ABSTRACT

BIO-METHANE POTENTIAL METHOD DEVELOPMENT AND PROCESS OPTIMIZATION OF A DRY BIODIGESTER

By Jorge A. Hernandez

Fossil fuels have been a crucial energy source for years. Their use come at a high cost with detrimental effects to the environment, including climate change, which affects both fauna and flora worldwide. Biogas production is a safe process by which products such as food and yard waste and feces are used as substrates for methane production. This serves as an alternative method to transform methane, a greenhouse gas, into electricity, thus benefiting both environment and population.

The German Institute of Standardization or Deutsches Institut für Normung (DIN) has developed a method for the analysis of biogas production. This method uses eudiometers, a laboratory glass device that measure changes in gas volume, to measure quantity and quality of biogas. The Automatic Methane Potential Test System (AMPTS) is a newly developed method, which makes use of an automated system to measure biogas production. The study looks at the AMPTS as a valid method for biomethane potential analysis (BMP)s, comparable to the DIN method. These methods were compared for analyzing biogas production with identical substrates over the course of 3-6 weeks. The substrates included, were microcrystalline cellulose, Smucker's ® jelly, lactose pellets, potato sludge, paper sludge, cyanobacterial biomass, parlor water, manure scrape, used bedding and fresh straw. Biogas production was quantified for both DIN and AMPTS. Statistical analysis showed that biogas produced using the DIN method was statistically similar to the AMPTS method ($p > 0.05$). This is important because it supports the hypothesis that the AMPTS method is valid for BMP analysis, which significantly decreases costs and increase efficiency, while reducing testing time to half. It also decreases the amount of methane produced from decomposing waste, by allowing rapid testing of feedstock.

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by

Jorge A. Hernandez

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

Master of Science

Microbiology

at

The University of Wisconsin Oshkosh
Oshkosh WI 54901-8621

May 2015

COMMITTEE APPROVAL

Advisor

5-26-15 Date Approved

Member

5-26-15 Date Approved

Member

5/26/2015 Date Approved

DEAN OF GRADUATE STUDIES

Susan Cramer

5/29/15
Date Approved

FORMAT APPROVAL

Marci Hoffman

5/15/15
Date Approved

I would like to dedicate this study to my family. Without them I wouldn't be where I am today. They have supported me through thin and thick with both my personal decision and my decisions as a father. My parents have always supported me professionally, and to them I dedicate this achievement. I also would like to dedicate this thesis to my wife and son. For all the countless hours I spent studying, at the lab, or in my computer, while she took care of the household. All of my efforts, blood and sweat are for you guys.

ACKNOWLEDGEMENTS

I would like to thank all of the people who in one way or another helped me with the completion of this project and thesis. All the input that I received over the course of this year from both professors and staff members was invaluable. This was a great time of learning and it has prepared me for my future as a dentist.

I would like to thank Dr. Kleinheinz, for always allowing me to “drop by” his office, and for being a great influence on this project. He had the patience to explain things to me that otherwise I wouldn’t have understood. I would also like to thank Dr. McDermott for all her guidance, and for always being readily available to help and to meet with me. She has been such a wonderful person to learn from. I would finally like to thank Dr. Kallas. I had him for class since the first day of graduate school, and he is one of the reasons why today, I am moving on with my life and going to Dental school. He has been a great professor and mentor since day 1, and he has taught me a lot of the things I know today. For all their support and the knowledge they provided throughout the course of the project. I sincerely feel grateful

I would also like to thank my project guides, and coordinators of the ERIC laboratory, Mr. Brian Langolf and Ms. Kimberly Busse. They provided knowledge and guidance on a daily basis for this project. Kim was a great mentor for both the project and the thesis.

Thank you,

Jorge

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INTRODUCTION

There are many factors that contribute to global warming. Some of these factors include fossil fuel extraction and purification, anthropogenic heat generation and the increase in waste biomass (13). These factors lead to the production of a detrimental greenhouse gas –methane. Methane is a greenhouse gas, synthesized by microorganisms known as methanogens. The gas holds 25 times more radiation in the atmosphere than carbon dioxide. This radiation is trapped in the form of heat, thus greatly contributing to global warming (8). These findings have contributed to the development of methods that use substrates that produce methane. Methane is used to generate electricity, with the purpose to lessen the continuous accumulation of methane in the atmosphere.

Fossil fuels have been a crucial energy source for years. These sources include coal, oil and natural gas. Natural gas, a product of petroleum extraction and refinement is the largest source of methane emissions in the United States (18). Anthropogenic waste from homes and businesses is another avid contributor the generation of methane (4). This human generated waste is commonly taken to landfills; structures that has been carefully built to act as disposal sites for waste. Methane is generated in these landfills as waste decomposes. Lastly, waste water from sewer waste, also an anthropogenic pollutant, leads to the generation of carbon dioxide, nitrous oxide and methane (9) from generated burning of fossil fuels to run water treatment facilities (2). Methane gas emission through waste water processing, amounts to over 75% of the total biogas

produced, when expressed as a carbon dioxide equivalent. (5). Also methane generated through methanogenesis, using biomass waste and agriculture waste as substrate, is being transformed into electricity; this process is conducted in anaerobic chambers referred to as bioreactors or Biodigester, in which waste undergoes bacterial metabolism of organic compounds and archaeal methanogenesis of those organic compounds to produce methane, in a process referred to as biodigestion.

All of the above mentioned processes cause detrimental effects to the environment, which affects both fauna and flora by contributing to global warming (15). However there are ways being used to handle waste, such as anaerobic digestion. Anaerobic digestion is a safe, natural biological process, by which feces originated from livestock, waste from agriculture and even human waste, are used as substrate for bacterial decomposition, which generates specific substrates used by archaea, to synthesize methane (13). Methane is the main component of natural gas. However this biogas generated through anaerobic digestion is being used as compressed natural gas (CNG), in the form of compressed methane, which serves as fuel for vehicles at nearly half the cost of production and distribution of petroleum (8). The use of these methods has contributed to reduction of greenhouse gas emissions into the environment, by burning methane and producing carbon dioxide, which holds less heat in the atmosphere than methane. This reduces the detrimental effects of greenhouse gases to the atmosphere, while benefiting the general population from this process.

Biodigestion

Biodigestion is the process through which substrates, usually waste, are used to produce biogas. Substrates used vary from manure from different livestock such as sheep, cows and pigs, to food waste, yard waste, and any other type of organic substrate from which methane can potentially be generated. This process of biodigestion was developed with the purpose of reducing waste, which can be problematic when produced on a large scale. Waste is characterized as being malodorous, having the potential to cause health problems, and it can be a source of air and water pollution (17). In addition, the process of biodigestion aids in bacterial and pathogen control, by degrading the microbes in substrates. For example, animal manure may contain pathogens, such as *E.coli* O157 or *Salmonella,* which could possibly be degraded through the process of biodigestion. However it is not yet known about microbial community changes associated with biomass inputs in percolate.

There are two different types of biodigestion; wet biodigestion, also referred to as low solid digestion, and dry biodigestion, referred to as high solids digestion. Wet biodigestion is used for the processing of organic material with total solids < 15% (14). Substrates that have higher solid percentages, have to be mixed first with water, re-circulated process water from the biodigester upkeep, or another organic liquid waste, such as milk house water waste (7). By adding water to these substrates, a wet slurry is often formed, with loss of organic volatile material. Other challenges that can arise from the biodegradation of municipal waste include the separation of the liquid waste into

layers; residue will accumulate at the bottom of the biodigester (14). Substrates with fibrous consistency tend to wind around mechanical components of the system, such as the stirrer. Finally the most prominent challenge is the conditioning of solid waste, into slurry of adequate consistency. A constant temperature of ~38°C is usually maintained throughout the biodigestion cycle; this allows for adequate bacterial growth and methanogenesis. The biodigestion process often involves complex mechanical components such as drums, pressers, breakers, pulpers and screens, which represent higher cost of building and maintaining a biodigester (14). There are many of these biodigesters currently being used in medium and big sized farms around the world, with the purpose of controlling animal and crop waste, and by taking advantage of anaerobic digestion to produce biogas, which serves as a way to generate electricity, and as fuel for vehicles and cooking (16).

The second type of biodigestion production process is dry biodigestion. This process is used for processing organic material with total solids between 15%-40%. This method for biodigestion is not as widely used as wet biodigestion, and it is relatively new. In dry biodigestion substrates do not need much pre-treatment before placement in the anaerobic chambers for digestion. The equipment required for substrate processing, although not required, can be very expensive, and it is only used with certain substrates that require special pre-treatment (14). This equipment is responsible for coarse impurity removal. While dry systems may still require the addition of water, or co-digestion of high and low solid waste, the use of water in dry systems is much lower than the wet systems. This translates into lower energy consumption, given that less energy is required

to heat the anaerobic chambers to ideal temperature when a smaller volume of water is utilized (14).

