket and a flange cap was bolted down. A wall PVC pipe cap was utilized at the bottom of the digester. In the digester flange cap, three holes were drilled in order to insert U tube waste pressure gage, gas output line and gas bag. Both gas output lines were connected to valves in order to open and close when needed. The gas output line was connected to a Wet Tip Gas Meter with a digital counter. The tips counted represented a specific volume of gas produced. This volume was known due to calibration procedures for these systems. A third ball cap valve was drilled and inserted in the middle of the pilot in order to feed and waste. The set up for the pilot systems is presented in Figure 3.1.



Figure 3.1. Pilot Set Up

# 3.4.2 Pilot Preparation

All pilots were cleaned with phosphorus free soap and rinsed with DI water. All pilots were tested for water leakage. The tip meters were calibrated utilizing a 150 mL syringe and introducing air until a consistent air volume would produce a tip. Connecting lines from the pilot to tip meter lines were measured in order to make sure all lines had an equal length of 53 cm.

# 3.4.3 Pilot Set Up and Seeding

Filtrate was collected from MSU SCAD. The following analysis were performed on the sample: pH, conductivity (EC), total solids (TS) and volatile solids (VS). A volume of 2,000 grams was weighted and utilized as inoculum for all six pilots. The six digesters were filled on Friday, July 24, 2020. The pilots were allowed to stabilize over the weekend while issues with leakage and tip meters were fixed. On Monday, July 27, 2020, after all pilots had stable pressures and readings, the pilots received their first feeding.

#### 3.4.4 Pilot Feeding

Cow manure from a local dairy farm was collected bi-weekly and used as feeding during a two-week period. The following analysis were performed on the sample after every collection: pH, EC, TS and VS. Based on a 45-day HRT, manual feedings were scheduled three times a week, every Monday, Wednesday, and Friday.

# 3.4.4.1 Feeding Volume

Feeding volume was calculated using Equation 6.

$$F_{\nu} = \frac{V}{\theta} * \frac{7 \text{ days}}{t}$$
(6)  

$$F_{\nu} \qquad \text{feeding volume, mL/day}$$

$$V \qquad \text{reactor volume, mL}$$

$$\theta \qquad \text{hydraulic retention time, days}$$

$$t \qquad \text{days of the week allotted for feeding, days}$$

# 3.4.4.2 Calculations

$$\frac{2,000 \text{ mL reactor volume}}{45 \text{ days HRT}} = 44.44 \frac{\text{mL}}{\text{day}} \approx 44 \frac{\text{mL}}{\text{day}}$$
$$\frac{44.44 \text{ mL}}{\text{day}} * \frac{7 \text{ days}}{1 \text{ week}} = 311.08 \text{ mL/week}$$
$$\frac{311.08 \text{ mL}}{\text{week}} * \frac{\text{week}}{3 \text{ days}} = 103.69 \frac{\text{mL}}{\text{week}} \text{ so } \approx 104 \text{ mL waste/feed}$$

# 3.4.4.3 Procedure

During the first week, raw cow manure was fed daily. After the first week of monitoring system stability, pH and gas production, feeding was reduced to 3 days a week: Monday, Wednesday and Friday with feedings all occurring roughly around 1 PM. All six pilots were wasted

and respectively fed 104 g of raw cow manure. The wasting and feeding occurred by weight measurements rather than by volume in order to minimize reading error between lab personnel. As a necessary precaution, the waste was measured for pH during every schedule feed. If pH was below 7.00, then the respective pilot would be buffered with 5% bicarbonate solution.

## 3.4.5 Pilot Monitoring

Pilots are monitored every day for pressure and gas production. During feedings, time, temperature, number of tips and pressure control was recorded.

## 3.4.6 Digestate

The digestate collected was tested for pH and EC with every feeding. The digestate collected every Wednesday was tested for TS, VS and SCOD. All remaining digestates were preserved in a refrigerator at 4°C categorized by pilot and by date.

# 3.4.7 Gas

#### 3.4.7.1 Gas Production

Cumulative gas production was calculated using Equation 7.

$$G_C = T * C \tag{7}$$

Gc	cumulative gas production, mL
Т	number of tips
С	calibration of tip meter, mL

#### 3.4.7.2 Gas Analysis

Gas analysis was performed weekly to record N<sub>2</sub>, CH<sub>4</sub>, CO<sub>2</sub>, and H<sub>2</sub>S. To collect a sample for GC analysis a 5 mL SGE Analytical Science syringe was used. The syringe was connected to the gas sampling port on the gas output line before the tip meter. Once connected, the syringe was flushed by pulling and plunging slowly three times. Five mL of sample was then drawn into the syringe and the syringe was connected to the gas chromatograph. This procedure was repeated three times for each individual pilot.

# **3.5 Statistical Analysis**

Statistical analysis was performed in the BMP and pilot data collected. A one-way ANOVA was utilized to calculate statistical parameters in the BMP data, while a two-way ANOVA was performed in the pilot data utilizing the analytical software R-4.0.3.

For the BMP data, a one-way ANOVA was performed for each temperature profile using the aov function. The following parameters were analyzed utilizing the software for the BMP: cumulative gas production, CH<sub>4</sub> concentration, and pre, post, destruction, and reduction of TS and VS. The codes utilized for pilot data can be found in Appendix A.

For the pilots, the analysis was performed for each HRT (1, 2, & 3) and the variations in temperature (L, B, H) using the R function aov. The following parameters were analyzed utilizing the software for the pilots: daily gas production, daily gas production per kg of vs, CH<sub>4</sub> concentration, hydrogen sulfide concentration, TS reduction and VS reduction. The codes utilized for pilot data can be found in Appendix B.

On all the ANOVA results, the Tukey pairwise comparison was performed to find statistically significant differences between the various operational parameters via the R function TukeyHSD.

# 4. CHARACTERIZATION AND BIOCHEMICAL METHANE POTENTIAL

# **4.1 Characteristics of Raw Samples**

Filtrate and dairy cow manure were collected prior to every BMP trial between the months of January and June. Microcrystalline cellulose was utilized during the trials to provide a positive control. Raw samples were tested for TS and VS. Table 4.1 presents the average with the respective standard deviations for the samples obtained. The number of samples (n) were averaged together to obtain the results presented in Table 4.1. For the filtrate samples, the TS ranged from 38,655 to 54,900 mg per L, while the VS ranged from 24,770 to 38,780 mg per L. The TS in manure samples ranged from 28,780 to 50,075 mg per L, while the VS ranged from 20,405 to 32,450 mg per L.

**Table 4.1. Raw Characterization** 

		TS	VS	TS	VS	
	Sample	( <b>mg/L</b> )	( <b>mg/L</b> )	(mg/kg)	(mg/kg)	n
	Seed	48,198±5,514	32,497±5,710	47,922±5,676	32,318±5,799	9
	Microcrystalline cellulose	1,017,250±31,419	1,017,207±31,455	958,989±50	958,947±58	3
	Cow Manure	37,157±9,102	24,963±5,237	37,035±9,065	24,881±5,214	9

# **4.2 BMP Test Results**

BMP was performed in order to compare the anaerobic biodegradability of dairy cow manure at different temperature profiles. The liquid cow manure was tested along with a control and a positive control in three separate BMP trials. The positive control was utilized as a form to assure the appropriate performance of the inoculum for BMP testing. Each BMP was tested in triplicate during each trial. All the statistical analysis' codes and results are presented in Appendix A. Additional data tables regarding individual trials are presented in Appendix C.

#### 4.2.1 Pre and post digestion analysis

Pre- and post-digestion analyses were carried out individually on every BMP bottle. The pre- and post-digestion analyses served as a comparison for the anaerobic biodegradability of dairy cow manure at different temperature profiles. Table 4.2 shows the pre- and post-digestion TS content, the TS reduction, and the percent reduction. The number of samples (n) were averaged together to obtain the results presented in table 4.2 and table 4.3. The pre-TS for all runs was approximately 13,000 mg per L. The post-TS ranged between 10,000 to 13,000 mg/L. The percent reduction average for 15°C non-mixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed were 5, 12, 16, 18 and 21%, respectively.

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Reduction Average ± Std. Dev. (mg/L)	Reduction Average ± Std. Dev. (%)	n
15°C, Non-Mixed	13,255±2,055	12,651±2,110	604±260	5±2	18
20°C, Non-Mixed	13,804±2,042	12,229±2,245	1,575±606	12±5	18
30°C, Non-Mixed	13,342±1,659	11,142±1,449	2,200±448	16±2	18
39°C, Non-Mixed	13,356±1,547	10,910±1,138	2,446±667	18±4	18
39°C, Mixed	13,371±1,795	10,507±1,210	2,864±846	21±4	18

Table 4.2. Pre- and post-digestion TS content in BMP bottles, Average of Trials 1, 2 & 3

Figure 4.1 presents the percent average reduction with the respective standard deviation for the TS reduction presented in Table 4.2. The letter category A through E represent 15°C nonmixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed, respectively. Mesophilic temperatures provided a greater TS reduction than the psychrophilic temperatures. The highest reduction was on the 39°C mixed BMP followed by 39°C non-mixed and 30°C non-mixed, respectively.



Figure 4.1. Percent Average Reductions with Standard Deviations for Total Solids in BMP bottles, Average of Trials 1, 2 & 3

Table 4.3 shows the pre- and post-digestion VS content, the VS reduction, and the percent reduction. The pre-VS for all runs was approximately 9,400 mg per L. The post-VS ranged between 6,000 to 9,000 mg per L. The percent reduction average for 15°C non-mixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed were 9, 17, 25, 28 and 32%, respectively.

Sample	Pre-digestion Average ± Std. Dev. (mg/L)	Post-digestion Average ± Std. Dev. (mg/L)	Reduction Average ± Std. Dev. (mg/L)	Reduction Average ± Std. Dev. (%)	n
15°C, Non-Mixed	9,498±1,678	8,741±1,842	757±232	9±4	18
20°C, Non-Mixed	9,871±1,752	8,279±1,807	1,591±411	17±5	18
30°C, Non-Mixed	9,430±1,490	7,032±1,161	2,398±401	25±2	18
39°C, Non-Mixed	9,446±1,393	6,719±1,046	2,727±427	28±2	18
39°C, Mixed	9,490±1,600	6,447±944	3,043±746	32±3	18

Table 4.3. Pre- and post-digestion VS content in BMP bottles, Average of Trials 1, 2 & 3

Figure 4.2 presents the percent average reduction with the respective standard deviation for the VS reduction presented in Table 4.3. The letter category A through E represent 15°C nonmixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed, respectively. Similar observations are presented with respect to TS reduction, the highest reduction was on the 39°C mixed BMP followed by 39°C non-mixed and 30°C non-mixed, respectively.



Figure 4.2. Percent Average Reductions with Standard Deviations for Volatile Solids in BMP bottles, Average of Trials 1, 2 & 3

Table 4.4 contains the pre- and post-digestion average pH characteristics and change presented between samples for all three trials. The ideal pH for anaerobic digestion is between 6.8 and 7.2. Any pH below 6.8 indicates inhibition in biogas quantity and quality due to the presence of an acidic environment unfavorable towards the methanogenic microbial community. The pre- and post-digestion samples for all three trials had pH readings in the optimal range for anaerobic digestion. Therefore, indicating a stable environment during the BMP test, which indicates no occurrence of inhibition correlated to temperature and lack of mixing (Liu et al., 2008).

Sample	Pre-digestion Average ± Std. Dev.	Post-digestion Average ± Std. Dev.	Change Average ± Std. Dev.	n
15°C, Non-Mixed	7.62±0.09	7.17±0.19	$0.44\pm0.11$	9
20°C, Non-Mixed	7.57±0.08	7.14±0.15	0.43±0.08	9
30°C, Non-Mixed	7.59±0.11	7.30±0.20	0.30±0.16	9
39°C, Non-Mixed	7.58±0.13	7.33±0.17	0.25±0.08	9
39°C, Mixed	7.55±0.15	7.29±0.21	0.26±0.10	9

Table 4.4. Pre- and post-digestion pH in BMP bottles, Average of Trials 1, 2 & 3

Figure 4.3 presents a visual representation of the average pH change with the respective standard deviations for the VS reduction presented in Table 4.4. The letter category A through E represent 15°C non-mixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed, respectively.



Figure 4.3. Average pH Change with Standard Deviations in BMP bottles, Average of Trials 1, 2 & 3

The statistical analysis presented in Appendix A, Section A.3, Section A.4 and Section A.5, provide a better insight in the correlation between the data obtained for all trials and the

characteristics analyzed. Table 4.5 contains the results of the one-way ANOVA performed for the percent TS reduction for all three BMP trials. There was a significant effect of TS reduction on the BMP trials based on temperature at the p<0.05 level for the five conditions [F (4, 40) = 26.46, p = 0.000].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	4	1,517.6	379.4	26.46	0.000
Residuals	40	573.6	14.3		

Table 4.5. One Way ANOVA Results for the Total Solids Reduction in BMP bottles

Figure 4.4 shows the results of the Tukey statistical tests utilized as a pairwise comparison of TS reductions between BMP conditions. The Tukey analysis for the TS reductions present no significant difference (p value>0.05) when comparing 30°C non-mixed; 39°C non-mixed; and 39°C mixed. Any confidence intervals that do not contain 0 provide evidence of a statistical difference in the groups.

#### 95% family-wise confidence level



Differences in mean levels of Temp

Figure 4.4. Tukey Honest Significant Difference Results for the percent TS reduction, Average of Trials 1, 2 & 3

Table 4.6 presents the results of the one-way ANOVA performed for the VS reduction for all three BMP trials. There was a significant effect of VS reduction on the BMP trials based on temperature at the p<0.05 level for the five conditions [F (4, 40) = 63.7, p = 0.000].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	4	3,263	815.6	63.7	0.000
Residuals	40	512	12.8		

Table 4.6. One Way ANOVA Results for the Volatile Solids Reduction in BMP bottles

Figure 4.5 provides the results of the Tukey statistical tests when comparing each BMP condition with respect to one another for VS reductions. The Tukey analysis for the VS reductions provided in summary no significant difference (p-value >0.05) when comparing 30°C non-mixed to 39°C non-mixed to 39°C mixed. There was a statistical significance when comparing 30°C non-mixed to 39°C mixed.

#### 95% family-wise confidence level



Figure 4.5. Tukey Honest Significant Difference Results for the percent VS reduction, Average of Trials 1, 2 & 3

Table 4.7 presents the results of the one-way ANOVA performed for the pH change for all three BMP trials. There was a significant effect of pH on the BMP trials based on temperature at the p<0.05 level for the five conditions [F (4, 40) = 6.069, p = 0.000].

Table 4.7. One Way ANOVA Results for the pH Change in BMP bottles

Figure 4.6 presents the results of the Tukey statistical tests when comparing each BMP condition with respect to one another for pH changes. The Tukey analysis for the pH change provided no significant difference (p value >0.05) for the pairwise comparison of 30°C non-mixed; 39°C non-mixed; and 39°C mixed.





Differences in mean levels of Temp

Figure 4.6. Tukey Honest Significant Difference Results for the pH change, Average of Trials 1, 2 & 3

## 4.2.2 Gas Production

The average cumulative biogas production from the BMP test demonstrated that during a 30-day test is presented in Table 4.8. The average cumulative biogas production in L per kg initial VS for 15°C non-mixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed were 86, 168, 440, 475 and 448, respectively. As presented in Table 4.8, 39°C non-mixed produced relatively closely the same biogas volume as 39 °C mixed. The 30°C non-mixed BMP produced approximately 80 mL less of biogas volume when compared to 39°C either mixed or non-mixed.

Table 4.8. Cumulative Biogas Production in BMP Bottles, Average of Trials 1, 2 & 3

Sample	Cumulative Biogas Production ± Std. Dev. (mL)	Cumulative Biogas Production ± Std. Dev. (L/kg Initial VS)	n
15°C, Non-Mixed	37±20	86±8	58
20°C, Non-Mixed	71±43	168±8	58
30°C, Non-Mixed	232±138	440±14	58
39°C, Non-Mixed	316±133	475±40	61
39°C, Mixed	307±130	448±29	61

Figure 4.7 presents the average cumulative biogas production in mL with the respective standard deviations for the values presented in Table 4.5 during all three trials. The letter category A through E represent 15°C non-mixed, 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed, respectively.



Figure 4.7. Average Cumulative Biogas Production in BMP bottles, Average of Trials 1, 2 & 3

Table 4.9 presents the results of the one-way ANOVA performed on the data for the three BMP trails. There was a significant effect on the cumulative biogas production for the BMP trials based on temperature at the p<0.05 level for the five conditions [F (4, 276) = 92.67, p = 0.000].

	<b>Table 4.9. O</b>	ne Way ANC	<b>OVA Results for</b>	the Cumulative	<b>Biogas Produc</b>	ction in BMP bottl
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	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	4	4'175,174	1'043,794	92.67	0.000
Residuals	276	3'108,698	11,263		

Figure 4.8 presents the Tukey statistical analysis with respect to the cumulative biogas production. From the analysis, there seems to be no statistical significance (p-value>0.05) between the mesophilic conditions with or without mixing.

#### 95% family-wise confidence level



Differences in mean levels of Temp

Figure 4.8. Tukey Honest Significant Difference Results for the Cumulative Biogas Production, Average of Trials 1, 2 & 3

Figures 4.9, 4.10 and 4.11 present the cumulative biogas plot lines for each of BMP trials performed. Gas volumes are corrected for change in temperature. Gas is counted at 22°C and corrected to STP (0°C, 1 atm) by utilizing Equation 1 in Section 3. As shown in the figures, the plot lines for 30°C non-mixed, 39°C non-mixed and 39°C mixed provided very similar results with respect to biogas production. Therefore, provide intriguing results if whether 39°C is actually necessary for the ultimate production of biogas.



Figure 4.9. Cumulative Biogas Production (Average of Triplicates) for Trial 1



Figure 4.10. Cumulative Biogas Production (Average of Triplicates) for Trial 2



Figure 4.11. Cumulative Biogas Production (Average of Triplicates) for Trial 3

## 4.2.3 Methane Concentration

The CH<sub>4</sub> concentration was measured using gas chromatography as explained in Section 3.3.2. Table 4.10 presents the average CH<sub>4</sub> concentrations, and the standard deviations collected during the 30-day BMP trial. The average methane content for  $15^{\circ}$ C non-mixed,  $20^{\circ}$ C non-mixed,  $30^{\circ}$ C non-mixed, and  $39^{\circ}$ C mixed were 21, 34, 53, 54 and 54%, respectively.

 Table 4.10. Methane Concentration in BMP bottles, Average of Trials 1, 2 & 3

Sample	Methane ± Std. Dev. (%)	Min (%)	Max (%)	n
15°C, Non-Mixed	21±10	3	39	12
20°C, Non-Mixed	34±12	16	54	12
30°C, Non-Mixed	53±3	44	57	12
39°C, Non-Mixed	54±2	51	58	12
39°C, Mixed	54±2	51	58	12

Figure 4.8 presents the average methane content the average CH<sub>4</sub> concentrations, and the standard deviations collected during the 30-day BMP trials. The BMPs samples at mesophilic temperatures with or without mixing (30°C non-mixed, 39°C non-mixed and 39°C mixed) produced the highest values of CH<sub>4</sub> concentrations with similar standard deviations, while the BMPs at psychrophilic temperatures without mixing provided lower concentrations of CH<sub>4</sub>.



Figure 4.12. Average Methane Content in BMP bottles, Average of Trials 1, 2 & 3

Table 4.11 presents the results of the one-way ANOVA performed on the data for the three BMP trails. There was a significant effect of methane content on the BMP trials based on temperature at the p<0.05 level for the five conditions [F (4, 55) = 51.7, p = 0.000].

Table 4.11. One Way ANOVA Results for Methane Content in BMP bottles

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	4	10,725	2,681.3	51.7	0.000
Residuals	55	2,825	51.9		

Figure 4.13 presents the results of the Tukey statistical analysis performed between BMP conditions for methane content. Based on the results shown from the Tukey analysis, there seems

to be no statistical significance (p-value>0.05) between the mesophilic conditions with or without mixing; additionally, there is a significant difference (p value<0.05) between 15°C non-mixed and 20°C non-mixed.



#### 95% family-wise confidence level



# Figure 4.13. Tukey Honest Significant Difference Results for the Cumulative Biogas Production, Average of Trials 1, 2 & 3

Figures 4.14, 4.15, and 4.16 present the average CH<sub>4</sub> concentrations with standard deviations for each of the BMP trials. As shown in the figures, the plot lines for 30°C non-mixed, 39°C non-mixed and 39°C mixed provided very similar results with respect to CH<sub>4</sub> concentration. The 15°C non-mixed and 20°C non-mixed provided an increasing trend with respect to time.



Figure 4.14. Biogas Methane Content (Average of Triplicates) for Trial 1



Figure 4.15. Biogas Methane Content (Average of Triplicates) for Trial 2



Figure 4.16. Biogas Methane Content (Average of Triplicates) for Trial 3

# **4.3 Discussion**

Performance data is limited on unheated and unmixed covered lagoon digesters. The lack of supplemental heating or mixing has created a misconception that there is reduction in biogas quantity and quality, therefore favoring other digester types such as the CSTR. Digesters such as the CSTR require supplemental mixing and heating systems according to standard practice. By including supplemental heating and mixing systems, the capital and operating costs can escalate to a great extent therefore creating deter to investors. The vast monetary investment provides unattractiveness to this waste management solution if covered lagoons are not presented as a possible solution.

Digester temperatures as discussed previously have three major categories: psychrophilic, mesophilic, and thermophilic. Thermophilic temperature was not studied in this research due that is mainly utilized in wastewater treatment plants. Mesophilic digesters have been heavily studied
in literature and throughout literature 37°C to 39°C has been presented not only as the ideal temperature for digester operations but it has become standard practice in the field, resulting in the lack of research for temperature variations.

Standard BMP protocols are considered ideal scenario situations due that they are constantly mixing and maintained at constant temperatures of approx. 39°C during the 30-day trail. BMP trials have been used mainly to determine the ideal biodegradability of the material. For purposes of this analysis, two parameters were modified: temperature and mixing. During the trials, constant mixing for was maintained for one BMP category while the remaining four categories were maintained non-mixed. The key reason for doing this is analyzing the impacts of temperature and mixing in the biodegradability of dairy cow manure. Therefore, creating a preliminary analysis about whether the effects of temperature and mixing in anaerobic digestion do support the ideology of 39°C and constant mixing is ideal for biogas acquisition.

Four factors were analyzed between the five categories: pH, TS and VS reduction, biogas quantity and quality. All samples demonstrated to be anaerobically biodegradable, but there were key differences discovered between psychrophilic and mesophilic temperatures with respect to biogas quality and quantity. For all the samples, pH was maintained in the ideal ranges and therefore discarding the idea of negative effects on pH based on temperature.

TS and VS reductions provide clear answer with respect to the biodegradability of the material. The reduction in TS was above 10% for four categories: 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed. The highest TS reduction occurred at 39°C mixed. The statistical analysis demonstrated no significant difference (p-value>0.05) between 30°C non-mixed, 39°C non-mixed, and 39°C mixed. TS reduction did occur in all the samples, therefore, providing the basis for biogas to be produced. It can be concluded that under anaerobic conditions

between psychrophilic and mesophilic temperatures, destruction of TS can occur. There is variation in the concentrations destroyed; but above 20°C, the destruction seems to be relatively similar even with higher variations in temperature.

The VS reduction was highest for 39°C mixed and followed subsequently by 39°C nonmixed, 30°C non-mixed, 20°C non-mixed, 15°C non-mixed, respectively. The reduction in VS was above 10% for four categories: 20°C non-mixed, 30°C non-mixed, 39°C non-mixed, and 39°C mixed. The highest VS reduction was presented in 39°C mixed. The statistical analysis demonstrated no significant difference (p value>0.05) between 30°C non-mixed, 39°C non-mixed, and 39°C mixed. All the bottles precented a percentage of VS reduction and it presented an increase with increase in temperature and the addition of mixing. The destruction of VS demonstrates the ability to produce biogas; therefore, biogas production is still possible between mesophilic and psychrophilic variation

Cumulative biogas production was observed for the scenarios during this experiment. The category with highest production was 39°C non-mixed followed by 39°C mixed and 30°C non-mixed, respectively. The psychrophilic temperatures, 15°C and 20°C, presented approx. 25% or less biogas production than bottles maintained at mesophilic temperatures with or without mixing. According to the statistical analysis, there was no statistical significance (p-value>0.05) when comparing 30°C non-mixed, 39°C non-mixed, and 39°C mixed. This indicates that the cumulative biogas production was following similar performance. The plot lines presented in this section also provide a visual perspective of both numerical and statistical analysis.

The biogas quality observed in all the mesophilic bottles was above 45% CH<sub>4</sub>, which is considered the ignitable minimum for biogas. When comparing 30°C non-mixed, 39°C non-mixed, and 39°C mixed, all these bottle categories produced an average of approx. 53% of CH<sub>4</sub>

concentration with standard deviation of approx. 2%. Psychrophilic bottles, 15°C and 20°C, produced average CH<sub>4</sub> concentrations below the 45% threshold. Although CH<sub>4</sub> is still available within the sample, lower quality biogas would require higher energy inputs to upgrade into a gas sample of 90% CH<sub>4</sub>.

A parameter altered from the standard BMP scenarios was mixing. When comparing the results obtained from this analysis, in an ideal scenario such as BMPs, mixing appears to not provide a difference in biogas quantity and quality when compared between 30°C and 39°C with or without mixing. When comparing TSVS reduction's mixing appeared to provide a greater reduction in TSVS concentrations at higher temperatures. Mixing for purposes for this experiment was treated as a binary scenario. In industry, mixing throughout digesters depending on type and operational parameters is treated as an individualized operational parameter specific to that individual digester. It is difficult from three trials and treatment as a binary component to produce a final decision on whether mixing is crucial or not for biogas quality and quantity. Due to industry scenarios, expert advice and time constraints, mixing will not be utilized as a parameter in the pilot scale testing. Mixing might not have an influence in small ideal scenarios; but it might be a parameter to consider in larger scale research with additional testing parameters and requirements. For purposes of subsequent sections, mixing was not considered or implemented during the testing to in the end be able to compare and focus on temperature variations in systems without supplemental heating systems to represents similarities to covered lagoons.

With the results presented in section 4, the idea that an additional 9°C within digester temperature might not be necessary in order to obtain higher biogas quantity and quality. The BMPs provided no significance difference (p-value>0.05) when comparing temperature profiles within the mesophilic range. For a CSTR, heating requirements for a digester is an economic

intensive activity and therefore if 39°C are not necessarily required for ultimate biogas quality and quantity, lowering the digester to a lower temperature would reduce the energy inputs and operational costs of the system. The energy requirement for heat requirements would be reduced by 40% if a digester were run from 35°C to 22°C (Arikan, Mulbry, & Lansing, 2015). In comparison, to heat one liter of water from 5°C to 39°C, it would require an energy input of 0.040 kWh; while to heat it instead to 30°C would require 0.029 kWh. This would represent a theoretical energy reduction of approximately 30% for a change in 9 degrees. On the other hand, it could provide incentives to opt for covered lagoons in environments that will still provide similar biogas quantity and qualities as a CSTR in the same location. Covered lagoons have been believed to not be able to produce the same biogas quality and quantity as a CSTR; but through this BMPs, an initial hypothesis can be presented with respect to this idea. Covered lagoons might be able to provide the same biogas quality and quantity if an operating temperature of 30°C can be maintained to promote the growth of methanogens at the lower end of the mesophilic range.

The next chapter will introduce the testing of small-scale pilots to analyze this hypothesis. The following chapter and testing will provide a greater insight on the actual variations in biogas quantity and quality when a non-ideal scenario is introduced. Instead of performing batch testing like BMPs, the pilot testing will function as a continuous reactor where fresh feedstock is introduced to the pilots at regular intervals. The end benefit is analyzing the efficiency to operate a lagoon at lower temperatures than presented in literature while still obtaining the benefits of biogas and GHG emissions reductions. In additions, CSTRs could reduce operational costs by running the system at lower temperatures and directing biogas to other valuable uses or processes.

#### **5. PILOT TESTING**

# **5.1 Purpose and Conditions**

There is a lack of data availability explaining the variations in temperature with respect to biogas production and biogas quality. The BMP results presented in Chapter 4 provide an opportunity to investigate the effects of temperature with respect to these topics. The BMPs testing was utilized to investigate the biodegradability of the dairy manure under ideal conditions at different temperatures and mixing regimens. The pilot testing allowed for comparisons of biogas production from cow manure while trying to represent lagoon conditions with variations in temperature and the lack of supplemental mixing. Three temperature profiles were analyzed during the pilot studies: constant 20°C and 39°C, and unregulated, ambient. Duplicated pilots were operated at each temperature profile. The unregulated pilots were allowed to fluctuate with ambient temperature so biogas production and quality could be analyzed without a controlled environment. Both 20°C and 39°C were maintained in environments with controlled temperature. All six pilots were operated as non-mixed systems due to the results presented in Chapter 4 with the BMPs and due to time constraints. Mixing is a multifaceted process and due to time constraints and the inability to consider mixing as a binary process, it was not considered for aspects of this research. The pilot characteristics and environments are summarized in Table 5.1. Additional supplementary data and graphs presenting biogas production can be found in Appendix D.

Pilots	Condition	Temperature
1 2	Psychrophilic	20°C
3 4	Unregulated	9°C to 28°C
5 6	Mesophilic	39°C

**Table 5.1. Summary of Pilots Testing Conditions** 

# 5.2 Results and discussion

### 5.2.1 Characterization

In this section, the results and discussion for the material characteristics from the pilots such as raw material, effluent pH, TS, and VS reduction will be provided. The number of samples collected (n) were averaged together in order to obtain results delivered in the tables within this section.

#### 5.2.1.1 Dairy Cow Manure

Dairy cow manure was collected biweekly in order to provide sample variety and freshness throughout the duration of the project. A total of 9 manure collections occurred for the project. An average and standard deviation of the raw characterization results are presented in Table 5.2. The pH for the liquid manure ranged from a minimum of 7.34 to a maximum of 8.57. The pH average presented is 7.77. The average TS collected was 44,374 mg per L or 44,026 mg per kg, while average VS collected was 29,005 mg per L or 28,773 mg per kg. The manure collected, with a TS percentage between 3% and 6%, represents similarly the concentrations that would be available in farms interested in utilizing an anaerobic covered lagoon.

 Table 5.2. Dairy Cow Manure Characterization

Sample	рН	TS (mg/L)	VS (mg/L)	TS (mg/kg)	VS (mg/kg)	n
Liquid Dairy Cow Manure	7.77±0.4	44,374±6,907	29,005±3,665	44,026±6,978	28,773±3,705	9

#### 5.2.1.2 Organic Loading Rate

As discussed previously, OLR is the amount of organic material fed into the digester. All the pilots were fed equal mass volume per feeding of 104 grams of liquid cow manure. Due to biweekly collections of fresh feedstocks, variations in OLR occurred over the timeline of the project. Table 5.3 presents the OLR variations per HRT. During the project timeline, the average OLR was 1.45±0.23 g VS/L per day. The minimum OLR was 1 g VS/L per day, while the maximum was 1.78 g VS/L per day.

HRT	Average OLR ± Std. Dev. (kg VS/m³/day)	Min (g/L/day)	Max (g/L/day)	n
1	1.45±0.26	1.00	1.68	23
2	1.51±0.23	1.30	1.78	19
3	1.39±0.18	1.15	1.55	19

Table 5.3. Organic Loading Rate for the Project based on HRT

Typical HRT for CSTR digesters is between 1 to 10 kg VS/m<sup>3</sup>/day. It can be noted that for a CSTR, which is typical at mesophilic temperatures between 37°C and 39°C, the organic loading rate utilized during this experiment was at the lower end of the scale. Any effects due to this will be discussed in the following sections with respect to biogas quantity and quality. For a covered lagoon, typical OLR are between 0 to 0.2 kg VS/m<sup>3</sup>/day, indicating that there was a higher introduction of solids for our systems when compared to typical covered lagoons. A high or low feeding rate can lead to poor performance. High feeding rates can cause an accumulation of volatile fatty acids, while under feeding a system can starve the microbial community of needed energy. Both causing effects in the methanogenic community.

5.2.1.3 pH

pH was measured for every effluent collected during feedings for each of the pilots. Table 5.4 presents the average pH collected for each pilot category. The average pH for psychrophilic (20°C), unregulated and mesophilic (39°C) conditions were 7.42, 7.46, and 7.81, respectively. The minimum pH for all the pilot categories was approximately 7.10; while the maximum was approx. 7.90.

Condition	Average pH ± Std. Dev.	Min	Max	n
Psychrophilic	7.42±0.17	7.11	7.91	120
Unregulated	7.46±0.17	7.14	7.98	120
Mesophilic	7.81±0.12	7.09	8.17	120

Table 5.4. Average pH Effluent Measurements based on Temperature Profile, Average of 3HRT's

Table 5.5 presented the average pH for psychrophilic, unregulated, and mesophilic conditions with respect to HRTs. For the psychrophilic condition, the pH was maintained between 7.30 and 7.50 during the timeline of the project. The second HRT demonstrates slight decreases in pH, but it did not present a concern with regards to inhibition. For both, the unregulated and mesophilic conditions, the pH decreased over the time between HRTs. Overall, none of the pilots during the HRTs fell below a pH of 7.00 indicating the presence of inhibitory conditions.

