IMPROVING THE METHANE PRODUCTION IN THE CO-DIGESTION OF MICROALGAE AND CATTLE MANURE

A Thesis

by

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ABSTRACT

The objective of this thesis is to evaluate the effects from various treatments in the anaerobic digestion of cattle manure when mixed with microalgae. The analysis would focus on two primary subjects: the effects of different treatments on the microalgae sludge, and the balancing of the carbon-to-nitrogen ratio. The results of this experiment would give a viable estimate on the possible methane production from co-digestion of these resources.

At the conclusion of the experiment, it was found that biogas production increased when algae was added to the digester. The highest methane production in the control groups, containing only manure, digestion sludge, and newsprint was 48120 L, while the highest in the mixtures containing algae and pretreated algae were 71170 L and 87715 L, respectively. Based on volatile solids, the highest production in the control groups was $0.36\,\frac{L\,CH_4}{g\,VS}$, while the production rates in the algae and pretreated algae mixtures were $0.22\,\frac{L\,CH_4}{g\,VS}$ and $0.44\,\frac{L\,CH_4}{g\,VS}$, respectively. This shows that the presence of algae increases the overall methane production, but is hindered by inhibitory factors contributing to ineffectiveness in the overall digestion process. The effects of carbon balancing for the carbon-to-nitrogen ratio also showed that overall, mixtures balanced at 25:1 carbon-to-nitrogen yielded more biogas. The exception is the normal algae mixture, in which the optimal ratio was 20:1. In conclusion, the anaerobic co-digestion of cattle manure with

pretreated algae, when balanced for carbon and nitrogen, can severely increase methane production rates.

DEDICATION

For my father, my mother, my family, and all those who have guided me.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

The simultaneous growth of the human population and the dependence on energy and fuels has increased the need for research into alternative energy resources. Coupled with the increasing threat of climate changes, an effective energy source is greatly desired.

Many sources of alternative energies come from natural resources. Solar energy, hydroelectricity, geothermal power, and wind power can all generate energy using natural occurrences when coupled with technology. One of the many types of renewable energy that has been developed is the use of converting biological materials into usable fuels. This bioenergy can come in many forms. Resources such as char, bio-oil, or gas can be obtained through gasification and pyrolysis. Liquid fuels such as ethanol and biodiesel are obtained through fermentation reactions and esterification. Many of these fuels are comparable to the established fossil fuels in the modern market, and with the proper equipment, can be used as a replacement.

A useful energy material is methane. Methane is a carbon-based gas primarily made from biological reactions. The reactions take place with microorganisms in the absence of oxygen in a process called anaerobic digestion. Anaerobic digestion takes place when

bacteria convert a biomass feedstock into various other organic compounds, ultimately ending in a mixture of carbon dioxide and methane called biogas. This biogas is a mixture approximately made of 60% methane and 40% carbon dioxide, with other trace gases found. While anthropogenic carbon dioxide is a concern with greenhouse gas emission, the carbon dioxide released in this reaction is considered carbon neutral. The methane can be purified and used for purposes of generating heat or electricity (Ward et al., 2008). The energy provided from anaerobic digestion not only is considered a net positive resource, but also a useful carbon reduction method (Batstone et al., 2002). Anaerobic digestion serves a dual purpose in both providing the methane and reduction in volatile solids, lowering the risk of possible pollution when the slurry is disposed. The solids can also be used for various agricultural purposes such as fertilization.

Anaerobic digestion reactors can be designed in various ways. Structures typically include a closed tank system, though can include lagoons when water levels are deep enough to assume oxygen is negligible. Virtually any organic compound can be converted into methane through anaerobic digestion, including wastewater streams, animal manures, food wastes, crop wastes, and biomass resources. Buildup of animal manures on farm property is an issue that may have to be handled individually, and anaerobic digestion is a simple enough process to treat them.

A major resource in bioenergy research has been microalgae. Microalgae is composed of unicellular algae species as well as bacteria (Samson and Leduy, 2003). Algae is a

favored biomass resource due to a high production rate and carbon sequestration. Primarily, algae is grown as a resource in the production of biodiesel due to high lipid counts. As an anaerobic digestion feedstock, however, research can still be done to optimize methane production. Algae can provide high amounts of nutrients and volatile solids to potentially emerge as a viable anaerobic digestion resource.

Co-digestion is a technique of combining multiple feed sources into the same anaerobic digestion system to increase overall methane content. By finding a proper balance of volatile solids for microbes, an increase in methane amount and production rate may be found (Angelidaki and Ellegaard, 2003). This balance can be found through the readings of carbon and nitrogen in the digestion process. Carbon is the primary food source for microbes in the reactor, while nitrogen is a key nutrient that can be toxic in high amounts. A high carbon-to-nitrogen ratio may lead to overwhelming the microbes, while a low ratio would result in a toxic environment. A proper balance is found at approximately 20:1 or 30:1, when methane production can be optimized.

This experiment utilizes four resources in anaerobic digestion: cattle manure, microalgae, newsprint, and inoculum sludge. The cattle manure was obtained from an agricultural research facility to provide the basis for the digestion stream. Microalgae was provided after harvesting from a research pond to act as a co-digestion feedstock for the microbes in the cattle manure. Newsprint was used to provide high amounts of carbon to balance the carbon-to-nitrogen ratio. Inoculum sludge from an anaerobic

wastewater treatment reactor was used to provide activated microbes for the digestion process to accelerate during initial testing.

Objectives

The primary objective of this research was to analyze the effects of various co-digestion techniques to find a possible means of increasing methane production when using cattle manure and microalgae. The co-digestion of the products was compared with cattle manure alone to find a possible increase when microalgae was present. This was done in tandem with two techniques to potentially increase methane yield. The first was to thermally pretreat the algae to disrupt the resistant cell walls in the slurry. The second was to balance the reactors to varying carbon-to-nitrogen ratios to find an optimal level. The biogas yields of the digestion mixtures were compared to find possible means to increase energy yield from the process.

Anaerobic Digestion Background

Anaerobic digestion is defined as a natural process in degrading organic material in the absence of oxygen. This is done through microbial conversion of biomass through several processes, ultimately ending in the production of biogas. Biogas contains several gases, but primarily is a mixture of methane and carbon dioxide, with concentrations at approximately 60% and 40%, respectively. While multitudes of microorganisms are involved in the digestion process, the processes themselves can be easily identified and analyzed. The basic pathways involved in anaerobic digestion are shown in Figure 1.

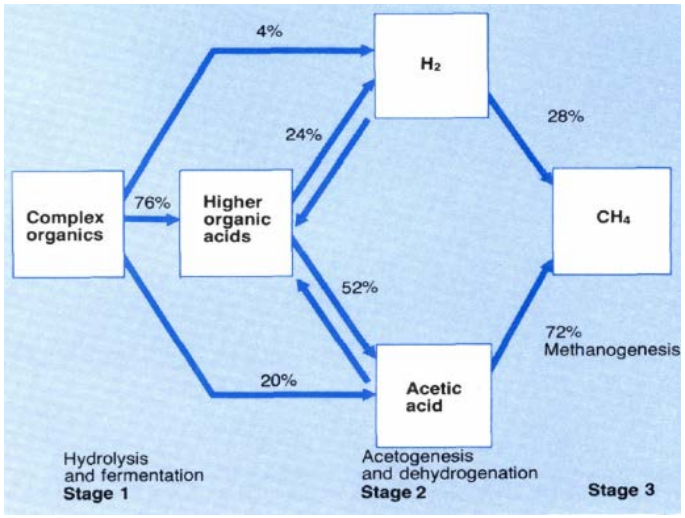

Figure 1. Basic anaerobic digestion pathways (Speece, 1983)

Anaerobic digestion is typically designed as a stream within an enclosed tank (Chynoweth et al., 2001). The influent stream is composed of an inoculum, found from wastewater treatment residues or active wastewater. This inoculum contains an initial point of microbial activity to begin digestion. Digestion can take place without inoculum, as microbes involved in the digestion process can be found virtually anywhere (this process is regularly found in composting). Using a stream of activated microbes, however, will accelerate the digestion process to increase the rate of initial methane production. Several weeks of residency time would be required for a fresh bacterial composition to reach the levels of activity found in a wastewater stream.

The process of anaerobic digestion is typically composed of three phases (McCarty and Smith, 1986). Digestion begins with microorganisms taking in organic matter from biomass. Complex organic molecules such as lipids, proteins, and polysaccharides are

broken into simple organic components through hydrolysis with the assistance of enzymes. Hydrolysis reactions will result in the production of monosaccharaides and acids.

The second phase of anaerobic digestion utilizes multiple types of bacteria to convert the simple organic molecules into various acids. Hydrolytic and acidogenic bacteria feed off of the initial components, and produce higher levels of other acids, such as acetic and propionic acids. During this phase, hydrogen production also becomes apparent, which can be used as another source of methane in the final step of digestion (McCarty and Mosey, 1991).

The final step in anaerobic digestion is methanogenesis, the formation of biogas. Methanogenic bacteria convert prior products, including acetic acid and hydrogen, to produce methane gas. The entire digestion process can take several days to several weeks, depending on the amount of feed in the inoculum and the potency of microbes, but typically at least an 80% reduction of volatile solids is seen when fully converted to methane (Gujer and Zehnder, 1983).

Factors influencing anaerobic digestion

While the setup of the actual anaerobic digestion process is fairly simple, several factors can be changed to optimize the reactions. Factors can include the variables within the digester as well as the digesters themselves. Temperature, pH levels, nutrient

amounts, and feed concentrations can all affect the anaerobic digestion process (Ward et al., 2008).