The University of Wisconsin Oshkosh currently runs a dry biodigester. The feedstocks added to this dry system are carefully selected based on both biomethane potential, known through the biomethane potential analysis, and based on both the total solids and volatile solids of the feedstock. Common feedstocks utilized at the UW-Oshkosh biodigester include jelly, lactose pellets, hot dog casings and food waste; this includes both cafeteria from the UW Oshkosh campus and food manufacturing waste, such as potato peels. These feedstocks are often used in co-digestion, meaning that many feedstocks are combined into a greater biomass, which is then used to produce methane. A constant temperature of ~38°C is usually kept throughout the biodigestion cycle; this allows for adequate bacterial growth and methanogenesis. However during the winter months it is more difficult to keep the temperature of the anaerobic chambers constant, given the low environmental temperatures, especially during the months of January and February.

The process of methanogenesis in a dry biodigester starts with feedstocks being loaded into an anaerobic concrete chamber. The feedstocks have been previously tested for biomethane potential analysis; different feedstocks are generally loaded together for co-digestion. After the feedstocks have been loaded, the door to the anaerobic chamber is sealed closed; this prevents anything from entering or leaving the chamber. The chamber heats up to ~38°C (6). This heating process is regulated by a built-in floor heating

system. Percolate, a mix of liquid, nutrients and microorganisms, is released from overhead sprinklers and evenly distributed from the top of the chamber throughout the organic matter. Run-through percolate is collected at the bottom of the chamber, where it is transported to a percolate liquid storage tank. This tank recirculates the percolate through the chambers repeatedly throughout the 28 day fermentation process. Biogas produced rises and it is transported and collected into a plastic receptacle, which is located on top of the chamber (Figure1). This biogas is then transported to a generator, where methane is burned, to produce electricity (6). Following the 28-day process, the chambers are decompressed and opened, given that pressure builds up in the chambers, and the left remaining organic matter, commonly referred to as digestate, is then taken out and often used as fertilizer. This allows for organic matter to be used to its maximum potential.

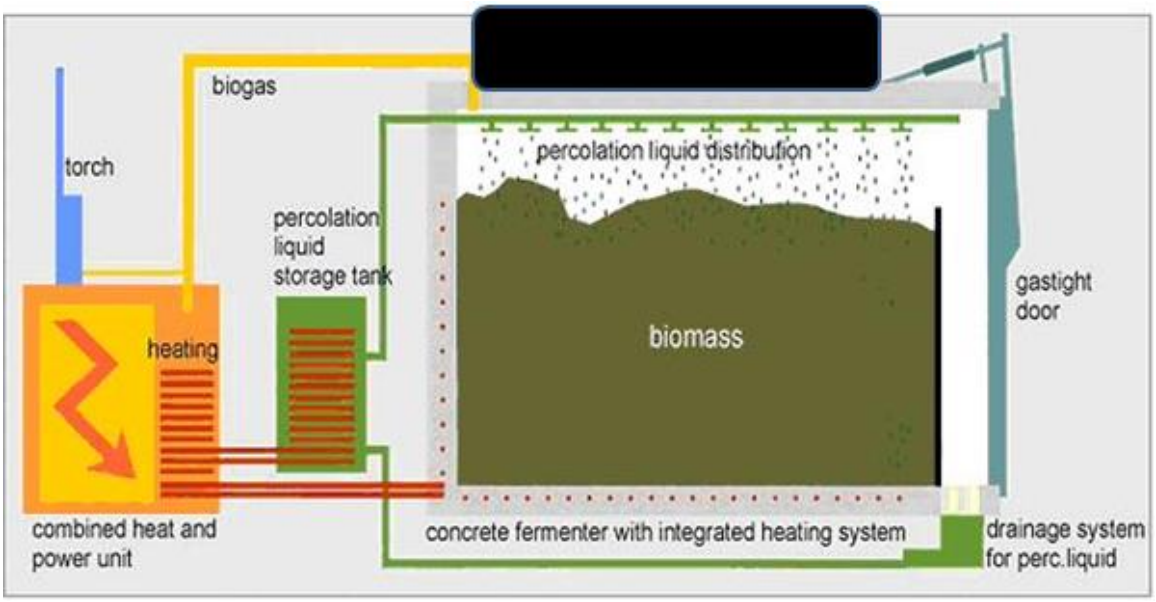

Figure 1. Diagram of a dry biodigester used for anaerobic fermentation of organic matter. The diagram shows the anaerobic concrete chamber containing biomass, the percolate storage tank (in green), the biogas storage chamber (in black), and the generator (in yellow). (modified from German Bio Energy Technology, n.d.)

Methanogenesis

Methanogenesis is a biological process characterized by the conversion of certain macromolecules, such as proteins, polysaccharides and lipids present in biomass into the end product, methane (1, 12). This is accomplished through many metabolic stages mediated by different groups of microorganisms. The first step of this process is the breakdown of the macromolecules. These molecules are transformed into simple products such as sugars, fatty acids, amino acids and glycerin, by fermentative bacterial enzymes,

through a process called hydrolysis (1, 11). Fermentative bacteria ferment the first step's soluble products into a mixture of carbon dioxide, hydrogen and organic acids. The second group of bacteria involved in methanogenesis is characterized for conversion of ethanol and volatile fatty acids (VFA's), generated through acidogenesis, into acetic acid, carbon dioxide, and hydrogen, also substrates for methanogenesis. Over 70% of methane production comes from the decarboxylation of acetate, while the rest of the methane comes from the reduction of carbon dioxide. The microorganisms responsible for methane production from acetate are commonly known as acetotrophic or acetoclastic methanogens (11).

The next group of bacteria in methanogenesis is referred to as hydrogen producing acetogenic bacteria. This step is known as acetogenesis. Some anaerobic species belonging to the family of Streptococcaceae and Enterobacteriaceae, and the genera of *Bacteroides, Clostridium, Butyrivibrio, Eubacterium, Bifidobacterium, and Lactobacillus,* are the most commonly involved species in this process (11). This group of bacteria metabolizes organic acids made of C3 or higher numbers of carbon molecules such as butyrate, ethanol, and some aromatic compounds, and converts them into acetate, H_2 and CO_2 (13). Hydrogen is crucial in this anaerobic process, given that hydrogen consuming methanogenic bacteria need readily available hydrogen for fast consumption, to keep hydrogen partial pressure level low, by providing a stable environment for the organisms.

After substrates have been synthesized for methanogenesis, Archaea finalize the methanogenesis process. The Archaeal Domain is comprised of single celled microorganisms with a glycoprotein and polysaccharide cell wall, commonly referred to as an S-layer, which is one of the main characteristics that distinguish this Archaea Domain from the Domain Bacteria. These cell walls allow Archaea to thrive in environments in which other organisms wouldn't be able to survive such as the bottom of the ocean and in hot springs. The cell wall of this Domain also differs from that of eubacteria, because it is composed of isoprenoid alcohols, which are linked by ether to glycerol. These organisms are obligate anaerobes, and are considered to be the rate-limiting species in methanogenesis (11).

Methanogenesis is characterized for having three major pathways; hydrogenotrophic, acetotrophic and methylotrophic pathways. The hydrogenotrophic pathway reduces CO_2 or HCO_3^- to methane. This process is characterized by using H_2 as a source of electrons, as shown in the reaction below (1, 10).

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O$$

Most of these methanogens use H_2 as a source of electrons. The sources of H_2 are the above mentioned fermentative bacteria, and hydrogen producing acetogenic bacteria. This pathway often contributes ~30% of the methane generated in anaerobic systems. There are many H_2 using methanogens that use formate as a source of electrons for the reduction of CO_2 to methane (11).