Table 5.5. Average pH Effluent Measurements based on HRT

HRT	Average pH ± Std. Dev.	Min	Max	n	
	Psychi	ophilic			
1	7.48±0.23	7.11	7.91	46	
2	7.34±0.11	7.18	7.79	36	
3	7.44±0.03	7.39	7.53	38	
	Unreg	gulated			
1	7.63±0.16	7.42	7.98	46	
2	7.41±0.05	7.31	7.56	36	
3	7.31±0.05	7.14	7.39	38	
Mesophilic					
1	7.84±0.17	7.09	8.17	46	
2	7.80±0.06	7.72	8.01	36	
3	7.79±0.05	7.72	7.91	38	

Figure 5.1 presents the pH measurements collected for each pilot. As shown in Table 5.4, Table 5.5 and Figure 5.1, the pH for none of the pilots fell below the 7.0 threshold. The psychrophilic pilots presented a decrease during the first HRT that was associated due to the slow growth of the methanogenic community, but the pH increases and stabilized during the second and third HRT. For the unregulated condition, the pH slowly increased during the timeline of the project; but the pH was maintained above 7.0. If the unregulated condition had been studied for longer HRTs, pH measurements below 7.0 would have probably been detected. The mesophilic pilots presented a stable pH during the timeline of the project. Overall, all the pilots never reached an inhibitory condition and microbial communities were stable during the duration of the project. Temperature did not indicate to cause effects on the pH of the pilots; moreover, disregarding the idea of inhibition occurring and creating a reduction on biogas quality or quantity. Additionally, the results indicate that dairy cow manure has the buffering capacity to maintain pH in the systems without the addition of substrates.



Figure 5.1. pH measurement for Pilot Effluents

#### 5.2.1.2 Total Solids and Volatile Solids Reduction

TS and VS were measured for every effluent sample collected during the duration of the project. The statistical analysis' codes and results performed for TSVS data are included in Appendix B.4. For the statistical analysis, a two-way ANOVA was performed to understand the overall statistical significance of HRT and temperature, while a Tukey Honest Significant Difference statistical analysis was performed in order to demonstrate the pairwise differences between temperature and HRT. The TS and VS reductions were calculated by comparing the TS and VS present in the feedstock to the effluent collected during each feeding. TS and VS reduction is correlated to biogas production and settling.

Table 5.6 represents the average TS reductions for the timeline of the project for each condition. All the pilots indicated an average TS reduction of above 45%. The TS reductions observed for psychrophilic, unregulated, and mesophilic conditions were 48, 57 and 65%, respectively.

Condition	Total Solids Reduction ± Std. Dev. (%)	Min (%)	Max (%)	n
Psychrophilic	48±12	18	69	40
Unregulated	57±6	46	71	40
Mesophilic	65±5	56	76	40

Table 5.6. Total Solids Reduction based on Temperature Profile, Average of 3 HRT's

Table 5.7 presents the variations in TS reduction with respect to HRT for the three conditions. The psychrophilic condition presented an increased in TS reduction between HRTs. The TS reduction for the psychrophilic condition was an average of 45% by the first HRT and increased to an average of 54% by the third HRT. The unregulated and mesophilic condition presented relatively stable reductions between all the HRTs. The unregulated pilots maintained an

average TS reduction between 55% and 62% over the timeline of the project. The mesophilic pilots presented average TS reduction of above 60% for all the HRTs. The psychrophilic pilots presented the lowest TS reduction when compared to unregulated and mesophilic pilots.

HRT	Total Solids Reduction ± Std. Dev. (%)	Min (%)	Max (%)	n	
	Psychi	rophilic			
1	45±7	33	54	14	
2	44±10	26	61	12	
3	54±14	18	69	14	
	Unreg	gulated			
1	55±5	46	63	14	
2	61±6	53	71	12	
3	56±5	47	62	14	
Mesophilic					
1	66±4	59	72	14	
2	69±4	63	76	12	
3	62±5	58	68	14	

Table 5.7. Average Total Solids Reduction based on HRT

Table 5.8 presents the results of the two-way ANOVA performed for the TS reductions for each condition with respect to temperature and HRT. There was a significant effect with respect to TS reduction on the pilots based on temperature at the p<0.05 level for the three conditions [F (2, 111) = 52.276, p = 0.000]. Moreover, there was no significant effect with respect TS reduction on the pilots based on HRT at the p>0.05 level for the three conditions [F (2, 111) = 1.624, p = 0.202]. This indicates that there are differences in the value of TS reductions associated to variations in temperature, but not necessarily to HRT. The results demonstrated a significant interaction (p-value<0.05) between temperature and HRT [F (4, 111) = 5.012, p = 0.000].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	2	6,148	3,074.0	52.276	0.000
HRT	2	191	95.5	1.624	0.202
Temperature: HRT	4	1,179	294.7	5.012	0.000
Residuals	111	6,527	58.8		

Table 5.8. Two Way ANOVA Results for Total Solids Reduction for each Condition

The letter category L, B and H represent the psychrophilic, unregulated, and mesophilic conditions, respectively. The values 1, 2, and 3 represent the first, second and third HRT, respectively. The Tukey analysis performed for the three conditions with respect to TS reduction is presented in Figure 5.2 and 5.3.

#### 95% family-wise confidence level



Differences in mean levels of Temp

# Figure 5.2. Tukey Honest Significant Difference Results for the Total Solids Reduction based on Temperature

Figure 5.2 presents the results of the Tukey analysis with respect to temperature. The statistical analysis indicated a statistical significance (p-value<0.05) between all the conditions

presented when comparing temperature profiles. This indicates that there are differences in the value of TS reductions associated to variations in temperature. Figure 5.3 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there were no statistical differences (p-value>0.05) when comparing TS reductions between all of the HRTs. This indicates that there are no differences in the value of TS reductions associated to variations in HRT.



95% family-wise confidence level

Differences in mean levels of HRT

Figure 5.3. Tukey Honest Significant Difference Results for the Total Solids Reduction based on HRT

Figure 5.4 represents a timeline of the reduction over all the pilots. In Figure 5.4, it is observed that throughout HRTs every pilot category performed relatively equal. In the second HRT, the psychrophilic pilots (1 & 2), as observed in Figure 5.4, presented a sudden decrease in reduction. This decrease in reduction could have been associated to a sudden change in solids present in the feed when compared to previous weeks or the occurrence of a settling event. Overall, the mesophilic condition had the highest TS reduction, indicating higher gas production see in those pilots.



Figure 5.4. Total Solids Reduction for Pilots during project timeline

Table 5.9 presents the average VS reductions measured during the project timeline. All the pilots presented an average reduction of above 50%. The average VS reduction for psychrophilic, unregulated, and mesophilic were 52, 62, and 70%, respectively.

Condition	Volatile Solids Reduction ± Std. Dev. (%)	Min (%)	Max (%)	n
Psychrophilic	52±13	22	76	40
Unregulated	62±6	42	74	40
Mesophilic	70±5	57	80	40

Table 5.9. Volatile Solids Reduction based on Temperature Profile, Average of 3 HRT's

Table 5.10 presents the changes in VS reduction with respect to HRT presented for each condition. The psychrophilic condition presented and increased in VS reduction between HRTs. The psychrophilic VS reduction was an average of 46% by the first HRT and increased to an average of 61% by the third HRT. The unregulated and mesophilic condition presented relatively stable reductions between all the HRTs. The unregulated pilots maintained an average VS

reduction between 58 and 66% over the timeline of the project. The mesophilic pilots presented average VS reduction of above 69% for all the HRTs.

HRT	Volatile Solids Reduction ± Std. Dev. (%)	Min (%)	Max (%)	n	
	Psychrop	hilic			
1	46±7	34	56	14	
2	47±12	22	61	12	
3	61±14	22	76	14	
	Unregul	ated			
1	58±7	42	68	14	
2	66±5	60	74	12	
3	61±4	53	66	14	
	Mesophilic				
1	69±6	57	77	14	
2	74±3	69	80	12	
3	69±4	63	75	14	

 Table 5.10. Volatile Solids Reduction based on HRTs

Table 5.11 presents the results of the two-way ANOVA performed for the VS reductions for each condition with respect to temperature and HRT. There was a significant effect on VS reduction on the pilots based on temperature at the p<0.05 level for the three conditions [F (2, 111) = 53.539, p = 0.000]. Additionally, there was significant effect on VS reduction on the pilots based on HRT at the p<0.05 level for the three conditions [F (2, 111) = 5.338, p = 0.000]. The results demonstrated a significant interaction (p-value<0.05) between temperature and HRT [F (4, 111) = 6.334, p = 0.000].

The Tukey analysis performed for the three conditions with respect to VS reduction is presented in Figure 5.5 and 5.6. Figure 5.5 presents the results of the Tukey analysis with respect to temperature. The Tukey statistical analysis indicated statistical significance (p-value<0.05)

between all the conditions presented when comparing temperature profiles. This indicates that there are differences in the value of VS reductions associated to variations in temperature. Figure 5.6 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there were statistical differences (p-value<0.05) when comparing VS reductions between the first and the third HRT.

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	2	7,041	3,521	53.539	0.000
HRT	2	702	351	5.338	0.006
Temperature: HRT	4	1,666	417	6.334	0.000
Residuals	111	7,299	66		

Table 5.11. Two Way ANOVA Results for Volatile Solids Reduction for each Condition

#### 95% family-wise confidence level



Differences in mean levels of Temp

Figure 5.5 Tukey Honest Significant Difference Results for the Volatile Solids Reduction based on Temperature

### 95% family-wise confidence level



Differences in mean levels of HRT

Figure 5.6. Tukey Honest Significant Difference Results for the Volatile Solids Reduction based on HRT

Figure 5.7 represents a timeline of the reduction for the individual pilots. In both, Figure 5.4 and 5.7, there is a noticeable decrease in both TS and VS reduction for the psychrophilic pilots during the second HRT. This phenomenon could have been associated to the introduction of a dairy manure sample with approx. 25% more TS and VS than previous samples collected or the occurrence of a settling event. Overall, the mesophilic had the highest TS and VS reduction, indicating higher gas production for pilots maintained at that condition.



**Figure 5.7. Volatile Solids Reduction for Pilots during project timeline** 5.2.2 Gas Production

Gas production was measured with a tip meter, as mentioned in the Material and Methods (Section 3). Gas volumes were counted using a tip counter and conversions were made based on the calibration volumes before initializing the experiment. The number of measurements collected (n) were averaged together in order to obtain results delivered in the tables within this section. The statistical analysis' codes and results for the data obtained corresponding to biogas quantity are presented in Appendix B.1. Additionally, supplementary data and graphs corresponding to pilots have been included in Appendix D.

The cumulative biogas production for each condition is presented in Table 5.12. Average cumulative biogas production for psychrophilic, unregulated, and mesophilic condition was 25 L, 31 L and 56 L, respectively. The mesophilic pilots produced the highest cumulative daily biogas production, followed by the unregulated and psychrophilic conditions, respectively.

Table 5.12. Cumulative Biogas Production based on Temperature Profile, Average of 3HRT's

Condition	Cumulative Biogas Production ± Std. Dev. (L)	n
Psychrophilic	25±17	125
Unregulated	31±18	124
Mesophilic	56±36	125

Table 5.13 presents the cumulative biogas production for each condition by each HRT. The psychrophilic condition presented an increase of approximately 12 L between the first and second HRT. During the second and the third HRT, the psychrophilic condition presented and increase of approximately 26 L which is roughly double when compared to the first and the second HRT. The unregulated condition presented an increase of approximately 21 L between the first and second HRT. During the second and the third HRT, the unregulated condition presented and increase of approximately 15 L which was lower when compared to the first and the second HRT. This would indicate in a reduction of biogas production due to temperature changes that can be noted with lower temperatures presented in the second and third HRT. The mesophilic condition presented an increase of approximately 36 L between the first and second HRT. During the second and the third HRT, the unregulated condition presented an increase of approximately 36 L between the first and second HRT. The mesophilic condition presented and increase of approximately 36 L between the first and second HRT. During the second and the third HRT, the mesophilic condition presented and increase of approximately 36 L between the first and second HRT. During the second and the third HRT, the mesophilic condition presented and increase of approximately 60 L which is roughly double when compared to the first and the second HRT. The mesophilic condition presented greater biogas production throughout the timeline of the project when compared to the psychrophilic and unregulated conditions.

HRT Cumulative Biogas Production ± Std. Dev. (L)		n		
	Psychrophilic			
1	8±4	43		
2	20±5	38		
3	46±8	46		
Unregulated				
1	12±7	44		
2	33±5	38		
3	48±4	46		
Mesophilic				
1	18±11	44		
2	54±9	38		
3	114±15	46		

Table 5.13. Average Cumulative Biogas Production after each HRT

Figure 5.8 presents the cumulative biogas production for the individual pilots. The three highest producing pilots were 5, 6 and 4, respectively. For the psychrophilic pilots during the first HRT, there is an initial low biogas production that stabilizes. During the second HRT, a steady increase in biogas production can be observed continuing into the third HRT. The pilots 3 and 4, during the first 500 hours, present close plot lines and similar volumes with regards to biogas production. During the second HRT, both pilots steady increased their cumulative production; and once the third HRT is achieved, both pilots appear to achieve a plateau in biogas production. For pilots 5 and 6, during the first HRT, the pilots presented close plot lines and similar volumes with regards to biogas production. The plot lines for these pilots continued to increase at a stable rate, therefore providing no indication of plateau or inhibition with respect to biogas production. Pilot 6 presents a lower plot line when compared to pilot 5 during the duration of the second and third

HRT. This could have been attributed to a leak or issues that could not be detected with day-today operations or with measurements such as pH and gas chromatography. It can also be noted that the steady increase between the first and second HRT for all pilots could have been associated to higher organic loading rates introduced in the second HRT in comparison to the first HRT.



Figure 5.8. Cumulative Biogas Production for Psychrophilic, Unregulated and Mesophilic Pilots

The daily biogas production for each condition is presented in Table 5.14. Average daily biogas production for psychrophilic, unregulated, and mesophilic condition was 0.9 L, 0.9 L and 1.8 L, respectively. The daily biogas production in L per initial VS for psychrophilic, unregulated, and mesophilic conditions was 324, 291 and 604, respectively. The mesophilic pilots produced the highest average daily biogas production, while the psychrophilic and unregulated conditions produced approximately half the biogas volume as mesophilic condition.

Condition	Daily Biogas Production ± Std. Dev. (L/kg Initial VS)	Daily Biogas Production ± Std. Dev. (L/day)	Min (L/day)	Max (L/day)	n
Psychrophilic	324±161	0.9±0.5	0.16	2.24	127
Unregulated	291±194	0.9±0.6	0.10	3.00	126
Mesophilic	604±276	1.8±0.8	0.20	4.20	126

Table 5.14. Daily Biogas Production based on Temperature Profile, Average of 3 HRT's

Table 5.15 presents the daily biogas production based on HRTs presented by each condition. For the psychrophilic condition, the daily biogas production increased at a relative steady pace between each HRT. For the unregulated condition, we can notice changes and decrease in biogas production as time progressed. The decreases in biogas production seem to be correlated to the temperature variations in the unregulated conditions associated to seasonal changes. It can also be noted that the unregulated pilots had higher daily biogas production than pilots in the psychrophilic condition when warmer temperatures were presented for the unregulated pilots. During the second HRT, the psychrophilic and unregulated pilots had higher daily biogas production; and in the third HRT, the psychrophilic pilots had higher daily biogas production than the unregulated pilots. During the third HRT, the unregulated pilots were experiencing temperatures around or below 15°C. The mesophilic pilots produced a relatively stable daily biogas production across all HRTs.

HRT	Daily Biogas Production ± Std. Dev. (L/kg Initial VS)	Daily Biogas Production ± Std. Dev. (L/day)	Min (L/day)	Max (L/day)	n	
		Psychrophilic				
1	205±137	0.6±0.6	0.16	1.96	43	
2	316±122	1.0±0.5	0.32	2.24	38	
3	429±130	1.2±0.4	0.24	2.04	46	
	Unregulated					
1	387±193	1.1±0.5	0.48	3.00	44	
2	284±131	0.9±0.4	0.38	2.00	37	
3	189±190	0.5±0.5	0.10	2.70	44	
Mesophilic						
1	593±250	1.7±0.7	0.20	4.20	43	
2	603±295	1.8±0.7	0.21	3.80	38	
3	600±291	1.7±0.6	0.20	3.40	45	

Table 5.15. Daily Biogas Production based on HRTs

Table 5.16 presents the results of the two-way ANOVA performed for the daily biogas production for each condition with respect to temperature and HRT. There was a significant effect on daily biogas production for the pilots based on temperature at the p<0.05 level for the three conditions [F (2, 365) = 77.236, p = 0.000]. This indicates that there are differences in daily biogas production associated to variations in temperature. Moreover, there was no significant effect on daily biogas production for the pilots based on HRT at the p>0.05 level for the three conditions [F (2, 365) = 0.321, p = 0.726]. The results demonstrated a significant interaction (p-value<0.05) between temperature and HRT [F (4, 365) = 10.478, p = 0.000].

The Tukey analysis performed for the three conditions with respect to daily biogas production is presented in Figure 5.9 and 5.10. Figure 5.9 presents the results of the Tukey analysis with respect to temperature. The Tukey statistical analysis indicated statistical significance (p-

value<0.05) when comparing psychrophilic and unregulated conditions to the mesophilic condition; but there was no statistical significance (p-value>0.05) between psychrophilic and unregulated conditions with respect to temperature. Figure 5.10 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there were no statistical differences (p-value>0.05) when comparing daily biogas production between HRTs.

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (< <b>F</b> )
Temperature	2	61.66	30.831	77.236	0.000
HRT	2	0.26	0.128	0.321	0.726
Temperature: HRT	4	16.73	4.183	10.478	0.000
Residuals	365	145.70	0.399		

Table 5.16. Two Way ANOVA Results for the Daily Biogas Production for each Condition

#### 95% family-wise confidence level



Differences in mean levels of Temp

Figure 5.9. Tukey Honest Significant Difference Results for the Daily Biogas Production based on Temperature

## 95% family-wise confidence level



Differences in mean levels of HRT

#### Figure 5.10. Tukey Honest Significant Difference Results for the Daily Biogas Production based on HRT

Table 5.17 presents the results of the two-way ANOVA performed for the daily biogas production per kg initial VS for each condition with respect to temperature and HRT. There was a significant effect on daily biogas production per kg initial VS for the pilots based on temperature at the p<0.05 level for the three conditions [F (2, 365) = 88.756, p = 0.000]. This indicates that there are differences in daily biogas production per kg initial VS associated to variations in temperature. Moreover, there was no significant effect on daily biogas production per kg initial VS for the pilots based on HRT at the p>0.05 level for the three conditions [F (2, 365) = 0.645, p = 0.525]. The results demonstrated a significant interaction (p-value<0.05) between temperature and HRT [F (4, 365) = 12.274, p = 0.000].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> ( <b>&lt;F</b> )
Temperature	2	7'397,233	3'698,616	88.756	0.000
HRT	2	53,740	26,870	0.645	0.525
Temperature: HRT	4	2'045,847	511,462	12.274	0.000
Residuals	365	15'210,220	41,672		

 Table 5.17. Two Way ANOVA Results for the Daily Biogas Production per kg Initial VS

 for each Condition

The Tukey analysis performed for the three conditions with respect to daily biogas production per kg initial VS is presented in Figure 5.11 and 5.12. Figure 5.11 presents the results of the Tukey analysis with respect to temperature. The Tukey statistical analysis indicated statistical significance (p-value<0.05) when comparing psychrophilic and unregulated conditions to the mesophilic condition; but there was no statistical significance (p-value>0.05) between psychrophilic and unregulated conditions with respect to temperature. Figure 5.12 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there were no statistical differences (p-value>0.05) when comparing daily biogas production per kg initial VS between HRTs.

# 95% family-wise confidence level



Differences in mean levels of Temp

Figure 5.11. Tukey Honest Significant Difference Results for the Daily Biogas Production per kg Initial VS based on Temperature

# 95% family-wise confidence level



Differences in mean levels of HRT

Figure 5.12. Tukey Honest Significant Difference Results for the Daily Biogas Production per kg Initial VS based on HRT

Figure 5.13 presents the daily biogas production for the pilots during the project timeline. The 39°C pilots produced roughly twice the volume of biogas when compared to psychrophilic and unregulated pilots.



Figure 5.13. Daily Biogas Production for Psychrophilic, Unregulated and Mesophilic Pilots

Figure 5.14 presents the daily biogas productions for pilot 1 and 2, which were maintained at 20°C. The daily biogas production for these pilots was an average of 0.9 L/day. The slow increase in biogas production might have been related to the effects of temperature in the microbial community. Methanogenesis is one of the major rates limiting processes within anaerobic digestion. At lower temperatures, there is the speculation that the process of hydrolysis does not occur as rapidly as higher temperatures and therefore limiting the material availability for the acetogenic and methanogenic bacteria (Patel, Pandit, & Chandrasekhar, 2017). Although the TS and VS reduction for these pilots was lower than unregulated and mesophilic pilot, these pilots managed to produce the same volume of biogas as the unregulated pilots.



**Figure 5.14. Daily Biogas Production for Psychrophilic Pilots** 

Pilots 3 and 4 were operated with no temperature control. Figure 5.15 represents the daily biogas production for each pilot in the primary axis and the temperature measurements for the room during the duration of the project in the secondary axis. During the first HRT, temperature ranged from 20°C to 28°C, corresponding to the months of July to September. During the second HRT, temperature ranged from 14°C to 23°C, which had fluctuations between the mesophilic and psychrophilic temperature ranges, corresponding to the months of September to October. During the third HRT, we observed temperatures from 9°C to 23°C, corresponding to the months of 0ctober to December. In Figure 5.15, clear decrease in temperature can be seen from the transition of the seasons of summer to fall and beginning of winter.

The overall average daily biogas production for unregulated pilots was 0.9 L/day. The biogas production was maintained relatively similar between the first and second HRT. During the third HRT there was a significant decrease in the daily biogas production which can be related to the transitions of mesophilic temperatures to psychrophilic temperatures between the second and

third HRT. The statistical analysis (Appendix B.1) supported this idea by demonstrating no statistical significance (p-value>0.05) between the first and second HRT, but statistical significance (p-value<0.05) between the first and third HRT. During the first HRT of the psychrophilic and unregulated, the unregulated pilots produced higher daily volumes. During the first HRT, the unregulated pilots were maintained at higher temperatures than 20°C, therefore suggesting that an advantage might have been provided in the microbial development. During the second HRT, psychrophilic and unregulated pilots produced relatively similar daily biogas volumes due to a similar temperature profile in the unregulated to the psychrophilic area. During the third HRT, with sudden changes in temperature due to seasonal changes, the unregulated produced less daily biogas volumes than psychrophilic pilots. The data indicates that biogas production was slowly decreasing through HRTs with a decrease in temperature. In a study presented by Wang et al. (2019), biogas production and CH<sub>4</sub> concentration were affected by disturbances in temperature from 35°C to 20°C. It was observed during the study that severe changes did not occur with biogas quality and quantity until the reactors were maintained at temperatures below 25°C. According to the results presented, although changes were presented to the reactors from 35°C to 20°C, biogas production efficiency and operation stability was maintained by the methanogenic community that had been developed at higher temperatures; but once the reactors were maintained below 20°C, severe decrease in biogas production was observed. This study provides a possibility of the similar occurrences in the unregulated pilots during the third HRT. As observed in Figure 5.15, once a temperature of 15°C was reached, biogas production became compromised in relationship to temperature changes. During the first and second HRTs, the methanogenic community was able to maintain biogas production and operation parameters,

while during the third HRT, the sudden decrease in biogas production can be associated to the inhibition of the metabolic activity of the methanogens.



Figure 5.15. Daily Biogas Production for Unregulated Pilots

Figure 5.16 represents the daily biogas production for pilots 5 and 6 maintained at 39°C. The average daily biogas production for mesophilic pilots was 1.8 L/day, which was approx. double the biogas volume of psychrophilic and unregulated pilots. There was no statistical significance (p-value>0.05) between the HRTs. Biogas production stabilized since the initial weeks of operation. In similarity to the cumulative biogas production graph (Figure 5.4), pilot 6 presents a lower plot line when compared to pilot 5; and as mentioned previously, this could have been attributed to a leak or issues that could not be detected with day-to-day operations or with measurements such as pH and gas chromatography. In conjunction with the finding for TS and VS reduction, it supports the idea that at 39°C, higher reduction correlates to higher biogas production.



Figure 5.16. Daily Biogas Production for Mesophilic Pilots

# 5.2.3 Gas Quality

Gas quality was obtained through weekly gas chromatography. The two gas quality parameters being considered are  $CH_4$  and hydrogen sulfide. The number of samples collected (n) were averaged together in order to obtain results delivered in the tables within this section. The statistical analysis' codes and results for the data obtained corresponding to biogas quality are presented in Appendix B.2 and Appendix B.3 for methane and hydrogen sulfide, respectively.

#### 5.2.3.1 Methane

Table 5.18 present the average, minimum and maximum CH<sub>4</sub> concentrations obtained for the samples collected. All the pilots provided an average CH<sub>4</sub> concentration of above 50% during the entire timeline of the project. The unregulated digesters reached an average of 62% followed by psychrophilic and mesophilic with 61 and 58%, respectively. The unregulated pilots produced the highest methane concentration of 67% over the lifetime followed by psychrophilic and mesophilic with 66% and 62% respectively.

Condition	Methane Content ± Std. Dev. (%)	Min (%)	Max (%)	n
Psychrophilic	61±3	54	66	42
Unregulated	62±2	56	67	42
Mesophilic	58±2	53	62	42

 Table 5.18. Methane Content based on Temperature Profile, Average of 3 HRT's

Table 5.19 presents the methane content over each HRT for each condition. Overall, the methane content for each condition over each HRT averaged to be relatively the same throughout the timeline of the project. The psychrophilic condition and the unregulated condition provided relatively similar methane content of approximately 60% during the three HRTs. The mesophilic pilot in comparison to the other two conditions produced lower methane content of approximately 58% over the timeline of the project.

HRT	Methane Content ± Std. Dev. (%)	Min (%)	Max (%)	n	
	Psychi	rophilic			
1	61±1	54	63	14	
2	62±2	57	66	14	
3	61±4	59	66	14	
Unregulated					
1	63±2	60	66	14	
2	62±2	58	64	14	
3	63±3	56	67	14	
Mesophilic					
1	58±2	53	62	14	
2	57±2	53	60	14	
3	58±1	56	60	14	

 Table 5.19. Methane Content based on HRTs

Table 5.20 presents the results of the two-way ANOVA performed for the CH<sub>4</sub> content for each condition with respect to temperature and HRT. There was a significant effect CH<sub>4</sub> content for the pilots based on temperature at the p<0.05 level for the three conditions [F (2,117) = 46.881, p = 0.000]. This indicates that there are differences in CH<sub>4</sub> content associated to variations in temperature. Moreover, there was no significant effect on CH<sub>4</sub> content for the pilots based on HRT at the p>0.05 level for the three conditions [F (2, 117) = 0.236, p = 0.790]. The results demonstrated no significant interaction (p-value>0.05) between temperature and HRT [F (4, 117) = 0.767, p = 0.549].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	2	554.2	277.09	46.881	0.000
HRT	2	2.8	1.40	0.236	0.790
Temperature: HRT	4	18.1	4.54	0.767	0.549
Residuals	117	691.5	5.91		

Table 5.20. Two Way ANOVA Results for Methane Content for each Condition

The Tukey analysis performed for the three conditions with respect to  $CH_4$  content is presented in Figure 5.17 and 5.18. Figure 5.17 presents the results of the Tukey analysis with respect to temperature. The Tukey statistical analysis indicated statistical significance (pvalue<0.05) when comparing psychrophilic and unregulated conditions to the mesophilic condition; but there was no statistical significance (p-value>0.05) between psychrophilic and unregulated conditions with respect to temperature. Figure 5.18 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there were no statistical differences (pvalue>0.05) when comparing to  $CH_4$  content between HRTs.

# 95% family-wise confidence level



Differences in mean levels of Temp

Figure 5.17. Tukey Honest Significant Difference Results for the Methane Content based on Temperature

# 95% family-wise confidence level



Differences in mean levels of HRT

Figure 5.18. Tukey Honest Significant Difference Results for the Methane Content based on HRT

Figure 5.19 presents the CH<sub>4</sub> concentrations collected per pilot during the project timeline. All the pilots presented CH<sub>4</sub> contents between 50 and 70%. As an overall comparison, as shown in Figure 5.19, psychrophilic and unregulated pilots maintained higher CH<sub>4</sub> content than mesophilic pilots during the second and third HRT. The statistical analysis demonstrated that there is a significance difference between mesophilic pilots with respect to unregulated and psychrophilic pilots with respect to temperature. There was no statistical significance when comparing HRTs within categories.



Figure 5.19. Methane Content from Weekly Gas Chromatography for Psychrophilic, Unregulated and Mesophilic Pilots

Figure 5.20 presents the CH<sub>4</sub> content for pilots 1 and 2 maintained at 20°C. The psychrophilic pilots presented a higher CH<sub>4</sub> concentration than the mesophilic pilots. The psychrophilic pilots presented an average CH<sub>4</sub> concentration of 61%, with a minimum of 54% and a maximum of 66%. Even though psychrophilic pilots had a lower biogas quantity in comparison to mesophilic pilots, these pilots had a higher CH<sub>4</sub> content with respect to mesophilic pilots. During
the first HRT, there is a decrease from approx. 65% CH<sub>4</sub> to 54%. This initial decrease during the first HRT could have also provided an additional form of information to infer the idea that at lower temperature ranges, there is a slower formation of methanogens and therefore influencing both biogas quantity and quality. After the first HRT, an increase and stabilization in CH<sub>4</sub> production occurs during the second and third HRT. From the statistical analysis, no statistical significance (p-value>0.05) was presented between HRTs.



**Figure 5.20. Methane Content for Psychrophilic Pilots** 

Figure 5.21 presents the CH<sub>4</sub> content for pilots 3 and 4 maintained in the uncontrolled temperature room. The average CH<sub>4</sub> content for the unregulated was 62% with a minimum of 56% and a maximum of 67%. The unregulated pilots produced the highest average CH<sub>4</sub> content when compared to psychrophilic and mesophilic pilots. Pilots at 37°C to 39°C are typically recommended for CH<sub>4</sub> production. Hawkes et al. (1984) presented results similar to the ones obtained during this research. The results reported presented less than a 10% difference in CH<sub>4</sub> production between psychrophilic scales pilots maintained at 20°C, 25°C, 30°C and 35°C. Other

studies suggest equal CH<sub>4</sub> production can be achieved at psychrophilic and mesophilic temperatures. Similar CH<sub>4</sub> productions were accredited to the higher biomass yield in digesters operated at lower mesophilic temperatures (Guo et al., 2013; Pandey and Soupir, 2012). Additionally, research has continued to explore the possibility of syntropy occurring within microbial communities in digesters. The CH<sub>4</sub>, CO<sub>2</sub> and hydrogen formed between acetogens and methanogens. Specialized communities of methanogens are able to convert the hydrogen and CO<sub>2</sub> molecules into CH<sub>4</sub> molecules by proton reduction. Therefore, creating biogas with higher CH<sub>4</sub> to CO<sub>2</sub> ratios (Dyksma, Jansen, & Gallert, 2020; Shimada et al., 2011). Even though these studies have been presented and demonstrated by the identification of microbial communities through RNA analysis, evidence has only supported this ideology in thermophilic digesters or in two stage anaerobic processes.

As show in Figure 5.21, CH<sub>4</sub> content fluctuated between measurements and can be correlated to sudden temperature changes from the seasonal change. From the statistical analysis, there was no statistical significance (p-value>0.05) between the HRTs for the unregulated. The TS and VS reduction was relatively similar to mesophilic pilots, probably indicating that even though not as high of biogas production can be achieves at lower mesophilic temperatures, similar biogas quality can be achieved.



**Figure 5.21. Methane Content for Unregulated Pilots** 

Figure 5.22 represents the CH<sub>4</sub> content for pilots 5 and 6 maintained in the mesophilic at 39°C. The mesophilic pilots produced an average CH<sub>4</sub> content of 58%, with a minimum of 53% and a maximum of 62%. The mesophilic pilots although had the highest biogas production, contained the lowest average CH<sub>4</sub> concentration. At higher temperatures, it is estimated that reaction rates are faster than at lower temperatures and therefore achieving TS and VS reductions in a shorter timeline (Kim et al., 2006). There could the possibility of insufficient organic material for the pilots to maintain a high CH<sub>4</sub> concentration and therefore, although TS and VS reduction and high daily biogas production can be achieved, there is a limitation on the gas quality.



**Figure 5.22. Methane Content for Mesophilic Pilots** 

### 5.2.3.2 Hydrogen Sulfide

According to MSU extension, hydrogen sulfide (H<sub>2</sub>S) is "one of the most dangerous gases associated with manure" digestion. CH<sub>4</sub> and CO<sub>2</sub> are usually the main components discussed about biogas. Typical biogas will contain CH<sub>4</sub>, CO<sub>2</sub>, water vapor and H<sub>2</sub>S. Any other material apart from CH<sub>4</sub> will have to be removed or scrubbed in order to obtain renewable natural gas. H<sub>2</sub>S has to be removed whether biogas is being upgraded or converted to electricity due to wear and tear on the engine and air quality concerns. The cleanup of H<sub>2</sub>S can be costly and therefore brings a concern to the possibility of increasing or decreasing with changes in temperature. One of the sources of sulfide production in digesters is the biological concentration of sulfates in the influent. Studies have presented a proportional correlation between organic loading rates and H<sub>2</sub>S concentration, and an inversely proportional correlation between pH and H<sub>2</sub>S. Research discovered that the higher the initial pH of the digester seed, the lower the survival of sulfhate reducing bacteria present and therefore a reduction in the production of H<sub>2</sub>S (Chen et al., 2014). Table 5.21 presents the average H<sub>2</sub>S present in each category. The H<sub>2</sub>S production for psychrophilic, unregulated, and mesophilic was 817, 1,448 and 1,271, respectively.

Condition	Hydrogen Sulfide Content ± Std. Dev. (ppm)	Min (ppm)	Max (ppm)	n
Psychrophilic	817±847	26	2,891	38
Unregulated	1,448±1,370	5	4,390	42
Mesophilic	1,271±1,029	4	3,881	42

Table 5.21. Hydrogen Sulfide Content based on Temperature Profile, Average of 3 HRT's

Table 5.22 presents the  $H_2S$  content per HRT for each condition. The  $H_2S$  in the psychrophilic condition had the highest change in production between the second HRT. The unregulated and mesophilic conditions had steady increases in  $H_2S$  content throughout the project. The unregulated condition had higher  $H_2S$  content in each HRT when compared to mesophilic and psychrophilic conditions.