Temperature can be one of the leading factors affecting the end methane output. Primarily, most reactors operate nominally at mesophilic and thermophilic temperatures, between 35° C and 55° C, respectively. While most research agrees that thermophilic reactors result in higher methane yields, most digestion mixtures should be handled individually to find an optimum temperature. The energy requirement to heat the reactors to the higher temperature should also be taken into account.

The ideal pH for anaerobic digestion if 6.8-7.2. The narrow range is mainly due to the varying optimal pH levels for the varying microbes involved in the system (Kim et al., 2003). The optimal level for methanogenesis is 7.0, while the optimal level for hydrolysis and acidogenesis is between 5.5 and 6.5. While the aforementioned pH range can provide a steady level of methane production, many designers opt to divide the process into two phases to optimize them individually.

Feedstock can have the greatest impact on methane production amounts, as it governs the amount of volatile feed given to microbes in the system. Feedstock can be divided into multiple categories, including municipal solid wastes, manures, fruit and vegetable wastes, and miscellaneous biomass (Ward et al., 2008). Municipal solid wastes are found through commercial streams, and can vary greatly in terms of consistency and solids

content. Inhibitory materials such as nonbiodegradable waste and toxic additives may also be found in these streams, so a preliminary separation should be utilized. Manures from varying animal sources show promise as a methane source, and have been tested extensively for methane production. Fruit and vegetable wastes in anaerobic digestion function similarly to composting, and can be easily degraded within the digester. However, acid content of these wastes can lead to inhibition. Other biomass sources can be used in a digestion system, though on an individual basis, inhibitory factors may include recalcitrance from fibers, harvesting time, and low energy yields.

Pretreatment conditions can greatly affect the potential yield of methane, both in the rate of gas production and the overall accumulation. Pretreatment tends to have a large impact on the degradability of biomass feedstock, as hindrances such as lignin or cellulose are overcome in cell lysis (Tiehm et al., 2001). Pretreatment can be divided into chemical, physical, and thermal methods. Chemical pretreatment finds a high methane yield in alkaline treatment; through the addition of NaOH, lignin can be degraded in biomass to provide microbes easier access to the feedstock (Gunaseelan, 1994). However, NaOH levels should be controlled to avoid toxicity. Thermal pretreatment involves the heating of the digestion substrate to breakdown cells in thermal hydrolysis. Introducing feedstock to a high temperature prior to input to the reactor can increase access to volatile compounds, but at too high a temperature, risks degradation of the solids (Mladenovska et al., 2006). Physical pretreatment includes mechanical means of lysing cells, such as ultrasonication or centrifugation. Methane was

found to increase by 34% due to ultrasonication pretreatment in sludge (Kim et al., 2003)

Potential inhibitors of anaerobic digestion

Several parameters within the digestion reactor may have a negative impact on the methane production. Some factors such as presence of toxic material or suboptimal conditions may lead to hindered gas production or microbial death. Toxic concentrations, light and heavy metals, and organic pollutants can all hinder the anaerobic digestion reactions (Chen et al., 2009).

Ammonia is produced as a byproduct of several organic processes, primarily from the degradation of nitrogen-based materials. Methanogens within the anaerobic digestion process are seen as the least tolerant of all microorganisms in the system (Kayhanian, 1994). As a result, ammonia concentration may lead to hindered growth or death of these bacteria. Ammonia levels should be kept to a minimum, but may become unavoidable as a product in several reactions. Ammonia levels can be controlled through neutral pH levels, increased temperatures, and utilization of ions such as sodium and potassium.

Methane inhibition may also be found in the presence of sulfides. Sulfate is converted to sulfide, typically through organisms also employed in the conversion of acetate used for methane production. Several bacteria may also be inhibited through toxicity of sulfide. Levels of sulfate and sulfide is typically controlled through dilution of the incoming

stream, or through more strenuous means of physically or chemically extracting sulfur from the influent.

Light metals include nutrients such as sodium, potassium, manganese, calcium, and aluminum. All of these metals are crucial as nutrients to microbes, but in high concentrations, can become detrimental to the anaerobic digestion process (Soto et al., 1993). These salts may be released into the system unavoidably from breakdown of organic compounds, but can be controlled through dilution. Heavy metals such as copper, zinc, and nickel are primarily undesired in digestion mixtures, as they are not only toxic in most cases, but are also nonbiodegradable and can accumulate to high concentrations. Heavy metals are primarily removed through precipitation, sorption, and chelation by organic ligands (Oleskiewicz and Sharma, 1990).

Organic compounds as many kinds may become inhibitors in the digestion system after accumulation. Organic chemicals with low solubility or adsorbed to solids within the digester can become inhibitory with high concentration, leading to eventual hindered cell activity and death. Removal of these compounds are mostly handled on an individual basis, but include practices such as filtration or dilution.

Digestion of Cattle Manure

Manure has proven to be a reliable and efficient source of methane. Among the various animal sources, cattle manure remains one of the strongest feedstock for biogas

production (Holm-Nielsen et al., 2009). Due to the abundance of livestock, manure is plentiful resource by default. Slurries from the waste of cattle, swine, fish, and poultry can be used in an anaerobic digestion system to produce a steady stream of methane. This provides a double benefit, as not only can methane be used as an energy source, but anaerobic treatment can also be used as a means of purifying the waste products prior to disposal. This can reduce the risks in pollution found when improperly disposing animal waste. The spent solids can also be utilized as a fertilizer product and recycled by the agricultural business.

The source of the manure can have an impact on the end methane yield. Swine manure shows to have a slightly higher methane yield than beef cattle, though is not as plentiful (Ward et al., 2008). Poultry manure has also shown promise as a methane source, but is susceptible to ammonia toxicity (Bujoczek et al., 2000). Several factors can influence the amount of methane found from manure: species of livestock, bedding material, feed, and livestock growth stage.

Cattle manure is of note due to its bountiful nature. The high population of cattle in the world makes it a near permanent resource in anaerobic digestion. Of note, however, is the high fiber content in cattle waste (Nielsen et al., 2004). Due to the resistant nature of lignocellulosic fiber in the manure mixture, methane conversion will be slowed or hindered completely. A common practice to overcome this obstacle is thermal treatment of the cattle manure.

Digestion of Microalgae

Microalgae refers to one of two types of algal biomass: while macroalgae includes large aquatic multicellular organisms such as kelp, microalgae are unicellular algae and bacteria (Zamalloa et al., 2011). Biomass derived from microalgae demonstrate many uses as an, energy feedstock outside of power. Algal growth is remarkably higher and more efficient than other energy crops, can be controlled in harsh environments throughout the year, can use both salt water and wastewater systems, and demonstrate high carbon dioxide reductions (Schenk, et al. 2008). Microalgae has become a notable biomass resource in the field of renewable energy for its use as a biodiesel feedstock. However, by the nature of anaerobic digestion, algae can be used as a digestion substrate.

Microalgae is notable for having a high production rate compared to other biomass. Average production rates can be estimated at $27 - 62 \frac{g}{m^2 d}$ in dry basis, depending on the species used and growth conditions. Typically, production is lower than theoretically maximized. A more realistic estimate would be $19 - 25 \frac{g}{m^2 d}$ in dry basis when grown in a common raceway pond. While composition within the algae may vary between species and growth systems, a general view of the algae would show a high concentration of proteins and lipids. The high lipid count is of note in methane production, as these lipids can provide high acid feed sources for microbes. A problem in using algae as a digestion resource, however, is the high resistance of the cell wall (Sialve et al., 2009). Pretreatment is almost required to reach effective levels of methane production when

utilizing microalgae. Due to the viable uses as a liquid fuel source, research on the use of microalgae as a sole methane resource is minimal. Algal residue from the conclusion of oil extraction is still a viable substrate. Digestion of the algal cell is still possible, however, though long startup times and low breakdown has been observed (Golueke et al., 1957).

Theoretical Yield

In the utilization of biomass substrates for anaerobic digestion, predictions of methane yield are valuable in selection. As the digester is a closed system, the entirety of the potential biogas yield from specific substrates is dependent on the input chemical composition. Previous research has been conducted on the biomethane potential (BMP) of many different biomass types and compositions. Results from experiments show that virtually any organic material can be used as a digestion substrate and be converted into methane.

Theoretical methane yield from dairy cattle manure can be approximated as $0.468\,\frac{L}{g\,VS}$(Møller et al, 2004). The estimation was compared to the ultimate methane yield of $0.148\,\frac{L}{g\,VS}$. The experiment was conducted as a BMP test for several manure types, one of which was dairy cattle manure. The theoretical yield was found through the hypothetical conversion of all organic carbon to methane and carbon dioxide following a stoichiometric balance. This conversion utilizes the volatile fatty acid content as well as other organic molecules Cattle manure was shown to have high volatile solids

composition, ranging from 79% - 94% of dry matter. The experiment was conducted utilizing 1100-mL bottles filled with approximately 122 g/L of dry matter. Over the course of 60 days, methane production ceased and the ultimate methane potential was listed. The experiment also listed a method of using varying straw content to affect the total volatile solids composition in the digester, thus affecting potential methane production. The experiment found that for every 1kg of straw added to 100 kg of manure, methane productivity increased by 10%, accounting for the high volatile solids content.