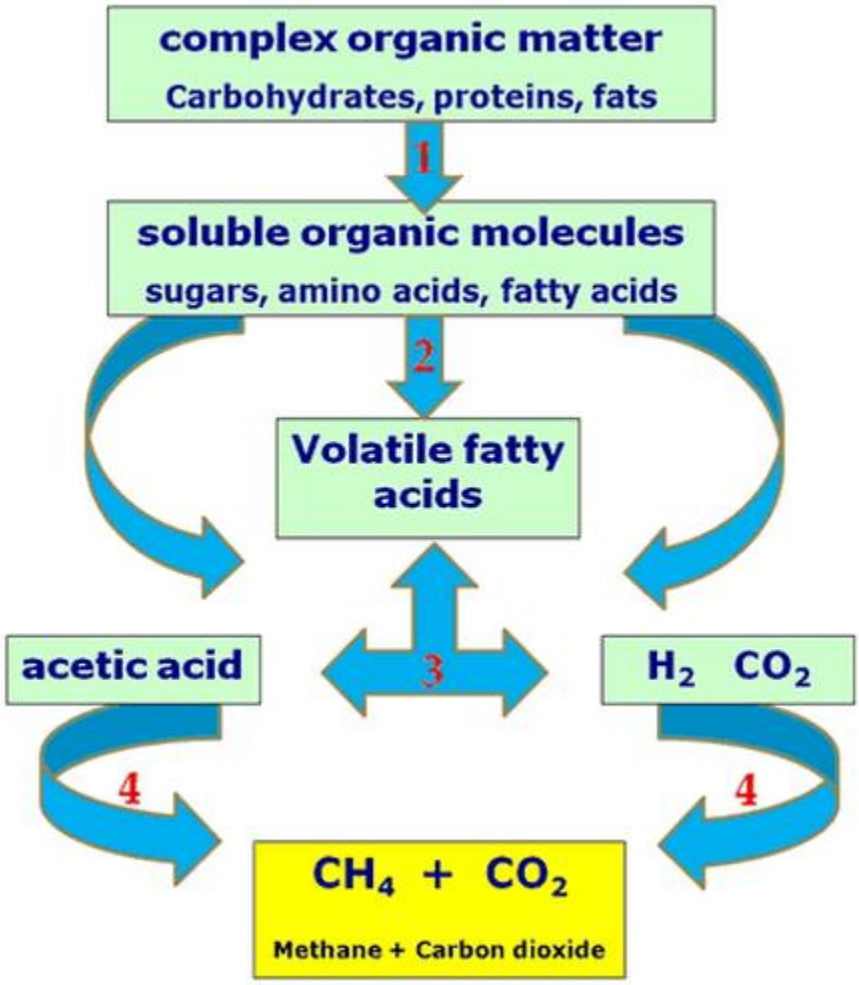

Figure 2. Diagram of the acetotrophic methanogenesis. Organic matter (top) as substrate , and methane (bottom), as the end product. (modified from Amaya et. al., 2013)

The acetotrophic, also known as acetogenic pathway contributes the remaining 70% of the total methane production. This pathway converts of acetate to methane. The two genera widely recognized for being acetotrophic are *Methanosarcina* and *Methanosaeta.* The formula below describes the acetogenesis chemical reaction. The

electron donor, acetate, is a byproduct of previous bacterial metabolism. This process is known for being driven by the decarboxylation of acetate (10).

$$CH_3COO^- + H_2O \longrightarrow CH_4 + 2HCO_3$$

The third pathway, also known as methylotrophic, is characterized by archaeal use of substrates such as methanol, methylamines, and sometimes H_2/CO_2, as shown below. The methyl group of the substrate is transferred to a methyl carrier and finally reduced to methane. The electrons for this methyl reduction are usually obtained through the oxidation of a fraction of the methyl groups to CO_2, or by using H_2 as an electron donor (10).

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

70% of biological methane is produced through the second pathway, using the methyl group from acetate, and turning it into methane, after the completion of the biochemical process. Bacterial decomposition in anaerobic scenarios also yield hydrogen sulfide (H_2S), which is characteristic for having a putrid smell.

One of the challenges with methanogenesis using a Biodigester is the difficulty in assessing how much biogas can be produced from a particular substrate (the biomethane potential or BMP). The analysis needs to be conducted in a short period of time, for the lowest possible cost in a specialized laboratory. It is important to collect accurate BMP data, to better understand how the economics of a biodigester system would work, or how various feedstocks may contribute to operations. The German Institute of Standardization

or Deutsches Institut für Normung (DIN) has developed a standard laboratory DIN 38414-17 method for the analysis of biogas production (3). This method uses a glass device call an eudiometer that measures changes in gas volume, allowing the assessment of biogas production over a six week period. In this method, a glass jar or bioreactor holds percolate sludge, the inoculum, for two weeks to allow for digestion of any organic material present in the sludge. The procedure generally mimics that of a liquid digester system as the bulk phase is liquid. After two weeks, a specific substrate is added and anaerobic digestion is allowed to take place for four additional weeks. The amount of substrate added is calculated based on dry matter (DM), also referred to as total solids (TS). The total solids, is the percentage of dry matter in a fresh matter sample. Measurements of both biogas production and quality are made daily. Gas quality provides composition of biogas samples, which often includes carbon dioxide, methane, oxygen and hydrogen. Daily biogas production and composition data is manually entered into a computer, which normalizes the data on the biogas production. This method is reliable when the quality of the volume readings are kept constant and accurate, by approximating to the closest milliliter, throughout the six-week experiment. If readings are not precisely measured, the biomethane potential analysis could give misleading results. This method is costly, given that most of the equipment is imported from Germany.

The Automated Methane Potential Testing System II (AMPTS) is a newly developed BMP testing system and method developed in Sweden. This method consists of a device that automatically measures biogas production in bioreactors holding both

inoculum and substrate at the start of the experiment. The amount of organic matter present in the percolate sludge at the start of the experiment is corrected for and normalized by the Bioprocess Control® software. Inoculum and substrate added to each bio-reactor are automatically calculated with the software. These amounts are based on the organic dry matter (oDM), commonly referred to as volatile solids (VS); the organic dry matter is the percentage of organic matter in a fresh sample. The amount of VS is obtained by drying a fresh sample, and then placing the sample in a furnace at high temperature, until only ashes remain; total solids is represented as the percentage of oDM in the sample. As biogas is produced, a carbon dioxide fixing unit in the AMPTS system binds carbon dioxide, which is a major product in anaerobic digestion. This CO_2 fixing unit holds a thymolphthalein pH-indicator solution, which changes from blue to colorless, when saturation with CO_2 occurs. Biogas production recordings are done by the gas volume measuring device. This unit has injection mold flow cells for each bioreactor with compartments filled with biogas. When the compartment is full, the flow cells release the biogas, and record the time required to fill the flow cell. This provides the amount of biogas produced by each substrate, per unit time. Collected data is automatically recorded into a computer file. The data provided by the computer is normalized by the Bioprocess Control®. The experiment lasts three weeks from start to finish, which makes it a faster method for substrate testing. However, this laboratory method is not yet officially recognized for standard biomethane potential analysis.

While both the AMPTS and DIN standard methods have been used by a number of laboratories for BMP determinations, there is no clear relationship between the results

from each method. Additionally, many research and academic laboratories have custom systems to determine BMPs. With various methods being reported and no clear comparisons of methods it makes it a challenge, if not impossible, to compare results from one study to another. Confounding these results is the fact that each commercial system has different operational conditions that are not easily mimicked by other lab test systems. Thus, there is great need to understand the relationship between BMP values from different methods and move toward a common testing method. The results of these BMP tests can influence the construction and/or operation of multi-million dollar biogas facilities.

OBJECTIVES

The overall objective of this study is to compare BMP results obtained from the AMPTS and DIN standard methods to determine if there is a consistent relationship between the methods. Specifically, ten unique feedstocks, or substrates, will be evaluated for BMP using both the DIN standard and the AMPTS method. Additionally, the overall biogas produced as well as the quality of the biogas produced will be compared. This will provide evidence on whether or not the AMPTS method can be utilized as a standard procedure for biomethane potential analysis, as an alternative to the DIN method. The results of this study could potentially translate into cheaper laboratory costs, human error elimination, given that measurements for the DIN method biogas production are performed by a laboratory analyst. This will also reduce testing time from six to three weeks, by using the AMPTS method for BMP analysis.

The specific objectives of the study were

1. To evaluate bio-methane generation (quantity and quality) from ten unique feedstocks using eudiometers and the German DIN standard for wet digester testing and a modified dry testing method using the same eudiometer systems.

2. To compare the AMPTS system bio-methane testing system on the same ten unique feedstocks (as above) to determine the output biogas similarities and differences between this test method and the eudiometer systems.

3. To assess the Automatic Methane Potential Test System (AMPTS II) as a viable method for rapid bio-methane potential analysis, by performing statistical analyses comparing the biogas production between the AMPTS and DIN methods.

METHODS

Feedstock Analysis

Collection and storage.

Samples for this study were supplied by the Environmental Research and Innovation Center (ERIC). Samples were supplied by both clients and incoming samples for the University of Wisconsin Oshkosh Biodigester. Samples were collected in Ziploc® freezer bags and stored at 4°C for up to 48 hours before feedstock analysis was done. pH, total solids (TS) and volatile solids (VS) were tested for each of the feedstocks. The same parameters also were measured for the percolate sludge, used as a negative control, and the cellulose microcrystalline, used as positive control. A portion of the feedstock samples were kept fresh in a refrigerator at 4°C, and the other portion of the feedstock samples were kept in a freezer at -4°C. These samples were used "fresh" and "thawed" as needed for biomethane potential analysis. Substrate analysis was performed as a basis for DIN and AMPTS methods loading amount calculations. Analysis was done after allowing the "fresh" samples to reach room temperature before testing.

Total solids (TS).