HRT	Hydrogen Sulfide Content ± Std. Dev. (ppm)	Min (ppm)	Max (ppm)	n		
Psychrophilic						
1	122±129	26	394	10		
2	160±212	193	1,002	14		
3	806±900	423	2,091	14		
Unregulated						
1	223±260	5	707	11		
2	695±749	76	2,327	14		
3	914±1,162	389	4,390	14		
Mesophilic						
1	188±201	4	536	7		
2	427±486	146	1,720	14		
3	672±807	654	3,881	14		

Table 5.22. Hydrogen Sulfide Content based on HRTs

Table 5.23 presents the results of the two-way ANOVA performed for the H<sub>2</sub>S content for each condition with respect to temperature and HRT. There was a significant effect H<sub>2</sub>S content for the pilots based on temperature at the p<0.05 level for the three conditions [F (2,103) = 7.963, p = 0.000]. This indicates that there are differences in H<sub>2</sub>S content associated to variations in temperature. Additionally, there was a significant effect on H<sub>2</sub>S content for the pilots based on HRT at the p<0.05 level for the three conditions [F (2, 103) = 79.964, p = 0.000]. The results demonstrated no significant interaction (p-value>0.05) between temperature and HRT [F (4, 103) = 1.962, p = 0.106].

	Df	Sum Sq	Mean Sq	F Value	<b>Pr</b> (> <b>F</b> )
Temperature	2	8'090,288	4'045,144	7.963	0.000
HRT	2	81'238,768	40'619,384	79.964	0.000
Temperature: HRT	4	3'986,279	996,570	1.962	0.106
Residuals	103	52'321,088	507,972		

Table 5.23. Two Way ANOVA Results for the Hydrogen Sulfide for each Condition

The Tukey analysis performed for the three conditions with respect to  $H_2S$  content is presented in Figure 5.23 and 5.24. Figure 5.23 presents the results of the Tukey analysis with respect to temperature. The Tukey statistical analysis indicated statistical significance (pvalue<0.05) when comparing unregulated and mesophilic conditions to the psychrophilic condition; but there was no statistical significance (p-value>0.05) between unregulated and mesophilic conditions with respect to temperature. Figure 5.24 represents the results of the Tukey analysis with respect to HRT, which demonstrates that there was a statistical difference (pvalue<0.05) when comparing to  $H_2S$  content between HRTs.

# 95% family-wise confidence level



Figure 5.23. Tukey Honest Significant Difference Results for the Hydrogen Sulfide Content based on Temperature

# 95% family-wise confidence level



Differences in mean levels of HRT

Figure 5.24. Tukey Honest Significant Difference Results for the Hydrogen Sulfide Content based on HRT

Figure 5.25 present the hydrogen sulfide content from weekly gas chromatography for the individual pilots. Every pilot presented levels above detection limits after 300 hours of operation. As shown in figure 5.14, hydrogen sulfide had a steady increase during the first and second HRT for all the pilots independently of temperature profile. There is statistical significance presented for all pilots for HRTs indicating drastic changes in H<sub>2</sub>S concentrations as time progressed. The development of hydrogen sulfide is not uncommon for digester. At lower concentrations, methods such as oxygen injections within digesters are availability to reduce this component. It has to be taken into consideration for digester design in order to remove from biogas and diminish the damage to mechanical components.



Figure 5.25. Hydrogen Sulfide Content from Weekly Gas Chromatography for Psychrophilic, Unregulated and Mesophilic Pilots

# **5.3 Summary**

Table 5.24 presents a summary of the results presented in his section. The main purpose of the pilot testing was to compare the biogas production from cow manure while trying to represent lagoon conditions with variations in temperature and the lack of supplemental mixing. Three temperature profiles were analyzed during the pilot studies: constant 20°C and 39°C, and unregulated, ambient.

Do more store	Condition			
rarameters	20°C	Unregulated	39°C	
Temperature (°C)	20	9 to 28	39	
Cumulative Biogas Production $\pm$ Std. Dev. (L)	25±17	31±18	56±36	
pН	7.42±0.17	7.46±0.17	7.81±0.12	
Total Solids Reduction ± Std. Dev. (%)	48±12	57±6	65±5	
Volatile Solids Reduction ± Std. Dev. (%)	52±13	62±6	70±5	
Daily Biogas Production $\pm$ Std. Dev. (L/day)	0.9±0.5	0.8±0.6	1.8±0.8	
Methane Content ± Std. Dev. (%)	61±3	62±2	58±2	
Hydrogen Sulfide Content ± Std. Dev. (ppm)	739±850	1,344±1,389	1,059±1,065	

 Table 5.24. Summary Table for Results obtained based on Temperature Profile

During the duration of the trial, measurements, such as pH, TSVS and GC, were performed in order to compare anaerobic reactions and verify the proper functioning of the digesters throughout the duration of the project. For all the pilots, pH was maintained in the ideal ranges and therefore discarding the idea of negative effects on pH based on temperature. In both the BMP trials and the pilots, low pH was never an issue encountered, therefore, disregarding all idea of inhibition in gas production. In pilot testing, the pH did lower across time in the psychrophilic and unregulated conditions so inhibition might have occurred around a fourth to fifth HRT possibly.

All the pilots presented TSVS reductions during the testing. There was an increase in both TSVS reductions with respect to temperature. All the pilots presented TS reduction of above 45% and VS reductions of above 50%. VS reduction is correlated to biogas production and settling. A higher VS reduction indicates a proportional relationship to biogas production and enhanced digestion. The mesophilic pilots produced the highest reduction in comparison to the other two conditions. Although the other two conditions, psychrophilic and unregulated, produced lower TSVS reductions, biogas production was still observed.

For biogas production, the mesophilic digesters had by almost doubled the biogas production of the other two conditions, psychrophilic and unregulated. The cumulative biogas production of the mesophilic pilots presented stable increase throughout the timeline of the project with a stable daily biogas production. A very interesting aspect was comparing the plot lines for biogas production between the psychrophilic and unregulated conditions. The unregulated pilots were able to provide higher biogas production during the first HRT due that the temperature was higher when compared to psychrophilic pilots. During the second HRT, both conditions were presented with similar temperature profiles and biogas production was relatively similar. During the third HRT, the unregulated pilots were experiencing temperatures below the ones for the psychrophilic pilots and therefore biogas production plummeted for the unregulated pilots. It could be noted that the biogas production for the unregulated pilots was dropping slowly with temperature but once a temperature of 15°C was reached, the biogas production experienced more drastic reduction in biogas production.

For gas quality, two components were analyzed: methane and hydrogen sulfide. The methane content between the conditions presented intriguing results. The unregulated and psychrophilic conditions presented higher methane content when compared to the mesophilic condition. The TS and VS reduction was relatively similar to mesophilic pilots, probably indicating that even though not as high of biogas production can be achieves at lower mesophilic temperatures, similar biogas quality can be achieved. There have been studies presenting the idea that biogas quality can be similar at lower temperatures other than 37 to 39°C. Certain studies have explained mechanisms on why this can occur but there is a lack of general consensus on why this occurs.

Temperature has been studied scarcely in literature and this research was able to provide insight on the idea that digesters without supplemental heating are still viable. Temperature had greater influence in biogas quantity when temperatures below 20°C were presented. There is background to establish the viability of covered lagoons efficiency at lower temperatures than presented in literature while still obtaining the benefits of biogas and GHG emissions reductions. These data have opened the idea to explore more variability in the temperature range for digesters to something other than 39°C which has become standard practice.

## 6. LIFE CYCLE ANALYSIS

## **6.1 Introduction**

Life cycle analysis (LCA) is a tool utilized to evaluate the environmental impacts of goods, processes, or services. ISO 14040 defines the approach by which the environmental impacts and burdens associated with a product can be presented to create an environmentally conscious decision. The approach analyzes the environmental tradeoffs associated with a process or system. This tool can be used to identify the system components that have the highest environmental impact and replace them with solutions, alternatives, or processes that are sustainable and environmentally friendly (Azapagic et al., 2006).

Throughout this assessment, two anaerobic digestion systems will be compared: a continuous stirred tank reactor (CSTR) and a covered lagoon. Additionally, the impact of current manure management systems, which do not use a digester, will be presented during the discussion of impacts. The comparison of a CSTR and a covered lagoon system are based on the biogas and digestate production, water consumption, and electricity consumption. These inputs and outputs represent the major variables that lead to environmental impacts for both scenarios.

### 6.1.1 Supply Chain

The systems' supply chains (Figure 6.1) are closed loops that start with cultivation. The crops are consumed by both humans and cattle. From cattle farming, the "harvest" leads to meat and dairy products that proceed to human consumption, while cattle manure is treated through an anaerobic digester. Anaerobic digestion produces biogas and digestate; biogas will be purified into RNG. The RNG can be utilized by multiple sectors, such as transportation, where it is used to fuel trucks and city buses. The digestate would be separated into liquid and solid fractions. The liquid can be used as fertilizer, while the solids can be used as compost or animal bedding.



Figure 6.1. Anaerobic Digestion Supply Chain system

### 6.1.2 Rural Biorefinery Classification

Under the Cherubini biorefinery classification, the biorefinery presented (Figure 6.2) is classified as two-platform biogas and digestate biorefinery that produces renewable natural gas, fertilizer, and animal/compost bedding from cow manure through anaerobic digestion, upgrading and separation. This classification system helps to differentiate biorefineries based on key components and functions.

This comparison reveals which AD type has the lowest environmental impacts for bioenergy production. There have been various studies about CH<sub>4</sub> production from the AD process; however, comparing the environmental impacts of a CSTR and a covered lagoon have

not been studied. This study provides a new perspective for decision makers to present AD systems as a waste management solution to mitigate the environmental impacts of dairy cow manure. In the end, this study intends to provide more insight about how to utilize livestock manure at its maximum value by producing valuable products, mitigating environmental and health risks, and generating more revenue for the livestock farm industry.



Figure 6.2. Biorefinery Classification for Both Systems: a CSTR and a Covered Lagoon 6.2 Goal and Scope

The goal is to compare the environmental impacts of a CSTR anaerobic digester to a covered lagoon as a waste management solution for dairy cow manure. The spatial, temporal, and geographical scopes are selected to ensure a fair comparison between the two systems.

The temporal scope covers the waste management needed in twenty years due to the typical lifetime of this system. The geographic scope is the county of Maricopa, AZ. This geographic area is selected due to the favorable temperature conditions for a covered lagoon system.

The spatial scope includes many components that span from manure collection to purification and digestate production as shown in Figure 6.3. The system designs for both a covered lagoon and a CSTR can be found in Appendix E and Appendix F, respectively. The scope does not include cattle management, sand and bedding recycling, solids to compost, digestate treatment and digestate application, long term storage lagoon, and the utilization of RNG in transportation. Cattle management and sand and bedding recycling are not included in the scope as they are similar for both systems. Solids for compost, long-term lagoon storage, and digestate treatment are not included in the scope due to time constraints and complexity, in conjunction, an assumption is made that the digestate will be applied as-is. In order to compare manure management strategies, the emissions associated with manure storage were accounted only in the system boundary for the current waste management system.

The functional unit (FU) is 95,813 kg (TS) per day. It is useful for the functional units to be based on TS because TS can be used to reflect the biodegradable material in the waste stream. The reference flow is equal to the functional unit because it refers to the flow of dry manure that requires treatment.

The data used to conduct this LCA were acquired from government annual reports, scientific articles, technical consulting reports, laboratory measurements, and assumptions based on previous research. The data are ranked from 1 (highest) to 5 (lowest) based on the Weidema method which has 6 key categories: acquisition method, independence of data supplier,

representativeness, data age, geographical correlation, and technological correlation. DQIs will be further discussed in Section 6.3.



Figure 6.3. LCA Scope and Associated Boundaries

Since the system creates digestate that can be used for agricultural applications, air acidification and water eutrophication have been chosen as impact categories to assess. Water consumption is assessed as it is consumed throughout certain processes such as the milking parlor and slope screens. Lastly, untreated cow manure produces CH<sub>4</sub> that is often released into the atmosphere; therefore, global warming potential has been assessed between these systems in comparison to untreated manure.

All systems considered, including the CSTR, lagoon, and current waste management practices, are compared using the same functional unit. All systems are being compared as a waste management solution for dairy cow manure, due that all the impacts are evaluated on the basis of kg TS treated, there is no need for allocation with respect to this LCA.

# **6.3 Life Cycle Data Inventory**

The life cycle inventory (LCI) has been split into three key sections. The raw material and handling section provides information regarding the material input into the system and processes before the manure enters the digester. The raw material and handling section also includes the emissions associated with manure storage if a digester was not in place. The processes section provides data regarding the process of anaerobic digestion for a CSTR and a covered lagoon. Furthermore, the third section will cover the processes and outputs after anaerobic digestion has occurred, such as digestate land application. The overall LCI presented in this section includes various data sets that have been identified as key parameters for mass balances (Appendix E & F). The LCI has been formatted to easily divide key components of data as inputs, processes, or outputs; therefore, allowing to easily understand the transition between all components and identify necessary data and data gaps to calculate the impacts associated with each scenario.

Data quality was evaluated using the Weidema method. There are six indicators to evaluate for data quality: acquisition method, independence of data supplier, representativeness, data age, geographical correlation, and technological correlation. The score ranges from one to five, where one is the best quality and five is the most uncertain. Table 6.1 presents how to apply the indicators based on the pedigree matrix.

Indicator Score	1	2	3	4	5
Acquisition method	Measured data	Calculated data based on measurements	Calculated data partly based on assumptions	Qualified estimate (by expert)	Nonqualified estimate
Independence of data supplier	Verified data, information from public or other independent source	Verified information from enterprise with interest in the study	Independent source but based on nonverified information from industry	Nonverified information from industry	Nonverified information from the enterprise interested in the study
Representative ness	Representative data form enough samples of sites over an adequate period to even out normal fluctuations	Representative data from smaller number of sites but for adequate periods	Representative data from smaller number of sites, but from shorter periods	Data from adequate number of sites but shorter periods	Representativeness unknown or incomplete data from smaller number of sites and/or from shorter periods
Data Age	Less than 3 years	Less than 5 years	Less than 10 years	Less than 20 years	Age unknown or more than 20 years
Geographical correlation	Data from area under study	Average data from larger area in which the area under study is included	Data from area with similar production conditions	Data from area with slightly similar production conditions	Data from unknown area with very different production conditions
Technological correlation	Data from enterprises, processes, and materials under study	Data from processes and materials under study but from different enterprises	Data on related processes and materials under study but from different technology	Data on related processes or materials but same technology	Data on related processes or materials but from different technology

 Table 6.1 Data Quality Evaluation Using the Weidema Method (Weidema et al., 2004)

## 6.3.1 Raw Material and Handling

Table 6.2 presents the inventory for raw material and handling. The first component of the inventory table includes the information and values associated with dairy cow manure production and chemical compositions. Cow manure was chosen as the input for the digester, a process that has been well researched in literature and offers vast data availability from ASABE standards, governmental organizations, and scholarly articles.

Key data such as the amount of manure produced per cow per day, the herd size for the study, and average TS and moisture content per kilogram of manure were used to calculate multiple parameters such as tank sizing, daily manure treatment volumes, and energy required to handle a wet ton of manure. These parameters have been utilized to back-calculate initial energy requirements for the sand separation system and pumps necessary to move the manure through the system.

For the impact assessments, a key parameter identified are the emissions from cow manure if left untreated. These parameters play key roles in two impact categories: air acidification potential and global warming potential. In both impact categories, the effects of manure emissions are being compared to a CSTR and a covered lagoon; therefore, providing a different perspective to this LCA. Other key parameters are the phosphorus and nitrogen concentrations within the cow manure. Phillys2 online biomass database and ASABE Standards were used to identify the chemical composition of cow manure and digestate to perform the stoichiometric calculations for the presented scenarios. One key assumption that will affect the scenarios analyzed is that all manure is introduced to the digester per day. Most AD systems within farms have a pump system from the storage tanks to the digester, but if the farm scales up, all the manure will not be possible to introduce to the digester every day.

Component	Value	Unit	Source	DQI
	I	Dairy Cow Manure		
Cows	10,000	Head	Assumption	4,1,2,3,2,1
Manure	68	kg/day/cow		
Total Solids	8.9	kg/day/cow	ASABE Standards	2,1,1,4,2,2
Volatile Solids	7.5	kg/day/cow		
TS:VS Ratio	0.8	Dimensionless	Calculated	2,1,1,4,2,2
Carbon	42.8		Engler et al.	
Hydrogen	6.1			1,2,5,4,3,3
Nitrogen	2.2	0/		
Oxygen	47.7	%0	(2010)	
Sulfur	0.6			
Phosphate	0.6			
CO <sub>2</sub> emissions	0.31	t CO <sub>2</sub> -e/t TS/y	Rotz et al. (2012)	2,1,2,3,2,2

Table 6.2. Life Cycle Data Inventory for Raw Material and Handling

# Table 6.2 (continued).

CH <sub>4</sub> emissions	78	kg CH4/cow/yr	<b>D</b> (2010)			
N <sub>2</sub> O emissions	0.1	kg N <sub>2</sub> O/kg N excreted	Rotz (2018)	3,1,5,1,5,1		
NH <sub>3</sub> emissions	0.265	kg NH3/kg N excreted	Bai et al. (2020)	1,1,5,1,4,3		
P <sub>2</sub> O <sub>5</sub> emissions	15.9	lbs/ton land applied	McGuire (2017)	3,1,1,1,2,5		
		Milking Parlor				
Flow Rate	12	gal/day/cow	Dr. Dana Kirk	4,1,5,1,3,1		
Moisture	98.50	%				
Total Solids	1.50	%	MWPS (2004)	1,1,1,4,2,1		
Volatile Solids	1.20	%				
Recycled Water for Free Stall Barn						
Volume	100	gal/day/cow				
Pump	50	HP	Dr. Dana Kirk	4,1,5,1,3,1		
Running Time for a CSTR	12	hrs				
Running Time for a lagoon	24	hrs				
		Slope Screen				
Water Consumption	1.0	gal/min				
Pump	35	HP				
Running Time for a CSTR	12	hrs	Dr. Dana Kirk	4,1,5,1,3,1		
Running Time for a lagoon	24	hrs				
		Sand Lane				
Volume	50	%				
TS	25	%	Tier 1 Model (2018)	2,1,1,1,2,1		
VS	20	%	()			

Slope Screen Thickening for a CSTR					
Volume	20	%	Tier 1 Model (2018)	2,1,1,1,2,1	
TS	6	%			
VS	5	%			
Slope Screen for Solids Separation for a Lagoon					
Volume	20	%			
TS	6	%	Tier 1 Model	2,1,1,1,2,1	
VS	5	%	(2018)		
Water Consumption	0.5	gal/min			

### Table 6.2 (continued).

# 6.3.2 Process

Table 6.3 holds information and values associated with the anaerobic digestion process for the CSTR and covered lagoon systems. Both systems were scaled to receive the same input of total solids per day. In practice, most digesters are loaded based on total solids, volatile solids, or chemical oxygen demand ratios (AD Operator Training, 2019). As the temporal scope covers the lifetime of these systems, the inventory and impacts associated with building the system were not measured or taken into consideration. Data have been gathered from environmental agencies such as the U.S. Environmental Protection Agency (EPA) and U.S. Department of Agriculture (USDA). From EPA, the Tier 1 Model has provided insight in calculations for certain aspects of the system design.

CSTR digesters are widely studied in literature and the access to various forms of data have allowed the required calculations to be made for this LCA. On the other hand, valuable covered lagoon data has been difficult to obtain since these systems are less properly studied in the anaerobic digestion field. Covered lagoons lack mixing, and this should be considered as it relates to particle settling. This characteristic of covered lagoons will affect the availability of total solids and volatile solids for methanogenic and acetogenic bacteria during the biogas conversion process.

Within the impact categories chosen for this LCA, electricity will have a higher impact for the CSTR than the lagoon. CSTR digesters require electricity for supplemental mixing and heating systems, while these systems are rarely seen in covered lagoons. For air acidification potential and global warming potential, part of the impact will be associated with biogas leakage as these are not perfectly sealed systems. Additionally, water consumption has been obtained as part of the design processes or data recovered from standard practice in the free stall barn and the milking parlors.

CSTR						
HRT	20	days	Assumption	4,1,1,1,3,1		
	971,633	kg/day				
Feed Rate	58,298	kg TS/day	Calculated	2,1,2,1,2,2		
	52,420	kg VS/day				
Heating	6,658,400	kWh/yr	Calculated	212122		
Mixing	224,290	kWh/yr		2,1,2,1,2,2		
Biogas to RNG	70	% VS	Assumption	2,1,5,1,3,1		
	0.42	% TS	Dr. Dono Kirk	4,1,5,1,3,1		
Recycle Liquid	0.34	% VS	Dr. Dana Kirk			
		Covered Lagoon				
HRT	30	days	Assumption	4,1,1,1,3,1		
	4,839,407	kg/day				
Feed Rate	67,026	kg TS/day	Calculated	2,1,2,1,2,2		
	61,964	kg VS/day				
Heating	0	kWh/yr	Accumption	411121		
Mixing	0	kWh/yr	Assumption	4,1,1,1,2,1		

 Table 6.3. Life Cycle Data Inventory Anaerobic Digestion Process

Biogas to RNG	50	%	Assumption	2,1,5,1,3,1
Recycle Liquid	1.5	% TS	Dr. Dr. r. Kirl	415121
	1	% VS	Dr. Dana Kirk	4,1,5,1,5,1

Table 6.3 (continued).

### 6.3.3 Outputs and Land Application

Table 6.4 contains relevant information and values regarding the two main anaerobic digestion products: digestate and biogas. Digestate has many different uses such as animal bedding, compost bedding, and land application. In the LCA analysis, digestate is considered as a direct land application fertilizer for calculations in impact categories. When the digestate is applied, it is replacing the need for a chemical fertilizer, and with soil application, water eutrophication potential will be considered. As part of this potential, the availability of key elements in digestate, such as nitrogen and phosphorus, are presented in the LCI. These values will be compared to the values obtained from the stoichiometric calculations (Appendix G). By using the stoichiometric calculations presented in Appendix G, nitrogen, and phosphorus conversions from the original cow manure concentrations to digestate concentrations are identified and found to be converted approximately 25% to ammonia and phosphate forms readily available for soil. Digestate will also have an impact when it comes to air acidification potential and global warming potential. Digestate produces SO<sub>2</sub>-equivalents and CO<sub>2</sub>-equivalents.

Biogas production will be calculated based on the stoichiometric equations and TS availability for both systems. Since both systems are in Arizona as described in the goal and scope section, a key assumption is that both systems will remain relatively within the mesophilic range (68 °F to 113 °F). Although temperature profiles affect biogas quantity, the pilot research presented in Section 5 showed that biogas quality remained relatively similar between both systems. One of the highest global warming potential parameters within our biogas is methane. According to the

EPA, methane has a GWP value of 25 kg CO<sub>2</sub>-eq./kg CH<sub>4</sub>. In both scenarios, biogas is considered a renewable form of energy, so it is displacing the use of natural gas or fossil fuels. Although displacement of fossil fuels is occurring, it was not accounted for in this LCA as the comparisons are based on input manure rather than products.

Biogas						
CH <sub>4</sub>	40-75	%				
CO <sub>2</sub>	25-40	%				
Nitrogen	0.5-2.5	%	Estefandari et al.,	1,1,2,3,5,1		
Oxygen	0.1-1	%	(2011)			
Hydrogen Sulfide	0.1-0.5	%				
Hydrogen	1-3	%				
Separated Solids to Compost						
Volume	70	%	Tier 1 Model (2018)	2,1,1,1,2,1		
TS	20	%				
VS	17	%	(2010)			
		Land Application				
Digestate Desired	43.5	lbs N/acre	Wrap (2016)	4,1,4,2,2,3		
Gasoline Used	0.28	gal/acre	Parsons (1980); Downs (1998)	1,1,1,5,2,2		
Fuel Emission	0.00236	t CO <sub>2</sub> -eq./L	EPA (2011)	1,1,1,3,2,1		
		Digestate				
Carbon	19.7					
Hydrogen	2.44		Phyllis 2 Database			
Nitrogen	1.25	%	% McCarty et al.	1,1,1,3,2,3		
Sulfur	0.2		(2011)			
Oxygen	19.6					

 Table 6.4. Life Cycle Data Inventory for Outputs and Land Application

Table 6.4 (continued).

Phosphate	0.005	%	Phyllis 2 Database, McCarty et al.	1.1.1.3.2.3
	01000	,.	(2011)	-,-,-,-,-,-

### 6.3.4 Data Quality Evaluation

For data acquisition methods, the data were acquired from valid sources, such as research publications, technical consulting reports, government annual reports, and personal laboratory results. Calculations were performed for a lagoon system based on partial assumptions from consulting data and lab results due to the difficulty to find a reliable source for this specific scenario. The data quality evaluation for the life cycle inventory is shown in Table 6.5. According to the evaluation, the independence of data supplier reached a DQI score of 1; while the other categories reached a DQI score between 2 and 3.

The data were supplied from verified institutions, such as EPA, USDA, ASABE, and research institutions. For data age, there were two sources from American Society of Agricultural and Biological Engineers (ASABE) and EPA which are less than 20 years old. These data were used because the current research related to this topic still refers to those data sets. Laboratory experiments suggest the data continues to be used as a standard within the bioenergy field. Most of the data is geographically in the US, which included the southern US.

 Table 6.5. Data Quality Evaluation Summary for LCI

Indicator	DQI Score	Discussion
Acquisition Method	2	Calculated data based on measurements
Independence of Data Supplier	1	Verified data, information from public or other independent source
Representativeness	3	Representative data from smaller number of sites, but from shorter periods

Table 6.5 (continued).

Data Age	2	Less than five years
Geographical Correlation	3	Data from similar production conditions
Technological Correlation	2	Data from processes and materials under study but from different enterprises

## **6.4 Impact Assessment**

Four impact categories were chosen for Life Cycle Impact Assessment (LCIA): Global Warming Potential (GWP), Air Acidification Potential (AAP), Water Consumption Potential (WCP) and Water Eutrophication Potential (WEP). The classification of each category is defined by the ISO 1998. The LCIA phase provides an examination of the impact categories mentioned previously as a form to analyze environmental impacts of both scenarios in comparison to the emissions of current waste management systems for dairy cow manure. The LCIA provides the analysis of the environmental effects due to processes or products associated with the systems in question.

### 6.4.1 Global Warming Potential (GWP)

Global warming is defined as the change in Earth's temperature due to the release of GHGs such as CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub>. Our current world energy systems are maintained running by fossil fuels such as coal and oil. As a rough approximation, 65% of GHG emissions are due to the utilization of fossil fuels (EPA, 2019). There is a concern across the world to find new renewable energy forms. Cattle management produces roughly 18% GHG emissions around the world (Esfandiari, Khosrokhavar & Sekhavat, 2011). AD systems have been promoted as a renewable energy system that can reduce global warming potential caused by fossil fuel uses and control emissions from cow manure.

Global warming potential (GWP) is the amount of GHG released during the life cycle of a process. This potential can be presented as a metric to compare the various greenhouse impacts to a reference gas, the most common being  $CO_2$  (Shine, 2009). From the data obtained for both systems, the variables analyzed are electricity consumption, leakage, fuel for land application, and effects of digestate in land application. Conversions utilized for these variables can be found in Table 6.6.

Components		<b>Global Warming Potentials</b>		
		Value	Units	
Biogas	CH <sub>4</sub>	25	kg CO <sub>2</sub> -eq./kg CH4	
	CO <sub>2</sub>	1	kg CO <sub>2</sub> -eq./kg CO2	
	Nitrous Oxide	0.03	kg CO <sub>2</sub> -eq./kg TS	
Land Application	CH <sub>4</sub>	0.000308		
	CO <sub>2</sub>	0.000671	kg CO <sub>2</sub> -eq./kg TS/y	
	Nitrous Oxide	0.000154		
Electricity Consumption		0.707	kg CO <sub>2</sub> -eq./kWh	
Fuel Emission		0.00236	t CO <sub>2</sub> -eq./L	

 Table 6.6. Global Warming Potential Conversion Values obtained from the TRACI Model and Chen et al., 2015

The contribution analysis for both scenarios in comparison to the impacts associated with a typical manure management system is presented in Figure 6.4. The results are presented in tons of CO2-equivalents (CO2-eq.). A key assumption proposed before analyzing the systems was that for both the CSTR and covered lagoon systems, all manure will be treated through the digester and without storage, while for current manure management strategies, the manure will be stored before land applying. Through current waste management practices, the majority of emissions will be released through manure storage, recognizing that manure can produce approximately 74 kg of CH4 per cow per year during storage conditions (Rotz, 2018). Manure storage was only considered for current waste management practices, which is outside of the system boundary, and disregarded for the CSTR and the covered lagoons, i.e., the storage of manure or digestate was not included in the system boundary. The emissions associated with manure storage could possibly be reduced by the CSTR and covered lagoon digesters, as potent GHGs like methane are captured in biogas and converted to electricity.



Figure 6.4. Global Warming Potential for Various Parameters for a CSTR, a Covered Lagoon and Dairy Cow Manure per FU<sup>1</sup>

Figure 6.5. is introduced to compare only the impacts of a CSTR and a covered lagoon as waste management systems. The least impactful parameter is the land application of digestate for a CSTR and a covered lagoon. The parameter with the highest impact when considering a CSTR is electricity consumption. CSTR systems require vast energy requirements to sustain operations

<sup>&</sup>lt;sup>1</sup> The scenario for current waste management practices includes storage which is not within the system boundary.

such as mixing and heating. In comparison, for a covered lagoon, the most impactful parameter is the leakage rate. As demonstrated in the contribution analysis, the potential emissions for a CSTR and a covered lagoon are approximately 80% or below when compared to dairy cow manure. In a similar LCA, the impacts of common waste management versus anaerobic digestion or algae treatment for cow manure were presented the GWP for the scenario of anaerobic digestion presented 337-ton CO<sub>2</sub>-eq. per 100 cows per 20 years. When that value is converted for this LCA, it would provide an approximate value of 33,700-ton CO<sub>2</sub>-e. This value is lower fin the study as they accounted for carbon sequestration, electricity produced was consumed in site for operations and the offset of commercial fertilizer by utilizing digestate (Zhang et al., 2013). Overall, a CSTR system has higher GWP when compared to a covered lagoon, and both systems mitigate the harmful impacts of manure compared to leaving it untreated.



Figure 6.5. Global Warming Potential for Various Parameters for a CSTR and a Covered Lagoon per FU

#### 6.4.2 Air Acidification Potential (AAP)

According to the EPA, atmospheric acidification can be defined as: "the result of the oxidation of sulfur, nitrogen, and organic compounds to form their corresponding acids." (Durham, 1985). When absorbed by the atmosphere, these acids can lead to conditions such as acid rain. Air acidification potential (AAP) is an impact category used to convert processes or materials that form acid rain into common unitsof sulfur dioxide equivalents (SO2-eq.).

Some of the inputs and outputs included in AAP are electricity consumption, digestate production, and biogas leakage. AAP is computed using the conversion values Chen et al. (2015) presented in Table 6.7. The energy requirements and TS destruction calculations were utilized paired with the conversions provided by Chen et al. (2015) to convert to acidification values for electricity, leakage, fuel consumption to apply digestate and the effects of digestate on land application.

 Table 6.7. Air Acidification Potential Conversion Values for Anaerobic Digestion (Chen et al., 2015)

Processes	Air Acidification Potentials		
Trocesses	Value	Units	
1 kWh electricity consumed	0.067	g SO <sub>2</sub> -eq.	
1 kg of fuel consumed	0.00054	kg SO <sub>2</sub> -eq.	
1 dry ton during AD process	0.17	kg SO <sub>2</sub> -eq.	
1 dry ton AD effluent in land application	0.073	kg SO <sub>2</sub> -eq.	

The contribution analysis for AAP is presented in Figure 6.6 in kilograms of sulfur dioxide equivalents (SO<sub>2</sub>-eq.). Figure 6.6 presents the impacts of both systems when compared to current manure management strategies. It can be denoted from the contribution analysis that current

manure management systems provide significant AAP when compared to a CSTR and a covered lagoon. Manure can be land applied as an organic fertilizer and the main parameter for AAP is its land application. For purposes of this analysis, the emissions related to manure storage in current manure management systems was not included in the system boundary for the CSTR and the covered lagoon. Manure itself provides a significant amount of emissions to AAP if not captured by a CSTR or a covered lagoon and converted to electricity or RNG.



Figure 6.6. Air Acidification Potential for Various Parameters in a CSTR, a Covered Lagoon and Dairy Cow Manure per FU<sup>2</sup>

Figure 6.7 is presented to compare the impacts between a CSTR and a covered lagoon closely. The least impactful parameter for both systems presented is the fuel utilized for land application. In both systems, the two most impactful parameters are leakage and the land application of digestate, respectively. The covered lagoon has a larger air acidification potential in

<sup>&</sup>lt;sup>2</sup> The scenario for current waste management practices includes storage which is not within the system boundary.

digestate application due that a lagoon produces larger volumes of digester with higher TS concentrations. For both systems, the most impactful category is biogas leakage. Overall, the covered lagoon has approximately twice the impact in this category when compared to a CSTR.



Figure 6.7. Air Acidification Potential for Various Parameters in a CSTR and a Covered Lagoon per FU

### 6.4.3 Water Consumption Potential (WCP)

Only a rough approximation of 2% of all water on earth is freshwater. It is estimated that one out of six people on Earth does not have access to drinking water. Water consumption in either direct or indirect manner provides a highly indicative aspect of the environmental impacts in a product's life cycle. According to LCA methodologies mentioned in ISO 14040:2006, water consumption potential (WCP) is described as water that has been removed from the watershed and cannot be returned. This is impact is presented in volumes of freshwater consumed such as gallons or liters. Water is a scarce resource and even though it does not seem obvious, digesters require vast amounts of water for different purposes, such as heating systems or power washing. For example, MSU SCAD only utilizes fresh water for power washing the digester. For digester heating, the system utilizes a glycol mix that is replaced every 2 years. Even though this glycol mix requires water to be produced, it is outside of the referent scope in this project. Lagoons do not require a supplemental heating system; therefore, heating system water consumption is not being considered in this impact assessment.