Calculating the theoretical yield of algae can take a number of approaches. As algae is a high-value biomass material in the production of biodiesel, the conversion of raw algae to methane is less covered than the conversion of residual algal sludge. A previous experiment in the BMP analysis of *Spirulina maxima* found viable results in the methane yield of microalgae (Samson and Leduy, 1982). The experiment was conducted similarly to most batch processes, though additional biomass was added to the system was productivity became too low. The end result after 210 days using a 12-L culture with replenishment yielded approximately $0.26\frac{L}{g\ VS}$. The approximate ratios of methane and carbon dioxide were also listed, with methane ranging from 68%-72%, and carbon dioxide ranging from 28%-32%.

Co-digestion

Co-digestion is defined as the mixing of two or more organic feeds in a digestion system. This method of combining solids contents and nutrients can improve both sources as a methane feedstock. Cattle manure alone has a low solids content (roughly 7%-9%), most of which is composed of lignocellulosic material (Angelidaki and Ellegaard, 2003). However, the low concentration of manure combined with high bacterial count make it an excellent carrier resource to mix with other materials. Manure provides a high water content, buffering capacity for pH, nutrients crucial to microbial growth, and can be easily replenished. By mixing manure with other streams of organic solids, methane production can be increased. In the combination of cattle manure and kitchen wastes, a 44% increase in methane was found when compared to methane produced kitchen wastes solely (Li et al., 2009). The experiment also found that NaOH pretreatment increased the methane yield even further.

Co-digestion has also been used as a technique to convert mixtures containing microalgae into methane. When properly balanced, microalgae when mixed with waste paper increases the overall methane yield by more than double, from $573\,\frac{ml}{L\,Day}$ to $1170\,\frac{ml}{L\,Day}$ through increasing the volatile solids count with waste paper (Yen and Brune, 2007). Digesters utilizing a mixture of paper and algae also saw an increase in cellulase activity.

Of note in co-digestion is the ratio of carbon to nitrogen. Microorganisms in the digester will convert organic carbon into the products used for methane. As such, a high organic carbon content is desired. However, a ratio of carbon-to-nitrogen that is too high will result in washout of bacteria. Conversely, a ratio that is too low signifies a high nitrogen content that can develop into toxic levels of ammonia. While the actual value varies depending on digestion mixture and literature, the ratio of carbon-to-nitrogen should be between 20:1 and 30:1 (Parkin and Owen, 1986). This ratio can be met through the use of virtually any organic material.

CHAPTER II

EXPERIMENT METHODOLOGY

Algae

Harvested *Spirulina* algae was sent from the Texas Agrilife algae research facility in Pecos, TX. The algal material was grown through a raceway pond setup and harvested after proper oil content was found. The sludge was centrifuged to a watered, concentrated form prior to being sent to the laboratory. Algae was kept frozen during shipment and storage, and thawed prior to analysis and implementation in the experiment. Algae samples were taken after thawing and dewatered to measure solids content and density, as well as carbon and nitrogen content.

Thermal pretreatment was used for several reactors in the experiment. This was done through the use of a Parr 4848 pressure reactor. Typically used for pyrolysis, the reactor served as a heating mechanism when valves were closed to prevent vapor escape. Algae was heated in 1-L batches at 100° C for 3 hours. Pressure increase was minimal, and moisture losses were negligible. The resultant slurry was noticeably different in color, as much of the green chlorophyll had faded to a brown color.

Cattle Manure

Fresh cattle manure was collected from research dairy cattle from the Texas A&M Animal Science Research and Extension Science Complex (ASTREC). The manure was collected in 25-L buckets and used the same day as collection to maintain

freshness. Care was taken that samples were as fresh as possible to maximize methane production potential.

The cattle used were raised on a measured grain diet. Compared to the hay diet of other cattle at the facility, grain-based manure was noted to have a slightly higher methanogenesis potential. The manure itself was noted to have a fairly even consistency. Manure was dried and analyzed for solids content and moisture, as well as carbon and nitrogen content.

Inoculum Sludge and Carbon Input

Anaerobic digester inoculum sludge was gathered from the Texas A&M wastewater treatment facility. Similar to the cattle manure, 25-L buckets were used to contain the harvested sludge. The sludge was collected directly from the recycle stream of the anaerobic digesters, and as such contained high moisture content, yet also high amounts of activated microbes for methane production.

The inoculum sludge was used the same day as harvesting to maintain microbial activity. It was noted that extended residence time caused settling of the sludge and separation of components into layers. Proper mixing and quick use prevented this becoming an issue in the digesters. Samples were dewatered to measure solids content, density, and pH, as well as carbon and nitrogen content.

Carbon balancing was provided by shredded newspaper gathered from the Texas A&M campus. Paper was shredded to a uniform size using a standard paper shredder. The paper scraps were measured in varying amounts to be introduced to each digester individually. While moisture content of the paper was low, the samples were still dried prior to measuring solids content, as well as carbon and nitrogen content.

Digester Setup

The laboratory setup consisted of 9 reactors, each with approximate working volumes of 6 L. Digesters were used in a previous anaerobic digestion study by a former student at Texas A&M. The digesters were constructed of clear PVC pipe with an inside diameter of 15.2 cm (6 in.) and with lengths of 30.5 cm (1 ft). Clear PVC allowed for visual investigation of the digesters while the experiment was being conducted. A properly sized 15.2 cm PVC cap was used to seal the reactor on the bottom. A threaded PVC fitting was used on the top of each reactor, with a 15.2 cm threaded PVC plug sealing the reactor. PVC cement was used to seal all fitting on the reactor, not including the top threading, allowing for opening. A sealing compound putty was used in the threaded connection to prevent gas leaks from the pressure inside the reactors.
The digester caps were drilled to allow for outlets. The bottom of each reactor consisted of a 0.635 cm valve to allow for liquid samples to be taken. The top was drilled for two outlets; one consisted of a 0.635 cm valve to allow for sample feeding, while a second outlet used a 0.635 tube connection to transfer gas into the connected gas collector. All fittings were sealed using a thread sealant tape to prevent leakage.

Gas collectors were primarily built as glass containers used in previous anaerobic digestion studies; some collectors had to be replaced with constructed PVC pipe collectors. All collectors were measured to have an internal diameter of 7.6 cm (3 in.) and 122 cm (48 in.) long. Tube connections on both ends were measured to be 0.635 cm (0.25in) in diameter on the top of the collectors, and 1.27 cm (0.5 in.) on the bottom. The PVC gas collectors used fitted caps, drilled to allow for tube connections of similar sizes to the glass collectors, and sealed with PVC cement.

Gas collectors were connected in parallel using 1.27 cm plastic tubing along the bottom connections. The main line was connected to two carboys in the experiment: an overhead refilling carboy and an overflow carboy below the collectors. The refilling carboy was used to fill the collectors with water initially to a zero-level. When the digesters were sealed from the atmosphere and the overflow carboy opened, any biogas produced displaced water into the overflow carboy, allowing for gas measurements. After several days, when the water levels of the collectors neared empty, gas samples were taken to release pressure as the overhead carboy refilled the collectors to repeat the process.

The experiment was conducted in an environmental chamber to allow for control of atmospheric conditions. Humidity and pressure remained constant throughout the experiment, however the heating element in the chamber was nonfunctional. A space heater was used to maintain a temperature of 35° C in the room.

Analysis Techniques

Analysis of the biomass utilized in the experiment consisted of two types: proximate and ultimate analysis. Proximate analysis yields results giving the volatile solids content, combustible matter, and ash content of the organic compounds used, while ultimate analysis gives the carbon, hydrogen, nitrogen, and sulfur contents as a percentage of dry matter.

The proximate analysis was conducted in several parts. Firstly, biomass samples are dried in a heating oven at 105° C for at least 12 hours, to ensure minimal moisture. The comparison of the weight before and after this process yields the moisture content of the raw sample. Dried samples are then measured in 1-g samples in metal crucibles and capped. They are first placed in a muffle furnace at 950° C purged with nitrogen gas for 15 minutes. This results in the biomass vaporizing, but not undergoing full combustion. The weight difference yields the volatile combustible matter. The remaining samples are then placed in a muffle furnace uncapped at 550° C for 4 hours. This results in complete conversion of volatile matter, and gives results of total volatile solids as a percentage of the original dry matter, as well as the ash content when the remaining residue is measured.

Ultimate analysis was conducted utilizing a VarioMICRO Cube Ultimate Analyzer. The machine uses a combustion tube and reduction tube to combust 2 -mg samples under a gaseous environment of oxygen and helium, and is calibrated to give calculations for the

percentage content of carbon, nitrogen, oxygen, and sulfur. The machine is first run with several blanks to ensure a zero starting point. Sulfuric acid samples are then used as a standard and compared to normal content values. When the samples meet the standard requirements, digester samples are run.

Initial biogas measurements are taken as the full amount of gas produced within the collectors. Samples are taken to measured the exact nature of the gas, primarily the amount of methane found. This is done through the use of a 8610C SRI gas chromatograph. Within the chromatograph were a 1.8-m (6-ft) silica gel packed column and a 13X packed column with a 1.8-m molecular sieve. The machine began analysis at 67° C, and analyzed gases of hydrogen, oxygen, nitrogen, carbon monoxide, methane, and carbon dioxide. After 15 minutes, temperature was increased to 220° to analyze additional alkane gases, such as ethane, butane, and hexane. Helium was used as a carrier gas within the chromatograph as samples were input from 500-mL tedlar bags.

pH was analyzed using an ion meter calibrated with samples measuring at pH 4 and 7. Samples were mixed with a magnetic stirrer when measured, and replaced after measuring. When pH required balancing, a 5-M NaOH solution was prepared using a mixture of solid sodium hydroxide pellets and deionized water.