All feedstocks were tested for total solids, including those used as controls, microcrystalline cellulose and percolate sludge. The sample was removed from the Ziploc® bag and two previously weighed crucibles were filled ¾ full with the feedstock.

The crucibles were weighed once again. The crucibles containing the samples were then placed in an oven at 105°C for 12-24 hours.

The following day both crucibles were taken out of the oven and placed in a desiccator. Crucibles were cooled to room temperature and were then weighed again with a Mettler Toledo Classic PB153-S® scale. Data was recorded into a MS Excel® spreadsheet. The samples were then analyzed for volatile solids. This procedure was repeated for all of the ten feedstocks, and positive and negative controls.

Volatile solids (VS).

All feedstocks were analyzed for volatile solids, including the controls, microcrystalline cellulose and percolate sludge. The previously weighed crucibles containing dry samples (previously analyzed for total solids analysis), were then placed in a 550°C muffle furnace for three hours. After three hours, the crucibles cooled inside the muffle for 3-4 hours.

The crucibles were taken out of the muffle and placed in a desiccator. Crucibles were then cooled to room temperature. The crucibles were weighed again with a Mettler Toledo Classic PB153-S® scale. Data was recorded into a MS Excel® spreadsheet.

Automated Methane Potential Testing System II

CO_2 fixing unit.

The initial step of this experiment was preparing the CO_2 fixing unit in the AMPTS. This unit is responsible for holding CO_2 fixing medium. The fume hood was used to prepare a 3M NaOH indicator solution (240g of NaOH pellets were added to 2000ml of milliQ water). The pellets were dissolved in the water by adding small amounts of pellets and water at a time, until the 240 grams had been dissolved. 40mg of thymolphthalein indicator were added to 9ml of 99.5% ethanol. After the thymolphthalein was dissolved, 1ml of milliQ water was added to the solution for a total of 10ml, given that the indicator is insoluble in water, but it is soluble in ethanol. The 10ml of the 0.4% Thymolphthalein solution was mixed with the 2000ml of the 3M NaOH solution. The blue solution was saved in tightly sealed 1 liter bottles. Before use in the experiment, approximately 80ml of the indicator 3M NaOH solution was added to the 100ml bottles in the AMPTS system. The phenolphthalein solution was dark blue in color. The medium's efficacy was tested by adding CO_2, and observing changes in the medium's color from dark blue to colorless. Crystals in the medium were formed as expected, given that the media had been saturated with CO_2. After confirmation of the medium's efficacy a total of fourteen bottles were filled for each experiment. Bottles containing the medium were held at room temperature for no more than two weeks before being used. Two bottles were used for each of the substrates, and a positive and negative control were tested in each of the experiments

A total of fourteen bottles were filled for each experiment. The bottles were tightly closed with rubber stoppers **(See appendix)**; rubber stoppers were lubricated with Dow Corning High Vacuum Grease ®, on the sides in contact with the glass bottle to prevent gas leaks. Plastic screw caps with holes were then screwed on top of the rubber stoppers until the lid was properly sealed **(See appendix)**. Fourteen CO_2 fixing bottles were then placed in their rack.

Reactors.

The amount of inoculum and substrate needed in each reactor, was determined by using the Bioprocess Control software; the experiment tab was selected, and data regarding oDM for both the inoculum and substrate were added to the left side of the table. The number of the reactor was selected, according to the placement of the reactors in the water baths. This placement was noted for reactors, CO_2 fixing units, and tubing connection to the AMPTS. Substrates were weighed using plastic weight boats. Reactor bottles were loaded with the appropriate amount of inoculum and substrate, as shown in the guidelines of the experiment section in the Bioprocess Control website **(*Table 1*)**. The reactor bottles were tightly closed with rubber stoppers containing two metal tubes and a bent stir rod **(See appendix)**; rubber stoppers were lubricated with Dow Corning High Vacuum Grease ®, on the sides in contact with the glass bottle to prevent gas leaks. A plastic cap/motor was screwed on the glass bottle until tight. Pieces of Tygon® tubing were used to connect the metal tube in the motor screw cap of the reactors, to the corresponding CO_2 fixing units. This was done for all of the fourteen reactors for each

trial. Motor cables were connected in series to each of the reactors, starting from reactor one through reactor 14. Reactor one motor cable was then connected to the motor module. This motor module was then connected to the gas measuring device (AMPTS). The tubing was purged with nitrogen to ensure that water was completely removed from the tubes.

Table 1. Weight of inoculum and substrate added to the AMPTS bioreactors. Values are displayed in grams.

Feedstock	Substrate amount (g)	Inoculum amount (g)
Erving paper sludge	76.04	323.96
Smuckers jelly	26.28	373.72
Lactose Pellets	22.25	377.75
Used bedding	20.51	379.49
Manure scrape	27.68	372.32
Potato sludge	71.13	328.87
Parlor water	24.71	375.29
Fresh straw	19.25	380.75
Cyanobacterial biomass	33.36	366.64
Hot dog casings	40.63	359.37
Positive Control	15.94	384.06
Negative Control	400	0

Thermostatic water bath.

The reactors were placed in the thermostatic water bath unit **(See appendix)**, in the same order used for the CO_2 fixing unit placement. The water bath was filled with milliQ water, to prevent bacterial and mold growth, until the level of the level of the

water had surpassed the level of the contents in the reactors. The plastic glass with 15 circular openings was placed on top of the water bath, which served as a barrier to prevent evaporation of the milliQ water.

Gas volume measuring device.

The gas volume measuring device **(See appendix)** was filled with milliQ water to the indicated water level mark. twelve inch long pieces of Tygon® tubing were used to connect the metal tubes in the CO_2 fixing units, to the respective input connection in the gas volume measuring device. This was done for units from 1-14.

Reactors and thermostatic water bath.

The water bath was allowed to reach temperature (38°C) before reactors were placed in the bath. A leakage test was performed for the bioreactors; none of the 14 bioreactors had gas leaks prior to starting the experiment.

Gas volume measuring device.

The measuring device was flushed with gas in order to create anaerobic conditions. This was done by unplugging the Tygon® tubing from one of the bioreactors metal openings. The unplugged Tygon® tube was the connected to a tank containing nitrogen. The nitrogen tank valve was opened with a low gas flow for 30 seconds. The Tygon® tubing was then closed with a tube clamp, to prevent oxygen from entering the bioreactor. The flush gas was closed and disconnected. The procedure was repeated for each of the 14

bioreactors in the water bath. Each of the flow cells in the gas volume measuring device was opened manually, in order to release the remaining gas in the unit.

Starting the software.

The Ethernet cable was connected from the computer to the gas measuring device. The AMPTS® software provided by Bioprocess Control™ was opened. The control panel was opened and the motors for each of the bioreactors were activated. The bioreactor motors were set to stir the contents every 30 minutes for 30.

Monitoring the experiment.

The devices used in the experiment were monitored twice each week. The water bath and the gas measuring device's water levels were kept to manufacturer standards, throughout the 21 day experiments. MilliQ water was used to fill these devices. The CO_2 fixing units were monitored daily for changes in color from blue to colorless. A change in color indicated saturation of the CO_2 medium. Clamps were used to prevent flow of gas in and out of the system. CO_2 fixing units were washed and filled to the 80ml mark with the NaOH indicator medium. The CO_2 fixing unit was re-connected to the Tygon® tubing.

End of experiment.

The experiment was conducted over 21 days. At the end of the operation, logging of the data was stopped by clicking the pause button in each of the bioreactor cells. Bioreactors motors were stopped by clicking the "stop" button, under the control tab. The

thermostatic water bath and the gas measuring device units were turned off, and disconnected. Motor cables and tubing was disconnected from the devices. Equipment was disassembled, washed, and set up for the second set of feedstocks to be tested. Data was recorded throughout the operation by the Bioprocess Control™ software. Raw data of biogas production per day for each feedstock was recorded in duplicate. A report was generated by the Bioprocess Control™ software, in the report tab. The report was downloaded and saved for data analysis. Average biogas production in ml was calculated from the duplicate tested feedstocks. Raw data for the AMPTS systems was analyzed for both 21 days and 28 days.

Deutsches Institut für Normung (DIN) Standard Method

Eudiometers.

Eudiometer set up, following the DIN standard method 38414, or DIN EN ISO 11734 (DIN 38414-18., n.d.). Eudiometers are 800 mL gas measuring devices made of glass that hold a red buffer solution **(See appendix)**. The eudiometer is graduated from the top downwards with 10mL scale divisions, and with a glass joint at the bottom, which is placed on a glass jar with a volume of 2L glass jar.