For this impact assessment, the three contributions taken into consideration are: milking parlor, slope screen and power washing. Power wash data was obtained from calculations from digester design and system size calculations, while the slope screen and milking parlor were assumed from similar system design parameters.

The contribution analysis for WCP is presented in Figure 6.8. The unit presented for the contribution is gallons. For all the systems, milking parlor will have a higher potential when compared to power washing and slope screens. The water utilized in the milking parlor is utilized to spray the manure deposited when milking the cow and avoid bacteria spreading from manure to milking equipment. The slope screens and power washing are directly related to digester design and operations. The lagoon will require less water to clean the slope screens due that there is only one slope screen present in covered lagoon designs. In the CSTR, there are two slope screens present from one of which is utilized to thicken the influent before entering the digester. Power washing will be based on digester size and individual operations. It can be noted that when comparing all the systems, the main concern is the amount of water utilized for the milking parlor. Although cow manure scenario provides less WCP, the slope screen and power washing are associated to digester design and not to is the waste management procedures at barns. Overall, the

WCP of digester operations is only a small percentage when compared to manure management strategies at barns.



Figure 6.8. Contribution Analysis for Water Consumption in a CSTR, a Covered Lagoon and Dairy Cow Manure per FU<sup>3</sup>

## 6.4.4 Water Eutrophication Potential (WEP)

Eutrophication is defined as the excess of nutrients available in a water body that cause catastrophic events such as algal blooms. When phosphorus or nitrogen are introduced to a water system, algal blooms develop due to nutrient accumulation. The bloom is not necessarily the major problem. The main issue occurs when the algae is broken down by bacteria present in the water and consuming oxygen for the decay to occur. The decay can leave "dead zone" in water bodies, which are defined as low oxygen areas causing harm in marine life (Mueller & Helsel, 1996).

<sup>&</sup>lt;sup>3</sup> The scenario for current waste management practices includes storage which is not within the system boundary.

The primary cause of eutrophication within the U.S. is the runoff of nitrogen and phosphorus from chemical fertilizers or septic systems (NOAA, 2017). Chemical fertilizers are often derived from materials such as petroleum or other forms of fossil fuels. Chemical fertilizers have been used for decades due to their fast release of nutrients into the soil. On the other hand, organic fertilizers have slowly gained popularity since they are derived from animal or plant matter but require the availability of various microorganisms in the ground in order to release nutrients into the soil (Tisdale et al., 1985).

According to Guinée et al. (2002), water eutrophication potential (WEP) is defined as: "the impacts on terrestrial and aquatic environments due to over-fertilization or excess supply of nutrients, particularly focusing on the most important substances nitrogen (N) and phosphorus (P)." WEP can be presented as either mass of nitrogen equivalents (kg N-eq.) or phosphate equivalents (PO<sub>4</sub>-eq.).

In the scenarios presented, digestate is a form of non-chemical or organic fertilizer being utilized as a substitution for current chemical fertilizers. Digestate has been studied in literature as an adequate substitute for chemical fertilizer due to concentrations of both nitrogen and phosphorus. Based on the TRACI model, the conversion utilized are listed in Table 6.8. Stoichiometric equations and additional assumptions with regards to conversions of compounds in solid are presented in Appendix G, Section G.2 and Section G.3.

 Table 6.8. Water Eutrophication Potential Conversion Values Obtained from the TRACI

 Model

Element	Water Eutrophication Potentials		
	Value	Unit	
Nitrogen	0.9864	kg N-eq./kg substance	
Phosphorus	7.290		

The contribution analysis for WEP in both scenarios in comparison to cow manure is presented in Figure 6.9. The contributions are presented as kilograms of nitrogen equivalents (kg N-e). Phosphorus has the highest impact in all the scenarios when compared to nitrogen. Phosphorus represents a vast proportion of the contribution analysis due to higher potential of impact when compared to nitrogen. Both the CSTR and the covered lagoon had less WEP when compared to the cow manure scenario. For both scenarios, the output whether it was digestate or post storage manure, was assumed to be land applied as-is. Although certain values have been expressed for the conversion of phosphorus and nitrogen during manure storage in literature, there is a lack of data available with regards to the transformation of certain molecules in cow manure during storage. Through the AD process, elemental nitrogen and phosphorus are converted to compounds with ammonia and phosphate, respectively. Ammonia and phosphate compounds are readily available for soil conversions, further investigation should be performed depending on soil type and composition within the specified geographical scope.


Figure 6.9. Contribution Analysis for Water Eutrophication Potential in a CSTR, a Covered Lagoon and Dairy Cow Manure per FU<sup>4</sup>

# **6.5 Interpretation**

### 6.5.1 Sensitivity Analysis

A sensitivity analysis is a tool utilized to measure the change in impacts based in changes in key parameters influencing the model and reporting which parameters within each impact are influenced greatly by changes in the model.

#### 6.5.1.1 Global Warming Potential (GWP)

The sensitivity analysis for the CSTR and covered lagoon system was performed by subtracting or adding 50% of methane yield to the stoichiometric formula utilized in the base case scenario. The parameters analyzed for global warming potential were leakage, electricity

<sup>&</sup>lt;sup>4</sup> The scenario for current waste management practices includes storage which is not within the system boundary.

consumption, fuel for land application and effects of digestate on land application. The sensitivity analysis for a CSTR is presented in Figure 6.10 (top); while the sensitivity for a covered lagoon, Figure 6.11 (bottom). The variation in methane yield has also been plotted in both figures to present correlation between parameters. For both system, electricity consumption, fuel for land application and effects of digestate on land application will show either none or minimally sensitive changes with regards to methane yield. The variation in methane yield influenced parameters that were directly correlated to biogas quality such as leakage. For both scenarios, the most sensitive parameter is leakage. Leakage is associated to the presence of gases in biogas such as methane and carbon dioxide. The variations in methane concentration had inversely proportional relations to carbon dioxide concentrations; and therefore, changes in biogas composition affected the AAP of biogas leakage.



Figure 6.10. CSTR Sensitivity Analysis for Global Warming Potential per FU



**Figure 6.11. Covered Lagoon Sensitivity Analysis for Global Warming Potential per FU** 6.5.1.2 Air Acidification Potential (AAP)

The sensitivity analysis for a CSTR and a covered lagoon was performed by subtracting or adding 50% of cow manure concentration to the base case scenario. The following parameters represented: leakage, electricity consumption, fuel for land application and effects on land application from digestate. The sensitivity analysis for a CSTR is presented in Figure 6.12 (top); while the sensitivity for a covered lagoon, Figure 6.13 (bottom). In the CSTR system, all the parameters showed a sensitivity to increase or decrease in manure concentrations to some extent. The least sensitive parameter for a CSTR and a covered lagoon is fuel for land application. The second least sensitive parameter for both systems is electricity consumption. Electricity consumption presented a greater sensitivity for a CSTR than a covered lagoon. The electricity consumption in covered lagoons is related to components such as pumps, but its less sensitive than CSTR, due that it does not include heating or mixing systems. For both systems, leakage presented the most sensitive parameter, followed by land application of digestate. Both CSTR and lagoons provide digestate as a by-product, which is correlated to the concentration of manure introduced to the system. As mentioned previously, lagoon systems produce higher amounts of digestate when compared to CSTR.



Figure 6.12. CSTR Sensitivity Analysis for Air Acidification Potential per FU



Figure 6.13. Covered Lagoon Sensitivity Analysis for Air Acidification Potential per FU

#### 6.5.1.3 Water Consumption Potential (WCP)

The sensitivity analysis for the CSTR and lagoon system was performed by subtracting or adding 50% of cow manure concentration to the base case scenario. The three processes assessed are: milking parlor, power wash and slope screens. The sensitivity analysis for a CSTR is presented in Figure 6.14 (top); while the sensitivity for a covered lagoon, Figure 6.15 (bottom). Power wash and slope screens has none or minimal sensitivity changes with respect to cow manure. Both of these parameters are highly dependent on system design, rather than herd size. For both systems, the most sensitive parameter is related to the milking parlor due that water consumption in the milking parlor is based in a per cow basis.



Figure 6.14. CSTR Sensitivity Analysis for Water Consumption Potential per FU



**Figure 6.15. Covered Lagoon Sensitivity Analysis for Water Consumption Potential per FU** 6.5.1.4 Water Eutrophication Potential (WEP)

The sensitivity analysis for the CSTR and lagoon system was performed by subtracting or adding 50% of cow manure concentration to the base case scenario. The sensitivity analysis for a CSTR is presented in Figure 6.16 (top); while the sensitivity for a covered lagoon, Figure 6.17 (bottom). Both systems have a higher sensitivity towards changes in phosphorus. In both systems, phosphorus has a higher potential for eutrophication than nitrogen.



Figure 6.16. CSTR Sensitivity Analysis for Water Eutrophication Potential per FU



Figure 6.17. Covered Lagoon Sensitivity Analysis for Water Eutrophication Potential per FU

## 6.5.2 Consistency and Completeness Check

The consistency check is a form to verify that assumptions, methods, and data used throughout the LCA process is consistent with the goal and scope of the study. It allows use to revise the consistency of the data used to compare systems to one another. The consistency check and explanations of inconsistency are explained within Table 6.9. The overall data adequately shows consistency to support goal and scope of the study.

Category	Checklist and Inconsistencies				
Data Source	The CSTR scenario was heavily based on literature, while the covered lagoon was heavily based on assumptions and studies of individual scenarios				
Data Accuracy	For both alternatives, a detailed process flow diagram was presented but in real life scenario, system design will be highly dependent on individual site needs.				
Technological Representation	Both scenarios are available at the full scale, but the covered lagoon has a less DQI for technological representation due to the lack of commercial scale data available when compared to a CSTR.				
Temporal Representation	Both technologies are utilized up to date.				
Geographical Representation	Both technologies include data from the United States, but data is also included from Europe where these systems are more predominant at the industry scale.				
System Boundary, Assumption and Model	Both systems serve as a waste management system and produce the same co-products.				

Table 6.9. Checklist and Inconsistencies based on Data Quality

Completeness check aims to assure that the required data for interpretation are available and complete. If there is a case that data is not completed, a verification must be done whether the incomplete data will affect the goal and scope of the study. A control list has been made that include all life cycle stages and the impact assessment indicators which are AAP, GWP, WEP and WCP. Table 6.10 presents a summary of the results for the CSTR and table 6.11 presents a summary of the results for the covered lagoon.

Throughout both studies, there might be incompletion with regards to the effects of settling in a covered lagoon and the impact of digestate for land application in both scenarios. At the moment, there is a lack of data available with regards to settling rates and effects on a covered lagoon with regards to feedstocks. The settling might affect parameters such as biogas production and digestate quality. Moreover, there was a lack of data available presenting elemental changes between the original cow manure introduced into a digester and the digestate retrieved. The Phyllis2 database did not contain elemental analysis from the same location. There was a lack of studies available comparing the elemental conversion between cow manure and digestate. Additionally, digestate contains compounds such as ammonia and phosphate in which there is a lack of research available towards the presence of this within digestate and their forms and their conversions within soil in comparison to chemical fertilizers.

Life cycle stage	CSTR	Complete	<b>Required Actions</b>	
Input: Cow Manure	Х	Yes	-	
Process:				
Anaerobic	Х	Yes	-	
Digester				
Output: Digestate	Х	Data Gap	There is lack of data available with regards to elemental analysis of digestate. Additionally, there is a lack of data comparing the infiltration of digestate into soil and the interactions of elements available in digestate and the availability for plant use.	
Output: Biogas	Х	Yes	-	
X: data available		n.a.: not applicable		

 Table 6.10. Completeness Check for a CSTR

Life cycle stage	Covered Lagoon	Complete	<b>Required Actions</b>	
Input: Cow Manure	Х	Yes	-	
Process: Anaerobic Digester	Х	Data Gap	There is a lack of data explaining how events such as settling affect the co-products of biogas and digestate.	
Output: Digestate	Х	Data Gap	There is lack of data available with regards to elemental analysis of digestate. Additionally, there is a lack of data comparing the infiltration of digestate into soil and the interactions of elements available in digestate and the availability for plant use.	
Output: Biogas	Х	Yes	-	
X: data available		n.a.: not applicable		

Table 6.11. Completeness Check for a Covered Lagoon

# 6.6 Overall Life Cycle Comparison and System Recommendation

The purpose of this life cycle assessment was to understand the environmental impacts of different anaerobic digester types in comparison to current manure management systems. The two types chosen for this analysis were a CSTR and a covered lagoon. Contribution analyses for the impacts are presented in Section 6.4 and sensitivity analysis are presented in Section 6.5.1. A summary of the total impacts for each scenario can be found in Table 6.12.

Table 6.12. Overall System Comparison for all Impact Categories analyzed for a CSTR,Covered Lagoon and Dairy Cow Manure per Functional Unit

Impact	Units	Scenario			
	(Per FU)	CSTR	Covered Lagoon	Dairy Cow Manure	
GWP	t CO <sub>2</sub> -eq.	136,344	102,323	928,065	
AAP	kg SO <sub>2</sub> -eq.	63,511	127,681	5,727,598	
WCP	gal	902,446,978	966,295,093	876,000,000	
WEP	kg N-eq.	31,305	35,992	41,314	

This LCA found both systems, a CSTR and a covered lagoon, have less environmental burdens when compared to current waste management systems. When comparing a CSTR and a covered lagoon, the covered lagoon provides less environmental burdens with respect to GWP; while the CSTR with respect to AAP and WEP. In GWP and AAP, electricity consumption played a key role for the CSTR scenario. CSTR digesters are well studied in literature and have been widely implemented across the United States as the second most common digester type. This type of digesters has supplemental heating and mixing systems which have been demonstrated in literature to require vast amounts of energy input. The lagoon system does not require this supplemental heating or mixing systems; but this can lead to settling which has not been well recorded in literature. Also, there is a lack of literature presenting case studies with regards to lagoon operations. As an overall conclusion, the CSTR seems to possess less environmental burdens than a covered lagoon, but both systems possess less environmental burdens than current manure management systems.

Both systems analyzed in this LCA provide a waste management solution. Both systems produce organic fertilizer and biogas that can be converted to renewable electricity or RNG. Based on the overall comparison, either system can be chosen based on stakeholder needs and resource availability. A covered lagoon can be implemented as a low-cost low technology waste management system instead of a CSTR. Both systems have various benefits that can be associated with different interests' groups and therefore both systems can be recommended depending on the target audience.

### 7. OVERALL CONCLUSIONS AND RECOMMENDATIONS

# 7.1 Biochemical methane potential testing

The BMP tests for all conditions were anaerobically biodegradable. There was no difference in biogas quantity and quality between 30°C non-mixed, 39°C non-mixed, and 39°C mixed. BMP trials have been used mainly to determine the ideal biodegradability of the material. Temperature did not influence the biodegradability of the material to great extent when temperatures where in the mesophilic range.

The idea that an additional 9°C within digester temperature might not be necessary in order to obtain higher biogas quantity and quality. This would represent a theoretical energy reduction of approximately 30% for a change in 9 degrees. The energy reduction in heating requirements for digesters could provide incentives to opt for covered lagoons. Covered lagoons have been believed to not be able to produce the same biogas quality and quantity as a CSTR; but through this BMPs, might be able to provide the same biogas quality and quantity if an operating temperature of 30°C can be maintained to promote the growth of methanogens at the lower end of the mesophilic range.

The BMPs allowed to provide a backbone for the idea that covered lagoons are due offer material biodegradability and therefore should not be disregarded as an optional waste management system when compared to a CSTR.

### 7.2 Pilot data

The main purpose of pilot testing was to run pilots at similar conditions to a covered lagoon and compare the effects of biogas quantity and quality due to temperature. The pilot research provided intriguing results for the purposes of this research. The mesophilic condition was able to produce higher biogas yield in comparison to psychrophilic and unregulated conditions. The unregulated pilots were able to provide higher methane yields than the pilots maintained at mesophilic temperatures.

There was no indication of inhibition due to changes in pH; but inhibitions associated to pH could have probably be encountered if the project were run for a longer timeline. TSVS reduction were achieved in all the pilots above 45% indicated the degradation of material associated to biogas production.

The pilots provided relevant information with regards to the idea that biogas quality can be achieved at lower temperatures. The biogas quantity aspect might require more in-depth analysis of variations in other parameters, or the simulation of geographical temperatures where covered lagoons might be located.

## 7.3 Life Cycle Analysis

The life cycle analysis provided a foundation for future work regarding LCA analysis on these systems. The CSTR had lowest environmental impacts when compared to a covered lagoon. Both systems provided reduction of emissions when compared to current manure waste management systems. Both systems provided cleaner solutions with additional benefits that can be associated to revenue. Although the CSTR had lower impacts, covered lagoon proves to be a viable solution with a low cost as a waste management system.

In both systems, leakage proved to be a sensitive factor for both scenarios when it came to global warming potential. Digesters have been believed to be carbon negative systems and further research should be performed in order to identify how this leakage can be reduced and possibly avoided. In another aspect, leakage represents monetary losses due that a volume of biogas towards electricity or RNG is lost. No system will be leakage free, but a reduction of these values obtained

by EPA should be analyzed in order to obtain minimum leakage and therefore reduction in global warming potential.

### 7.4 Future Work

Given the limited data availability with respect to covered lagoons, the implementation of new studies to fill the data gap could be appreciated. The pilot testing provided interesting results with regard to gas quality. There is the availability of studies presenting the phenomenon but lack on the why of this occurrence. The COVID-19 pandemic provided a shorten amount of time for testing. The pandemic limited the availability to run the testing for 60 days instead of 45 or run 5 HRTs instead of 3. Longer HRTs and experimentation time could have provided a more in-depth analysis of parameters presented. If more HRTs have been analyzed, extra data for unregulated pilots during the winter season could have been obtained. Additionally, it would have been interesting analyzing the mixing patterns for these systems; but the time allotted for a master program did not allowed for the complexity of studying effects of mixing on digester systems.

There is a lack of case studies comparing the performance of different digester types to one another. There is a lack of data available comparing individual digester operations and conveying results about how operations might be affecting biogas quantity and quality. Although most of the data available for anaerobic digestion research is based on lab scale models, there should be more research on already implemented commercial scale sites.

For future studies, it would be beneficial for the industry to compare these systems to a power plant, or the impacts associated with chemical fertilizers; due that both systems, a CSTR and a covered lagoon, will provide renewable energy forms and organic fertilizer. A possible future study could be the impacts associated with producing 1 kWh in an anaerobic digestion system versus 1 kWh in a power plant

APPENDICIES

# Appendix A. R-Script and Results for BMP data

A.1 R-script and Results for Cumulative Biogas Production

```
## Statistical analysis for BMP Biogas
## Feb 17, 2021 CREATED MIB
# Loading Library and Tables -----
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package -----
_ _
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
_ _
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summarized
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary_func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
 }
```

## the .txt file needs to be saved as the type of "Tab delimited".

##load biogas.txt

```
con <-file.choose(new = FALSE)
metadata <- read.table(con, header = T, row.names = 1)</pre>
```

**## DEFINING FACTORS** 

### Abbreviations
## A- 10 C, non-mixed
## B- 20 C, non-mixed
## C- 30 C, non-mixed
## D- 39 C, non-mixed
## E- 39 C, mixed

metadata\$Temp <- factor(metadata\$Temp) ##Factor statement</pre>

#### # 1. Effects of Temp

#### ## one-way ANOVA

```
fit1 <- aov(Cumul_Gas~Temp, data = metadata)</pre>
summary(fit1)
##
                Df Sum Sq Mean Sq F value Pr(>F)
## Temp
                4 4175174 1043794 92.67 <2e-16 ***
## Residuals 276 3108698
                             11263
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey1
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = Cumul_Gas ~ Temp, data = metadata)
##
## $Temp
```

diff lwr upr padj ## ## B-A 34.181818 -21.38975 89.75338 0.4424396 ## C-A 244.745455 189.17389 300.31702 0.0000000 ## D-A 278.456113 223.60785 333.30437 0.0000000 ## E-A 270.249216 215.40096 325.09748 0.0000000 ## C-B 210.563636 154.99207 266.13520 0.0000000 ## D-B 244,274295 189,42603 299,12256 0,000000 ## E-B 236.067398 181.21914 290.91566 0.0000000 ## D-C 33.710658 -21.13760 88.55892 0.4432553 ## E-C 25.503762 -29.34450 80.35202 0.7057045 ## E-D -8.206897 -62.32219 45.90839 0.9936747 *#Biogas data summary* Gas\_data1 <- data\_summary(metadata, varname="Cumul\_Gas", groupnames="Temp</pre> ") ## Loading required package: plyr ## ## Attaching package: 'plyr' ## The following object is masked from 'package:ggpubr': ## ## mutate Gas data1 ## Temp Cumul Gas sd ## 1 A 37.18182 20.35799 ## 2 B 71.36364 42.97086 C 281.92727 137.58726 ## 3 ## 4 D 315.63793 132.97588 ## 5 E 307.43103 129.53806 #2. Plot Gas\_production1 <- data\_summary(metadata, varname="Cumul\_Gas",</pre> groupnames=c("Temp")) Gas production1\$Temp=as.factor(Gas production1\$Temp) Gas\_production1 Temp Cumul Gas ## sd ## 1 A 37.18182 20.35799 ## 2 B 71.36364 42.97086 C 281.92727 137.58726 ## 3 D 315.63793 132.97588 ## 4 ## 5 E 307.43103 129.53806 box\_1 <- ggplot(Gas\_production1, aes(x=Temp, y=Cumul\_Gas, fill=Temp)) +</pre> geom\_bar(stat="identity", position=position\_dodge(0.9), width=0.5)+ geom\_errorbar(aes(ymin=Cumul\_Gas-sd, ymax=Cumul\_Gas+sd), width=0.2, pos ition=position\_dodge(0.9))+



Figure A.1. Average Cumulative Biogas Production in BMP bottles, Average of Trials 1, 2 & 3

A.2 R-script and Results for Methane Concentration

```
## Statistical analysis for BMP Methane
## Feb 17, 2021 CREATED MIB
# Loading Library and Tables -----
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package ------
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summarized
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
  }
 data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data sum <- rename(data sum, c("mean" = varname))</pre>
```

```
return(data_sum)
}
```

## the .txt file needs to be saved as the type of "Tab delimited".

```
##load methane.txt
```

```
con <-file.choose(new = FALSE)
metadata <- read.table(con, header = T, row.names = 1)</pre>
```

**## DEFINING FACTORS** 

### Abbreviations
## A- 10 C, non-mixed
## B- 20 C, non-mixed
## C- 30 C, non-mixed
## D- 39 C, non-mixed
## E- 39 C, mixed

```
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
```

#### # 1. Effects of Temp

#### ## one-way ANOVA

```
fit1 <- aov(Methane~Temp, data = metadata)</pre>
summary(fit1)
##
               Df Sum Sq Mean Sq F value Pr(>F)
                                    51.7 <2e-16 ***
## Temp
                4 10725
                         2681.3
## Residuals
               55
                    2852
                             51.9
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey1
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = Methane ~ Temp, data = metadata)
##
## $Temp
##
             diff
                         lwr
                                   upr
                                           p adj
## B-A 13.5000000 5.208074 21.791926 0.0002443
## C-A 32.4166667 24.124741 40.708592 0.0000000
```

```
## D-A 32.8333333 24.541408 41.125259 0.0000000
## E-A 32.5833333 24.291408 40.875259 0.0000000
## C-B 18.9166667 10.624741 27.208592 0.0000003
## D-B 19.3333333 11.041408 27.625259 0.0000002
## E-B 19.0833333 10.791408 27.375259 0.0000003
## D-C 0.4166667 -7.875259 8.708592 0.9999062
## E-C 0.1666667 -8.125259 8.458592 0.9999976
## E-D -0.2500000 -8.541926 8.041926 0.9999878
#Biogas data summary
Methane data1 <- data summary(metadata, varname="Methane", groupnames="Te
mp")
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
       mutate
Methane data1
##
     Temp Methane
                          sd
      A 21.00000 10.099505
## 1
## 2
        B 34.50000 11.658005
## 3
       C 53.41667 3.342790
## 4
       D 53.83333 2.480225
## 5
        E 53.58333 2.020726
#2. PLot
Methane production1 <- data summary(metadata, varname="Methane",
                                groupnames=c("Temp"))
Methane production1$Temp=as.factor(Methane production1$Temp)
Methane_production1
##
    Temp Methane
                          sd
## 1
       A 21.00000 10.099505
        B 34.50000 11.658005
## 2
## 3
      C 53.41667 3.342790
## 4
       D 53.83333
                   2.480225
## 5
      E 53.58333 2.020726
box_1 <- ggplot(Methane_production1, aes(x=Temp, y=Methane, fill=Temp)) +</pre>
 geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=Methane-sd, ymax=Methane+sd), width=0.2, positio
n=position dodge((0.9))+
 xlab("Temperature")+
ylab("Methane Concentration (%)") + ylim(0, 65) + labs(title = "", sub
```



Figure A.2. Methane Content in BMP bottles, Average of Trials 1, 2 & 3

A.3 R-script and Results for Total Solids

```
## Statistical analysis for TS
## Feb 17, 2021 CREATED MIB
# Loading Library and Tables -----
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package -----
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summarized
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
  }
 data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data sum <- rename(data sum, c("mean" = varname))</pre>
```

```
return(data sum)
}
# ANALYSIS-----
## the .txt file needs to be saved as the type of "Tab delimited".
con <-file.choose(new = FALSE)</pre>
metadata <- read.table(con, header = T, row.names = 1)</pre>
## DEFINING FACTORS
### Abbreviations
## A- 10 C, non-mixed
## B- 20 C, non-mixed
## C- 30 C, non-mixed
## D- 39 C, non-mixed
## E- 39 C, mixed
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
## TS PRE ANALYSIS------
- - -
## one-way ANOVA
fit1 <- aov(TS_Pre~Temp, data = metadata)</pre>
summary(fit1)
##
               Df
                     Sum Sq Mean Sq F value Pr(>F)
                    1687224 421806
## Temp
                4
                                     0.126 0.972
## Residuals
               40 134093910 3352348
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey1
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = TS_Pre ~ Temp, data = metadata)
##
## $Temp
             diff
                        lwr
##
                                 upr
                                          p adj
## B-A 549.44444 -1915.689 3014.577 0.9681291
## C-A 87.00000 -2378.133 2552.133 0.9999756
## D-A 100.88889 -2364.244 2566.022 0.9999559
## E-A 115.88889 -2349.244 2581.022 0.9999234
## C-B -462.44444 -2927.577 2002.689 0.9830441
## D-B -448.55556 -2913.689 2016.577 0.9848636
```

```
150
```

```
## E-B -433.55556 -2898.689 2031.577 0.9866714
## D-C 13.88889 -2451.244 2479.022 1.0000000
## E-C
         28.88889 -2436.244 2494.022 0.9999997
## E-D
         15.00000 -2450.133 2480.133 1.0000000
# data summary
TS_data1 <- data_summary(metadata, varname="TS_Pre", groupnames="Temp")
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
       mutate
TS data1
##
    Temp
            TS Pre
                         sd
## 1 A 13255.11 2054.874
## 2
        B 13804.56 2042.183
## 3
      C 13342.11 1659.064
      D 13356.00 1546.983
## 4
## 5
      E 13371.00 1795.291
#2. Plot
TS1 <- data summary(metadata, varname="TS Pre",
                                    groupnames=c("Temp"))
TS1$Temp=as.factor(TS1$Temp)
TS1
##
    Temp
          TS Pre
                         sd
## 1 A 13255.11 2054.874
## 2
        B 13804.56 2042.183
## 3
      C 13342.11 1659.064
## 4
      D 13356.00 1546.983
## 5
      E 13371.00 1795.291
box_1 <- ggplot(TS1, aes(x=Temp, y=TS_Pre, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=TS_Pre-sd, ymax=TS_Pre+sd), width=0.2, position=
position dodge((0.9))+
  xlab("Temperature")+
  ylab("Total Solids Pre-digestion (mg/L)") + ylim(0, 20000) + labs(titl
e = "", subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=18, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
```

```
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("blue", "blue", "blue", "blue", "blue"))
box_1
```



Figure A.3. Average Pre-digestion Content with Standard Deviations for Total Solids in BMP bottles, Average of Trials 1, 2 & 3

```
## TS POST ANALYSIS------
_ _ _ _
## one-way ANOVA
fit2 <- aov(TS Post~Temp, data = metadata)</pre>
summary(fit2)
##
               Df
                     Sum Sq Mean Sq F value Pr(>F)
                   29857000 7464250
                                       2.601 0.0504 .
## Temp
                4
## Residuals
               40 114811330 2870283
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey2 <- TukeyHSD(fit2, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey2
```

```
Tukey multiple comparisons of means
##
##
       95% family-wise confidence level
##
## Fit: aov(formula = TS Post ~ Temp, data = metadata)
##
## $Temp
##
             diff
                        lwr
                                  upr
                                           p adj
## B-A -422.3333 -2703.349 1858.6820 0.9838506
## C-A -1509.1111 -3790.126 771.9042 0.3393481
## D-A -1740.6667 -4021.682 540.3487 0.2083140
## E-A -2144.2222 -4425.238 136.7931 0.0742095
## C-B -1086.7778 -3367.793 1194.2376 0.6555897
## D-B -1318.3333 -3599.349 962.6820 0.4750735
## E-B -1721.8889 -4002.904 559.1264 0.2173855
      -231.5556 -2512.571 2049.4598 0.9983897
## D-C
## E-C -635.1111 -2916.126 1645.9042 0.9304898
## E-D -403.5556 -2684.571 1877.4598 0.9863725
#data summary
TS data2 <- data summary(metadata, varname="TS Post", groupnames="Temp")
TS data2
##
     Temp TS Post
                         sd
        A 12651.11 2110.252
## 1
## 2
        B 12228.78 2244.987
## 3
       C 11142.00 1449.052
## 4
      D 10910.44 1137.758
## 5
       E 10506.89 1209.977
#2. Plot
TS2 <- data_summary(metadata, varname="TS_Post",</pre>
                    groupnames=c("Temp"))
TS2$Temp=as.factor(TS2$Temp)
TS2
     Temp TS Post
##
                         sd
## 1
        A 12651.11 2110.252
## 2
        B 12228.78 2244.987
## 3
       C 11142.00 1449.052
## 4
       D 10910.44 1137.758
## 5
      E 10506.89 1209.977
box_2 <- ggplot(TS2, aes(x=Temp, y=TS_Post, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=TS_Post-sd, ymax=TS_Post+sd), width=0.2, positio
n=position dodge((0.9))+
  xlab("Temperature")+
  ylab("Total Solids Post- Digestion (mg/L)") + ylim(0, 15000) + labs(ti
tle = "", subtitle=NULL) +
theme(title=element_text(size=20, family="Times New Roman"),
```

```
axis.text.x = element_text(size=20, family="Times New Roman"),
axis.text.y=element_text(size=18, family="Times New Roman"),
axis.title.y = element_text(size = 20, family="Times New Roman"),
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("blue", "blue", "blue", "blue", "blue"))
box_2
```



Figure A.4. Average Post-digestion Content with Standard Deviations for Total Solids in BMP bottles, Average of Trials 1, 2 & 3

```
## TS DESTROYED ANALYSIS--------
## one-way ANOVA
fit3 <- aov(TS_Destroyed~Temp, data = metadata)
summary(fit3)
## Df Sum Sq Mean Sq F value Pr(>F)
## Temp 4 27856208 6964052 19.37 6.22e-09 ***
## Residuals 40 14380091 359502
```

## ---## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1 Tukey3 <- TukeyHSD(fit3, conf.level=0.95) #Tukey multiple comparisons Tukey3 ## Tukey multiple comparisons of means ## 95% family-wise confidence level ## ## Fit: aov(formula = TS Destroyed ~ Temp, data = metadata) ## ## \$Temp p adi ## diff lwr upr ## B-A 971.3333 164.06736 1778.599 0.0114505 ## C-A 1596.0000 788.73403 2403.266 0.0000142 ## D-A 1841.6667 1034.40069 2648.933 0.0000009 ## E-A 2260.1111 1452.84514 3067.377 0.0000000 ## C-B 624.6667 -182.59931 1431.933 0.1969401 ## D-B 870.3333 63.06736 1677.599 0.0290328 ## E-B 1288.7778 481.51180 2096.044 0.0004369 ## D-C 245.6667 -561.59931 1052.933 0.9064558 ## E-C 664.1111 -143.15486 1471.377 0.1507497 ## E-D 418.4444 -388.82153 1225.710 0.5807905 #data summary TS data3 <- data summary(metadata, varname="TS Destroyed", groupnames="Te mp") TS\_data3 ## Temp TS Destroyed sd 604.000 260.4765 ## 1 Α ## 2 В 1575.333 606.5124 ## 3 С 2200.000 447.6933 2445.667 667.4684 ## 4 D ## 5 E 2864.111 846.0868 #2. Plot TS3 <- data summary(metadata, varname="TS Destroyed", groupnames=c("Temp")) TS3\$Temp=as.factor(TS3\$Temp) TS3 ## Temp TS\_Destroyed sd ## 1 А 604.000 260.4765 ## 2 В 1575.333 606.5124 ## 3 С 2200.000 447.6933 ## 4 D 2445.667 667.4684 ## 5 Е 2864.111 846.0868