CHAPTER III

RESULTS

Substrate Biomass Characterization

Results of the characterization of digestion substrate are given below in Table 1. Initial analysis shows a high volatile solids content in algae, manure, and newspaper. Digestion sludge from the wastewater treatment is significantly lower, demonstrating the spent microbial activity from the original process. Algae and anaerobic sludge showed fairly similar moisture contents, with newspaper having a very low content level. Density values for the algae and anaerobic sludge were measured at $1.01\frac{kg}{L}$ and $0.93\frac{kg}{L}$, respectively.

Table 1.Volatile solids characterization of digestion substrates. Values shown in parentheses represent standard error.

Substrate	VSS (% of Total Solids)
Manure	71.5 (1.003)
Algae	38.86 (0.443)
Newspaper	42.79 (0.035)
Sludge	25.89 (0.049)

Ultimate analysis results of the substrate components are shown below in Table 2. Variability was found in later result as the trials continued due to readings from the analyzer, though the end results were deemed accurate. Newspaper was found to have a significantly high carbon content, and the lowest nitrogen content of all components. Algae shows a high nitrogen content, primarily due to the high protein count of algal

cells. Microbial activity would also explain the high nitrogen content in digester sludge. The results from ultimate analysis includes findings on sulfur and hydrogen contents. These values are found in the Appendices.

Table 2. Carbon and nitrogen characterization of digestion substrates. Values shown in parentheses represent standard error.

Substrate	Carbon (%)	Nitrogen (%)	Hydrogen (%)	Sulfur (%)	C/N Ratio
Manure	36.13 (1.04)	2.30 (0.12)	4.67 (0.14)	0.11 (0.01)	16.01 (1.06)
Algae	38.86 (2.43)	7.66 (0.44)	5.44 (0.29)	0.69 (0.04)	5.07 (0.15)
Newspaper	42.79 (0.06)	0.83 (0.02)	5.61 (0.01)	0.06 (0.004)	51.81 (1.08)
Sludge	25.89 (0.11)	5.97 (1.14)	3.83 (0.01)	0.45 (0.007)	6.52 (0.009)

Gas Production Trial

During the digestion trials, an issue arose regarding the working volume of the digesters. The newspaper increased the bulk density of the mixture far higher than within acceptable levels in the digester. To compensate, 1-kg of the total medium was removed from each digester; this sample was dried and characterized to give accurate readings of the volatile solids content in the mixture prior to digestion. The result of the characterization of this is given below in Table 3. As expected, the mixture with higher carbon-to-nitrogen ratios show higher volatile solids content due to additional paper material added.

Table 3. Volatile solids characterization of digestion mixtures prior to anaerobic digestion process. Values shown in parentheses represent standard error.

Treatment	Moisture Content (%)	VSS (% of Total Solids)
C 17	91.39	76.99 (0.1366)
C 20	90.25	83.86 (0.2907)
C 25	87.7	88.76 (0.2438)
NA 17	85.15	70.59 (2.0622)
NA 20	84.56	86.84 (0.7492)
NA 25	83.25	88.62 (0.6795)
TA 17	85.96	75.43 (0.2766)
TA 20	85.16	81.53 (0.8228)
TA 25	82.87	87.20 (0.2476)

The experiment concluded after 92 days. Gas levels over the first 2 days were factored out, in part because of the aforementioned bulk issue. This gas is also typically not necessary as most of the gas is composed of sulfides as digestion begins. Therefore, the data collected shows 90 days of the digestion process after the digestion process began producing methane gas. Gas analysis after the 2 day period showed a steady increase in methane content. Cumulative gas production rates are shown in Figure 2. Figure 3 shown is an average cumulative gas production curve for the 3 groups of digestion trials based on pretreatment, with error bars produced from standard error.

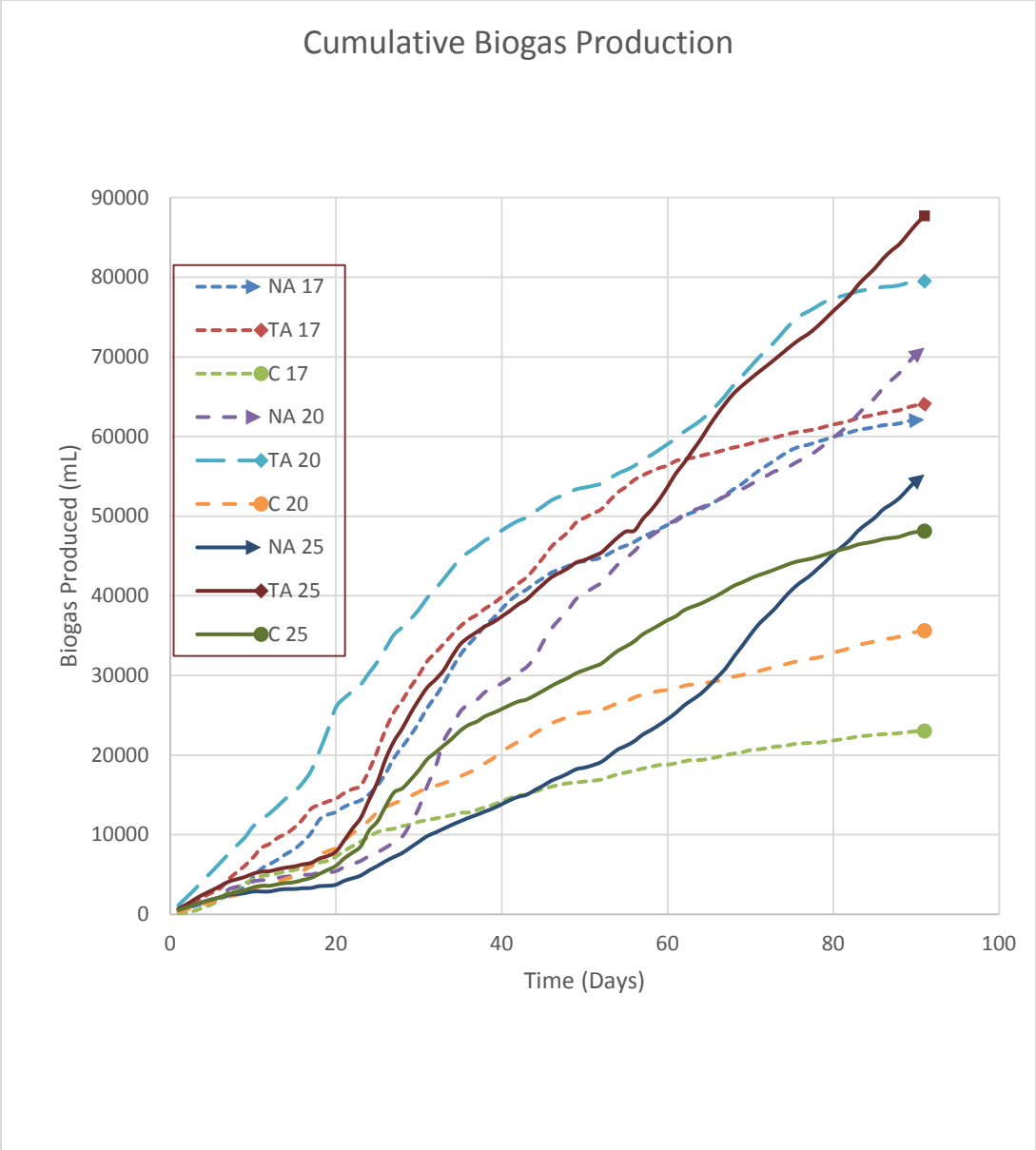

Figure 2. Cumulative gas production rates for nine digesters.

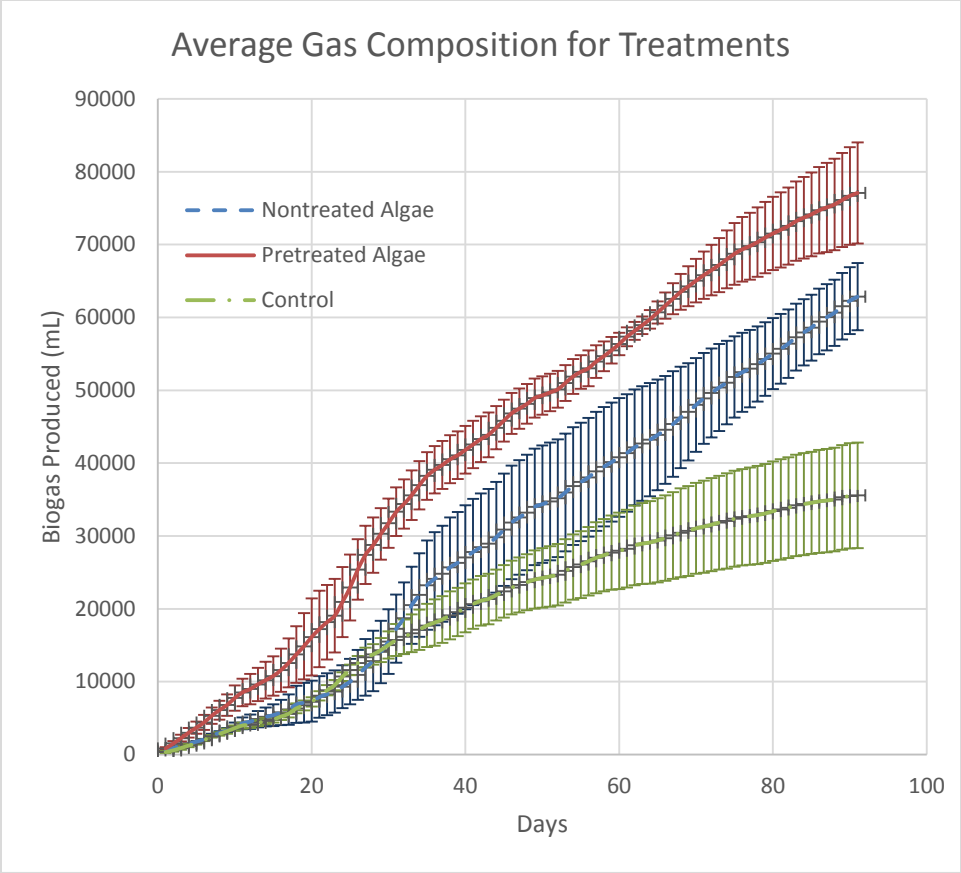

Figure 3. Cumulative gas production for nontreated, pretreated, and control algae mixtures averaged by carbon-to-nitrogen ratios.