3395 mL of milliQ water was added to a 4L Erlenmeyer flask. A stir bar was placed inside the Erlenmeyer flask. The fume hood and personal protective equipment was used to add 105 ml of 95% sulfuric Acid (H_2SO_4) to the Erlenmeyer flask. The

sulfuric acid was added slowly. The Erlenmeyer flask containing both milliQ water and sulfuric acid was then heated with stirring. 700g of sodium sulfate decahydrate (Na_2SO_4) were added to the 4L flask using a large plastic funnel, until the sodium sulfate decahydrate was completely dissolved.

The Erlenmeyer was then removed from the hot plate, and allowed to cool to room temperature. 0.05g of methyl orange indicator was added to the Erlenmeyer. pH for the red buffer solution was checked.

Medium storage bottles.

The bottom glass line in both the eudiometer and the media bottle were lubricated with Dow Corning High Vacuum Grease ®. Lubrication was done around the glass line in contact with the rubber tubing, to prevent gas leaks. 0.0127m diameter tubing was used to connect the lubricated bottom orifice of the eudiometer, to the glass line orifice in the media bottle. The media bottle was filled up to the mark line, using the previously prepared red buffer solution **(See appendix)**.

Fermentation jars.

Fermentation jars were thoroughly washed with tap water and soap. Jars were then rinsed three times with distilled water. The fermentation jars were tightly closed by fitting the bottom of the eudiometer device, into the glass jar. Both the bottom of the eudiometer, and the fermentation jar's opening were lubricated with Dow Corning High Vacuum Grease ®, on the sides that were in contact with glass, to prevent gas leaks. The

gas releasing valve, used for gas reading with the gas meter, were also taken apart and lubricated with Dow Corning High Vacuum Grease ®, to ensure that they were functioning throughout the experiment.

Water bath.

Each fermentation system (fermentation jar and eudiometer) was placed in the water bath. Each fermentation system's corresponding media bottle was placed in a shelf adjacent to the water bath. Both eudiometers and media bottles were numbered from 1-14. The water bath was filled with tap water until the level of the water had surpassed the level of the contents in the fermentation jars, containing both the inoculum and the substrate.

A 1:100 bleach solution was added to the water bath to prevent mineral deposit excess, odor, and mold growth. The Cole Parmer™ temperature controller was used to monitor the temperature in the water bath through the course of the experiment. The thermostat included a built-in thermometer, which was placed inside the water bath. A Ulanet® water heater was connected to the temperature controller. A universal plate stand was used to sustain the water heater, inside the water bath. A total of two water heater settings were used in the water bath, to maintain constant water temperature (38°C) throughout the bath. The thermometer was checked for placement in the middle of the water bath. The water heaters connected to the thermostats were checked, to make sure they were in working condition.

Gas quality meter.

A gas meter was used to measure the biogas quality throughout the experiment. The device consisted of an electronic display, and an inlet hose, which allowed for direct connection to the eudiometer. This device also had a filter, to prevent damage of the electronic device, by stopping condensation products from entering the electronic device.

Sludge collection.

Seven gallon water jugs were used for percolate collection on day 0 of the experiment. The water jugs were cleaned by filling the jugs one quarter of the total volume with distilled water. The water jugs were then closed with its respective screw cap, and the jug was shaken for 30 seconds. The water was disposed of, and the process was repeated 3 times total.

Following water jug cleaning, sludge collection was done. twenty four liters of sludge was collected from the University of Wisconsin Oshkosh biodigester, in each of the 7 gallon jugs for a total of ~73.5 liters of sludge per bath containing the 14 eudiometer set-ups. The sludge was collected specifically from the “perc-out” valve at the biodigester. Sludge was then taken to the laboratory for DM and oDM analysis.

Sludge incubation.

Sludge (1.9L) was incubated in the eudiometer base jars after laboratory analysis. The measured sludge was placed in each of the glass jars. The fermentation jars and eudiometers were tightly closed by fitting the bottom of the eudiometer device, into the

glass jar. Both the bottom of the eudiometer, and the fermentation jar's opening were lubricated with Dow Corning High Vacuum Grease ®, on the sides that were in contact with glass, to prevent gas leaks.

The eudiometer was placed on the glass joint at the top of the fermentation jar. Stopcock was checked for free movement. The eudiometer was twisted slightly to ensure good seal. Jars were allowed to acclimate to the water temperature water for 2 hours. After two hours had passed, the eudiometers were set to 0ml, by opening the gas valve and raising the media bottle up to the 0ml mark. The valve was closed. After four hours the fermentation jar was checked for leaks. This was done by observing homogeneous gas production (in mls) from each of the fermenters. All seals were checked to ensure that all the eudiometers were functioning properly. There was apparent biogas being produced, however this biogas was not measured, but rather monitored for equal biogas production in each duplicate set of eudiometers. Different sets of eudiometers showed ambiguous apparent biogas production; joints and valves were re-greased, with the purposed of covering any present leaks.

Sludge was incubated at 38°C for a total of two weeks from loading date. Homogeneous gas production was checked daily. Joints were greased using Dow Corning High Vacuum Grease ®, in the case of gas leaks being present.

Substrate input.

Following the two week sludge incubation period, the substrates were loaded into each of the fermentation jars. Substrates were loaded into each of the fermentation jars, based on suggested grams to add by the computer Excel spreadsheet provided by the ERIC **(Table 2)**. The amount added to each of the glass jars was based on dry matter of the substrate. Calculations were done using the formula 3g (%DM) x 1.9L /100. Substrates were weighed with a Mettler Toledo Classic PB153-S® using a weight boat. The appropriate amount of calculated substrate was added to each of the fermentation jars.

A positive control fermentation jar, loaded with microcrystalline cellulose, and a negative control jar was loaded with the percolate sludge only. Each of the feedstocks were run in duplicate during the fermentation process. A total of 12-14 fermentation jars were placed in each bath. This allowed for testing of positive control (2 jars), negative control (2 jars), and 4-5 feedstocks (8-10 jars).

Table 2. Suggested weights (in grams) of feedstocks needed in fermentation jars.

Feedstock	Target Grams to add
Erving paper sludge	12.34
Smuckers jelly	8.39
Lactose Pellets	6.41
Used bedding	21.85
Manure scrape	33.31
Potato sludge	22.20
Parlor water	100.00
Fresh straw	6.52
Cyanobacterial biomass	100.00
Hot dog casings	14.32
Positive Control	5.93
Negative Control	0.00

Eudiometers monitoring.

Eudiometers were monitored daily. Readings on biogas production were done every 22-26 hours. Biogas production was recorded by holding the media bottle up to the eudiometer media mark. The amount of biogas produced was logged in a production sheet. Eudiometers with biogas production greater than 300ml at the time of gas monitoring, were also analyzed for biogas quality using the portable gas meter. This was done for the pair of eudiometers containing the same substrate.

Following biogas quality analysis, the pair of eudiometers was set to 0ml. This process was done daily throughout the 28 day experiment. Biogas production monitoring,

gas quality readings and lubrication of the eudiometer devices were always done for both of the fermenters containing the same substrate. This allowed for homogeneity in readings and sample treatment.

Confining liquid storage bottles.

Confining liquid storage bottles were checked daily. Fresh media was added to the bottles when the quantity was not supplying enough media for the eudiometer 0ml mark.

Water bath.

The water bath was monitored daily for water evaporation. The water bath measuring water level was kept to DIN standards, throughout the 42 day experiment. Faucet water was used to fill the water bath. Temperature was checked daily using the temperature displayed in the thermostat, and with a laboratory thermometer. This was done to assure precision in water temperature standards.

Portable gas meter.

The gas quality meter was used daily to assess gas quality prior to zeroing volume of gas measured in eudiometers. This was done by connecting the device's input hose to the valve in the top of the eudiometer. The apparatus was turned on and set to measure. The eudiometer valve was opened, and gas was allowed to flow from the eudiometer to the gas measuring device for 30 seconds. After 30 seconds the device was paused and the eudiometer valve was closed.

Gas quality of the biogas was displayed on the screen of gas meter, providing composition of the gas sample. Oxygen, carbon dioxide, hydrogen sulfide and methane percentages were recorded for the respective eudiometer. The gas measuring device was zeroed by allowing air to enter the device.

End of experiment.

The experiment was conducted for a total of 42 days, which included 14 days of sludge incubation, and 28 days of fermentation and biogas production monitoring. A last reading on biogas production was done for all the eudiometers. Gas quality was assessed for all substrates following the gas production reading.

The electronic thermostat in the water bath and water heaters were turned off and disconnected. Equipment was disassembled, washed, and set up for the second set of feedstocks to be tested. All data was logged into a computer, and a report was generated using the "Biogas production log" Excel™ spreadsheet from the Environmental Research and Innovation Center (ERIC).

Data Analysis

Data was analyzed using the data analysis tool from MS Excel™ software.

Eudiometers.

Total biogas production for each analyzed feedstock was calculated. Methane production percentages for feedstocks were calculated using the median. The mean was not used to calculate methane production, given that methane production does not peak until few days after biodigestion starts. The percentage of methane produced from each feedstock was used to assess overall methane production, from the previously averaged total biogas production.