Figure A.5. Average Reductions with Standard Deviations for Total Solids in BMP bottles, Average of Trials 1, 2 & 3



```
fit4 <- aov(TS Reduc~Temp, data = metadata)</pre>
summary(fit4)
##
               Df Sum Sq Mean Sq F value
                                           Pr(>F)
## Temp
                                   26.46 9.01e-11 ***
               4 1517.6
                           379.4
## Residuals
               40 573.6
                            14.3
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey4 <- TukeyHSD(fit4, conf.level=0.95) #Tukey multiple comparisons
Tukey4
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = TS Reduc ~ Temp, data = metadata)
##
## $Temp
##
            diff
                         lwr
                                   upr
                                           p adj
## B-A 7.000000 1.90172125 12.098279 0.0029451
## C-A 11.666667 6.56838792 16.764945 0.0000008
## D-A 13.555556 8.45727681 18.653834 0.0000000
## E-A 16.666667 11.56838792 21.764945 0.0000000
## C-B 4.666667 -0.43161208 9.764945 0.0867740
## D-B 6.555556 1.45727681 11.653834 0.0059861
## E-B 9.6666667 4.56838792 14.764945 0.0000297
## D-C 1.8888889 -3.20938986 6.987168 0.8264666
## E-C 5.000000 -0.09827875 10.098279 0.0568934
## E-D 3.111111 -1.98716764 8.209390 0.4203284
#data summary
TS data4 <- data summary(metadata, varname="TS Reduc", groupnames="Temp")
TS data4
##
     Temp TS Reduc
                          sd
        A 4.666667 2.236068
## 1
## 2
        B 11.666667 5.431390
## 3
       C 16.333333 2.397916
## 4
       D 18.222222 3.929942
        E 21.333333 4.000000
## 5
#2. PLot
TS4 <- data_summary(metadata, varname="TS_Reduc",
                    groupnames=c("Temp"))
TS4$Temp=as.factor(TS4$Temp)
TS4
##
     Temp TS_Reduc
                          sd
## 1 A 4.666667 2.236068
```

```
## 2
        B 11.666667 5.431390
## 3
        C 16.333333 2.397916
        D 18.222222 3.929942
## 4
## 5
        E 21.333333 4.000000
box_4 <- ggplot(TS4, aes(x=Temp, y=TS_Reduc, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=TS_Reduc-sd, ymax=TS_Reduc+sd), width=0.2, posit
ion=position dodge(0.9))+
 xlab("Temperature")+
 ylab("Total Solids Reduction (%)") + ylim(0, 30) + labs(title = "", su
btitle=NULL) +
 theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=18, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "red", "green", "green", "green"))
box 4
```



Figure A.6. Percent Average Reductions with Standard Deviations for Total Solids in BMP bottles, Average of Trials 1, 2 & 3

A.4 R-script and Results for Volatile Solids

```
## Statistical analysis for VS
## Feb 17, 2021 CREATED MIB
# Loading Library and Tables ------
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package -----
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summarized
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
  }
 data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data sum <- rename(data sum, c("mean" = varname))</pre>
```

```
return(data sum)
}
# ANALYSIS-----
## the .txt file needs to be saved as the type of "Tab delimited".
con <-file.choose(new = FALSE)</pre>
metadata <- read.table(con, header = T, row.names = 1)</pre>
## DEFINING FACTORS
### Abbreviations
## A- 10 C, non-mixed
## B- 20 C, non-mixed
## C- 30 C, non-mixed
## D- 39 C, non-mixed
## E- 39 C, mixed
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
## VS PRE ANALYSIS------
- - -
## one-way ANOVA
fit1 <- aov(VS_Pre~Temp, data = metadata)</pre>
summary(fit1)
##
               Df
                     Sum Sq Mean Sq F value Pr(>F)
                    1209945 302486
## Temp
                4
                                      0.12 0.975
## Residuals
               40 100872205 2521805
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey1
##
     Tukey multiple comparisons of means
       95% family-wise confidence level
##
##
## Fit: aov(formula = VS_Pre ~ Temp, data = metadata)
##
## $Temp
##
              diff
                          lwr
                                  upr
                                           p adj
## B-A 372.777778 -1765.291 2510.846 0.9870989
## C-A -67.777778 -2205.846 2070.291 0.9999841
## D-A -52.333333 -2190.402 2085.735 0.9999943
## E-A
       -8.444444 -2146.513 2129.624 1.0000000
## C-B -440.555556 -2578.624 1697.513 0.9760345
## D-B -425.111111 -2563.179 1712.957 0.9789755
```

```
## E-B -381.222222 -2519.291 1756.846 0.9859695
## D-C 15.444444 -2122.624 2153.513 1.0000000
## E-C
         59.333333 -2078.735 2197.402 0.9999906
## E-D
        43.888889 -2094.179 2181.957 0.9999972
# data summary
VS_data1 <- data_summary(metadata, varname="VS_Pre", groupnames="Temp")</pre>
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
       mutate
VS data1
##
    Temp
            VS Pre
                         sd
## 1 A 9498.000 1677.644
## 2
        B 9870.778 1752.217
## 3
      C 9430.222 1490.467
      D 9445.667 1393.249
## 4
## 5
      E 9489.556 1600.511
#2. Plot
VS1 <- data summary(metadata, varname="VS Pre",</pre>
                    groupnames=c("Temp"))
VS1$Temp=as.factor(VS1$Temp)
VS1
##
     Temp
           VS Pre
                         sd
## 1 A 9498.000 1677.644
## 2
        B 9870.778 1752.217
## 3
      C 9430.222 1490.467
## 4
      D 9445.667 1393.249
## 5
      E 9489.556 1600.511
box_1 <- ggplot(VS1, aes(x=Temp, y=VS_Pre, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=VS_Pre-sd, ymax=VS_Pre+sd), width=0.2, position=
position dodge((0.9))+
  xlab("Temperature")+
  ylab("Volatile Solids Pre-digestion (mg/L)") + ylim(0, 15000) + labs(t
itle = "", subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=18, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
```


Figure A.7. Average Pre-digestion Content with Standard Deviations for Volatile Solids in BMP bottles, Average of Trials 1, 2 & 3

```
## VS POST ANALYSIS-----
## one-way ANOVA
fit2 <- aov(VS_Post~Temp, data = metadata)</pre>
summary(fit2)
##
               Df
                    Sum Sq Mean Sq F value Pr(>F)
                                      4.584 0.00385 **
                4 36639511 9159878
## Temp
## Residuals
               40 79931799 1998295
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey2 <- TukeyHSD(fit2, conf.level=0.95) #Tukey multiple comparisons
Tukey2
```

```
Tukey multiple comparisons of means
##
##
       95% family-wise confidence level
##
## Fit: aov(formula = VS Post ~ Temp, data = metadata)
##
## $Temp
##
             diff
                        lwr
                                  upr
                                          p adj
## B-A -461.7778 -2365.027 1441.4713 0.9568605
## C-A -1709.2222 -3612.471 194.0268 0.0966009
## D-A -2022.5556 -3925.805 -119.3065 0.0324048
## E-A -2294.4444 -4197.694 -391.1954 0.0112492
## C-B -1247.4444 -3150.694 655.8046 0.3486025
## D-B -1560.7778 -3464.027
                            342.4713 0.1529818
## E-B -1832.6667 -3735.916
                             70.5824 0.0639846
      -313.3333 -2216.582 1589.9157 0.9896056
## D-C
## E-C -585.2222 -2488.471 1318.0268 0.9032272
## E-D -271.8889 -2175.138 1631.3602 0.9939429
#data summary
VS data2 <- data summary(metadata, varname="VS Post", groupnames="Temp")
VS data2
##
     Temp VS Post
                          sd
## 1
        A 8741.222 1842.0779
## 2
        B 8279.444 1806.8145
       C 7032.000 1160.8508
## 3
      D 6718.667 1046.0535
## 4
## 5
       E 6446.778 944.3742
#2. Plot
VS2 <- data_summary(metadata, varname="VS_Post",</pre>
                    groupnames=c("Temp"))
VS2$Temp=as.factor(VS2$Temp)
VS2
     Temp VS Post
##
                          sd
## 1
        A 8741.222 1842.0779
## 2
        B 8279.444 1806.8145
## 3
       C 7032.000 1160.8508
## 4
      D 6718.667 1046.0535
## 5
      E 6446.778 944.3742
box_2 <- ggplot(VS2, aes(x=Temp, y=VS_Post, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=VS_Post-sd, ymax=VS_Post+sd), width=0.2, positio
n=position dodge((0.9))+
  xlab("Temperature")+
  ylab("Volatile Solids Post- Digestion (mg/L)") + ylim(0, 13000) + labs
(title = "", subtitle=NULL) +
theme(title=element_text(size=20, family="Times New Roman"),
```





Figure A.8. Average Post-digestion Content with Standard Deviations for Volatile Solids in BMP bottles, Average of Trials 1, 2 & 3

```
## VS DESTROYED ANALYSIS

## one-way ANOVA

fit3 <- aov(VS_Destroyed~Temp, data = metadata)

summary(fit3)

## Df Sum Sq Mean Sq F value Pr(>F)

## Temp 4 30902222 7725556 34.42 1.85e-12 ***
```

## Residuals 40 8978493 224462 ## ---## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1 Tukey3 <- TukeyHSD(fit3, conf.level=0.95) #Tukey multiple comparisons Tukey3 ## Tukey multiple comparisons of means ## 95% family-wise confidence level ## ## Fit: aov(formula = VS Destroyed ~ Temp, data = metadata) ## ## \$Temp ## diff lwr upr p adj ## B-A 834.3333 196.455437 1472.2112 0.0050104 ## C-A 1641.2222 1003.344326 2279.1001 0.0000001 ## D-A 1970.1111 1332.233215 2607.9890 0.0000000 ## E-A 2285.8889 1648.010993 2923.7668 0.0000000 ## C-B 806.8889 169.010993 1444.7668 0.0070688 ## D-B 1135.7778 497.899882 1773.6557 0.0000848 ## E-B 1451.5556 813.677660 2089.4335 0.0000009 ## D-C 328.8889 - 308.989007 966.7668 0.5857252 ## E-C 644.6667 6.788771 1282.5446 0.0465181 ## E-D 315.7778 -322.100118 953.6557 0.6225541 #data summary VS data3 <- data summary(metadata, varname="VS Destroyed", groupnames="Te mp") VS\_data3 Temp VS Destroyed ## sd ## 1 756.8889 231.9140 А ## 2 1591.2222 410.8673 В ## 3 С 2398.1111 400.9372 ## 4 D 2727.0000 426.7807 Е ## 5 3042.7778 746.2059 #2. Plot VS3 <- data\_summary(metadata, varname="VS\_Destroyed",</pre> groupnames=c("Temp")) VS3\$Temp=as.factor(VS3\$Temp) VS3 ## Temp VS Destroyed sd ## 1 А 756.8889 231.9140 ## 2 1591.2222 410.8673 В ## 3 С 2398.1111 400.9372 ## 4 2727.0000 426.7807 D ## 5 E 3042.7778 746.2059

```
box 3 <- ggplot(VS3, aes(x=Temp, y=VS Destroyed, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
 geom_errorbar(aes(ymin=VS_Destroyed-sd, ymax=VS_Destroyed+sd), width=0.
2, position=position_dodge(0.9))+
 xlab("Temperature")+
  ylab("Volatile Solids Destroyed (mg/L)") + ylim(0, 4300) + labs(title
= "", subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=18, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "red", "green", "green", "green"))
box 3
## Warning in grid.Call(C textBounds, as.graphicsAnnot(x$label), x$x, x$y
, : font
```

```
## family not found in Windows font database
```



Figure A.9. Average Reductions with Standard Deviations for Volatile Solids in BMP bottles, Average of Trials 1, 2 & 3

## VS REDUCTION ANALYSIS------\_ \_ \_ \_ \_ \_ \_ \_ \_ \_ ## one-way ANOVA fit4 <- aov(VS\_Reduc~Temp, data = metadata)</pre> summary(fit4) ## Df Sum Sq Mean Sq F value Pr(>F) ## Temp 3263 815.6 63.7 <2e-16 \*\*\* 4 ## Residuals 12.8 40 512 ## ---## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1 Tukey4 <- TukeyHSD(fit4, conf.level=0.95) #Tukey multiple comparisons Tukev4 ## Tukey multiple comparisons of means ## 95% family-wise confidence level ## ## Fit: aov(formula = VS Reduc ~ Temp, data = metadata) ## ## \$Temp ## diff lwr upr p adj ## B-A 8.222222 3.404242 13.040203 0.0001648 ## C-A 16.777778 11.959797 21.595758 0.0000000 ## D-A 20.222222 15.404242 25.040203 0.0000000 ## E-A 23.333333 18.515353 28.151314 0.0000000 ## C-B 8.555556 3.737575 13.373536 0.0000885 ## D-B 12.000000 7.182019 16.817981 0.0000001 ## E-B 15.111111 10.293131 19.929092 0.0000000 ## D-C 3.444444 -1.373536 8.262425 0.2653634 ## E-C 6.555556 1.737575 11.373536 0.0032615 ## E-D 3.111111 -1.706869 7.929092 0.3634301 #data summary VS\_data4 <- data\_summary(metadata, varname="VS\_Reduc", groupnames="Temp")</pre> VS data4 ## Temp VS Reduc sd A 8.555556 3.844188 ## 1 ## 2 B 16.777778 5.426274 ## 3 C 25.333333 1.936492 ## 4 D 28.777778 2.223611 ## 5 E 31.888889 3.333333 #2. Plot VS4 <- data summary(metadata, varname="VS Reduc", groupnames=c("Temp"))

```
VS4$Temp=as.factor(VS4$Temp)
VS4
##
     Temp VS_Reduc
                          sd
       A 8.555556 3.844188
## 1
## 2
        B 16.777778 5.426274
      C 25.333333 1.936492
## 3
## 4
      D 28.777778 2.223611
## 5
        E 31.888889 3.333333
box_4 <- ggplot(VS4, aes(x=Temp, y=VS_Reduc, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=VS_Reduc-sd, ymax=VS_Reduc+sd), width=0.2, posit
ion=position_dodge(0.9))+
  xlab("Temperature")+
  ylab("Volatile Solids Reduction (%)") + ylim(0, 40) + labs(title = "",
 subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=18, family="Times New Roman"),
        axis.title.y = element text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "red", "green", "green", "green"))
box 4
```



Figure A.10. Percent Average Reductions with Standard Deviations for Volatile Solids in BMP bottles, Average of Trials 1, 2 & 3

A.5 R-script and Results for Pre and Post Digestion pH

```
## Statistical analysis for pH
## Feb 17, 2021 CREATED MIB
# Loading Library and Tables -----
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package -----
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ----
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summarized
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary_func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
 }
 data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data_sum <- rename(data_sum, c("mean" = varname))</pre>
 return(data sum)
}
```

# ANALYSIS------

## the .txt file needs to be saved as the type of "Tab delimited".

```
con <-file.choose(new = FALSE)
metadata <- read.table(con, header = T, row.names = 1)</pre>
```

**## DEFINING FACTORS** 

### Abbreviations
## A- 10 C, non-mixed
## B- 20 C, non-mixed
## C- 30 C, non-mixed
## D- 39 C, non-mixed
## E- 39 C, mixed

metadata\$Temp <- factor(metadata\$Temp) ##Factor statement</pre>

## one-way ANOVA

```
fit1 <- aov(Pre~Temp, data = metadata)
summary(fit1)</pre>
```

##Df Sum Sq Mean Sq F value Pr(>F)## Temp4 0.0216 0.0053970.4 0.808## Residuals40 0.5403 0.013508

Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparisons
Tukey1</pre>

```
Tukey multiple comparisons of means
##
##
       95% family-wise confidence level
##
## Fit: aov(formula = Pre ~ Temp, data = metadata)
##
## $Temp
##
              diff
                          lwr
                                     upr
                                             p adj
## B-A -0.04555556 -0.2020384 0.11092728 0.9192355
## C-A -0.02333333 -0.1798162 0.13314950 0.9928639
## D-A -0.03333333 -0.1898162 0.12314950 0.9729471
## E-A -0.06555556 -0.2220384 0.09092728 0.7533016
## C-B 0.02222222 -0.1342606 0.17870506 0.9940790
## D-B 0.01222222 -0.1442606 0.16870506 0.9994257
## E-B -0.02000000 -0.1764828 0.13648284 0.9960534
## D-C -0.01000000 -0.1664828 0.14648284 0.9997405
```

```
## E-C -0.04222222 -0.1987051 0.11426061 0.9375546
## E-D -0.03222222 -0.1887051 0.12426061 0.9760930
# data summary
pH data1 <- data summary(metadata, varname="Pre", groupnames="Temp")
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
       mutate
pH_data1
##
     Temp
               Pre
                           sd
## 1
       A 7.615556 0.09234597
## 2
        B 7.570000 0.08544004
## 3
      C 7.592222 0.10929064
## 4
      D 7.582222 0.13169831
## 5
        E 7.550000 0.14974979
#2. Plot
pH1 <- data_summary(metadata, varname="Pre",</pre>
                    groupnames=c("Temp"))
pH1$Temp=as.factor(pH1$Temp)
pH1
##
     Temp
               Pre
                           sd
       A 7.615556 0.09234597
## 1
## 2
        B 7.570000 0.08544004
## 3
       C 7.592222 0.10929064
       D 7.582222 0.13169831
## 4
        E 7.550000 0.14974979
## 5
box 1 <- ggplot(pH1, aes(x=Temp, y=Pre, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
 geom_errorbar(aes(ymin=Pre-sd, ymax=Pre+sd), width=0.2, position=positi
on_dodge((0.9))+
 xlab("Temperature")+
 ylab("pH Pre-digestion") + ylim(0, 8) + labs(title = "", subtitle=NULL
) +
 theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=18, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element_text(size=20, family="Times New Roman"),
        legend.position="none")+
```

scale\_fill\_manual(values=c("blue", "blue", "blue", "blue", "blue"))
box\_1



Figure A.11. Average Pre-digestion pH with Standard Deviations in BMP bottles, Average of Trials 1, 2 & 3

```
## POST ANALYSIS-----
## one-way ANOVA
fit2 <- aov(Post~Temp, data = metadata)</pre>
summary(fit2)
##
               Df Sum Sq Mean Sq F value Pr(>F)
## Temp
                4 0.2496 0.06241
                                   1.841
                                            0.14
## Residuals
               40 1.3560 0.03390
Tukey2 <- TukeyHSD(fit2, conf.level=0.95) #Tukey multiple comparisons</pre>
Tukey2
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
##
## Fit: aov(formula = Post ~ Temp, data = metadata)
```

## ## \$Temp diff ## lwr upr p adj ## B-A -0.035555556 -0.28344936 0.2123383 0.9938489 ## C-A 0.1188888889 -0.12900492 0.3667827 0.6500231 ## D-A 0.154444444 -0.09344936 0.4023383 0.3993672 ## E-A 0.1166666667 -0.13122714 0.3645605 0.6658176 ## C-B 0.154444444 -0.09344936 0.4023383 0.3993672 ## D-B 0.190000000 -0.05789381 0.4378938 0.2047049 ## E-B 0.152222222 -0.09567158 0.4001160 0.4139990 ## D-C 0.035555556 -0.21233825 0.2834494 0.9938489 ## E-C -0.002222222 -0.25011603 0.2456716 0.9999999 ## E-D -0.037777778 -0.28567158 0.2101160 0.9922458 # data summary pH\_data2 <- data\_summary(metadata, varname="Post", groupnames="Temp")</pre> pH data2 ## Temp Post sd A 7.176667 0.1918333 ## 1 ## 2 B 7.141111 0.1499537 ## 3 C 7.295556 0.1955832 ## 4 D 7.331111 0.1673652 ## 5 E 7.293333 0.2096426 #2. Plot pH2 <- data summary(metadata, varname="Post",</pre> groupnames=c("Temp")) pH2\$Temp=as.factor(pH2\$Temp) pH2 ## Temp Post sd A 7.176667 0.1918333 ## 1 ## 2 B 7.141111 0.1499537 C 7.295556 0.1955832 ## 3 ## 4 D 7.331111 0.1673652 ## 5 E 7.293333 0.2096426 box\_2 <- ggplot(pH2, aes(x=Temp, y=Post, fill=Temp)) +</pre> geom\_bar(stat="identity", position=position\_dodge(0.9), width=0.5)+ geom errorbar(aes(ymin=Post-sd, ymax=Post+sd), width=0.2, position=posi tion\_dodge(0.9))+ xlab("Temperature")+ ylab("pH Post-digestion") + ylim(0, 8) + labs(title = "", subtitle=NUL L) + theme(title=element text(size=20, family="Times New Roman"), axis.text.x = element text(size=20, family="Times New Roman"), axis.text.y=element\_text(size=18, family="Times New Roman"), axis.title.y = element\_text(size = 20, family="Times New Roman"),



Figure A.12. Average Post-digestion pH with Standard Deviations in BMP bottles, Average of Trials 1, 2 & 3

```
Tukey multiple comparisons of means
##
##
       95% family-wise confidence level
##
## Fit: aov(formula = Change ~ Temp, data = metadata)
##
## $Temp
##
               diff
                           lwr
                                         upr
                                                 p adj
## B-A -0.010000000 -0.1617088 0.141708770 0.9997066
## C-A -0.142222222 -0.2939310 0.009486547 0.0754363
## D-A -0.187777778 -0.3394865 -0.036069008 0.0087587
## E-A -0.182222222 -0.3339310 -0.030513453 0.0116376
## C-B -0.132222222 -0.2839310 0.019486547 0.1134493
## D-B -0.177777778 -0.3294865 -0.026069008 0.0145536
## E-B -0.172222222 -0.3239310 -0.020513453 0.0191507
## D-C -0.045555556 -0.1972643 0.106153214 0.9104805
## E-C -0.040000000 -0.1917088 0.111708770 0.9423217
## E-D 0.005555556 -0.1461532 0.157264325 0.9999717
# data summary
pH data3 <- data summary(metadata, varname="Change", groupnames="Temp")
pH data3
##
     Temp
             Change
                            sd
        A 0.4388889 0.11285438
## 1
## 2
        B 0.4288889 0.07896905
## 3
       C 0.2966667 0.16568042
      D 0.2511111 0.08146233
## 4
## 5
       E 0.2566667 0.10210289
#2. Plot
pH3 <- data_summary(metadata, varname="Change",</pre>
                    groupnames=c("Temp"))
pH3$Temp=as.factor(pH3$Temp)
pH3
##
     Temp
             Change
                            sd
## 1
        A 0.4388889 0.11285438
## 2
        B 0.4288889 0.07896905
## 3
        C 0.2966667 0.16568042
## 4
       D 0.2511111 0.08146233
## 5
      E 0.2566667 0.10210289
box_3 <- ggplot(pH3, aes(x=Temp, y=Change, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=Change-sd, ymax=Change+sd), width=0.2, position=
position dodge((0,9))+
  xlab("Temperature")+
  ylab("pH Change") + ylim(0, 0.6) + labs(title = "", subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
```

```
axis.text.y=element_text(size=18, family="Times New Roman"),
axis.title.y = element_text(size = 20, family="Times New Roman"),
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("blue", "blue", "green","green","green"))
box_3
```



Figure A.13. Average pH Change with Standard Deviations in BMP bottles, Average of Trials 1, 2 & 3

# **Appendix B. R-Scripts and Results for Pilot Data**

#### B.1 R-script and Results for Biogas Production

```
## Statistical analysis for Biogas
## Maria Bariosarosemena's data
## Feb 9, 2021 created
## Feb 9, 2021 update WL
## Feb 9, 2021 update MIB
## Feb 15, 2021 update MIB
## Mar 4, 2021 update MIB
# Loading Library and Tables ------
- -
library (MASS)
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package ------
_ _
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summariezed
# groupnames : vector of column names to be used as
# grouping variables
```

# ANALYSIS------

## the .txt file needs to be saved as the type of "Tab delimited".

#### ##Load biogas.txt

con <-file.choose(new = FALSE)
metadata <- read.table(con, header = T, row.names = 1)</pre>

#### **## DEFINING FACTORS**

```
metadata$HRT <- factor(metadata$HRT) ##Factor Statement
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
```

#### # 1. Effects of HRT and temp on Daily Biogas production

```
## two-way ANOVA
```

```
# Daily biogas
fit1 <- aov(Daily_Gas~Temp*HRT, data = metadata)</pre>
summary(fit1)
##
                Df Sum Sq Mean Sq F value Pr(>F)
## Temp
                 2 61.66 30.831 77.236 < 2e-16 ***
## HRT
                 2
                     0.26 0.128
                                  0.321
                                            0.726
               4 16.73 4.183 10.478 4.8e-08 ***
## Temp:HRT
## Residuals 365 145.70 0.399
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparions</pre>
Tukey1
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
##
```

## Fit: aov(formula = Daily Gas ~ Temp \* HRT, data = metadata) ## ## \$Temp ## diff lwr p adj upr ## H-B 0.90138581 0.7129359 1.0898357 0.0000000 ## L-B 0.08754581 -0.1009041 0.2759957 0.5188596 ## L-H -0.81384000 -1.0019111 -0.6257689 0.0000000 ## ## \$HRT ## diff lwr upr p adi ## 2-1 0.06480571 -0.1259744 0.2555858 0.7036300 ## 3-1 0.03400822 -0.1504107 0.2184271 0.9014415 ## 3-2 -0.03079749 -0.2215776 0.1599826 0.9235542 ## ## \$`Temp:HRT` ## diff lwr upr p adj ## H:1-B:1 0.61048097 0.18770746 1.03325449 0.0003032 ## L:1-B:1 -0.54510042 -0.96787394 -0.12232691 0.0022511 ## B:2-B:1 -0.27120813 -0.70782198 0.16540571 0.5875091 ## H:2-B:1 0.68431818 0.24770434 1.12093203 0.0000526 ## L:2-B:1 -0.14620813 -0.58282198 0.29040571 0.9811310 ## B:3-B:1 -0.56330087 -0.98861220 -0.13798953 0.0014566 ## H:3-B:1 0.59500000 0.17466324 1.01533676 0.0004458 ## L:3-B:1 0.12954545 -0.29079130 0.54988221 0.9889461 ## L:1-H:1 -1.15558140 -1.58077770 -0.73038509 0.0000000 ## B:2-H:1 -0.88168911 -1.32064936 -0.44272886 0.0000000 ## H:2-H:1 0.07383721 -0.36512304 0.51279746 0.9998539 ## L:2-H:1 -0.75668911 -1.19564936 -0.31772886 0.0000048 ## B:3-H:1 -1.17378184 -1.60150159 -0.74606209 0.0000000 ## H:3-H:1 -0.01548097 -0.43825449 0.40729254 1.0000000 ## L:3-H:1 -0.48093552 -0.90370903 -0.05816200 0.0128267 ## B:2-L:1 0.27389229 -0.16506796 0.71285254 0.5814163 ## H:2-L:1 1.22941860 0.79045835 1.66837886 0.0000000 ## L:2-L:1 0.39889229 -0.04006796 0.83785254 0.1086868 ## B:3-L:1 -0.01820044 -0.44592019 0.40951930 1.0000000 0.71732691 1.56287394 0.0000000 ## H:3-L:1 1.14010042 ## L:3-L:1 0.67464588 0.25187236 1.09741939 0.0000345 ## H:2-B:2 0.95552632 0.50322077 1.40783186 0.0000000 ## L:2-B:2 0.12500000 -0.32730554 0.57730554 0.9946735 ## B:3-B:2 -0.29209273 -0.73349775 0.14931228 0.4990831 ## H:3-B:2 0.86620813 0.42959429 1.30282198 0.0000001 ## L:3-B:2 0.40075359 -0.03586025 0.83736743 0.1010383 ## L:2-H:2 -0.83052632 -1.28283186 -0.37822077 0.0000008 ## B:3-H:2 -1.24761905 -1.68902406 -0.80621403 0.0000000 0.34729566 0.9993719 ## H:3-H:2 -0.08931818 -0.52593203 ## L:3-H:2 -0.55477273 -0.99138657 -0.11815888 0.0028255 ## B:3-L:2 -0.41709273 -0.85849775 0.02431228 0.0809046 ## H:3-L:2 0.74120813 0.30459429 1.17782198 0.0000072 ## L:3-L:2 0.27575359 -0.16086025 0.71236743 0.5647862 ## H:3-B:3 1.15830087 0.73298953 1.58361220 0.0000000

## L:3-B:3 0.69284632 0.26753499 1.11815765 0.0000209 ## L:3-H:3 -0.46545455 -0.88579130 -0.04511779 0.0176206 *#Biogas data summary* Daily Gas data1 <- data summary(metadata, varname="Daily Gas", groupnames ="HRT") ## Loading required package: plyr ## ## Attaching package: 'plyr' ## The following object is masked from 'package:ggpubr': ## ## mutate Daily Gas data1 ## HRT Daily Gas sd ## 1 1.152308 0.7904366 ## 2 2 1.219649 0.7634747 ## 3 3 1.193923 0.7755893 Daily Gas data2 <- data summary(metadata, varname="Daily Gas", groupnames ="Temp") Daily\_Gas\_data2 Temp Daily Gas ## sd ## 1 B 0.8567742 0.6122377 ## 2 H 1.7581600 0.8327552 ## 3 L 0.9443200 0.4966782 #2. Plot for Daily Biogas Production *#Daily gas production based on HRT* Daily gas\_production1 <- data\_summary(metadata, varname="Daily\_Gas", groupnames=c("HRT")) Daily\_gas\_production1\$HRT=as.factor(Daily\_gas\_production1\$HRT) Daily\_gas\_production1 ## HRT Daily Gas sd ## 1 1 1.152308 0.7904366 2 1.219649 0.7634747 ## 2 ## 3 3 1.193923 0.7755893 box\_1 <- ggplot(Daily\_gas\_production1, aes(x=HRT, y=Daily\_Gas, fill=HRT))</pre> geom\_bar(stat="identity", position=position\_dodge(0.9), width=0.5)+ geom\_errorbar(aes(ymin=Daily\_Gas-sd, ymax=Daily\_Gas+sd), width=0.2, pos ition=position\_dodge(0.9))+





#Daily gas production based on Temp

Daily\_gas\_production2\$Temp=as.factor(Daily\_gas\_production2\$Temp)
Daily\_gas\_production2

```
##
    Temp Daily Gas
                           sd
## 1
        B 0.8567742 0.6122377
## 2
       H 1.7581600 0.8327552
## 3
        L 0.9443200 0.4966782
box_2 <- ggplot(Daily_gas_production2, aes(x=Temp, y=Daily_Gas, fill=Temp</pre>
)) +
 geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom errorbar(aes(ymin=Daily_Gas-sd, ymax=Daily_Gas+sd), width=0.2, pos
ition=position_dodge(0.9))+
 xlab("Temperature")+
 ylab("Daily biogas production (L/day)") + ylim(0, 3) + labs(title = ""
, subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=18, family="Times New Roman"),
        axis.title.y = element text(size = 20, family="Times New Roman"),
        axis.title.x=element_text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "red", "blue"))
box_2
```



Figure B.2. Average Daily Biogas Production based on Temperature Profile, Average of 3 HRT's

### 95% family-wise confidence level



Differences in mean levels of Temp:HRT

### Figure B.3. Tukey Honest Significant Difference Results for the Daily Biogas Production based on HRT and Temperature

```
## Section 2------
_ _ _ _ _ _ _ _
# 3. Effects of HRT and temp on Biogas Production per kg Initial VS
## two-way ANOVA
# Biogas per Kg Initial VS
fit2 <- aov(Gas_kgVS~Temp*HRT, data = metadata)</pre>
summary(fit2)
##
                Df
                     Sum Sq Mean Sq F value
                                               Pr(>F)
                 2
                    7397233 3698616 88.756
                                             < 2e-16 ***
## Temp
## HRT
                      53740
                               26870
                                       0.645
                                                 0.525
                 2
## Temp:HRT
                 4
                    2045847
                              511462 12.274 2.25e-09 ***
## Residuals
               365 15210220
                               41672
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey2 <- TukeyHSD(fit2, conf.level=0.95) #Tukey multiple comparions</pre>
Tukey2
##
     Tukey multiple comparisons of means
       95% family-wise confidence level
##
##
```

## Fit: aov(formula = Gas\_kgVS ~ Temp \* HRT, data = metadata) ## ## \$Temp ## diff lwr p adj upr ## H-B 313.56819 252.67972 374.45667 0.0000000 -27.32828 ## L-B 33.56019 94.44867 0.3977175 ## L-H -280.00800 -340.77409 -219.24191 0.0000000 ## ## \$HRT ## diff lwr upr p adj ## 2-1 5.380373 -56.26097 67.02171 0.9770097 ## 3-1 27.268243 -32.31781 86.85429 0.5289960 ## 3-2 21.887871 -39.75347 83.52921 0.6811269 ## ## \$`Temp:HRT` ## diff lwr upr p adj ## H:1-B:1 204.405391 67.806598 341.004184 0.0001464 ## L:1-B:1 -182.501586 -319.100379 -45.902792 0.0012582 ## B:2-B:1 -103.642344 -244.712970 37.428281 0.3493112 ## H:2-B:1 215.647129 74.576504 356.717755 0.0000924 -71.458134 -212.528759 69.612492 0.8149030 ## L:2-B:1 ## B:3-B:1 -191.003247 -328.422013 -53.584481 0.0006266 ## H:3-B:1 230.795455 94.983981 366.606928 0.0000070 61.386364 -74.425110 197.197837 0.8934187 ## L:3-B:1 ## L:1-H:1 -386.906977 -524.288577 -249.525376 0.0000000 ## B:2-H:1 -308.047736 -449.876489 -166.218982 0.0000000 ## H:2-H:1 11.241738 -130.587015 153.070491 0.9999996 ## L:2-H:1 -275.863525 -417.692278 -134.034772 0.0000001 ## B:3-H:1 -395.408638 -533.605567 -257.211709 0.0000000 ## H:3-H:1 26.390063 -110.208730 162.988857 0.9995882 ## L:3-H:1 -143.019027 -279.617821 -6.420234 0.0321991 -62.969512 220.687995 0.7244409 ## B:2-L:1 78.859241 ## H:2-L:1 398.148715 256.319961 539.977468 0.0000000 ## L:2-L:1 111.043452 -30.785302 252.872205 0.2639602 ## B:3-L:1 -8.501661 -146.698590 129.695268 0.9999999 549.895833 0.000000 ## H:3-L:1 413.297040 276.698247 ## L:3-L:1 243.887949 107.289156 380.486742 0.0000017 ## H:2-B:2 319.289474 173.148835 465.430113 0.0000000 ## L:2-B:2 32.184211 -113.956428 178.324849 0.9989246 ## B:3-B:2 -87.360902 -229.979562 55.257758 0.6062381 ## H:3-B:2 334.437799 193.367174 475.508425 0.0000000 ## L:3-B:2 165.028708 23.958083 306.099334 0.0090409 ## L:2-H:2 -287.105263 -433.245902 -140.964624 0.0000001 ## B:3-H:2 -406.650376 -549.269036 -264.031716 0.0000000 ## H:3-H:2 15.148325 -125.922300 156.218951 0.9999954 ## L:3-H:2 -154.260766 -295.331391 -13.190140 0.0203076 ## B:3-L:2 -119.545113 -262.163773 23.073547 0.1836643 ## H:3-L:2 302.253589 161.182963 443.324214 0.000000 ## L:3-L:2 132.844498 -8.226128 273.915123 0.0831233 ## H:3-B:3 421.798701 284.379935 559.217467 0.0000000