Initial observations of biogas production shows a higher production rate in systems containing algae, primarily in systems containing thermally pretreated algae. Average daily production rates are also higher in these systems. The reactors containing normal algae showed a long startup time to produce methane in comparison to the other digesters, decreasing over the first 14 days of the experiment and steadily increasing afterwards. This had been accounted previously due to low pH. During testing, pH in this digester was approximately 4.8; using a 5M NaOH solution, this pH was restored to 7.0, after which gas production increased.

Pretreatment Comparisons

Gas production was compared based on the different levels balanced on carbon-to-nitrogen ratios, and plotted for gas production in Figures 4-6. In all systems, the cumulative gas production followed a model with treated algae producing the most algae and the control group producing the least. This shows evidence that algae increases the biogas production due to increase of organic solids, and that pretreated algae increases production due to cell lysis. As mentioned previously, the normal algae mixture had a long startup time possibly due to pH levels. This can be seen in the cumulative production in normal algae systems when compared to control mixtures at 20:1 and 25:1 carbon-to-nitrogen. In the 17:1 mixtures, normal algae produced less methane than the control mixture until day 31, when normal algae began to cumulatively produce more biogas than the control run. This is similar in the 25:1 systems, when the normal algae mixture began producing more biogas on day 83. This may be because of the high recalcitrance of algal cells preventing faster microbial digestion.

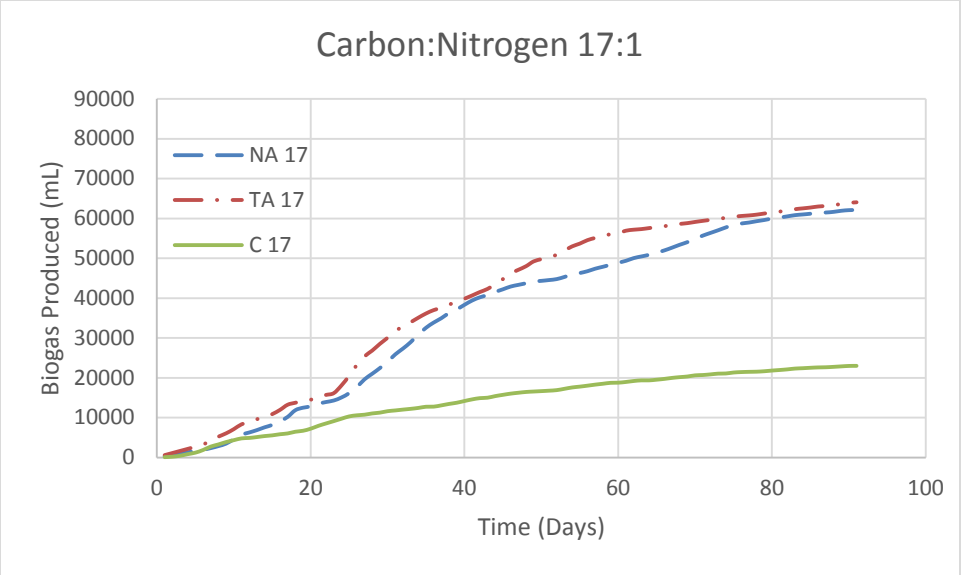

Figure 4. Cumulative biogas production for digesters balanced at 17:1 carbon-to-nitrogen.

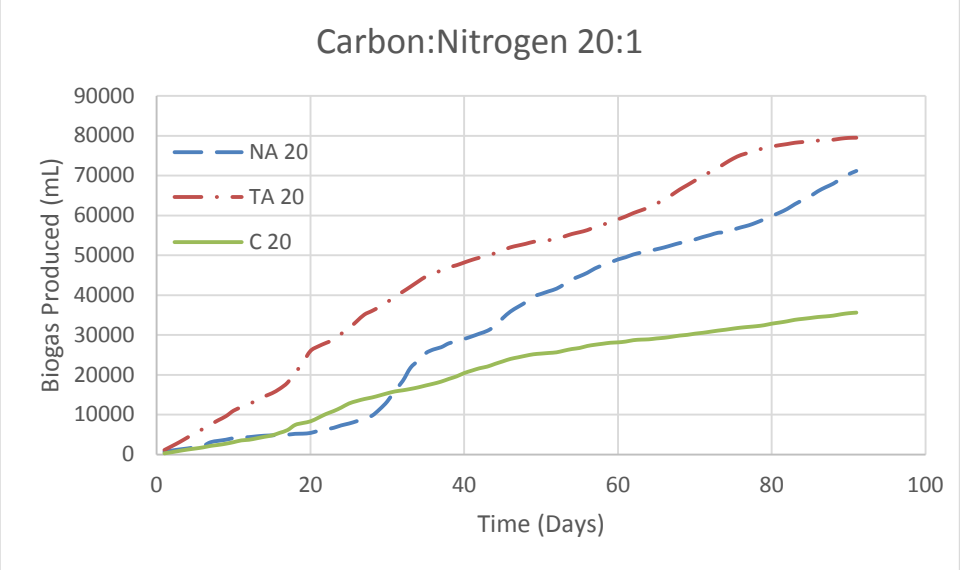

Figure 5. Cumulative biogas production for digesters balanced at 20:1 carbon-to-nitrogen.

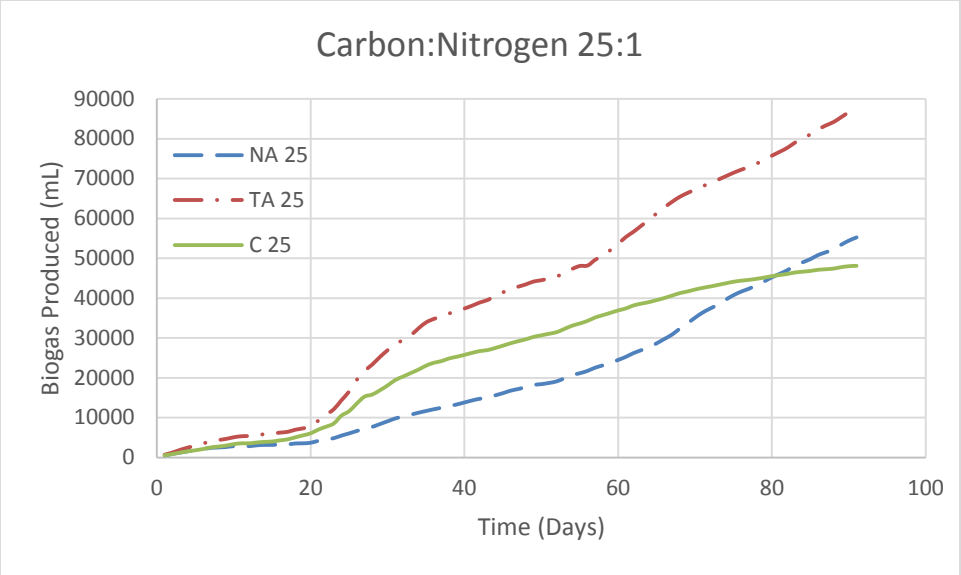

Figure 6. Cumulative biogas production for digesters balanced at 25:1 carbon-to-nitrogen.

Carbon-to-Nitrogen Comparisons

The three treatments were internally compared at the varying carbon-to-nitrogen ratios and plotted for gas production in Figures 7-9. This gives results on the carbon balancing potentially having an impact on cumulative gas production. Results from the experiment show that for the pretreated and control mixtures, balancing at a ratio of 25:1 results in highest potential biogas production. This is understandable by the increase in volatile solids provided by the added paper. Both systems also experienced the lowest biogas production in reactors balanced to 17:1 carbon-to-nitrogen. The nontreated algae gas production shows different results, with the highest production being shown in the reactor balanced to 20:1, with the lowest being seen in 25:1. The gas production in the 25:1 reactor shows a high slope late in the experiment, showing that the actual potential

biogas production may continue far longer than the 90 day experiment allowed. In this case, the 25:1 reactor would eventually provide more biogas than the other two systems.