Each feedstock's methane production was calculated by dividing the overall methane production of the feedstock, by the amount of fresh material added in grams. Each feedstock's methane production per gram of organic dry matter was calculated by dividing the overall methane production of the feedstock, by the organic dry matter percent of the sample.

AMPTS.

Total biogas production for each analyzed feedstock was calculated. Methane production from each feedstocks in the AMPTS was normalized by the Bioprocess Control® software. The methane percentages for each feedstock were used to assess overall methane production, from the previously averaged total biogas production.

Each feedstock's methane production was calculated by dividing the overall methane production of the feedstock by over the amount of fresh material added in grams. Each feedstock's methane production per gram of organic dry matter was calculated by dividing the overall methane production of the feedstock by over the organic dry matter percent of the sample.

Method comparison.

Daily methane production data was compiled for both the DIN and the AMPTS method. Biogas production from the AMPTS method was determined for 21 days, while from the DIN method was determined for both 21 and 28 days of methane production. Two tailed T-tests were used to compare methane production between the AMPTS and DIN methods for each individual feedstock, at 21 days of methane production.

A paired T-test was performed using both "R" statics software, and the built-in statistical tool in MS Excel™, to compare methane production (ml) per gram of organic dry matter (ml/g oDM) for all feedstocks. A comparison of methane production by the AMPTS system at day 21, and at day 28 was also made. A paired T-test was performed to compare the methane production from the AMPTS method at day 21, and the methane production using the DIN method at day 28.

RESULTS AND DISCUSSION

The overall goal of this project was to determine if the AMPTS method was appropriate for biomethane potential analysis of feedstocks used in dry biodigestion. This project was conducted because the AMPTS method is a more rapid method than the DIN method, which could translate into cheaper and faster testing times for biogas production analysis. This study was successful in providing data that could be useful in determining whether or not the AMPTS method can be used as a standard laboratory method for BMP analysis.

Feedstock Analysis

Total solids (TS).

The total solid analysis provided data on dry matter as a percentage of fresh bio-matter. Total solids were calculated for each of ten feedstocks (**Table 3).**

Table 3. Feedstock's total solid analysis. Total solids were calculated as dry matter as percentages of fresh biomass.

Feedstock	DM (%FM)
Erving paper sludge	46.21
Smuckers jelly	67.94
Lactose Pellets	88.87
Used bedding	26.08
Manure scrape	17.11
Potato sludge	25.68
Parlor water	0.19
Fresh straw	87.42
Cyanobacterial biomass	5.18
Hot dog casings	39.80
Positive Control	96.04
Negative Control	2.68

Parlor water and cyanobacterial biomass, both liquid feedstocks, had the lowest dry matter percentage of the feedstocks (0.19% and 5.18% of the fresh matter, respectively). Fresh straw and lactose pellets, both dry feedstocks, had the highest dry matter percentage of the feedstocks (87.42% and 88.87% of the fresh matter, respectively). The other analyzed feedstocks, with mid-ranging total solid percentages, were characterized by being viscous or aqueous substances. The positive control, microcrystalline cellulose had the highest dry matter of all substrates, which is expected given that cellulose has high carbon content, which translates into readily available

organic solubles for methanogenesis. The negative control, percolate sludge, had a lower dry matter percentage as expected, given that the negative control is the baseline for the lowest production of biogas possible.

Volatile solids (VS).

The volatile solids analysis performed on the feedstocks, provided information regarding the percentage of organic dry matter present in the samples and were used as a baseline for calculations on the amount off fresh material added to the reactors in the AMPTS method. Volatile solids were calculated for each of the ten feedstocks (**Table 4**).

Table 4. Feedstock's volatile solid analysis. Both organic dry matter as a percentage of fresh matter and organic dry matter as a percentage of dry matter were determined.

Feedstock	oDM (%FM)	oDM (%DM)
Parlor water	0.1305	68.354
Cyanobacterial biomass	2.91	49.446
Manure scrape	10.359	60.535
Erving Paper Sludge	19.173	42.415
Potato sludge	20.805	81.017
Used bedding	21.713	83.249
Hot dog casings	39.166	98.3
Smuckers jelly	62.974	92.651
Lactose Pellets	76.4	85.963
Fresh straw	89.016	100
Positive Control	96.135	100
Negative Control	1.323	49.333

Parlor water and cyanobacterial biomass, both liquid feedstocks, had the lowest organic dry matter as a percentage of dry matter (0.13% and 2.91% of the fresh matter, respectively). Fresh straw and lactose pellets, both dry feedstocks, had the highest organic dry matter as a percentage of dry matter (87.42% and 88.87% of the fresh matter, respectively). The other analyzed feedstocks, with mid-ranging organic dry matter percentages, were characterized as being viscous or aqueous substances. Organic dry matter, as a percentage of dry matter values, was higher for feedstocks that were dry, and lower for liquid or aqueous feedstocks. The positive control, microcrystalline cellulose, the positive control, had the highest organic dry matter percentage values of the feedstocks. Percolate sludge, the negative control, had a very low organic dry matter (%FM) value.

Feedstocks with the lowest total solid percentages had the lowest percentages of volatile solids, or oDM, as well. Although high dry matter does not necessarily translate into high organic dry matter, it is usually the case that feedstocks with high dry matter percentages have more dry matter to potentially be turned into ash or organic dry matter. Fresh straw, for example, had high TS percentage and it also had a higher VS percentage (87.42% of VS (%FM)). On the other hand, the positive control was expected to have the highest of all VS percentages, as high organic dry matter is readily available for cellular processes, such as bacterial fermentation and methanogenesis, as described previously.

Automated Methane Potential Testing System II

21 day AMPTS biogas production analysis.

The AMPTS system was developed in Sweden with the aim to provide a more cost effective and faster method for testing BMP in feedstocks. The system is comprised of bio-reactors that hold both inoculum and substrate for 21 days, which quantifies biogas production using an automated system. This method, however, has not been accepted as a standardized method, which is why there was a need to validate this method as an alternative to the DIN standard for BMP analysis.

Smucker's ® jelly, a feedstock that did not meet quality control standards, was used for BMP analysis. This feedstock had the highest biogas production of the tested feedstocks. A total of 4,558mL of methane were produced over the course of 21 days. Average methane production per day, peaked at 596.45mL between day 0 and day 1. The highest amount of methane was produced between day 0 and day 8, with 3,868mL total. The remaining biogas was produced over last 13 days of the experiment **(Figure 3).**

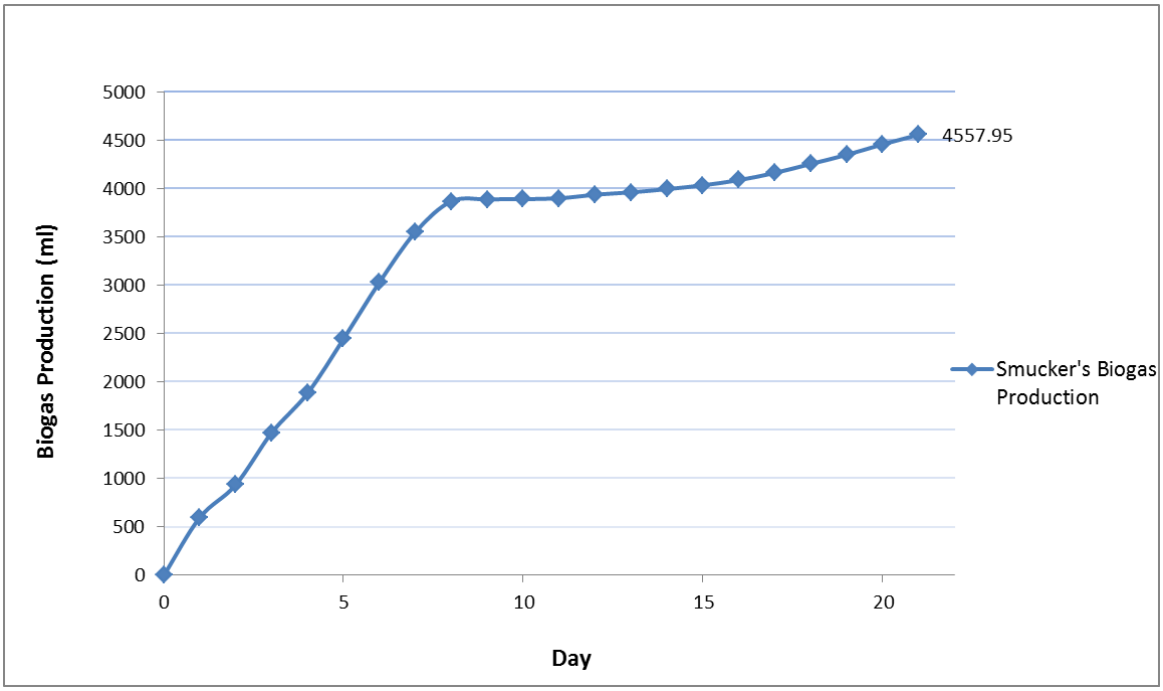

Figure 3. Biogas production from Smucker's jelly, measured using the AMPTS method, over a 21-day period. Total methane production was 4557.95ml.