## L:3-B:3 252.389610 114.970844 389.808376 0.0000007 ## L:3-H:3 -169.409091 -305.220565 -33.597617 0.0037287 *#Biogas data summary* Gaskg data1 <- data summary(metadata, varname="Gas kgVS", groupnames="HRT ") Gaskg\_data1 ## HRT Gas kgVS sd 1 394.6769 254.4945 ## 1 ## 2 2 400.9474 246.6602 ## 3 3 424.6154 270.1238 Gaskg data2 <- data summary(metadata, varname="Gas kgVS", groupnames="Tem p") Gaskg\_data2 Temp Gas\_kgVS ## sd ## 1 B 290.9758 193.9921 ## 2 H 604.5440 276.3760 ## 3 L 324.5360 160.8791 #2. Plot for Biogas Production per kg Initial VS #Gas per VS production based on HRT Gaskg\_production1 <- data\_summary(metadata, varname="Gas\_kgVS", groupnames=c("HRT")) Gaskg production1\$HRT=as.factor(Gaskg production1\$HRT) Gaskg production1 ## HRT Gas\_kgVS sd ## 1 1 394.6769 254.4945 ## 2 2 400.9474 246.6602 ## 3 3 424.6154 270.1238 box 3 <- ggplot(Gaskg production1, aes(x=HRT, y=Gas kgVS, fill=HRT)) + geom\_bar(stat="identity", position=position\_dodge(0.9), width=0.5)+ geom\_errorbar(aes(ymin=Gas\_kgVS-sd, ymax=Gas\_kgVS+sd), width=0.2, posit ion=position dodge(0.9))+ xlab("HRT")+ ylab("Daily biogas production per kg Initial VS (L/kg/day)") + ylim(0, 800) + labs(title = "", subtitle=NULL) + theme(title=element text(size=20, family="Times New Roman"), axis.text.x = element\_text(size=20, family="Times New Roman"), axis.text.y=element text(size=12, family="Times New Roman"), axis.title.y = element\_text(size = 15, family="Times New Roman"), axis.title.x=element\_text(size=20, family="Times New Roman"), legend.position="none")+



Figure B.4. Average Daily Biogas Production in L per kg Initial VS based on HRTs, Average of 3 Conditions

```
#Gas per VS production based on Temp
Gaskg production2 <- data summary(metadata, varname="Gas kgVS",
                                  groupnames=c("Temp"))
Gaskg production2$Temp=as.factor(Gaskg production2$Temp)
Gaskg_production2
##
     Temp Gas_kgVS
                         sd
## 1
        B 290.9758 193.9921
## 2
       H 604.5440 276.3760
## 3
       L 324.5360 160.8791
box_4 <- ggplot(Gaskg_production2, aes(x=Temp, y=Gas_kgVS, fill=Temp)) +</pre>
 geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
 geom_errorbar(aes(ymin=Gas_kgVS-sd, ymax=Gas_kgVS+sd), width=0.2, posit
ion=position_dodge(0.9))+
xlab("Temperature")+
```



Figure B.5. Average Daily Biogas Production in L per kg Initial VS based on Temperature Profile, Average of 3 HRT's

### 95% family-wise confidence level



Differences in mean levels of Temp:HRT

## Figure B.6. Tukey Honest Significant Difference Results for the Daily Biogas Production per kg Initial VS based on HRT and Temperature

```
##Section 3------
                                         _ _ _ _ _ _ _
# 4. Effects of HRT and temp on Cumulative Biogas Production
## two-way ANOVA
# Biogas per Kg Initial VS
fit3 <- aov(Total_Gas~Temp*HRT, data = metadata)</pre>
summary(fit3)
##
               Df Sum Sq Mean Sq F value Pr(>F)
                           32898 248.34 <2e-16 ***
                   65797
## Temp
                2
## HRT
                           80514 607.77 <2e-16 ***
                2 161029
## Temp:HRT
                   22659
                            5665
                                   42.76 <2e-16 ***
                4
## Residuals
                             132
              365 48353
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey3 <- TukeyHSD(fit3, conf.level=0.95) #Tukey multiple comparions
Tukey3
##
    Tukey multiple comparisons of means
       95% family-wise confidence level
##
##
## Fit: aov(formula = Total_Gas ~ Temp * HRT, data = metadata)
```

##					
##	\$Temp				
##		diff	lwr	upr p	adj
##	H-B 24.	912219 21	.479175 28.	345264 0.0000	0000
##	L-B -5.	560501 -8	.993545 -2.	127456 0.0004	1746
##	L-H -30.	472720 -33	.898864 -27.	046576 0.0000	0000
##					
##	\$HRT				
##	7	diff	lwr upr	n adi	
##	2-1 23.0	1358 19.53	809 26.48908	0	
##	3-1 49.7	2653 46.36	591 53,08614	0	
##	3-2 26 7	1294 23 23	745 30 18843	0	
##	5 2 20.7	1294 29.29	, -, -, -, -, -, -, -, -, -, -, -, -, -,	0	
ш. ##	\$`Temp·H	RT`			
##	\$ 1Cmp.m	diff	lwr	unn	n adi
## ##	∐•1_B•1	5 863833	_1 8370501	13 5656130	
## ##	$1 \cdot 1 - B \cdot 1$	_3 06128/	-11 6630664	3 7/0/077	0.3004730
## ##	L.I-D.I D.2 D.1	- 3. 901204	12 7400064	20 6570266	0.001/312
## ##	D.Z-D.I	42 211906	12.7499904		0.0000000
## ##	$\Pi: Z - D: I$	42.211000	0 2070011	JU.10J/21J	0.0000000
##	L:2-D:1	0.101000	0.20/8911	10.115/215	0.0392739
##	B:3-B:1	35.969037	28.2210227	43./1/0509	0.0000000
##	H:3-B:1	81.222955	/3.5655636	88.8803455	0.0000000
##	L:3-B:1	33.661364	26.0039/2/	41.318/546	0.0000000
##	L:1-H:1	-9.825116	-17.5710349	-2.0/919/6	0.0029014
##	B:2-H:1	14.840080	6.8434193	22.836/398	0.0000005
##	H:2-H:1	36.34/9/4	28.3513140	44.3446346	0.0000000
##	L:2-H:1	2.29/9/4	-5.6986860	10.2946346	0.9930586
##	B:3-H:1	30.105205	22.3133160	37.8970938	0.0000000
##	H:3-H:1	75.359123	67.6573406	83.0609046	0.000000
##	L:3-H:1	27.797532	20.0957497	35.4993137	0.000000
##	B:2-L:1	24.665196	16.6685356	32.6618561	0.000000
##	H:2-L:1	46.173091	38.1764303	54.1697508	0.000000
##	L:2-L:1	12.123091	4.1264303	20.1197508	0.0001107
##	B:3-L:1	39.930321	32.1384322	47.7222101	0.000000
##	H:3-L:1	85.184239	77.4824569	92.8860209	0.000000
##	L:3-L:1	37.622648	29.9208660	45.3244300	0.000000
##	H:2-B:2	21.507895	13.2681196	29.7476699	0.000000
##	L:2-B:2	-12.542105	-20.7818804	-4.3023301	0.0001014
##	B:3-B:2	15.265125	7.2239281	23.3063225	0.000003
##	H:3-B:2	60.519043	52.5651279	68.4729582	0.000000
##	L:3-B:2	12.957452	5.0035370	20.9113673	0.0000209
##	L:2-H:2	-34.050000	-42.2897752	-25.8102248	0.000000
##	B:3-H:2	-6.242769	-14.2839666	1.7984278	0.2748164
##	H:3-H:2	39.011148	31.0572332	46.9650634	0.000000
##	L:3-H:2	-8.550443	-16.5043577	-0.5965275	0.0244685
##	B:3-L:2	27.807231	19.7660334	35.8484278	0.000000
##	H:3-L:2	73.061148	65.1072332	81.0150634	0.000000
##	L:3-L:2	25.499557	17.5456423	33.4534725	0.000000
##	H:3-B:3	45.253918	37.5059036	53.0019319	0.000000

```
## L:3-B:3 -2.307673 -10.0556873 5.4403410 0.9911793
## L:3-H:3 -47.561591 -55.2189819 -39.9041999 0.0000000
#Biogas data summary
T data1 <- data summary(metadata, varname="Total Gas", groupnames="HRT")
T data1
##
    HRT Total Gas
                          sd
## 1 1 12.76408 8.994012
       2 35.82728 16.773643
## 2
## 3 3 62.63946 26.942374
T_data2 <- data_summary(metadata, varname="Total_Gas", groupnames="Temp")</pre>
T data2
##
     Temp Total Gas
                          sd
## 1
        B 30.66258 17.66933
       H 55.57480 35.58018
## 2
## 3 L 25.10208 17.19481
#2. Plot for Biogas Production per kg Initial VS
#HRT
T_production1 <- data_summary(metadata, varname="Total_Gas",</pre>
                                  groupnames=c("HRT"))
T_production1$HRT=as.factor(T_production1$HRT)
T production1
##
    HRT Total Gas
                          sd
## 1 1 12.76408 8.994012
## 2
       2 35.82728 16.773643
## 3
     3 62.63946 26.942374
box 5 <- ggplot(T production1, aes(x=HRT, y=Total Gas, fill=HRT)) +
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom errorbar(aes(ymin=Total Gas-sd, ymax=Total Gas+sd), width=0.2, pos
ition=position dodge(0.9))+
  xlab("HRT")+
  ylab("Cumulative Biogas Production (L)") + ylim(0, 100) + labs(title =
 "", subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=12, family="Times New Roman"),
        axis.title.y = element text(size = 15, family="Times New Roman"),
        axis.title.x=element_text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "green", "red"))
box 5
```

```
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```



Figure B.7. Average Cumulative Biogas Production based on HRTs, Average of 3 Conditions

## #TEMP

```
T production2 <- data summary(metadata, varname="Total Gas",</pre>
                                   groupnames=c("Temp"))
T_production2$Temp=as.factor(T_production2$Temp)
T_production2
##
     Temp Total Gas
                          sd
## 1
           30.66258 17.66933
        В
## 2
        H 55.57480 35.58018
## 3
           25.10208 17.19481
        L
box_6 <- ggplot(T_production2, aes(x=Temp, y=Total_Gas, fill=Temp)) +</pre>
 geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=Total_Gas-sd, ymax=Total_Gas+sd), width=0.2, pos
ition=position dodge(0.9))+
 xlab("Temperature")+
 ylab("Cumulative Biogas Production (L)") + ylim(0, 100) + labs(title =
 "", subtitle=NULL) +
 theme(title=element_text(size=20, family="Times New Roman"),
```

```
axis.text.x = element_text(size=20, family="Times New Roman"),
axis.text.y=element_text(size=12, family="Times New Roman"),
axis.title.y = element_text(size = 15, family="Times New Roman"),
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("blue", "red", "blue"))
box_6
```



Figure B.8. Average Cumulative Biogas Production based on Temperature Profile, Average of 3 HRT's

B.2 R-script and Results for Methane Production

```
library(ggplot2)
library(grid)
library(gridExtra)
library(ggpubr)
# Installing the font package ------
library(extrafont)
## Registering fonts with R
font_import() #It may take a few minutes to import.
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summariezed
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
  require(plyr)
  summary_func <- function(x, col){</pre>
    c(mean = mean(x[[col]], na.rm=TRUE),
      sd = sd(x[[col]], na.rm=TRUE))
  }
  data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data_sum <- rename(data_sum, c("mean" = varname))</pre>
 return(data_sum)
}
# ANALYSIS-----
## the .txt file needs to be saved as the type of "Tab delimited".
```

```
##Load Methane.txt
```

```
con <-file.choose(new = FALSE)</pre>
metadata <- read.table(con, header = T, row.names = 1)</pre>
## DEFINING FACTORS
metadata$HRT <- factor(metadata$HRT) ##Factor Statement</pre>
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
# 1. Effects of HRT and temp on Methane
## two-way ANOVA
# Methane
fit1 <- aov(Methane_percent~Temp*HRT, data = metadata)</pre>
summary(fit1)
##
                Df Sum Sq Mean Sq F value
                                              Pr(>F)
## Temp
                 2 554.2 277.09 46.881 1.11e-15 ***
## HRT
                       2.8
                              1.40
                                     0.236
                                               0.790
                 2
## Temp:HRT
                 4
                      18.1
                              4.54
                                     0.767
                                               0.549
## Residuals
               117 691.5
                              5.91
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparions</pre>
Tukev1
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = Methane_percent ~ Temp * HRT, data = metadata)
##
## $Temp
##
            diff
                        lwr
                                   upr
                                            p adj
## H-B -4.905000 -6.164401 -3.6455991 0.0000000
## L-B -1.130476 -2.389877 0.1289247 0.0880238
## L-H 3.774524 2.515123 5.0339247 0.0000000
##
## $HRT
##
             diff
                          lwr
                                   upr
                                            p adj
## 2-1 -0.2376190 -1.4970200 1.021782 0.8954284
## 3-1 0.1207143 -1.1386866 1.380115 0.9718685
## 3-2 0.3583333 -0.9010676 1.617734 0.7782203
##
## $`Temp:HRT`
##
                  diff
                                lwr
                                            upr
                                                    p adj
## H:1-B:1 -4.93214286 -7.83643749 -2.0278482 0.0000143
## L:1-B:1 -1.98428571 -4.88858034 0.9200089 0.4389207
## B:2-B:1 -0.95857143 -3.86286606 1.9457232 0.9806807
```

```
## H:2-B:1 -5.72642857 -8.63072320 -2.8221339 0.0000003
## L:2-B:1 -0.94428571 -3.84858034 1.9600089 0.9824231
## B:3-B:1 -0.03928571 -2.94358034 2.8650089 1.0000000
## H:3-B:1 -5.05428571 -7.95858034 -2.1499911 0.0000079
## L:3-B:1 -1.46071429 -4.36500892 1.4435803 0.8085541
## L:1-H:1 2.94785714 0.04356251 5.8521518 0.0437798
## B:2-H:1 3.97357143 1.06927680 6.8778661 0.0010496
## H:2-H:1 -0.79428571 -3.69858034 2.1100089 0.9943485
## L:2-H:1 3.98785714 1.08356251 6.8921518 0.0009895
## B:3-H:1 4.89285714 1.98856251 7.7971518 0.0000172
## H:3-H:1 -0.12214286 -3.02643749 2.7821518 1.0000000
## L:3-H:1 3.47142857
                       0.56713394 6.3757232 0.0074530
## B:2-L:1
           1.02571429 -1.87858034 3.9300089 0.9706820
## H:2-L:1 -3.74214286 -6.64643749 -0.8378482 0.0026658
## L:2-L:1 1.04000000 -1.86429463
                                  3.9442946 0.9681353
## B:3-L:1 1.94500000 -0.95929463 4.8492946 0.4672270
## H:3-L:1 -3.07000000 -5.97429463 -0.1657054 0.0297902
## L:3-L:1 0.52357143 -2.38072320 3.4278661 0.9997137
## H:2-B:2 -4.76785714 -7.67215177 -1.8635625 0.0000312
## L:2-B:2 0.01428571 -2.89000892 2.9185803 1.0000000
## B:3-B:2 0.91928571 -1.98500892 3.8235803 0.9851799
## H:3-B:2 -4.09571429 -7.00000892 -1.1914197 0.0006302
## L:3-B:2 -0.50214286 -3.40643749 2.4021518 0.9997905
## L:2-H:2 4.78214286 1.87784823 7.6864375 0.0000292
## B:3-H:2 5.68714286
                      2.78284823 8.5914375 0.0000003
## H:3-H:2 0.67214286 -2.23215177 3.5764375 0.9982298
## L:3-H:2 4.26571429 1.36141966 7.1700089 0.0003038
## B:3-L:2 0.90500000 -1.99929463 3.8092946 0.9865979
## H:3-L:2 -4.11000000 -7.01429463 -1.2057054 0.0005932
## L:3-L:2 -0.51642857 -3.42072320 2.3878661 0.9997416
## H:3-B:3 -5.01500000 -7.91929463 -2.1107054 0.0000096
## L:3-B:3 -1.42142857 -4.32572320 1.4828661 0.8307447
## L:3-H:3
          3.59357143 0.68927680 6.4978661 0.0047278
#Methane data summary
Methane data1 <- data_summary(metadata, varname="Methane_percent", groupn</pre>
ames="HRT")
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
       mutate
Methane data1
    HRT Methane_percent
                              sd
##
## 1 1
         60.53667 3.461578
```

 ## 2
 2
 60.29905
 3.070081

 ## 3
 3
 60.65738
 3.068768

Methane\_data2 <- data\_summary(metadata, varname="Methane\_percent", groupn ames="Temp") Methane\_data2

##		Temp	Methane_percent	sd
##	1	В	62.50952	2.578621
##	2	Н	57.60452	1.691031
##	3	L	61.37905	2.804994

#### #2. Plot for Methane

# based on HRT

```
Methane data production1 <- data summary(metadata, varname="Methane perce
nt",
                                      groupnames=c("HRT"))
Methane data production1$HRT=as.factor(Methane data production1$HRT)
Methane_data_production1
##
    HRT Methane_percent
                               sd
## 1 1
                60.53667 3.461578
## 2
       2
                60.29905 3.070081
## 3
     3
               60.65738 3.068768
box 1 <- ggplot(Methane data production1, aes(x=HRT, y=Methane percent, f
ill=HRT)) +
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
 geom errorbar(aes(ymin=Methane percent-sd, ymax=Methane percent+sd), wi
dth=0.2, position=position_dodge(0.9))+
 xlab("HRT")+
 ylab("Methane Concentration (%)") + ylim(0, 70) + labs(title = "", sub
title=NULL) +
 theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=20, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "blue", "blue"))
box 1
```


Figure B.9. Average Methane Content based on HRTs, Average of 3 Conditions

#### # based on Temp

```
Methane_data_production2 <- data_summary(metadata, varname="Methane_perce
nt",</pre>
```

```
groupnames=c("Temp"))
```

Methane\_data\_production2\$Temp=as.factor(Methane\_data\_production2\$Temp)
Methane\_data\_production2

##		Temp	Methane_percent	sd
##	1	В	62.50952	2.578621
##	2	Н	57.60452	1.691031
##	3	L	61.37905	2.804994

```
box_2 <- ggplot(Methane_data_production2, aes(x=Temp, y=Methane_percent,
fill=Temp)) +
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=Methane_percent-sd, ymax=Methane_percent+sd), wi
  dth=0.2, position=position_dodge(0.9))+
   xlab("Temperature")+
   ylab("Methane Concentration (%)") + ylim(0, 70) + labs(title = "", sub
  title=NULL) +
```



Figure B.10. Average Methane Content based on Temperature Profile, Average of 3 HRT's



Differences in mean levels of Temp:HRT

#### Figure B.11. Tukey Honest Significant Difference Results for the Methane Content based on HRT and Temperature

B.3 R-script and Results for Hydrogen Sulfide

```
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summariezed
# groupnames : vector of column names to be used as
# grouping variables
data_summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary_func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
 }
 data_sum<-ddply(data, groupnames, .fun=summary_func,</pre>
                 varname)
 data_sum <- rename(data_sum, c("mean" = varname))</pre>
 return(data_sum)
}
## the .txt file needs to be saved as the type of "Tab delimited".
##Load Methane.txt
con <-file.choose(new = FALSE)</pre>
metadata <- read.table(con, header = T, row.names = 1)</pre>
## DEFINING FACTORS
metadata$HRT <- factor(metadata$HRT) ##Factor Statement</pre>
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
# 1. Effects of HRT and temp
```

```
## two-way ANOVA
```

fit1 <- aov(H2S ppm~Temp\*HRT, data = metadata)</pre> summary(fit1) ## Df Sum Sq Mean Sq F value Pr(>F) 4045144 7.963 0.000608 \*\*\* ## Temp 2 8090288 ## HRT 2 81238768 40619384 79.964 < 2e-16 \*\*\* ## Temp:HRT 4 3986279 996570 1.962 0.105881 ## Residuals 103 52321088 507972 ## ---## Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1 Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparions</pre> Tukey1 ## Tukey multiple comparisons of means ## 95% family-wise confidence level ## ## Fit: aov(formula = H2S\_ppm ~ Temp \* HRT, data = metadata) ## ## \$Temp ## diff lwr upr p adj ## H-B -176.7617 -571.3948 217.87141 0.5377878 ## L-B -630.6642 -1017.0003 -244.32813 0.0005333 ## L-H -453.9025 -850.9840 -56.82103 0.0208535 ## ## \$HRT ## diff lwr upr p adj ## 2-1 552.2164 138.7032 965.7295 0.0055526 ## 3-1 2031.6423 1618.1291 2445.1555 0.0000000 ## 3-2 1479.4260 1109.5685 1849.2834 0.0000000 ## ## \$`Temp:HRT` ## diff lwr upr p adj -40.03416 -1131.98110 1051.9128 1.0000000 ## H:1-B:1 ## L:1-B:1 -96.14873 -1082.93694 890.6395 0.9999974 ## B:2-B:1 739.10084 -170.85494 1649.0566 0.2106568 ## H:2-B:1 559.91442 -350.04137 1469.8702 0.5813346 ## L:2-B:1 225.44870 -684.50709 1135.4045 0.9970591 ## B:3-B:1 2587.11799 1677.16220 3497.0738 0.0000000 2003.26656 1093.31077 2913.2223 0.0000000 ## H:3-B:1 ## L:3-B:1 1372.35727 462.40149 2282.3131 0.0001975 ## L:1-H:1 -56.11457 -1169.09131 1056.8622 1.0000000 ## B:2-H:1 779.13500 -266.32460 1824.5946 0.3163721 ## H:2-H:1 599.94857 -445.51103 1645.4082 0.6699726 ## L:2-H:1 265.48286 -779.97675 1310.9425 0.9965097 ## B:3-H:1 2627.15214 1581.69254 3672.6117 0.0000000 ## H:3-H:1 2043.30071 997.84111 3088.7603 0.0000004 366.93182 2457.8510 0.0013414 ## L:3-H:1 1412.39143 ## B:2-L:1 835.24957 -99.83793 1770.3371 0.1191939

## H:2-L:1 656.06314 -279.02435 1591.1506 0.3988493 ## L:2-L:1 321.59743 -613.49007 1256.6849 0.9745064 ## B:3-L:1 2683.26671 1748.17922 3618.3542 0.0000000 ## H:3-L:1 2099.41529 1164.32779 3034.5028 0.0000000 ## L:3-L:1 1468.50600 533.41850 2403.5935 0.0000888 ## H:2-B:2 -179.18643 -1032.80062 674.4278 0.9990964 ## L:2-B:2 -513.65214 -1367.26634 339.9620 0.6107062 ## B:3-B:2 1848.01714 994.40295 2701.6313 0.0000000 ## H:3-B:2 1264.16571 410.55152 2117.7799 0.0002781 633.25643 -220.35776 1486.8706 0.3224487 ## L:3-B:2 ## L:2-H:2 -334.46571 -1188.07991 519.1485 0.9451051 ## B:3-H:2 2027.20357 1173.58938 2880.8178 0.0000000 ## H:3-H:2 1443.35214 589.73795 2296.9663 0.0000179 ## L:3-H:2 812.44286 -41.17134 1666.0570 0.0752011 ## B:3-L:2 2361.66929 1508.05509 3215.2835 0.0000000 ## H:3-L:2 1777.81786 924.20366 2631.4320 0.0000001 ## L:3-L:2 1146.90857 293.29438 2000.5228 0.0014620 ## H:3-B:3 -583.85143 -1437.46562 269.7628 0.4346007 ## L:3-B:3 -1214.76071 -2068.37491 -361.1465 0.0005678 ## L:3-H:3 -630.90929 -1484.52348 222.7049 0.3274055 ##data summary H2S data1 <- data summary(metadata, varname="H2S ppm", groupnames="HRT") ## Loading required package: plyr ## ## Attaching package: 'plyr' ## The following object is masked from 'package:ggpubr': ## ## mutate H2S\_data1 ## HRT H2S ppm sd ## 1 1 209.5654 214.3300 ## 2 2 762.0674 577.8486 ## 3 3 2241.4933 1100.7416 H2S data2 <- data summary(metadata, varname="H2S ppm", groupnames="Temp") H2S\_data2 ## Temp H2S ppm sd ## 1 B 1447.9400 1388.3155 ## 2 H 1271.1783 1044.1035 ## 3 L 817.2758 858.0148 #2. Plot for H2S # based on HRT

```
H2S data production1 <- data summary(metadata, varname="H2S ppm",
                                         groupnames=c("HRT"))
H2S_data_production1$HRT=as.factor(H2S_data_production1$HRT)
H2S_data_production1
##
     HRT
         H2S ppm
                          sd
## 1 1 209.5654 214.3300
     2 762.0674 577.8486
## 2
## 3 3 2241.4933 1100.7416
box_1 <- ggplot(H2S_data_production1, aes(x=HRT, y=H2S_ppm, fill=HRT)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
 geom_errorbar(aes(ymin=H2S_ppm-sd, ymax=H2S_ppm+sd), width=0.2, positio
n=position dodge((0.9))+
 xlab("HRT")+
 ylab("Hydrogen Sulfide Concentration (%)") + ylim(-100, 3500) + labs(t
itle = "", subtitle=NULL) +
 theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=20, family="Times New Roman"),
        axis.title.y = element text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("green", "blue", "red"))
box 1
```

```
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```



Figure B.12. Average Hydrogen Sulfide Content based on HRTs, Average of 3 Conditions

```
# based on Temp
H2S_data_production2 <- data_summary(metadata, varname="H2S_ppm",
                                      groupnames=c("Temp"))
H2S data production2$Temp=as.factor(H2S data production2$Temp)
H2S_data_production2
##
     Temp
            H2S ppm
                           sd
## 1
        B 1447.9400 1388.3155
## 2
        H 1271.1783 1044.1035
## 3
           817.2758 858.0148
        L
box_2 <- ggplot(H2S_data_production2, aes(x=Temp, y=H2S_ppm, fill=Temp))</pre>
+
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=H2S_ppm-sd, ymax=H2S_ppm+sd), width=0.2, positio
n=position_dodge(0.9))+
  xlab("Temperature")+
  ylab("Hydrogen Sulfide Concentration (%)") + ylim(-200, 3000) + labs(t
itle = "", subtitle=NULL) +
 theme(title=element_text(size=20, family="Times New Roman"),
```

```
axis.text.x = element_text(size=20, family="Times New Roman"),
axis.text.y=element_text(size=20, family="Times New Roman"),
axis.title.y = element_text(size = 20, family="Times New Roman"),
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("green", "blue", "red"))
box_2
```



Figure B.13. Average Hydrogen Sulfide Content based on Temperature Profile, Average of 3 HRT's



Differences in mean levels of Temp:HRT

#### Figure B.14. Tukey Honest Significant Difference Results for the Hydrogen Sulfide Content based on HRT and Temperature

B.4 R-script and Results for Total Solids and Volatile Solids Reduction

```
## Importing fonts may take a few minutes, depending on the number of fon
ts and the speed of the system.
## Continue? [y/n]
## Exiting.
loadfonts(device="win")
# PROGRAM TO PLOT BAR CHART WITH STANDARD DEVIATION ------
- -
# Function to calculate the mean and the standard deviation
# for each group
# data : a data frame
# varname : the name of a column containing the variable
#to be summariezed
# groupnames : vector of column names to be used as
# grouping variables
data summary <- function(data, varname, groupnames){</pre>
 require(plyr)
 summary_func <- function(x, col){</pre>
   c(mean = mean(x[[col]], na.rm=TRUE),
     sd = sd(x[[col]], na.rm=TRUE))
 }
 data sum<-ddply(data, groupnames, .fun=summary func,</pre>
                 varname)
 data_sum <- rename(data_sum, c("mean" = varname))</pre>
 return(data sum)
}
# ANALYSIS------
                                     _____
## the .txt file needs to be saved as the type of "Tab delimited".
##Load TSVS.txt
con <-file.choose(new = FALSE)</pre>
metadata <- read.table(con, header = T, row.names = 1)</pre>
## DEFINING FACTORS
metadata$HRT <- factor(metadata$HRT) ##Factor Statement</pre>
metadata$Temp <- factor(metadata$Temp) ##Factor statement</pre>
## Section 1-----
# 1. Effects of HRT and temp on TS Reduction
```

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## two-way ANOVA

```
# TS Reduction
fit1 <- aov(TS_Reduction~Temp*HRT, data = metadata)</pre>
summary(fit1)
##
                Df Sum Sq Mean Sq F value
                                             Pr(>F)
## Temp
                                           < 2e-16 ***
                 2
                     6148
                           3074.0
                                  52.276
## HRT
                      191
                             95.5
                                    1.624 0.201717
                 2
                     1179
                            294.7
                                     5.012 0.000945 ***
## Temp:HRT
                 4
## Residuals
                     6527
                             58.8
               111
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey1 <- TukeyHSD(fit1, conf.level=0.95) #Tukey multiple comparions</pre>
Tukey1
##
     Tukey multiple comparisons of means
##
       95% family-wise confidence level
##
## Fit: aov(formula = TS_Reduction ~ Temp * HRT, data = metadata)
##
## $Temp
##
            diff
                        lwr
                                    upr
                                           p adj
         8.16225
                   4.088907
                             12.235593 1.74e-05
## H-B
## L-B -9.35700 -13.430343 -5.283657 9.00e-07
## L-H -17.51925 -21.592593 -13.445907 0.00e+00
##
## $HRT
             diff
##
                        lwr
                                 upr
                                          p adj
## 2-1 2.8255952 -1.311899 6.963090 0.2405365
## 3-1 2.4564286 -1.518748 6.431605 0.3102459
## 3-2 -0.3691667 -4.506661 3.768328 0.9755427
##
## $`Temp:HRT`
##
                  diff
                               lwr
                                            upr
                                                    p adj
## H:1-B:1 10.8142857
                         1.6442165 19.9843549 0.0088791
## L:1-B:1 -10.3900000 -19.5600692
                                    -1.2199308 0.0142747
## B:2-B:1
             6.0286905
                       -3.5158201 15.5732011 0.5477822
## H:2-B:1 13.5320238
                         3.9875132
                                    23.0765344 0.0005866
## L:2-B:1 -10.6596429 -20.2041535 -1.1151322 0.0167803
## B:3-B:1
             1.2528571 -7.9172120 10.4229263 0.9999645
## H:3-B:1
             7.3278571
                       -1.8422120 16.4979263 0.2301242
## L:3-B:1 -0.7871429 -9.9572120
                                    8.3829263 0.9999990
## L:1-H:1 -21.2042857 -30.3743549 -12.0342165 0.0000000
## B:2-H:1
           -4.7855952 -14.3301059
                                    4.7589154 0.8101924
## H:2-H:1
             2.7177381
                        -6.8267725
                                   12.2622487 0.9925108
## L:2-H:1 -21.4739286 -31.0184392 -11.9294180 0.0000000
## B:3-H:1 -9.5614286 -18.7314978
                                   -0.3913594 0.0340876
```

```
## H:3-H:1 -3.4864286 -12.6564978 5.6836406 0.9543015
## L:3-H:1 -11.6014286 -20.7714978 -2.4313594 0.0035083
                       6.8741799 25.9632011 0.0000111
## B:2-L:1 16.4186905
## H:2-L:1 23.9220238 14.3775132 33.4665344 0.0000000
## L:2-L:1 -0.2696429 -9.8141535 9.2748678 1.0000000
## B:3-L:1 11.6428571
                        2.4727880 20.8129263 0.0033355
## H:3-L:1 17.7178571
                      8.5477880 26.8879263 0.0000005
## L:3-L:1
           9.6028571
                        0.4327880 18.7729263 0.0326973
## H:2-B:2
           7.5033333 -2.4014734 17.4081401 0.2961640
## L:2-B:2 -16.6883333 -26.5931401 -6.7835266 0.0000181
## B:3-B:2 -4.7758333 -14.3203440 4.7686773 0.8119114
            1.2991667 -8.2453440 10.8436773 0.9999655
## H:3-B:2
## L:3-B:2 -6.8158333 -16.3603440 2.7286773 0.3757622
## L:2-H:2 -24.1916667 -34.0964734 -14.2868599 0.0000000
## B:3-H:2 -12.2791667 -21.8236773 -2.7346560 0.0027588
## H:3-H:2 -6.2041667 -15.7486773 3.3403440 0.5080252
## L:3-H:2 -14.3191667 -23.8636773 -4.7746560 0.0002092
## B:3-L:2 11.9125000 2.3679894 21.4570106 0.0042384
                        8.4429894 27.5320106 0.0000011
## H:3-L:2 17.9875000
## L:3-L:2 9.8725000
                      0.3279894 19.4170106 0.0367827
## H:3-B:3
           6.0750000 -3.0950692 15.2450692 0.4813677
## L:3-B:3 -2.0400000 -11.2100692 7.1300692 0.9986500
## L:3-H:3 -8.1150000 -17.2850692 1.0550692 0.1271923
#TS data summary
TS data1 <- data summary(metadata, varname="TS Reduction", groupnames="HR
T")
## Loading required package: plyr
##
## Attaching package: 'plyr'
## The following object is masked from 'package:ggpubr':
##
##
      mutate
TS_data1
##
     HRT TS Reduction
                            sd
## 1
      1
            55.17357 10.362400
## 2
       2
            57.99917 12.715902
## 3
      3
            57.63000
                     9.617261
TS data2 <- data summary(metadata, varname="TS Reduction", groupnames="Te
mp")
TS data2
##
     Temp TS_Reduction
                             sd
## 1
        В
             57.27925
                      6.216955
## 2
             65.44150 5.291935
       Н
## 3
        L
             47.92225 11.654731
```