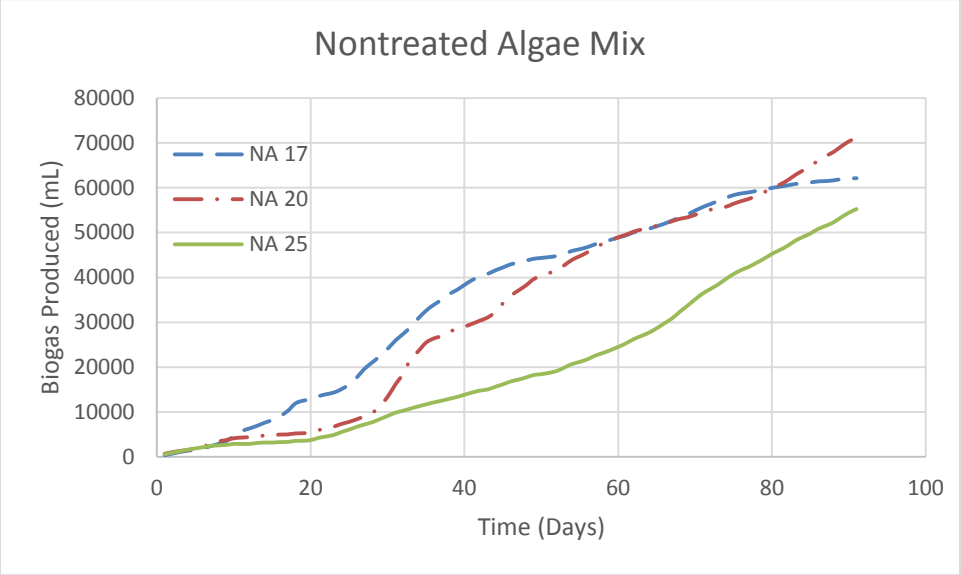

Figure 7. Cumulative biogas production for reactors containing nontreated algae.

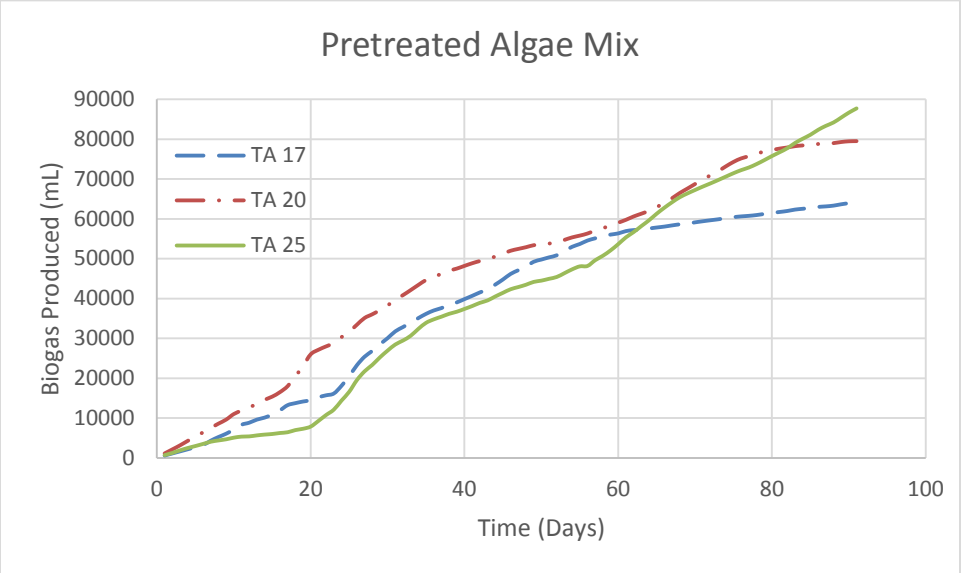

Figure 8. Cumulative biogas production for reactors containing pretreated algae.

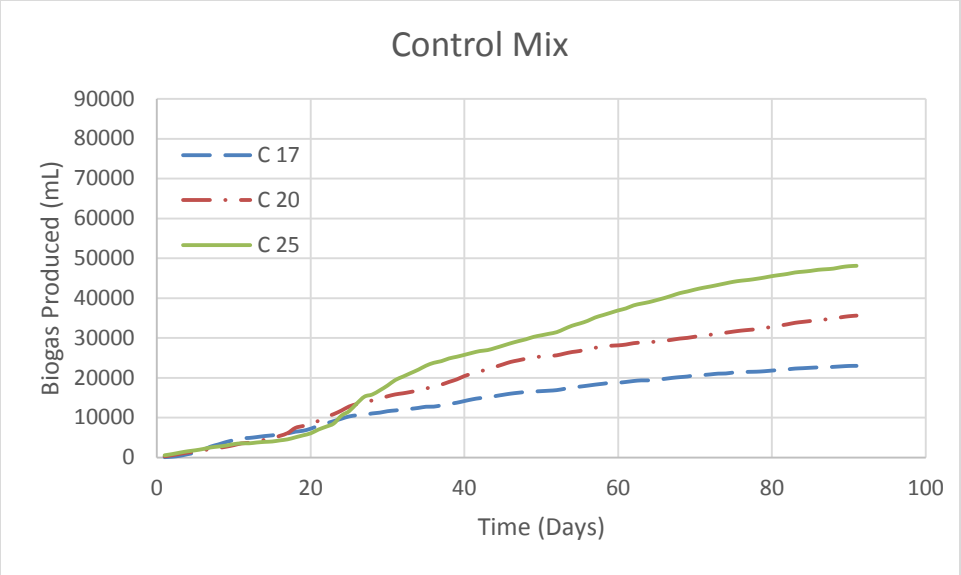

Figure 93. Cumulative biogas production for reactors containing control mixtures.

Volatile Solids Consumption

At the conclusion of the experiment, samples from the reactors were dried and analyzed similar to materials prior to digestion. Values were collected for the amount of volatile solids found in the digested mixtures. The solids composition was compared to gas production in Table 4.

Initial values show a decrease in total solids content. The percentage of volatile solids based on total solids also decreased. This is understandable as volatile solids would be primarily converted to methane. Volatile solids were not completely consumed, signifying that methane production for the reactors could potentially continue after further treatment. Values were compared on the initial and end solids contents, and when

calculated with the total amount of methane produced, can give an estimate for the methane production potential on the solids from the mixtures made.

Table 4. Gas production based on volatile solids consumed.

Mixture	Initial Moisture (%)	End Moisture (%)	Initial Total Solids (kg)	Initial VSS (%)	End Total Solids (kg)	End VSS (%)	VSS Consumed (kg)	Methane Produced (L)	L CH4 / g VSS
C 17	91.39	93.39	0.2621	76.99	0.135	68.45	0.1097	23016	0.2104
C 20	90.25	90.7	0.3027	93.86	0.1958	79.74	0.0977	35620	0.3645
C 25	87.7	89.51	0.3983	88.79	0.2349	84.89	0.1541	48120	0.3121
NA 17	85.15	96.43	0.4742	70.59	0.0784	68.17	0.2813	62125	0.2208
NA 20	84.56	95.53	0.5119	86.84	0.1034	83.91	0.3577	71170	0.1989
NA 25	83.25	93.03	0.6004	88.62	0.1801	73.73	0.3993	55250	0.1384
TA 17	85.96	90.02	0.4487	75.44	0.2191	70.37	0.1844	64060	0.3475
TA 20	85.16	88.35	0.4918	81.54	0.2697	81.85	0.1803	79505	0.4409
TA 25	82.86	90.44	0.6139	87.2	0.2473	83.44	0.329	87715	0.2665

Volatile solids consumptions runs different than the methane produced for most mixtures. Overall, the volatile solids consumption is higher in the normal algae systems, primarily in the system balanced at 25:1 carbon-to-nitrogen. Values in the digesters prior to digestion shows that volatile solids is slightly higher in these reactors compared to the pretreated algae mixtures. Total solids consumption, however, is higher in the pretreated algae mixtures than in the normal algae reactors. This may be a result of all solids becoming easily digested in the pretreated system due to thermal deconstruction. In all three types of mixtures, higher carbon contents result in the highest volatiles consumption, primarily due to the ease of digestion from the added newspaper mass.

Comparing the methane production in terms of volatile solids consumed, the normal algae systems are shown to have lower rates than the control groups. While methane produced is overall much higher, the required volatile solids to reach this level is also much higher. The thermal pretreatment shows to have a massive increase in production rates for these reactors, including the highest production rate in the treated algae mixture balanced at 20:1 carbon-to-nitrogen.

Biogas Composition

Gas analysis tests were run when gas collectors were to be emptied. On average, this was approximately every three days. Gas samples were collected and analyzed, primarily to check methane and carbon dioxide content. As some collectors contained less biogas than others when samples were collected, biogas concentration was lower by default. To account for this, a CH_4:CO_2 ratio was made for all samples. In biogas, a theoretical ratio of methane to carbon dioxide is approximately 1.5:1. Tables 5 and 6 show the average compositional analysis for the gases detected in the chromatograph.

Table 5. Average analysis of gas composition on percentage basis. Extraneous carbon gases (C2H4, C3H6, etc.) and Oxygen not included.

Mixture	% N2	% CH4	% CO2	CH4 / CO2	CH4 : CO2
C17	50.40	30.25	13.79	2.19	2.194:1
C20	58.32	18.37	14.63	1.26	1.255:1
C25	27.56	45.34	25.16	1.80	1.802:1
NA17	44.32	34.62	19.71	1.76	1.759:1
NA20	44.26	57.86	16.55	3.50	3.496:1
NA25	19.41	53.23	16.60	3.21	3.205:1
TA17	42.40	33.76	21.89	1.54	1.542:1
TA20	24.12	57.86	18.83	3.07	3.072:1
TA25	41.70	40.71	12.55	3.24	3.244:1

Table 6. Average analysis of gas composition, normalized to compensate for oxygen contamination.

Mixture	% N2	% CH4	% CO2
C17	74.37	23.11	2.52
C20	93.52	4.64	1.85
C25	20.55	62.87	16.58
NA17	57.29	33.49	9.22
NA20	34.54	62.90	2.57
NA25	9.74	83.40	6.86
TA17	54.30	33.16	12.54
TA20	11.91	81.49	6.60
TA25	51.08	48.25	0.67

Oxygen was detected in the gas chromatograph system, contradicting the presence of methane found from anaerobic digestion. Samples taken in the Tedlar bags may have provided a leakage possibility, and coupled with potential air pockets in the digesters during refilling, oxygen contamination would have occurred during analysis. Values were normalized to accommodate for the desired oxygen-less environment, and standard values are found in Table 6.