Lactose pellets had the second highest biogas production. A total of 3,838.65mL of methane were produced over the 21 day experiment. Methane production showed an upward trend until day 21. Methane production peaked between day 0 and day 1 with 774.60mL produced. Production over the 21 days was not consistent. A malfunctioning water bath between day 8 and day 11 resulted in little methane production during this period **(Figure 4).**

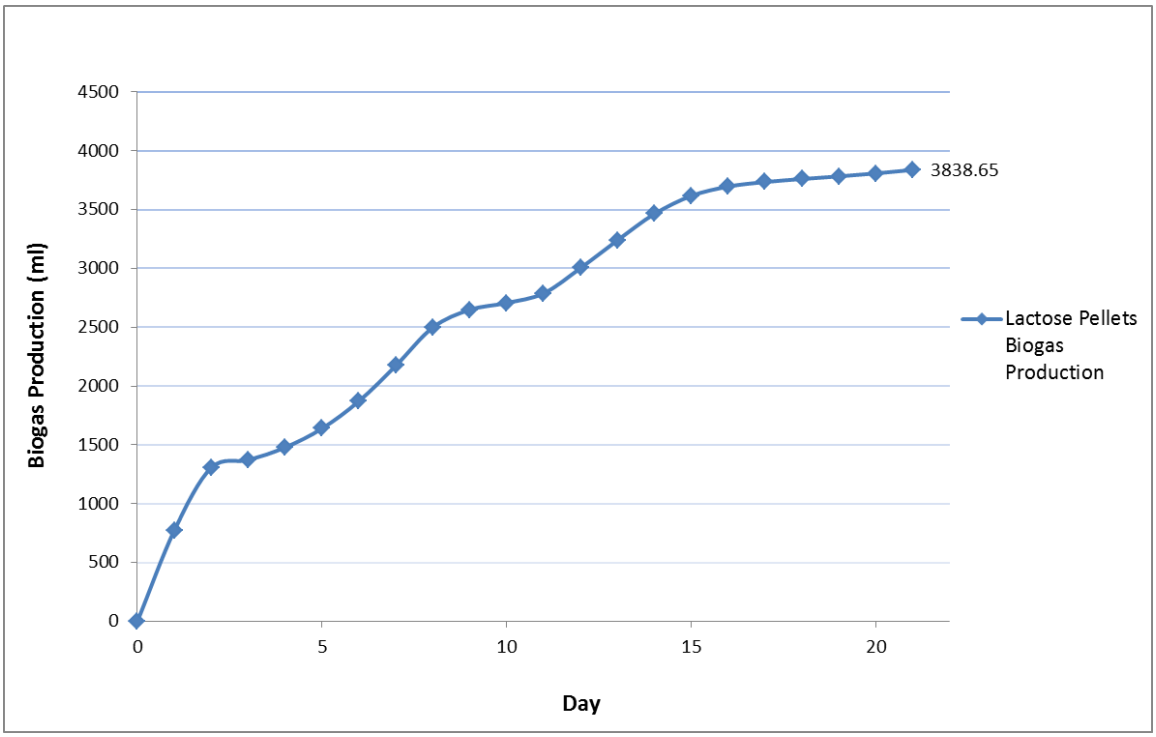

Figure 4. Biogas production from lactose measured using the AMPTS method, over a 21-day period. Total methane production was 3,838.65mL.

Paper sludge, the feedstock with the third highest biogas production of the tested feedstocks, produced a total of 3,695.7mL of methane over the course of 21 days. Average biogas production per day peaked between day 7 and 8, with 420.70mL of methane produced. **(Figure 5).**

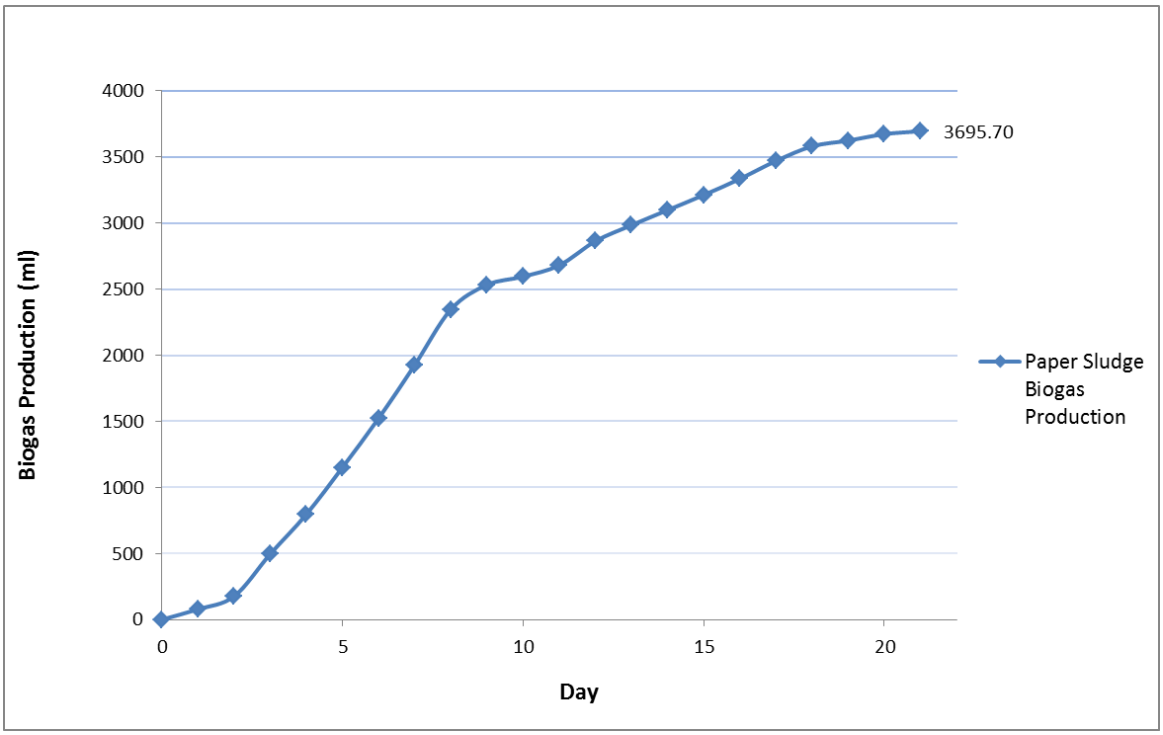

Figure 5. Biogas production from paper sludge measured using the AMPTS method, over a 21-day period. Total methane production was 3,695.7mL.

Hot dog casings were the feedstock with the fourth highest biogas production, with a total of 3,146.95mL of methane produced over a 21 day period. Biogas production peaked between day 7 and 8, with 276.7mL of methane produced. Methane production was not consistent, stalling at day 21. **(Figure 6)**