#### #2. Plot for TS Reduction

```
# based on HRT
```

```
TS_Reduction1 <- data_summary(metadata, varname="TS_Reduction",
                                        groupnames=c("HRT"))
TS Reduction1$HRT=as.factor(TS Reduction1$HRT)
TS_Reduction1
    HRT TS Reduction
##
                             sd
## 1 1
            55.17357 10.362400
## 2 2
            57.99917 12.715902
## 3 3
             57.63000 9.617261
box 1 <- ggplot(TS Reduction1, aes(x=HRT, y=TS Reduction, fill=HRT)) +
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=TS_Reduction-sd, ymax=TS_Reduction+sd), width=0.
2, position=position_dodge(0.9))+
  xlab("HRT")+
  ylab("Total Solids Reduction (%)") + ylim(0, 80) + labs(title = "", su
btitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element text(size=20, family="Times New Roman"),
        axis.text.y=element text(size=20, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
        legend.position="none")+
  scale_fill_manual(values=c("blue", "blue", "blue"))
box 1
```



Figure B.15. Average Total Solid Reductions based on HRTs, Average of 3 Conditions

```
# based on Temp
TS Reduction2 <- data summary(metadata, varname="TS Reduction",
                              groupnames=c("Temp"))
TS_Reduction2$Temp=as.factor(TS_Reduction2$Temp)
TS_Reduction2
##
     Temp TS_Reduction
                              sd
## 1
              57.27925 6.216955
        В
## 2
        Н
              65.44150 5.291935
## 3
       L
              47.92225 11.654731
box_2 <- ggplot(TS_Reduction2, aes(x=Temp, y=TS_Reduction, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=TS_Reduction-sd, ymax=TS_Reduction+sd), width=0.
2, position=position_dodge(0.9))+
  xlab("Temperature")+
  ylab("Total Solids Reduction (%)") + ylim(0, 80) + labs(title = "", su
btitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element_text(size=20, family="Times New Roman"),
```

```
axis.text.y=element_text(size=20, family="Times New Roman"),
axis.title.y = element_text(size = 20, family="Times New Roman"),
axis.title.x=element_text(size=20, family="Times New Roman"),
legend.position="none")+
scale_fill_manual(values=c("red", "blue", "green"))
box 2
```



Figure B.16. Average Total Solid Reductions based on Temperature Profile, Average of 3 HRT's



Differences in mean levels of Temp:HRT

# Figure B.17. Tukey Honest Significant Difference Results for the Total Solid Reductions based on HRT and Temperature

```
## Section 2------
# 1. Effects of HRT and temp on TS Reduction
## two-way ANOVA
# VS Reduction
fit2 <- aov(VS Reduction~Temp*HRT, data = metadata)</pre>
summary(fit2)
##
                Df Sum Sq Mean Sq F value
                                             Pr(>F)
## Temp
                 2
                     7041
                             3521 53.539 < 2e-16 ***
## HRT
                 2
                      702
                              351
                                    5.338 0.006118 **
                                    6.334 0.000125 ***
## Temp:HRT
                 4
                     1666
                              417
## Residuals
                     7299
                               66
               111
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Tukey2 <- TukeyHSD(fit2, conf.level=0.95) #Tukey multiple comparions</pre>
Tukey2
     Tukey multiple comparisons of means
##
## 95% family-wise confidence level
```

##					
## ##	Fit: aov(formula = )	VS_Reduction	~ Temp * H	RT, data =	metadata)
##	\$Temp				
##	diff	lwr	unr nad	i	
###	H_R 8 98550 / 6	78077 13 20	0073 7 80-00	5	
ππ ##		70722 5 46	1777 1 20 00	5	
##		/9/23 -3.404 55333 14 450	+/// 1.20-00		
##	L-H -18./5//5 -23.00	05223 -14.450	02// 0.00+00	0	
##	<i>4</i> 1157				
##	\$НКІ		. •		
##		Iwr upr	p adj		
##	2-1 4.066587 -0.308	/24 8.441899	0.0/42294		
##	3-1 5.614/62 1.4110	099 9.818425	0.0055004		
##	3-2 1.548175 -2.8273	137 5.923486	0.6786750		
##					
##	\$ Temp:HRT	_			
##	diff	lwr	upr	p adj	
##	H:1-B:1 11.0457143	1.3485637	20.742865	0.0134320	
##	L:1-B:1 -11.9735714	-21.6707220	-2.276421	0.0049071	
##	B:2-B:1 7.2595238	-2.8335905	17.352638	0.3658261	
##	H:2-B:1 15.2370238	5.1439095	25.330138	0.0001855	
##	L:2-B:1 -11.2246429	-21.3177572	-1.131529	0.0175829	
##	B:3-B:1 2.7435714	-6.9535792	12.440722	0.9928247	
##	H:3-B:1 10.5328571	0.8357065	20.230008	0.0226367	
##	L:3-B:1 2.6400000	-7.0571506	12.337151	0.9944636	
##	L:1-H:1 -23.0192857	-32.7164363	-13.322135	0.0000000	
##	B:2-H:1 -3.7861905	-13.8793048	6.306924	0.9577200	
##	H:2-H:1 4.1913095	-5.9018048	14.284424	0.9252056	
##	L:2-H:1 -22.2703571	-32.3634715	-12.177243	0.0000000	
##	B:3-H:1 -8.3021429	-17.9992935	1.395008	0.1568151	
##	H:3-H:1 -0.5128571	-10.2100077	9.184293	1.0000000	
##	L:3-H:1 -8.4057143	-18.1028649	1.291436	0.1452745	
##	B:2-L:1 19.2330952	9.1399809	29.326210	0.000008	
##	H:2-L:1 27.2105952	17.1174809	37.303710	0.0000000	
##	L:2-L:1 0.7489286	-9.3441858	10.842043	0.9999997	
##	B:3-L:1 14.7171429	5.0199923	24.414293	0.0001673	
##	H:3-L:1 22.5064286	12.8092780	32.203579	0.0000000	
##	L:3-L:1 14.6135714	4.9164208	24.310722	0.0001919	
##	H:2-B:2 7.9775000	-2.4966198	18.451620	0.2891969	
##	L:2-B:2 -18.4841667	-28.9582864	-8.010047	0.0000059	
##	B:3-B:2 -4.5159524	-14.6090667	5.577162	0.8895568	
##	H:3-B:2 3.2733333	-6.8197810	13.366448	0.9825423	
##	1·3-B·2 -4.6195238	-14,7126382	5.473591	0.8763223	
##	I · 2 - H · 2 - 26, 4616667	-36,9357864	-15,987547	0.0000000	
##	B·3-H·2 _12 /93/52/	-22 5865667	-2 400338	0.0000000	
##	H·3_H·2 _/ 70/1667	_1/ 7972810	5 3889/8	0.0047452	
π# ##	1.3-H.2 _12 5070220	-22 6001302	-2 503940	0.00+0+20	
##	R·3-I·2 12 06001/0	2 2750000	2. 061220	0.0042393	
##	$1 \cdot 3 - 1 \cdot 2$ 13.3002143	11 66/2056	24.001329	0.0000044	
## ##	1.3-L.2 21./3/3000	3 7715205	23 057757	0.0000000	
tt tt	L.J-L.Z 1J.0040429	J.//TJC03	22.22/12/	0.0010001	

```
## H:3-B:3 7.7892857 -1.9078649 17.486436 0.2241132
## L:3-B:3 -0.1035714 -9.8007220 9.593579 1.0000000
## L:3-H:3 -7.8928571 -17.5900077 1.804293 0.2091325
#TS data summary
VS data1 <- data summary(metadata, varname="VS Reduction", groupnames="HR
T")
VS_data1
    HRT VS_Reduction
##
                            sd
## 1
     1
            58.09786 11.76480
## 2
       2
             62.16444 13.55856
## 3 3
            63.71262 9.74957
VS data2 <- data summary(metadata, varname="VS Reduction", groupnames="Te
mp")
VS data2
##
     Temp VS Reduction
                              sd
## 1
        В
              61.54525 6.389300
## 2
        Н
              70.53075 5.355522
## 3
        L
              51.77300 13.355472
#2. Plot for TS Reduction
# based on HRT
VS_Reduction1 <- data_summary(metadata, varname="VS_Reduction",</pre>
                              groupnames=c("HRT"))
VS Reduction1$HRT=as.factor(VS Reduction1$HRT)
VS Reduction1
    HRT VS_Reduction
##
                            sd
## 1
     1
            58.09786 11.76480
       2
## 2
             62.16444 13.55856
## 3
     3
            63.71262 9.74957
box 3 <- ggplot(VS Reduction1, aes(x=HRT, y=VS Reduction, fill=HRT)) +
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom errorbar(aes(ymin=VS Reduction-sd, ymax=VS Reduction+sd), width=0.
2, position=position_dodge(0.9))+
  xlab("HRT")+
  ylab("Volatile Solids Reduction (%)") + ylim(0, 80) + labs(title = "",
 subtitle=NULL) +
  theme(title=element_text(size=20, family="Times New Roman"),
        axis.text.x = element text(size=20, family="Times New Roman"),
        axis.text.y=element_text(size=20, family="Times New Roman"),
        axis.title.y = element_text(size = 20, family="Times New Roman"),
        axis.title.x=element text(size=20, family="Times New Roman"),
```

```
legend.position="none")+
scale_fill_manual(values=c("blue", "blue", "blue"))
box_3
```



Figure B.18. Average Volatile Solid Reductions based on HRTs, Average of 3 Conditions

```
# based on Temp
VS_Reduction2 <- data_summary(metadata, varname="VS_Reduction",</pre>
                               groupnames=c("Temp"))
VS_Reduction2$Temp=as.factor(VS_Reduction2$Temp)
VS_Reduction2
##
     Temp VS_Reduction
                               sd
## 1
        В
              61.54525 6.389300
## 2
              70.53075 5.355522
        Н
## 3
        L
              51.77300 13.355472
box_4 <- ggplot(VS_Reduction2, aes(x=Temp, y=VS_Reduction, fill=Temp)) +</pre>
  geom_bar(stat="identity", position=position_dodge(0.9), width=0.5)+
  geom_errorbar(aes(ymin=VS_Reduction-sd, ymax=VS_Reduction+sd), width=0.
2, position=position_dodge(0.9))+
xlab("Temperature")+
```

```
ylab("Volatile Solids Reduction (%)") + ylim(0, 80) + labs(title = "",
subtitle=NULL) +
theme(title=element_text(size=20, family="Times New Roman"),
    axis.text.x = element_text(size=20, family="Times New Roman"),
    axis.text.y=element_text(size=20, family="Times New Roman"),
    axis.title.y = element_text(size = 20, family="Times New Roman"),
    axis.title.x=element_text(size=20, family="Times New Roman"),
    legend.position="none")+
    scale_fill_manual(values=c("red", "blue", "green"))
    box 4
```



Figure B.19. Average Volatile Solid Reductions based on Temperature Profile, Average of 3 HRT's



Differences in mean levels of Temp:HRT

Figure B.21. Tukey Honest Significant Difference Results for the Volatile Solid Reductions based on HRT and Temperature

# Appendix C. Additional BMP data

Appendix C give additional BMP data for individual triplicate samples.

## C.1 Raw Material Characterization

Table	C.1.	Raw	Sample	Characterization	Round	1
I abic	<b>U.I.</b>	<b>I</b> \a w	Sample	Character ization	Nounu .	L

Commle	TS	VS	TS	VS	TS	VS	TS:VS
Sample	(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29	41,843	25,327	41,333	25,014	4.1	2.5	61%
Cellulose Microcrystalline	1,017,250	1,017,207	958,989	958,947	95.9	95.9	100%
Cow Manure	33,060	22,152	32,978	22,097	3.3	2.2	67%

## Table C.2. Raw Sample Characterization Trial 2

Samula	TS	VS	TS	VS	TS	VS	TS:VS
Sample	(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20	54,008	38,253	53,803	38,108	5.4	3.8	71%
Cellulose Microcrystalline	1,017,250	1,017,207	958,989	958,947	95.9	95.9	100%
Cow Manure	49,080	31,885	48,905	31,771	4.9	3.2	65%

#### Table C.3. Raw Sample Characterization Trial 3

Samula	TS	VS	TS	VS	TS	VS	TS:VS
Sample	(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15	48,743	33,910	48,631	33,832	4.9	3.4	70%
Cellulose Microcrystalline	1,017,250	1,017,207	958,989	958,947	95.9	95.9	100%
Cow Manure	29,332	20,853	29,222	20,775	2.9	2.1	71%

# C.2 BMP Data for Trial 1

#### *C.2.1* 15°*C*, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.83	6,377	4,475	6,243	4,381	0.6	0.4	70%
Seed 1/29 (2)	7.86	6,180	4,353	5,984	4,214	0.6	0.4	70%
Seed 1/29 (3)	7.81	6,187	4,260	6,109	4,205	0.6	0.4	69%
Cellulose Microcrystalline (1)	7.89	9,288	7,088	9,054	6,910	0.9	0.7	76%
Cellulose Microcrystalline (2)	7.83	9,048	6,865	8,924	6,772	0.9	0.7	76%
Cellulose Microcrystalline (3)	7.88	8,975	6,875	8,690	6,657	0.9	0.7	77%
Cow Manure (1)	7.63	10,550	7,232	10,386	7,120	1.0	0.7	69%
Cow Manure (2)	7.65	10,735	7,428	10,649	7,368	1.1	0.7	69%
Cow Manure (3)	7.61	10,758	7,485	10,471	7,286	1.0	0.7	70%

## Table C.4. Trial 1 BMP Pre-digestion data for 15°C, non-mixed

## Table C.5. Trial 1 BMP Post-digestion data for 15°C, non-mixed

Somethe	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.63	6,330	4,110	6,291	4,085	0.6	0.4	65%
Seed 1/29 (2)	7.57	5,980	3,938	5,903	3,887	0.6	0.4	66%
Seed 1/29 (3)	7.58	6,143	3,963	6,123	3,950	0.6	0.4	65%
Cellulose Microcrystalline (1)	6.48	8,392	6,070	8,330	6,024	0.8	0.6	72%
Cellulose Microcrystalline (2)	6.43	8,732	6,200	8,623	6,123	0.9	0.6	71%
Cellulose Microcrystalline (3)	6.40	8,887	6,340	8,747	6,239	0.9	0.6	71%
Cow Manure (1)	7.29	10,253	6,540	10,135	6,464	1.0	0.6	64%
Cow Manure (2)	7.31	10,218	6,443	10,031	6,324	1.0	0.6	63%
Cow Manure (3)	7.27	9,780	6,252	9,718	6,213	1.0	0.6	64%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	6,377	6,330	48	1%
Seed 1/29 (2)	6,180	5,980	200	3%
Seed 1/29 (3)	6,187	6,143	45	1%
Cellulose Microcrystalline (1)	9,288	8,392	895	10%
Cellulose Microcrystalline (2)	9,048	8,732	315	3%
Cellulose Microcrystalline (3)	8,975	8,887	88	1%
Cow Manure (1)	10,550	10,253	297	3%
Cow Manure (2)	10,735	10,218	517	5%
Cow Manure (3)	10,758	9,780	977	9%

Table C.6. Trial 1 BMP Total Solids Reduction for 15°C, non-mixed

Table C.7. Trial 1 BMP Volatile Solids Reduction for 15°C, non-mixed

Sampla	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	4,475	4,110	365	8%
Seed 1/29 (2)	4,353	3,938	415	10%
Seed 1/29 (3)	4,260	3,963	297	7%
Cellulose Microcrystalline (1)	7,088	6,070	1,018	14%
Cellulose Microcrystalline (2)	6,865	6,200	665	10%
Cellulose Microcrystalline (3)	6,875	6,340	535	8%
Cow Manure (1)	7,232	6,540	692	10%
Cow Manure (2)	7,428	6,443	985	13%
Cow Manure (3)	7,485	6,252	1,232	16%

## C.2.2 20°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.75	7,585	5,088	7,544	5,060	0.8	0.5	67%
Seed 1/29 (2)	7.75	7,805	5,298	7,653	5,195	0.8	0.5	68%
Seed 1/29 (3)	7.72	7,560	5,085	7,424	4,994	0.7	0.5	67%
Cellulose Microcrystalline (1)	7.87	10,308	7,560	10,034	7,359	1.0	0.7	73%
Cellulose Microcrystalline (2)	7.75	10,823	8,270	10,651	8,139	1.1	0.8	76%
Cellulose Microcrystalline (3)	7.72	11,175	8,532	10,977	8,382	1.1	0.8	76%
Cow Manure (1)	7.50	11,448	7,705	11,438	7,698	1.1	0.8	67%
Cow Manure (2)	7.57	11,255	7,668	10,976	7,479	1.1	0.7	68%
Cow Manure (3)	7.55	11,793	7,870	11,589	7,734	1.2	0.8	67%

## Table C.8. Trial 1 BMP Pre-digestion data for 20°C, non-mixed

# Table C.9. Trial 1 BMP Post-digestion data for 20°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.27	7,337	4,837	7,308	4,818	0.7	0.5	66%
Seed 1/29 (2)	7.34	7,478	4,735	7,361	4,662	0.7	0.5	63%
Seed 1/29 (3)	7.29	7,393	4,745	7,286	4,677	0.7	0.5	64%
Cellulose Microcrystalline (1)	6.24	8,622	5,968	8,496	5,880	0.8	0.6	69%
Cellulose Microcrystalline (2)	5.97	9,000	6,418	8,852	6,312	0.9	0.6	71%
Cellulose Microcrystalline (3)	5.94	9,152	6,358	9,080	6,307	0.9	0.6	69%
Cow Manure (1)	7.08	9,417	5,942	9,171	5,787	0.9	0.6	63%
Cow Manure (2)	7.07	9,625	5,950	9,588	5,927	1.0	0.6	62%
Cow Manure (3)	7.04	9,312	5,955	9,264	5,924	0.9	0.6	64%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	7,585	7,337	248	3%
Seed 1/29 (2)	7,805	7,478	327	4%
Seed 1/29 (3)	7,560	7,393	168	2%
Cellulose Microcrystalline (1)	10,308	8,622	1,685	16%
Cellulose Microcrystalline (2)	10,823	9,000	1,823	17%
Cellulose Microcrystalline (3)	11,175	9,152	2,022	18%
Cow Manure (1)	11,448	9,417	2,030	18%
Cow Manure (2)	11,255	9,625	1,630	14%
Cow Manure (3)	11,793	9,312	2,480	21%

Table C.10. Trial 1 BMP Total Solids Reduction for 20°C, non-mixed

Table C.11. Trial 1 BMP Volatile Solids Reduction for 20°C, non-mixed

Sampla	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	5,088	4,837	250	5%
Seed 1/29 (2)	5,298	4,735	562	11%
Seed 1/29 (3)	5,085	4,745	340	7%
Cellulose Microcrystalline (1)	7,560	5,968	1,593	21%
Cellulose Microcrystalline (2)	8,270	6,418	1,852	22%
Cellulose Microcrystalline (3)	8,532	6,358	2,175	25%
Cow Manure (1)	7,705	5,942	1,763	23%
Cow Manure (2)	7,668	5,950	1,718	22%
Cow Manure (3)	7,870	5,955	1,915	24%

## C.2.3 30°C, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.43	7,663	5,285	7,603	5,244	0.8	0.5	69%
Seed 1/29 (2)	7.67	7,485	5,002	7,275	4,863	0.7	0.5	67%
Seed 1/29 (3)	7.69	7,652	5,467	7,437	5,314	0.7	0.5	71%
Cellulose Microcrystalline (1)	7.71	9,607	7,172	9,368	6,994	0.9	0.7	75%
Cellulose Microcrystalline (2)	7.59	9,572	7,140	9,497	7,084	0.9	0.7	75%
Cellulose Microcrystalline (3)	7.60	9,570	7,127	9,356	6,968	0.9	0.7	74%
Cow Manure (1)	7.56	11,190	7,485	10,990	7,352	1.1	0.7	67%
Cow Manure (2)	7.58	11,280	7,475	11,095	7,352	1.1	0.7	66%
Cow Manure (3)	7.64	11,115	7,410	10,886	7,257	1.1	0.7	67%

Table C.12. Trial 1 BMP Pre-digestion data for 30°C, non-mixed

## Table C.13. Trial 1 BMP Post-digestion data for 30°C, non-mixed

Samula	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.14	7,058	4,435	7,004	4,402	0.7	0.4	63%
Seed 1/29 (2)	7.19	6,902	4,210	6,876	4,194	0.7	0.4	61%
Seed 1/29 (3)	7.24	6,950	4,290	6,819	4,210	0.7	0.4	62%
Cellulose Microcrystalline (1)	6.97	7,487	4,617	7,416	4,573	0.7	0.5	62%
Cellulose Microcrystalline (2)	6.92	7,548	4,805	7,446	4,741	0.7	0.5	64%
Cellulose Microcrystalline (3)	7.00	7,888	5,008	7,776	4,937	0.8	0.5	63%
Cow Manure (1)	7.06	9,518	5,665	9,254	5,506	0.9	0.6	60%
Cow Manure (2)	7.11	9,457	5,440	9,263	5,328	0.9	0.5	58%
Cow Manure (3)	7.09	9,287	5,480	9,118	5,381	0.9	0.5	59%

Samula	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	7,663	7,058	605	8%
Seed 1/29 (2)	7,485	6,902	582	8%
Seed 1/29 (3)	7,652	6,950	702	9%
Cellulose Microcrystalline (1)	9,607	7,487	2,120	22%
Cellulose Microcrystalline (2)	9,572	7,548	2,025	21%
Cellulose Microcrystalline (3)	9,570	7,888	1,682	18%
Cow Manure (1)	11,190	9,518	1,673	15%
Cow Manure (2)	11,280	9,457	1,822	16%
Cow Manure (3)	11,115	9,287	1,828	16%

Table C.14. Trial 1 BMP Total Solids Reduction for 30°C, non-mixed

Table C.15. Trial 1 BMP Volatile Solids Reduction for 30°C, non-mixed

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	5,285	4,435	850	16%
Seed 1/29 (2)	5,002	4,210	792	16%
Seed 1/29 (3)	5,467	4,290	1,178	22%
Cellulose Microcrystalline (1)	7,172	4,617	2,555	36%
Cellulose Microcrystalline (2)	7,140	4,805	2,335	33%
Cellulose Microcrystalline (3)	7,127	5,008	2,120	30%
Cow Manure (1)	7,485	5,665	1,820	24%
Cow Manure (2)	7,475	5,440	2,035	27%
Cow Manure (3)	7,410	5,480	1,930	26%

# C.2.4 39°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.71	7,460	5,000	7,378	4,945	0.7	0.5	67%
Seed 1/29 (2)	7.70	8,030	5,392	7,835	5,262	0.8	0.5	67%
Seed 1/29 (3)	7.71	7,335	4,982	7,281	4,946	0.7	0.5	68%
Cellulose Microcrystalline (1)	7.71	10,620	8,175	10,448	8,042	1.0	0.8	77%
Cellulose Microcrystalline (2)	7.68	10,545	7,945	10,410	7,842	1.0	0.8	75%
Cellulose Microcrystalline (3)	7.63	10,365	7,533	10,219	7,427	1.0	0.7	73%
Cow Manure (1)	7.48	11,493	7,645	11,175	7,433	1.1	0.7	67%
Cow Manure (2)	7.51	11,000	7,500	10,742	7,324	1.1	0.7	68%
Cow Manure (3)	7.50	11,793	7,805	11,533	7,633	1.2	0.8	66%

Table C.16. Trial 1 BMP Pre-digestion data for 39°C, non-mixed

Table	C.17.	Trial	<b>1 BMP</b>	Post-dige	estion data	for 39	°C. non-	mixed
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Samula	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.22	6,548	3,963	6,514	3,942	0.7	0.4	61%
Seed 1/29 (2)	7.24	7,098	4,063	7,090	4,058	0.7	0.4	57%
Seed 1/29 (3)	7.23	6,775	4,063	6,658	3,991	0.7	0.4	60%
Cellulose Microcrystalline (1)	6.76	8,193	5,203	8,169	5,188	0.8	0.5	64%
Cellulose Microcrystalline (2)	6.94	8,325	5,217	8,314	5,210	0.8	0.5	63%
Cellulose Microcrystalline (3)	6.93	8,260	5,142	8,109	5,049	0.8	0.5	62%
Cow Manure (1)	7.16	9,833	5,480	9,645	5,377	1.0	0.5	56%
Cow Manure (2)	7.15	9,572	5,313	9,403	5,218	0.9	0.5	55%
Cow Manure (3)	7.16	9,583	5,375	9,305	5,220	0.9	0.5	56%

Samula	Initial	Final	Destroyed	Reduction	
Sample	(mg/L)	(mg/L)	(mg/L)	(%)	
Seed 1/29 (1)	7,460	6,548	913	12%	
Seed 1/29 (2)	8,030	7,098	932	12%	
Seed 1/29 (3)	7,335	6,775	560	8%	
Cellulose Microcrystalline (1)	10,620	8,193	2,428	23%	
Cellulose Microcrystalline (2)	10,545	8,325	2,220	21%	
Cellulose Microcrystalline (3)	10,365	8,260	2,105	20%	
Cow Manure (1)	11,493	9,833	1,660	14%	
Cow Manure (2)	11,000	9,572	1,427	13%	
Cow Manure (3)	11,793	9,583	2,210	19%	

Table C.18. Trial 1 BMP Total Solids Reduction for 39°C, non-mixed

Table C.19. Trial 1 BMP Volatile Solids Reduction for 39°C, non-mixed

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	5,000	3,963	1,037	21%
Seed 1/29 (2)	5,392	4,063	1,330	25%
Seed 1/29 (3)	4,982	4,063	920	18%
Cellulose Microcrystalline (1)	8,175	5,203	2,972	36%
Cellulose Microcrystalline (2)	7,945	5,217	2,728	34%
Cellulose Microcrystalline (3)	7,533	5,142	2,390	32%
Cow Manure (1)	7,645	5,480	2,165	28%
Cow Manure (2)	7,500	5,313	2,187	29%
Cow Manure (3)	7,805	5,375	2,430	31%

# C.2.5 39°C, mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.68	7,508	5,178	7,303	5,038	0.7	0.5	69%
Seed 1/29 (2)	7.62	7,615	5,080	7,432	4,959	0.7	0.5	67%
Seed 1/29 (3)	7.61	7,747	5,110	7,646	5,043	0.8	0.5	66%
Cellulose Microcrystalline (1)	7.57	10,143	7,682	9,938	7,527	1.0	0.8	76%
Cellulose Microcrystalline (2)	7.62	9,898	7,495	9,783	7,408	1.0	0.7	76%
Cellulose Microcrystalline (3)	7.58	9,558	7,143	9,442	7,056	0.9	0.7	75%
Cow Manure (1)	7.40	11,128	7,510	10,965	7,401	1.1	0.7	67%
Cow Manure (2)	7.40	11,330	7,495	11,108	7,349	1.1	0.7	66%
Cow Manure (3)	7.48	11,080	7,340	10,973	7,269	1.1	0.7	66%

Table C.20. Trial 1 BMP Pre-digestion data for 39°C, mixed

Table C.21. Trial 1 BMP	Post-digestion	data for	<sup>•</sup> 39°C, mixed
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Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 1/29 (1)	7.17	6,838	3,950	6,789	3,922	0.7	0.4	58%
Seed 1/29 (2)	7.17	6,675	4,017	6,437	3,875	0.6	0.4	60%
Seed 1/29 (3)	7.12	6,750	4,085	6,724	4,069	0.7	0.4	61%
Cellulose Microcrystalline (1)	6.50	8,300	5,515	8,259	5,488	0.8	0.5	66%
Cellulose Microcrystalline (2)	6.25	8,328	5,590	8,304	5,574	0.8	0.6	67%
Cellulose Microcrystalline (3)	6.62	8,552	5,662	8,368	5,540	0.8	0.6	66%
Cow Manure (1)	7.07	8,835	5,090	8,668	4,995	0.9	0.5	58%
Cow Manure (2)	7.06	9,210	5,240	9,039	5,144	0.9	0.5	57%
Cow Manure (3)	7.04	9,235	5,358	8,981	5,211	0.9	0.5	58%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	7,508	6,838	670	9%
Seed 1/29 (2)	7,615	6,675	940	12%
Seed 1/29 (3)	7,747	6,750	997	13%
Cellulose Microcrystalline (1)	10,143	8,300	1,843	18%
Cellulose Microcrystalline (2)	9,898	8,328	1,570	16%
Cellulose Microcrystalline (3)	9,558	8,552	1,005	11%
Cow Manure (1)	11,128	8,835	2,292	21%
Cow Manure (2)	11,330	9,210	2,120	19%
Cow Manure (3)	11,080	9,235	1,845	17%

Table C.22. Trial 1 BMP Total Solids Reduction for 39°C, mixed

Table C.23. Trial 1 BMP Volatile Solids Reduction for 39°C, mixed

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 1/29 (1)	5,178	3,950	1,227	24%
Seed 1/29 (2)	5,080	4,017	1,063	21%
Seed 1/29 (3)	5,110	4,085	1,025	20%
Cellulose Microcrystalline (1)	7,682	5,515	2,168	28%
Cellulose Microcrystalline (2)	7,495	5,590	1,905	25%
Cellulose Microcrystalline (3)	7,143	5,662	1,480	21%
Cow Manure (1)	7,510	5,090	2,420	32%
Cow Manure (2)	7,495	5,240	2,255	30%
Cow Manure (3)	7,340	5,358	1,983	27%

# C.3 BMP Data for Trial 2

#### *C.3.1* 15°*C*, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.56	8,080	6,095	7,925	5,978	0.8	0.6	75%
Seed 5/20 (2)	7.64	8,182	6,185	8,138	6,151	0.8	0.6	76%
Seed 5/20 (3)	7.67	9,703	7,285	9,444	7,089	0.9	0.7	75%
Cellulose Microcrystalline (1)	7.69	13,308	10,940	13,235	10,880	1.3	1.1	82%
Cellulose Microcrystalline (2)	7.75	13,350	10,940	13,045	10,691	1.3	1.1	82%
Cellulose Microcrystalline (3)	7.72	13,730	11,230	13,549	11,082	1.4	1.1	82%
Cow Manure (1)	7.50	15,212	11,347	15,018	11,201	1.5	1.1	75%
Cow Manure (2)	7.50	15,633	11,232	15,258	10,963	1.5	1.1	72%
Cow Manure (3)	7.51	15,085	10,840	14,637	10,517	1.5	1.1	72%

## Table C.24. Trial 2 BMP Pre-digestion data for 15°C, non-mixed

## Table C.25. Trial 2 BMP Post-digestion data for 15°C, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.17	7,670	5,720	7,652	5,707	0.8	0.6	75%
Seed 5/20 (2)	7.24	7,920	5,998	7,888	5,974	0.8	0.6	76%
Seed 5/20 (3)	7.25	8,757	6,250	8,670	6,188	0.9	0.6	71%
Cellulose Microcrystalline (1)	6.22	12,475	9,772	12,448	9,751	1.2	1.0	78%
Cellulose Microcrystalline (2)	6.13	12,170	9,615	12,106	9,565	1.2	1.0	79%
Cellulose Microcrystalline (3)	6.12	12,420	9,570	12,374	9,535	1.2	1.0	77%
Cow Manure (1)	6.84	14,777	10,710	14,742	10,684	1.5	1.1	72%
Cow Manure (2)	6.96	15,258	10,673	15,173	10,614	1.5	1.1	70%
Cow Manure (3)	6.98	14,638	10,265	14,601	10,239	1.5	1.0	70%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	8,080	7,670	410	5%
Seed 5/20 (2)	8,182	7,920	262	3%
Seed 5/20 (3)	9,703	8,757	945	10%
Cellulose Microcrystalline (1)	13,308	12,475	833	6%
Cellulose Microcrystalline (2)	13,350	12,170	1,180	9%
Cellulose Microcrystalline (3)	13,730	12,420	1,310	10%
Cow Manure (1)	15,212	14,777	435	3%
Cow Manure (2)	15,633	15,258	375	2%
Cow Manure (3)	15,085	14,638	448	3%

Table C.26. Trial 2 BMP Total Solids Reduction for 15°C, non-mixed

Table C.27. Trial 2 BMP Volatile Solids Reduction for 15°C, non-mixed

Sample	Initial	Final	Destroyed	Reduction
Sampie	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	6,095	5,720	375	6%
Seed 5/20 (2)	6,185	5,998	187	3%
Seed 5/20 (3)	7,285	6,250	1,035	14%
Cellulose Microcrystalline (1)	10,940	9,772	1,168	11%
Cellulose Microcrystalline (2)	10,940	9,615	1,325	12%
Cellulose Microcrystalline (3)	11,230	9,570	1,660	15%
Cow Manure (1)	11,347	10,710	638	6%
Cow Manure (2)	11,232	10,673	560	5%
Cow Manure (3)	10,840	10,265	575	5%

# C.3.2 20°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.69	10,385	7,925	10,045	7,665	1.0	0.8	76%
Seed 5/20 (2)	7.73	10,625	7,780	10,554	7,728	1.1	0.8	73%
Seed 5/20 (3)	7.73	9,722	7,162	9,499	6,997	0.9	0.7	74%
Cellulose Microcrystalline (1)	7.69	13,060	10,735	12,917	10,617	1.3	1.1	82%
Cellulose Microcrystalline (2)	7.69	13,717	11,375	13,405	11,117	1.3	1.1	83%
Cellulose Microcrystalline (3)	7.68	13,308	11,035	12,983	10,765	1.3	1.1	83%
Cow Manure (1)	7.46	16,008	11,645	15,644	11,381	1.6	1.1	73%
Cow Manure (2)	7.48	15,933	11,668	15,588	11,415	1.6	1.1	73%
Cow Manure (3)	7.55	16,598	11,983	15,988	11,543	1.6	1.2	72%