The highest methane concentration was overall found in the normal algae mixture balanced at 20:1 carbon-to-nitrogen. The concentration ratios, however, are comparable to the pretreated algae mixtures, being fairly similar. With the exception of the control mixtures, carbon balancing shows in increase in methane concentration with respect to carbon dioxide. This may be due to the smaller samples taken from the control mixtures due to less biogas produced from these reactors overall.

CHAPTER IV

CONCLUSIONS

The research conducted here set out to show a possible means of increasing methane production potential of cattle manure. In agreement with past studies, the two methods tested both had a positive impact on production rate. Results on both the testing of algae pretreatment strategies as well as carbon balancing show to have a positive influence on digestion. The cumulative biogas amounts for the digestion mixtures were: 62125 L, 71170 L, and 55250 L for normal algae mixtures; 64060 L, 79505 L, and 87715 L for pretreated algae mixtures; 23016 L, 35620 L, and 48120 L for control mixtures, with carbon balancing at ratios for carbon-to-nitrogen of 17:1, 20:1, and 25:1, respectively.

Biogas yield on a volatile solids basis also showed that the maximum potential was found with proper balancing with the use of pretreated algae. The maximum biogas yield for the control mixtures was $0.3645\,\frac{L}{g\,VS}$, compared to the yield of $0.2208\,\frac{L}{g\,VS}$ in normal algae mixtures and $0.4409\,\frac{L}{g\,VS}$ in pretreated algae mixtures. This demonstrates the effect that pretreatment has on the digestion of algae, as well as the recalcitrant nature of normal algae sludge in the digestion process. Therefore, when algae is desired as a co-digestion product, pretreatment is highly suggested to improve biogas production.

Biogas composition comparisons across the mixtures showed that while potency is fairly similar for the digestion mixtures, the highest methane-to-carbon dioxide ratios were

found in systems containing algae, both treated and nontreated. The highest ratio of biogas in the control mixtures was 2.194:1 for methane-to-carbon dioxide, while the highest in the treated and nontreated algae mixtures were 3.244:1 and 3.496:1, respectively.

Recommendations for Future Studies

Noted in this experiment is the fact that while the procedure concluded after 90 days of testing, gas production could have continued. For a full view of the methane production potential of these mixtures, in particular the mixtures containing nontreated algae, the experiment could be run similarly for a longer period of time. This can also be done through a reduction of the amount of solids introduced into the reactors at the start of the experiment.

Future studies in the anaerobic digestion and co-digestion of these materials can focus on further optimizing the digestion environment, through nutrient addition or additional pretreatment strategies. Nutrient balance could have an impact on the actions of algae within the system as well as the process of microbial digestion. Other pretreatment strategies may also find other means of lysing algal cells while increasing energy efficiency. These can be compared to thermal pretreatment to find if methane production can be further increased while balancing cost of production and treatment.

The experiment conducted focused on lab-scale batch operations. If this procedure is to be expanded, a pilot-scale experiment would show the viability of producing methane efficiently from co-digestion. Obtaining a steady supply of digestion material, producing a stream of methane, and potentially switching to a continuous system can all provide studies into the possibility of adapting this data to a larger scale. In this way, a potential efficient methane source could be derived.

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APPENDIX A

DAILY GAS PRODUCTION

Table A-1. First 30 days of gas production

Days	Not Treated Algae			Treated Algae			Control		
	20	25	17	20	25	17	20	25	17
1	680	560	355	1150	720	620	310	580	110
2	350	350	360	1000	540	520	300	300	110
3	270	340	350	1020	660	520	340	370	240
4	240	310	290	1120	580	520	310	330	400
5	300	310	250	1050	500	530	260	250	380
6	310	260	350	1100	530	530	280	320	660
7	950	310	420	1100	550	1100	380	400	860
8	350	100	510	1080	320	920	250	220	540
9	280	120	620	1010	290	830	300	250	600
10	400	210	1110	1400	410	1050	390	360	470
11	160	0	1100	850	260	1200	440	200	450
12	90	0	580	800	60	510	160	0	110
13	200	210	600	920	250	780	360	210	220
14	160	100	700	1000	200	500	380	150	250
15	80	0	600	800	150	780	360	100	180
16	140	100	900	1120	250	1050	640	280	280
17	30	30	1110	1500	190	1300	680	260	200
18	220	230	1680	2450	540	500	1300	500	430
19	40	40	630	2620	330	410	480	500	240
20	180	140	320	2920	550	340	420	510	520
21	520	490	670	1140	1410	720	890	940	720
22	380	320	440	850	1490	500	940	720	600
23	380	340	380	860	1340	440	740	800	580
24	620	650	760	1460	2270	1930	890	1980	600
25	460	550	1080	1380	2200	2420	1020	1120	580
26	580	610	1530	1720	2930	2730	610	1930	280
27	670	530	1940	1700	2210	2150	510	1720	150
28	720	490	1400	930	1610	1450	390	520	320
29	1620	710	1430	1040	1900	1690	510	1070	200
30	1980	700	1600	1170	1700	1520	540	1200	360

Table A-2. Second 30 days of gas production.

Days	Non-Treated Algae			Pre-Treated Algae			No Algae		
	20	25	17	20	25	17	20	25	17
30	1980	700	1600	1170	1700	1520	540	1200	360
31	2720	710	1830	1460	1530	1710	470	1370	190
32	2400	450	1460	1140	1000	1080	280	860	210
33	3280	490	1610	1260	1190	1110	340	880	190
34	1800	490	1910	1300	1730	1140	390	860	230
35	1900	440	1700	1240	1520	1060	480	1000	300
36	920	430	1310	820	870	850	450	720	26
37	560	380	1100	700	670	600	520	420	320
38	980	420	1300	800	720	800	680	680	360
39	500	410	910	540	530	630	630	420	300
40	600	500	1140	680	700	820	840	480	430
41	640	510	1080	660	720	820	620	500	410
42	700	420	800	560	800	860	590	450	310
43	700	250	570	420	610	780	410	240	110
44	1200	600	780	720	990	1180	680	530	400
45	1960	550	660	710	920	1280	620	580	320
46	1660	630	690	730	900	1400	620	600	300
47	1200	420	420	460	550	930	400	530	220
48	1140	480	400	430	580	900	410	480	200
49	1400	540	430	500	710	1220	360	600	110
50	700	200	180	200	320	560	170	390	100
51	660	300	200	220	460	550	160	390	110
52	650	400	240	270	460	520	160	400	140
53	1110	770	540	640	950	1080	430	820	330
54	1190	790	590	630	960	1050	400	800	340
55	820	480	390	450	750	700	260	560	210
56	860	580	460	520	100	870	500	640	240
57	1100	810	630	750	1440	560	310	860	260
58	790	580	480	590	1120	500	250	590	220
59	720	650	510	680	1340	430	250	590	230
60	750	720	510	700	1580	300	100	600	40

Table A-3. Final days of gas production and cumulative readings.

Days	Non-Treated Algae			Pre-Treated Algae			No Algae		
	20	25	17	20	25	17	20	25	17
61	550	780	480	720	1700	580	200	500	180
62	700	940	670	840	1320	250	350	760	240
63	460	720	420	690	1500	150	140	440	160
64	400	740	410	730	1460	250	70	380	0
65	440	980	540	940	1650	250	220	510	170
66	460	1090	570	1040	1500	230	170	520	200
67	550	1110	690	1180	1380	300	260	600	250
68	540	1470	730	1300	1250	270	290	620	190
69	340	1350	660	1120	960	200	180	420	160
70	560	1420	840	1150	870	300	300	490	290
71	600	1310	790	1150	850	270	210	420	80
72	530	1010	680	1020	800	250	280	350	160
73	560	1010	670	1050	840	240	280	390	190
74	100	1210	710	1205	865	280	230	400	40
75	680	1100	600	1120	860	260	280	390	240
76	580	900	380	880	770	190	240	270	110
77	510	720	240	540	660	140	180	200	60
78	700	910	340	600	860	200	175	270	40
79	800	940	300	610	980	280	245	310	110
80	840	1030	360	370	1030	260	390	360	170
81	820	860	260	340	960	230	280	280	140
82	940	910	290	280	1010	250	310	270	160
83	1200	1150	310	330	1310	350	380	370	200
84	1000	840	170	180	1060	200	230	190	90
85	990	790	180	160	1000	210	220	200	110
86	1260	1030	240	200	1230	260	250	280	90
87	1000	680	70	60	1000	130	150	140	40
88	900	780	160	160	850	190	200	160	100
89	1240	1120	270	280	1230	340	340	360	120
90	1120	1030	160	180	1240	260	260	240	110
91	830	810	40	40	1060	140	150	100	20
TOTAL	71170	55250	62125	79505	87715	64060	35620	48120	23016
AVERAGE	782	607	682	873	963	703	391	528	252

APPENDIX B

SOLIDS ANALYSIS, DIGESTION SUBSTRATES

Table B-1. Ultimate analysis results of substrate components.