Table C.28. Trial 2 BMP Pre-digestion data for 20°C, non-mixed

Table	C.29.	Trial 2	2 BMP	Post-di	gestion	data f	for 20°C	. non-mixed
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Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.26	9,620	6,858	9,464	6,747	0.9	0.7	71%
Seed 5/20 (2)	7.30	10,282	7,125	9,987	6,920	1.0	0.7	69%
Seed 5/20 (3)	7.32	9,115	6,412	8,893	6,258	0.9	0.6	70%
Cellulose Microcrystalline (1)	6.06	12,377	9,545	12,107	9,336	1.2	0.9	77%
Cellulose Microcrystalline (2)	5.98	12,510	9,435	11,952	9,014	1.2	0.9	75%
Cellulose Microcrystalline (3)	6.06	12,048	9,228	11,638	8,914	1.2	0.9	77%
Cow Manure (1)	6.93	13,763	9,563	13,592	9,443	1.4	0.9	69%
Cow Manure (2)	7.08	14,122	9,620	13,836	9,426	1.4	0.9	68%
Cow Manure (3)	7.08	15,417	10,465	15,206	10,320	1.5	1.0	68%
Sample	Initial	Final	Destroyed	Reduction				
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Sample	(mg/L)	(mg/L)	(mg/L)	(%)				
Seed 5/20 (1)	10,385	9,620	765	7%				
Seed 5/20 (2)	10,625	10,282	343	3%				
Seed 5/20 (3)	9,722	9,115	607	6%				
Cellulose Microcrystalline (1)	13,060	12,377	683	5%				
Cellulose Microcrystalline (2)	13,717	12,510	1,207	9%				
Cellulose Microcrystalline (3)	13,308	12,048	1,260	9%				
Cow Manure (1)	16,008	13,763	2,245	14%				
Cow Manure (2)	15,933	14,122	1,810	11%				
Cow Manure (3)	16,598	15,417	1,180	7%				

Table C.30. Trial 2 BMP Total Solids Reduction for 20°C, non-mixed

Table C.31. Trial 2 BMP Volatile Solids Reduction for 20 $^{\circ}C,$  non-mixed

Sampla	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	7,925	6,858	1,068	13%
Seed 5/20 (2)	7,780	7,125	655	8%
Seed 5/20 (3)	7,162	6,412	750	10%
Cellulose Microcrystalline (1)	10,735	9,545	1,190	11%
Cellulose Microcrystalline (2)	11,375	9,435	1,940	17%
Cellulose Microcrystalline (3)	11,035	9,228	1,807	16%
Cow Manure (1)	11,645	9,563	2,082	18%
Cow Manure (2)	11,668	9,620	2,047	18%
Cow Manure (3)	11,983	10,465	1,518	13%

## C.3.3 30°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	g) (mg/kg) ('		(%)	
Seed 5/20 (1)	7.75	9,960	7,465	9,905	7,424	1.0	0.7	75%
Seed 5/20 (2)	7.78	9,637	7,230	9,507	7,132	1.0	0.7	75%
Seed 5/20 (3)	7.77	9,333	7,020	9,248	6,956	0.9	0.7	75%
Cellulose Microcrystalline (1)	7.76	12,425	10,240	12,045	9,926	1.2	1.0	82%
Cellulose Microcrystalline (2)	7.79	12,610	10,418	12,251	10,122	1.2	1.0	83%
Cellulose Microcrystalline (3)	7.71	13,380	11,065	12,841	10,620	1.3	1.1	83%
Cow Manure (1)	7.49	14,575	10,578	14,476	10,506	1.4	1.1	73%
Cow Manure (2)	7.47	14,853	10,553	14,744	10,476	1.5	1.0	71%
Cow Manure (3)	7.45	15,098	10,690	14,909	10,556	1.5	1.1	71%

Table C.32. Trial 2 BMP Pre-digestion data for 30°C, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.21	8,195	5,703	7,996	5,564	0.8	0.6	70%
Seed 5/20 (2)	7.27	7,728	5,413	7,584	5,312	0.8	0.5	70%
Seed 5/20 (3)	7.40	8,132	5,745	8,002	5,653	0.8	0.6	71%
Cellulose Microcrystalline (1)	7.08	9,033	6,498	8,953	6,440	0.9	0.6	72%
Cellulose Microcrystalline (2)	7.06	9,372	6,680	9,133	6,509	0.9	0.7	71%
Cellulose Microcrystalline (3)	7.13	9,427	6,732	9,160	6,541	0.9	0.7	71%
Cow Manure (1)	7.21	12,173	7,732	11,938	7,585	1.2	0.8	64%
Cow Manure (2)	7.28	13,130	8,353	12,746	8,109	1.3	0.8	64%
Cow Manure (3)	7.32	12,798	8,023	12,513	7,844	1.3	0.8	63%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	9,960	8,195	1,765	18%
Seed 5/20 (2)	9,637	7,728	1,910	20%
Seed 5/20 (3)	9,333	8,132	1,200	13%
Cellulose Microcrystalline (1)	12,425	9,033	3,393	27%
Cellulose Microcrystalline (2)	12,610	9,372	3,238	26%
Cellulose Microcrystalline (3)	13,380	9,427	3,953	30%
Cow Manure (1)	14,575	12,173	2,403	16%
Cow Manure (2)	14,853	13,130	1,722	12%
Cow Manure (3)	15,098	12,798	2,300	15%

Table C.34. Trial 2 BMP Total Solids Reduction for 30°C, non-mixed

1 able C.35. 1 rial 2 BMP volatile Solids Reduction for 30°C, non-mixe
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Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	7,465	5,703	1,762	24%
Seed 5/20 (2)	7,230	5,413	1,818	25%
Seed 5/20 (3)	7,020	5,745	1,275	18%
Cellulose Microcrystalline (1)	10,240	6,498	3,743	37%
Cellulose Microcrystalline (2)	10,418	6,680	3,737	36%
Cellulose Microcrystalline (3)	11,065	6,732	4,333	39%
Cow Manure (1)	10,578	7,732	2,845	27%
Cow Manure (2)	10,553	8,353	2,200	21%
Cow Manure (3)	10,690	8,023	2,667	25%

# C.3.4 39°C, non-mixed

Sample	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		( <b>mg/L</b> )	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.67	9,293	7,108	9,004	6,887	0.9	0.7	76%
Seed 5/20 (2)	7.70	9,393	6,848	9,069	6,611	0.9	0.7	73%
Seed 5/20 (3)	7.69	9,848	7,318	9,621	7,149	1.0	0.7	74%
Cellulose Microcrystalline (1)	7.70	12,420	10,078	12,104	9,821	1.2	1.0	81%
Cellulose Microcrystalline (2)	7.70	12,888	10,518	12,618	10,298	1.3	1.0	82%
Cellulose Microcrystalline (3)	7.68	12,273	9,935	11,869	9,608	1.2	1.0	81%
Cow Manure (1)	7.51	14,505	10,368	14,457	10,333	1.4	1.0	71%
Cow Manure (2)	7.49	15,075	10,833	14,606	10,495	1.5	1.0	72%
Cow Manure (3)	7.48	15,040	10,905	14,813	10,740	1.5	1.1	73%

Table C.36. Trial 2 BMP Pre-digestion data for 39°C, non-mixed

Table	C.37.	<b>Trial</b> (	2 <b>BMP</b>	Post-digest	ion data	for 39°C.	non-mixed
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Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample	( <b>mg/L</b> )		(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.31	8,650	6,070	8,608	6,041	0.9	0.6	70%
Seed 5/20 (2)	7.30	9,005	6,220	8,973	6,198	0.9	0.6	69%
Seed 5/20 (3)	7.31	8,710	6,008	8,669	5,979	0.9	0.6	69%
Cellulose Microcrystalline (1)	7.14	10,313	7,575	10,226	7,511	1.0	0.8	73%
Cellulose Microcrystalline (2)	7.14	9,865	6,985	9,829	6,960	1.0	0.7	71%
Cellulose Microcrystalline (3)	7.17	9,232	6,490	9,215	6,478	0.9	0.6	70%
Cow Manure (1)	7.26	12,493	7,782	12,402	7,727	1.2	0.8	62%
Cow Manure (2)	7.31	12,143	7,640	12,049	7,581	1.2	0.8	63%
Cow Manure (3)	7.34	12,077	7,727	12,014	7,687	1.2	0.8	64%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	9,293	8,650	642	7%
Seed 5/20 (2)	9,393	9,005	388	4%
Seed 5/20 (3)	9,848	8,710	1,138	12%
Cellulose Microcrystalline (1)	12,420	10,313	2,107	17%
Cellulose Microcrystalline (2)	12,888	9,865	3,022	23%
Cellulose Microcrystalline (3)	12,273	9,232	3,040	25%
Cow Manure (1)	14,505	12,493	2,013	14%
Cow Manure (2)	15,075	12,143	2,933	19%
Cow Manure (3)	15,040	12,077	2,963	20%

Table C.38. Trial 2 BMP Total Solids Reduction for 39°C, non-mixed

Table C.39. Trial 2 BMF	<b>Volatile Solids Reduction</b>	for 39°C, non-mixed
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Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	7,108	6,070	1,037	15%
Seed 5/20 (2)	6,848	6,220	628	9%
Seed 5/20 (3)	7,318	6,008	1,310	18%
Cellulose Microcrystalline (1)	10,078	7,575	2,503	25%
Cellulose Microcrystalline (2)	10,518	6,985	3,533	34%
Cellulose Microcrystalline (3)	9,935	6,490	3,445	35%
Cow Manure (1)	10,368	7,782	2,585	25%
Cow Manure (2)	10,833	7,640	3,193	29%
Cow Manure (3)	10,905	7,727	3,178	29%

## C.3.5 39°C, mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.68	8,993	6,835	8,838	6,717	0.9	0.7	76%
Seed 5/20 (2)	7.67	9,365	7,145	9,249	7,057	0.9	0.7	76%
Seed 5/20 (3)	7.66	9,077	6,915	8,811	6,712	0.9	0.7	76%
Cellulose Microcrystalline (1)	7.68	12,600	10,295	12,311	10,059	1.2	1.0	82%
Cellulose Microcrystalline (2)	7.69	12,467	10,230	12,222	10,028	1.2	1.0	82%
Cellulose Microcrystalline (3)	7.68	13,525	11,155	13,294	10,965	1.3	1.1	82%
Cow Manure (1)	7.51	14,158	10,330	13,815	10,079	1.4	1.0	73%
Cow Manure (2)	7.46	15,905	11,418	15,623	11,215	1.6	1.1	72%
Cow Manure (3)	7.49	15,295	11,070	14,930	10,806	1.5	1.1	72%

Table C.40. Trial 2 BMP Pre-digestion data for 39°C, mixed

Table C.41. Trial 2 BMP Post-digestion data for 39°C, m	ixed
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Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 5/20 (1)	7.32	8,345	5,718	8,156	5,588	0.8	0.6	69%
Seed 5/20 (2)	7.32	7,545	5,200	7,532	5,191	0.8	0.5	69%
Seed 5/20 (3)	7.31	8,307	5,677	8,075	5,519	0.8	0.6	68%
Cellulose Microcrystalline (1)	7.15	9,075	6,553	8,981	6,484	0.9	0.6	72%
Cellulose Microcrystalline (2)	7.18	9,170	6,598	9,097	6,545	0.9	0.7	72%
Cellulose Microcrystalline (3)	7.08	9,680	7,060	9,385	6,845	0.9	0.7	73%
Cow Manure (1)	7.25	12,228	7,477	11,676	7,140	1.2	0.7	61%
Cow Manure (2)	7.30	11,795	7,330	11,559	7,182	1.2	0.7	62%
Cow Manure (3)	7.31	11,453	6,997	11,236	6,865	1.1	0.7	61%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	8,993	8,345	648	7%
Seed 5/20 (2)	9,365	7,545	1,820	19%
Seed 5/20 (3)	9,077	8,307	770	8%
Cellulose Microcrystalline (1)	12,600	9,075	3,525	28%
Cellulose Microcrystalline (2)	12,467	9,170	3,297	26%
Cellulose Microcrystalline (3)	13,525	9,680	3,845	28%
Cow Manure (1)	14,158	12,228	1,930	14%
Cow Manure (2)	15,905	11,795	4,110	26%
Cow Manure (3)	15,295	11,453	3,843	25%

Table C.42. Trial 2 BMP Total Solids Reduction for 39°C, mixed

	Table C.43.	<b>Trial 2 BMP</b>	<b>Volatile Solids</b>	<b>Reduction for</b>	: 39°C, m	ixed
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Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 5/20 (1)	6,835	5,718	1,117	16%
Seed 5/20 (2)	7,145	5,200	1,945	27%
Seed 5/20 (3)	6,915	5,677	1,238	18%
Cellulose Microcrystalline (1)	10,295	6,553	3,743	36%
Cellulose Microcrystalline (2)	10,230	6,598	3,632	36%
Cellulose Microcrystalline (3)	11,155	7,060	4,095	37%
Cow Manure (1)	10,330	7,477	2,853	28%
Cow Manure (2)	11,418	7,330	4,088	36%
Cow Manure (3)	11,070	6,997	4,073	37%

# C.4 BMP Data for Trial 3

#### *C.4.1* 15°*C*, non-mixed

Samula	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	8.17	8,987	6,845	8,858	6,746	0.9	0.7	76%
Seed 6/15 (2)	8.08	9,143	6,885	8,869	6,679	0.9	0.7	75%
Seed 6/15 (3)	8.04	8,772	6,472	8,611	6,355	0.9	0.6	74%
Cellulose Microcrystalline (1)	7.97	12,420	10,240	12,368	10,197	1.2	1.0	82%
Cellulose Microcrystalline (2)	7.97	12,975	10,578	12,768	10,409	1.3	1.0	82%
Cellulose Microcrystalline (3)	7.98	12,698	10,285	12,332	9,989	1.2	1.0	81%
Cow Manure (1)	7.72	13,943	10,170	13,550	9,885	1.4	1.0	73%
Cow Manure (2)	7.72	13,380	9,670	13,123	9,483	1.3	0.9	72%
Cow Manure (3)	7.70	14,000	10,078	13,956	10,046	1.4	1.0	72%

### Table C.44. Trial 3 BMP Pre-digestion data for 15°C, non-mixed

### Table C.45. Trial 3 BMP Post-digestion data for 15°C, non-mixed

Samula	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.80	8,570	6,495	8,394	6,361	0.8	0.6	76%
Seed 6/15 (2)	7.77	8,900	6,540	8,721	6,409	0.9	0.6	73%
Seed 6/15 (3)	7.70	8,408	6,313	8,307	6,237	0.8	0.6	75%
Cellulose Microcrystalline (1)	6.71	11,815	9,243	11,741	9,185	1.2	0.9	78%
Cellulose Microcrystalline (2)	6.68	11,542	9,015	11,235	8,775	1.1	0.9	78%
Cellulose Microcrystalline (3)	6.70	11,243	8,782	11,085	8,659	1.1	0.9	78%
Cow Manure (1)	7.31	13,235	9,485	12,763	9,147	1.3	0.9	72%
Cow Manure (2)	7.31	12,733	9,113	12,402	8,876	1.2	0.9	72%
Cow Manure (3)	7.32	12,968	9,190	12,830	9,093	1.3	0.9	71%

Samula	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	8,987	8,570	417	5%
Seed 6/15 (2)	9,143	8,900	242	3%
Seed 6/15 (3)	8,772	8,408	365	4%
Cellulose Microcrystalline (1)	12,420	11,815	605	5%
Cellulose Microcrystalline (2)	12,975	11,542	1,433	11%
Cellulose Microcrystalline (3)	12,698	11,243	1,455	11%
Cow Manure (1)	13,943	13,235	707	5%
Cow Manure (2)	13,380	12,733	647	5%
Cow Manure (3)	14,000	12,968	1,033	7%

Table C.46. Trial 3 BMP Total Solids Reduction for 15 °C, non-mixed

Table C.47. Trial 3 BMJ	Volatile Solids Reduction	for 15 °C, non-mixed
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Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	6,845	6,495	350	5%
Seed 6/15 (2)	6,885	6,540	345	5%
Seed 6/15 (3)	6,472	6,313	160	2%
Cellulose Microcrystalline (1)	10,240	9,243	997	10%
Cellulose Microcrystalline (2)	10,578	9,015	1,562	15%
Cellulose Microcrystalline (3)	10,285	8,782	1,503	15%
Cow Manure (1)	10,170	9,485	685	7%
Cow Manure (2)	9,670	9,113	557	6%
Cow Manure (3)	10,078	9,190	888	9%

## C.4.2 20°C, non-mixed

Sample pH	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	8.05	9,333	7,175	9,277	7,132	0.9	0.7	77%
Seed 6/15 (2)	7.96	9,108	7,008	8,895	6,843	0.9	0.7	77%
Seed 6/15 (3)	8.00	9,347	7,065	9,315	7,040	0.9	0.7	76%
Cellulose Microcrystalline (1)	7.97	12,345	10,160	11,914	9,805	1.2	1.0	82%
Cellulose Microcrystalline (2)	7.94	12,438	10,210	12,343	10,132	1.2	1.0	82%
Cellulose Microcrystalline (3)	7.95	12,090	9,852	11,865	9,669	1.2	1.0	81%
Cow Manure (1)	7.69	13,570	9,995	13,392	9,864	1.3	1.0	74%
Cow Manure (2)	7.66	13,918	10,195	13,499	9,888	1.3	1.0	73%
Cow Manure (3)	7.67	13,718	10,108	13,325	9,818	1.3	1.0	74%

Table C.48. Trial 3 BMP Pre-digestion data for 20°C, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.97	9,230	6,743	9,147	6,682	0.9	0.7	73%
Seed 6/15 (2)	7.93	8,965	6,570	8,830	6,472	0.9	0.6	73%
Seed 6/15 (3)	7.89	9,158	6,768	9,137	6,752	0.9	0.7	74%
Cellulose Microcrystalline (1)	6.61	10,458	8,048	10,266	7,900	1.0	0.8	77%
Cellulose Microcrystalline (2)	6.54	10,847	8,245	10,562	8,028	1.1	0.8	76%
Cellulose Microcrystalline (3)	6.53	11,073	8,520	10,834	8,336	1.1	0.8	77%
Cow Manure (1)	7.30	12,750	8,910	12,625	8,823	1.3	0.9	70%
Cow Manure (2)	7.33	12,963	9,085	12,823	8,987	1.3	0.9	70%
Cow Manure (3)	7.36	12,690	9,025	12,462	8,862	1.2	0.9	71%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	9,333	9,230	102	1%
Seed 6/15 (2)	9,108	8,965	143	2%
Seed 6/15 (3)	9,347	9,158	190	2%
Cellulose Microcrystalline (1)	12,345	10,458	1,887	15%
Cellulose Microcrystalline (2)	12,438	10,847	1,590	13%
Cellulose Microcrystalline (3)	12,090	11,073	1,017	8%
Cow Manure (1)	13,570	12,750	820	6%
Cow Manure (2)	13,918	12,963	955	7%
Cow Manure (3)	13,718	12,690	1,028	7%

Table C.50. Trial 3 BMP Total Solids Reduction for 20°C, non-mixed

Table C.51. Trial 3 BMP Volatile Solids Reduction for  $20^{\circ}$ C, non-mixed

Sampla	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	7,175	6,743	432	6%
Seed 6/15 (2)	7,008	6,570	438	6%
Seed 6/15 (3)	7,065	6,768	297	4%
Cellulose Microcrystalline (1)	10,160	8,048	2,112	21%
Cellulose Microcrystalline (2)	10,210	8,245	1,965	19%
Cellulose Microcrystalline (3)	9,852	8,520	1,332	14%
Cow Manure (1)	9,995	8,910	1,085	11%
Cow Manure (2)	10,195	9,085	1,110	11%
Cow Manure (3)	10,108	9,025	1,083	11%

## C.4.3 30°C, non-mixed

Sample pH	TS	VS	TS	VS	TS	VS	TS:VS	
Sample		( <b>mg/L</b> )	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	8.02	9,023	6,795	9,002	6,780	0.9	0.7	75%
Seed 6/15 (2)	7.95	9,425	7,063	9,410	7,051	0.9	0.7	75%
Seed 6/15 (3)	7.95	9,455	7,080	9,423	7,056	0.9	0.7	75%
Cellulose Microcrystalline (1)	8.00	12,792	10,500	12,747	10,463	1.3	1.0	82%
Cellulose Microcrystalline (2)	7.97	12,848	10,393	12,789	10,345	1.3	1.0	81%
Cellulose Microcrystalline (3)	7.98	12,893	10,542	12,740	10,417	1.3	1.0	82%
Cow Manure (1)	7.75	14,005	10,268	13,910	10,198	1.4	1.0	73%
Cow Manure (2)	7.71	13,853	10,130	13,823	10,108	1.4	1.0	73%
Cow Manure (3)	7.68	14,110	10,283	14,076	10,258	1.4	1.0	73%

Table C.52. Trial 3 BMP Pre-digestion data for 30°C, non-mixed

Table C.53.	<b>Trial 3 BMP</b>	Post-digestion	data for 30 °	C. non-mixed
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Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.67	7,965	5,670	7,901	5,624	0.8	0.6	71%
Seed 6/15 (2)	7.67	8,242	5,917	8,171	5,866	0.8	0.6	72%
Seed 6/15 (3)	7.68	8,100	5,773	8,038	5,729	0.8	0.6	71%
Cellulose Microcrystalline (1)	7.34	9,425	7,090	9,281	6,982	0.9	0.7	75%
Cellulose Microcrystalline (2)	7.33	8,847	6,400	8,519	6,162	0.9	0.6	72%
Cellulose Microcrystalline (3)	7.37	8,670	6,393	8,436	6,220	0.8	0.6	74%
Cow Manure (1)	7.51	11,143	7,550	11,044	7,484	1.1	0.7	68%
Cow Manure (2)	7.53	11,342	7,555	11,031	7,348	1.1	0.7	67%
Cow Manure (3)	7.55	11,430	7,490	11,310	7,411	1.1	0.7	66%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	9,023	7,965	1,058	12%
Seed 6/15 (2)	9,425	8,242	1,183	13%
Seed 6/15 (3)	9,455	8,100	1,355	14%
Cellulose Microcrystalline (1)	12,792	9,425	3,367	26%
Cellulose Microcrystalline (2)	12,848	8,847	4,000	31%
Cellulose Microcrystalline (3)	12,893	8,670	4,223	33%
Cow Manure (1)	14,005	11,143	2,862	20%
Cow Manure (2)	13,853	11,342	2,510	18%
Cow Manure (3)	14,110	11,430	2,680	19%

Table C.54. Trial 3 BMP Total Solids Reduction for 30°C, non-mixed

Table C.55. Trial 3 BMP Volatile Solids Reduction for 30 $^{\circ}C,$  non-mixed

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	6,795	5,670	1,125	17%
Seed 6/15 (2)	7,063	5,917	1,145	16%
Seed 6/15 (3)	7,080	5,773	1,308	18%
Cellulose Microcrystalline (1)	10,500	7,090	3,410	32%
Cellulose Microcrystalline (2)	10,393	6,400	3,993	38%
Cellulose Microcrystalline (3)	10,542	6,393	4,150	39%
Cow Manure (1)	10,268	7,550	2,718	26%
Cow Manure (2)	10,130	7,555	2,575	25%
Cow Manure (3)	10,283	7,490	2,793	27%

## C.4.4 39°C, non-mixed

[Sample p]	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.99	9,033	6,797	8,982	6,760	0.9	0.7	75%
Seed 6/15 (2)	7.97	8,993	6,803	8,748	6,618	0.9	0.7	76%
Seed 6/15 (3)	7.97	8,903	6,763	8,788	6,676	0.9	0.7	76%
Cellulose Microcrystalline (1)	8.00	12,398	9,995	12,186	9,824	1.2	1.0	81%
Cellulose Microcrystalline (2)	8.03	12,590	10,317	12,425	10,182	1.2	1.0	82%
Cellulose Microcrystalline (3)	8.04	12,162	10,115	11,899	9,895	1.2	1.0	83%
Cow Manure (1)	7.78	13,625	9,920	13,425	9,776	1.3	1.0	73%
Cow Manure (2)	7.74	13,970	10,130	13,581	9,847	1.4	1.0	73%
Cow Manure (3)	7.75	13,703	9,905	13,535	9,783	1.4	1.0	72%

Table C.56. Trial 3 BMP Pre-digestion data for 39°C, non-mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.62	7,790	5,375	7,663	5,287	0.8	0.5	69%
Seed 6/15 (2)	7.60	8,125	5,513	7,981	5,414	0.8	0.5	68%
Seed 6/15 (3)	7.59	8,075	5,558	8,054	5,543	0.8	0.6	69%
Cellulose Microcrystalline (1)	7.38	8,443	5,663	8,340	5,594	0.8	0.6	67%
Cellulose Microcrystalline (2)	7.37	8,437	5,915	8,284	5,808	0.8	0.6	70%
Cellulose Microcrystalline (3)	7.36	8,540	6,020	8,189	5,773	0.8	0.6	70%
Cow Manure (1)	7.48	11,225	7,323	10,940	7,137	1.1	0.7	65%
Cow Manure (2)	7.55	10,685	6,928	10,665	6,915	1.1	0.7	65%
Cow Manure (3)	7.57	10,583	6,900	10,501	6,847	1.1	0.7	65%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	9,033	7,790	1,243	14%
Seed 6/15 (2)	8,993	8,125	868	10%
Seed 6/15 (3)	8,903	8,075	828	9%
Cellulose Microcrystalline (1)	12,398	8,443	3,955	32%
Cellulose Microcrystalline (2)	12,590	8,437	4,153	33%
Cellulose Microcrystalline (3)	12,162	8,540	3,622	30%
Cow Manure (1)	13,625	11,225	2,400	18%
Cow Manure (2)	13,970	10,685	3,285	24%
Cow Manure (3)	13,703	10,583	3,120	23%

Table C.58. Trial 3 BMP Total Solids Reduction for 39°C, non-mixed

Table C.59. Trial 3 BMP	Volatile Solids Reduction	for 39°C, non-mixed
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Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	6,797	5,375	1,422	21%
Seed 6/15 (2)	6,803	5,513	1,290	19%
Seed 6/15 (3)	6,763	5,558	1,205	18%
Cellulose Microcrystalline (1)	9,995	5,663	4,333	43%
Cellulose Microcrystalline (2)	10,317	5,915	4,402	43%
Cellulose Microcrystalline (3)	10,115	6,020	4,095	40%
Cow Manure (1)	9,920	7,323	2,597	26%
Cow Manure (2)	10,130	6,928	3,203	32%
Cow Manure (3)	9,905	6,900	3,005	30%

## C.4.5 39°C, mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.99	9,513	7,100	9,473	7,070	0.9	0.7	75%
Seed 6/15 (2)	8.00	9,375	6,982	9,349	6,963	0.9	0.7	74%
Seed 6/15 (3)	8.00	9,480	7,062	9,419	7,017	0.9	0.7	74%
Cellulose Microcrystalline (1)	8.00	12,940	10,470	12,864	10,409	1.3	1.0	81%
Cellulose Microcrystalline (2)	7.99	12,493	10,108	12,443	10,068	1.2	1.0	81%
Cellulose Microcrystalline (3)	7.96	13,320	10,805	13,236	10,737	1.3	1.1	81%
Cow Manure (1)	7.72	13,848	10,070	13,795	10,032	1.4	1.0	73%
Cow Manure (2)	7.82	13,850	10,125	13,759	10,059	1.4	1.0	73%
Cow Manure (3)	7.67	13,745	10,048	13,663	9,988	1.4	1.0	73%

Table C.60. Trial 3 BMP Pre-digestion data for 39°C, mixed

Sampla	pН	TS	VS	TS	VS	TS	VS	TS:VS
Sample		(mg/L)	(mg/L)	(mg/kg)	(mg/kg)	(%)	(%)	
Seed 6/15 (1)	7.57	7,375	5,210	7,350	5,192	0.7	0.5	71%
Seed 6/15 (2)	7.54	7,592	5,315	7,577	5,304	0.8	0.5	70%
Seed 6/15 (3)	7.50	7,690	5,192	7,655	5,168	0.8	0.5	68%
Cellulose Microcrystalline (1)	7.41	8,182	5,860	8,069	5,779	0.8	0.6	72%
Cellulose Microcrystalline (2)	7.42	8,455	6,068	8,279	5,941	0.8	0.6	72%
Cellulose Microcrystalline (3)	7.41	8,717	6,332	8,675	6,301	0.9	0.6	73%
Cow Manure (1)	7.49	10,475	6,767	10,444	6,747	1.0	0.7	65%
Cow Manure (2)	7.56	10,823	7,000	10,744	6,950	1.1	0.7	65%
Cow Manure (3)	7.56	10,508	6,762	10,442	6,720	1.0	0.7	64%

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	9,513	7,375	2,138	22%
Seed 6/15 (2)	9,375	7,592	1,783	19%
Seed 6/15 (3)	9,480	7,690	1,790	19%
Cellulose Microcrystalline (1)	12,940	8,182	4,758	37%
Cellulose Microcrystalline (2)	12,493	8,455	4,038	32%
Cellulose Microcrystalline (3)	13,320	8,717	4,603	35%
Cow Manure (1)	13,848	10,475	3,372	24%
Cow Manure (2)	13,850	10,823	3,027	22%
Cow Manure (3)	13,745	10,508	3,238	24%

Table C.62. Trial 3 BMP Total Solids Reduction for 39°C, mixed

Table C.63. Trial 3 BMP Volatile Solids Reduction for 39°C, mixed

Sample	Initial	Final	Destroyed	Reduction
Sample	(mg/L)	(mg/L)	(mg/L)	(%)
Seed 6/15 (1)	7,100	5,210	1,890	27%
Seed 6/15 (2)	6,982	5,315	1,668	24%
Seed 6/15 (3)	7,062	5,192	1,870	26%
Cellulose Microcrystalline (1)	10,470	5,860	4,610	44%
Cellulose Microcrystalline (2)	10,108	6,068	4,040	40%
Cellulose Microcrystalline (3)	10,805	6,332	4,473	41%
Cow Manure (1)	10,070	6,767	3,303	33%
Cow Manure (2)	10,125	7,000	3,125	31%
Cow Manure (3)	10,048	6,762	3,285	33%

## Appendix D. Additional Data and Figures for Pilot Data

### D.1 Daily Biogas Production per kg Initial VS

Environment	Biogas Production per kg Initial VS ± Std. Dev. (L/ kg Initial VS)	Min (L/ kg Initial VS)	Max (L/ kg Initial VS)	n
Lab	324±161	53	682	125
Unregulated	291±194	32	894	124
Mesophilic	604±276	59	1,272	125

#### Table D.1. Biogas Production per kg of Initial VS based on Environment



Figure D.1. Daily Biogas Production for Lab, Unregulated and Mesophilic Pilots



Figure D.2. Daily Biogas Production for Lab Pilots



Figure D.3. Daily Biogas Production for Unregulated Pilots



Figure D.4. Daily Biogas Production for Mesophilic Pilots

D.2 Cumulative Biogas Production



Figure D.5. Cumulative Biogas Production for Lab Pilots



Figure D.6. Cumulative Biogas Production for Unregulated Pilots



Figure D.7. Cumulative Biogas Production for Mesophilic Pilots

**Appendix E. Covered Lagoon System Diagram and Table of System Stream Conditions** 



Figure E.1. System Diagram for a Covered Lagoon Anaerobic Digester

Stream	Volume	TS	VS
Stream		(kg/day)	
1	4,465,000	104,897	87,869
2	454,200	6,813	5,450
3	4,919,200	111,710	93,319
4	55,855	27,928	18,664
5	4,863,345	83,783	74,656
6	23,938	16,757	12,691
7	4,839,407	67,026	61,964
8	-	30,982	30,982
9	4,839,407	36,044	30,982
10	3,785,000	15,897	12,869
11	1,054,407	20,147	18,113

Table E.1. Stream Conditions for Figure E.1



Appendix F. CSTR System Diagram and Table of System Stream Conditions

Figure F.1. System Diagram for a CSTR Anaerobic Digester

Stream	Volume	TS	VS
	(kg/day)		
1	4,465,000	145,775	112,850
2	454,200	6,813	5,450
3	4,919,200	152,588	118,300
4	61,035	30,518	23,660
5	4,858,165	122,070	94,640
6	3,785,000	56,775	37,850
7	101,532	6,997	4,371
8	971,633	58,298	52,420
9	-	36,694	36,694
10	971,633	21,604	15,726
11	6,173	4,321	2,673
12	965,460	17,283	13,052
13	1,066,992	24,281	17,423

Table F.1. Stream Conditions for Figure F.1

#### **Appendix G. Stoichiometric Equations**

#### G.1 Stoichiometric Equation for the Anaerobic Digestion Process

The following equation was obtained by utilizing ratios from Phyllis2 database and Chen et al. (2015). In order to obtain the equation, procedures presented by McCarty et al. (2011) were utilized.

$$\begin{split} \mathrm{CH}_{1.71}\mathrm{N}_{0.044}\mathrm{O}_{0.836}\mathrm{S}_{0.005}\mathrm{P}_{0.005} + 0.0314~\mathrm{H_2O} + 0.026~\mathrm{H_2} \\ & \rightarrow 0.242~\mathrm{CH_4} + 0.225~\mathrm{CO_2} + 0.015~\mathrm{NH_3} + 0.003~\mathrm{H_2S} + 0.0049~\mathrm{H_3PO_4} \\ & + 0.5333~\mathrm{CH}_{1.486}\mathrm{N}_{0.054}\mathrm{O}_{0.836}\mathrm{S}_{0.004}\mathrm{P}_{0.0001} \end{split}$$

### G.2 Stoichiometric Equation for the Conversion of Ammonia in Soil

The following equations were obtained from the following literature: Kanter & Brownlie (2019), Meynell (1972) and Fontaine (2019) in which the process of the nitrogen cycle and ammonia conversions from digestate in soil are described.

$$2 \text{ NH}_3 + 3 \text{ O}_2 \rightarrow 2 \text{ NO}_2^- + 2 \text{ H}^+ + 2 \text{ H}_2\text{ O}$$

$$2 \operatorname{NO}_2^- + \operatorname{O}_2 \rightarrow 2 \operatorname{NO}_3^-$$

#### G.3 Stoichiometric Equation for the Conversion of Phosphate in Soil

The following equations were obtained from the following literature: Kanter & Brownlie (2019), and Mullins (2009) in which the process of the phosphorus cycle and phosphate conversions from digestate in soil are described.

$$H_3PO_4 \rightarrow 3 H^+ + H_2PO_4^-$$
$$H_2PO_4^- \rightarrow 3 H^+ + HPO_4^{2-}$$

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