Substrate	Carbon	Nitrogen	Hydrogen	Sulfur	C/N Ratio
Manure	32.98	2.54	4.26	0.134	12.9867
	39.191	2.495	5.078	0.129	15.7106
	36.207	1.872	4.673	0.073	19.338
Algae	38.032	6.87	4.949	0.811	5.535953421
	46.502	9.197	6.438	0.575	5.056
	32.011	6.925	4.92	0.683	4.6225
Newspaper	42.632	0.804	5.588	0.067	53.045
	43	0.792	5.654	0.044	54.2687
	42.74	0.888	5.607	0.062	48.1277
Sludge	25.784	3.975	3.868	0.47	6.4865
	25.638	9.931	3.811	0.431	6.5212
	26.244	4.013	3.806	0.442	6.5398

Table B-2. Moisture content analysis of substrate components.

Substrate	Tin (g)	Tin + Wet (g)	Tin + Dry (g)	Moisture (%)
Algae	1.3147	10.964	2.069	0.9218
	1.3098	12.562	2.41	0.9022
Manure	1.3092	26.389	5.8693	0.8182
	1.3045	27.839	5.8357	0.8292
Newspaper	1.3055	2.45	2.39	0.0524
	1.3045	2.5732	2.4896	0.0659
Sludge	1.3028	10.548	1.5425	0.9741
	1.3045	10.568	1.551	0.9734

Table B-3. Volatile solids analysis of substrate components.

Substrate	Crucible (g)	Crucible + Mass (g)	Dry Mass (g)	Cruc + Residue (g)	Residue (g)	% VSS
Algae	11.557	12.1227	0.5657	11.6584	0.1014	0.82075
	7.795	8.227	0.432	7.8969	0.1019	0.76412
Manure	14.4259	15.742	1.3161	14.8176	0.3917	0.70238
	14.7676	15.553	0.7854	14.9805	0.2129	0.72893
Newspaper	15.339	15.669	0.33	15.348	0.009	0.97273
	15.075	15.554	0.479	15.089	0.014	0.97077
Sludge	7.568	7.9677	0.3997	7.746	0.178	0.55467
	7.4918	8.0105	0.5187	7.7242	0.2324	0.55196

APPENDIX C

SOLIDS ANALYSIS, PRE-DIGESTION

Table C-1. Moisture content of digestion mixtures.

Mixture	Wet Sample (g)	Dried Sample (g)	Moisture Content (%)
C 17	154.13	13.269	0.9139
C 20	153.84	14.997	0.9025
C 25	231.2	28.421	0.8771
NA 17	196.43	29.155	0.8516
NA 20	210.42	32.496	0.8456
NA 25	248.65	41.645	0.8325
TA 17	222.23	31.211	0.8596
TA 20	224.74	33.344	0.8516
TA 25	215.53	36.909	0.8288

Table C-2. Volatile solids analysis of digestion mixtures.

Treatment	Crucible (g)	Cru + Mass (g)	Cru + Cap (g)	Cru + Mass + Cap (g)	Dry Mass (g)	W Cap @ 950 (g)	W/O Cap B 550 (g)	W/O Cap B 550 (g)	% VCM	% ASH	% FC	% VSS
C17	19.817	20.697			0.88			20.0168				77.295
	19.027	19.837			0.81			19.212				77.16
	15.1964		23.371	24.4016	1.031	23.7331	15.5587	15.4384	64.84	11.67	23.49	76.528
C20	19.31	20.519			1.209			19.515				83.044
	19.222	20.164			0.942			19.375				83.758
	14.0589		20.871	21.8615	0.9901	21.1659	14.3527	14.2096	70.256	14.45	15.29	84.779
C25	19.622	21.262			1.64			19.819				87.988
	19.767	20.941			1.174			19.891				89.438
	14.2121		20.962	22.0596	1.0981	21.2547	14.5052	14.3342	73.299	15.57	11.13	88.881
NA 17	18.182	20.412			2.23			18.794				72.556
	17.952	19.898			1.946			18.659				63.669
	14.9812		23.054	24.1353	1.0818	23.4589	15.3865	15.2455	62.525	13.03	24.44	75.568
NA 20	20.176	21.491			1.315			20.347				86.996
	18.773	20.272			1.499			19.005				84.523
	14.1272		21.135	22.1043	0.9691	21.405	14.3966	14.2337	72.16	16.81	11.03	89.01
NA 25	19.532	21.571			2.039			19.8				86.856
	17.561	20.331			2.77			17.889				88.159
	14.5102		22.837	23.9325	1.0955	23.152	14.7781	14.6104	71.246	15.31	13.45	90.853
TA 17	19.565	21.417			1.852			20.037				74.514
	23.531	25.491			1.96			23.999				76.122
	14.2357		21.789	22.8398	1.0507	22.1827	14.6288	14.4913	62.539	13.09	24.37	75.673
TA 20	17.116	19.687			2.571			17.662				78.763
	19.649	21.311			1.662			19.942				82.371
	14.1353		21.619	22.7136	1.0948	21.9599	14.454	14.3161	68.844	12.6	18.56	83.486
TA 25	18.775	21.41			2.635			19.126				86.679
	19.537	21.656			2.119			19.815				86.881
	14.4046		22.771	23.8169	1.0456	23.0581	14.6917	14.5295	72.571	15.51	11.92	88.055

APPENDIX D

SOLIDS ANALYSIS, POST-DIGESTION

Table D-1. Moisture content of digestion products.

Treatment	Initial Weight (g)	Dried Weight (g)	Moisture Content (%)
C17	262.96	9.18	96.51
	209.8	7.65	96.35
C20	313.83	13.94	95.56
	356.37	16.05	95.5
C25	258.13	26.82	92.94
	310.52	21.37	93.12
NA17	258.13	25.77	90.02
	209.74	39.03	81.39
NA20	329.25	32.47	90.14
	356.3	48	86.53
NA25	287.52	22.19	92.28
	310.54	35.42	88.59
TA17	335.99	22.18	93.4
	267.26	17.67	93.39
TA20	294.27	27.44	90.68
	369.01	34.24	90.72
TA25	335.04	39.92	88.68
	353.3	34.13	90.34

Table D-2. Volatile solids analysis of digestion products.

Treatment	CRC (g)	CRC+CAP (g)	CRC + CAP + MASS (g)	DRY MASS (g)	W/cap @950 (g)	W/O cap B550 (g)	W/O Cap @550 (g)	%VCM	%Ash	%FC	% VSS
C17	14.4082	22.5825	23.7957	1.2132	23.1149	14.9406	14.8097	56.12	10.79	33.09	66.91
	14.1607	20.9564	22.0072	1.0508	21.3742	14.5784	14.4743	60.24	9.91	29.85	70.16
	15.014	28.8824	30.1413	1.2589	29.405	15.5383	15.4133	58.49	9.93	31.58	68.28
C20	11.0031	19.4567	20.4695	1.0128	19.8087	11.355	11.2097	65.24	14.35	20.41	79.60
	14.7201	22.2038	23.2619	1.0581	22.5826	15.0965	14.9407	64.20	14.72	21.08	79.15
	14.9937	21.7866	22.8513	1.0647	22.1486	15.3557	15.2006	66.00	14.57	19.43	80.57
C25	14.2362	21.0026	22.0791	1.0765	21.3829	14.6171	14.4569	64.67	14.88	20.45	79.50
	14.1274	22.4945	23.5818	1.0873	22.8538	14.4876	14.3381	66.95	13.75	19.30	80.62
	14.4073	21.3387	23.8345	2.4958	21.6289	14.6975	14.5428	88.37	6.20	5.43	94.57
NA17	14.5178	22.3073	23.9006	1.5933	22.9558	15.1989	15.0349	59.30	10.29	30.41	67.55
	14.1918	20.9416	22.2296	1.288	21.5004	14.751	14.6256	56.61	9.74	33.65	66.32
	11.0189	24.7675	25.8054	1.0379	25.0727	11.472	11.3235	70.59	14.31	15.10	70.65
NA20	15.0818	21.8755	22.9022	1.0267	22.1922	15.3999	15.2604	69.15	13.59	17.26	82.60
	14.0781	21.4954	22.5642	1.0688	21.8218	14.406	14.2089	69.46	18.44	12.10	87.76
	14.2377	21.0505	22.2916	1.2411	21.523	14.7102	14.469	61.93	19.43	18.64	81.36
NA25	14.2312	22.6052	23.8013	1.1961	22.923	14.5505	14.3914	73.43	13.30	13.27	86.61
	14.339	25.5978	26.6054	1.0076	25.8747	14.6165	14.4704	72.52	14.50	12.98	86.96
	14.0193	19.0973	20.1135	1.0162	19.5318	14.8538	14.5516	57.24	29.74	13.02	47.62
TA17	15.2081	22.1389	23.2105	1.0716	22.5311	15.6001	15.5277	63.40	6.76	29.84	70.18
	14.9861	23.0592	24.1236	1.0644	23.4898	15.4165	15.2881	59.55	12.06	28.39	71.63
	14.2365	28.0085	29.1724	1.1639	28.5104	14.7379	14.5936	56.88	12.40	30.72	69.32
TA20	14.1837	20.9965	22.0137	1.0172	21.3726	14.5602	14.4293	63.03	12.87	24.11	75.86
	14.2218	21.2306	22.2756	1.045	21.6217	14.6126	14.4805	62.57	12.64	24.78	75.24
	14.1254	22.5591	26.6364	4.0773	25.959	17.5317	14.3513	16.61	78.00	5.38	94.46
TA25	14.1499	21.7035	22.7368	1.0333	22.0563	14.5024	14.3463	65.86	15.11	19.04	80.99
	13.5112	24.3288	25.3858	1.057	24.6704	13.8622	13.7186	67.68	13.59	18.73	80.38
	14.5193	22.7842	23.8345	1.0503	23.1993	14.781	14.6354	60.48	13.86	25.66	88.95