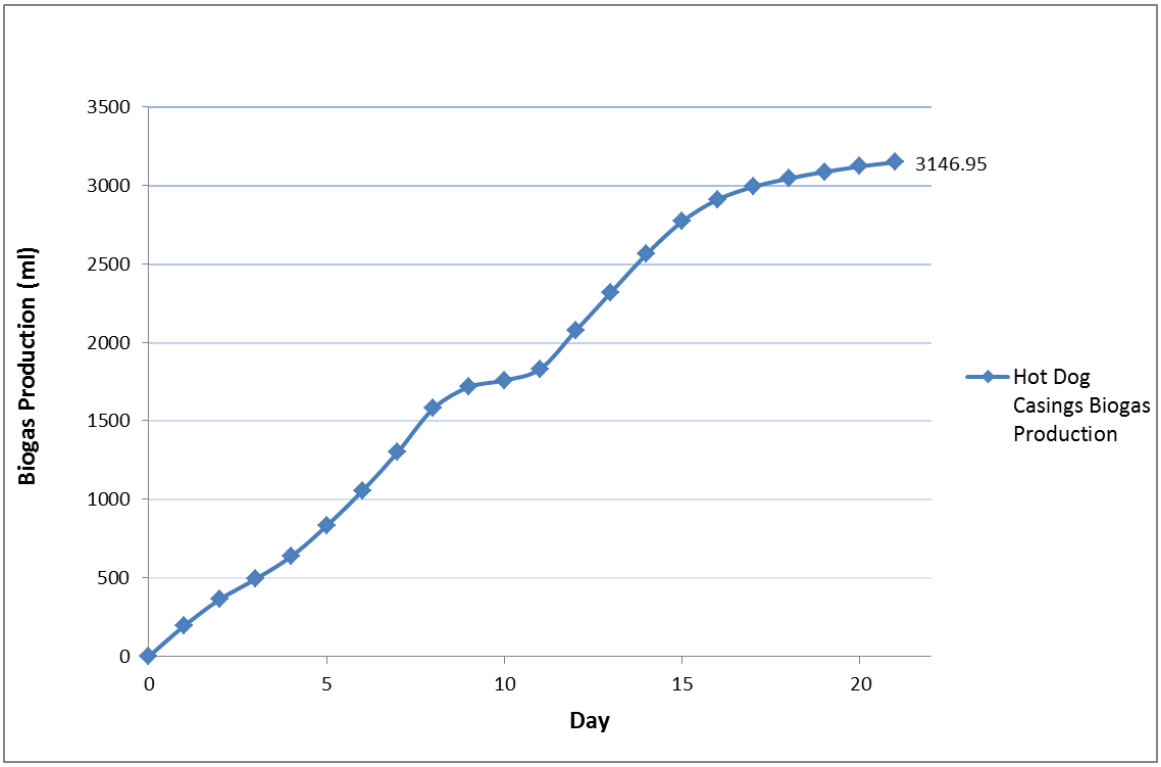

Figure 6. Biogas production from hot dog casings measured using the AMPTS method, over a 21-day period. Total methane production was 3,146.95mL.

Potato sludge had the fifth highest biogas production. A total of 2,071.55mL of methane were produced over the 21 day experiment. Biogas production peaked between day 1 and 2 with 462.1mL of methane produced. The amount of biogas produced per day, gradually decreased until day 21 was reached. Overall biogas production peaked at day 21 with a projection of decrease onwards. **(Figure 7)**

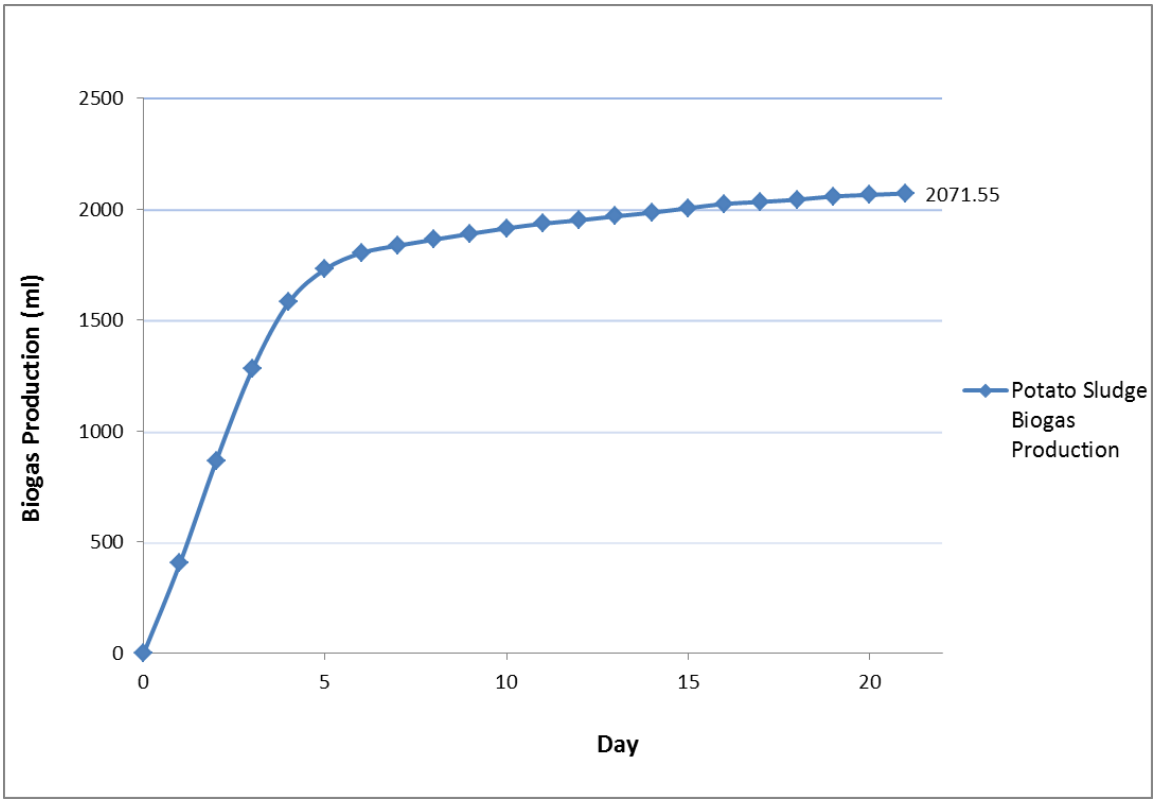

Figure 7. Biogas production from potato sludge measured using the AMPTS method, over a 21-day period. Total methane production was 2,071.55mL.

Fresh straw showed a total production of 2,019.35mL of methane using the AMPTS system, over the 21 day experiment. Methane production decreased gradually, with top highest methane production between day 0 and day 1 (350.95mL produced). Methane production increased until day 21. An upward trend appears to continue passed day 21 **(Figure 8).**

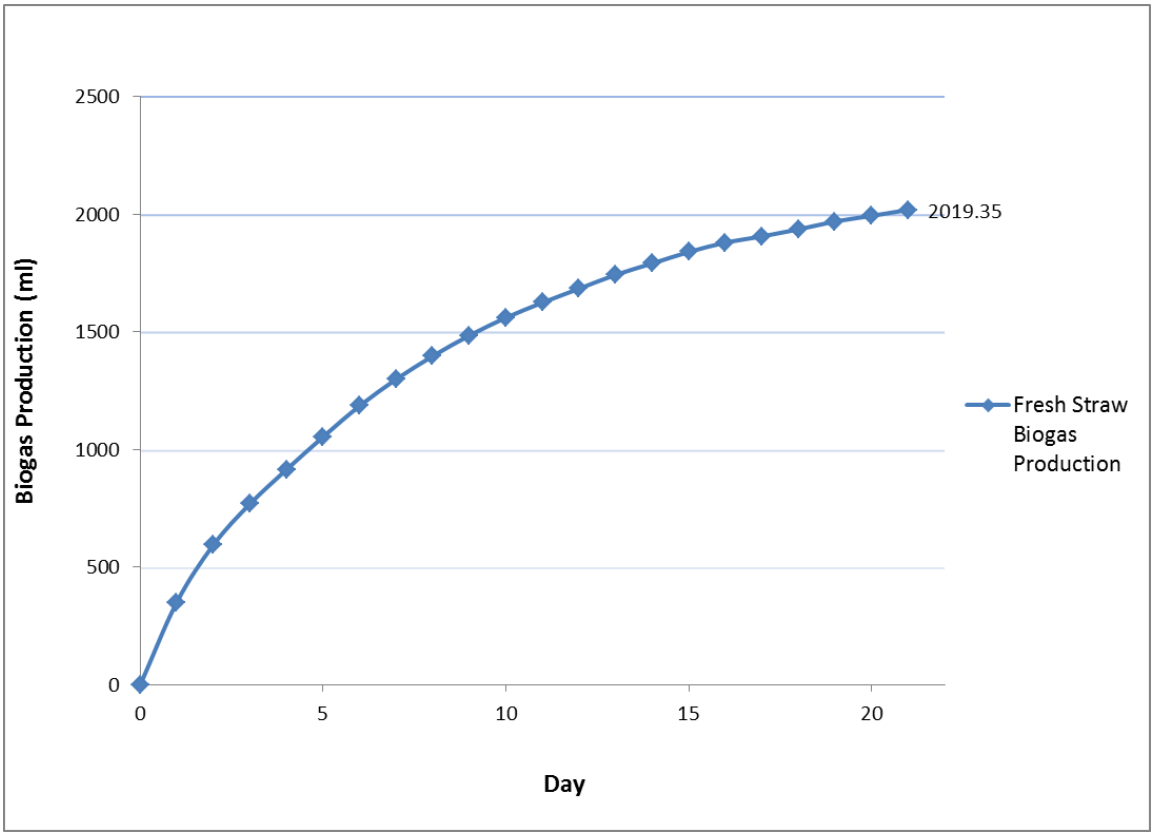

Figure 8. Biogas production from fresh straw measured using the AMPTS method, over a 21-day period. Total methane production was 2,019.35mL.

Used bedding was one of the substrates with lower biogas production. A total of 1,005.85mL of methane were produced over the 21 day experiment. Used bedding substrate analysis showed a gradual decrease in biogas production. The 21 day experiment did not seem to be long enough for the substrate's methane production to peak. The highest average methane production for used bedding was between day 0 and day 1 with 154.05mL of methane produced. **(Figure 9)**

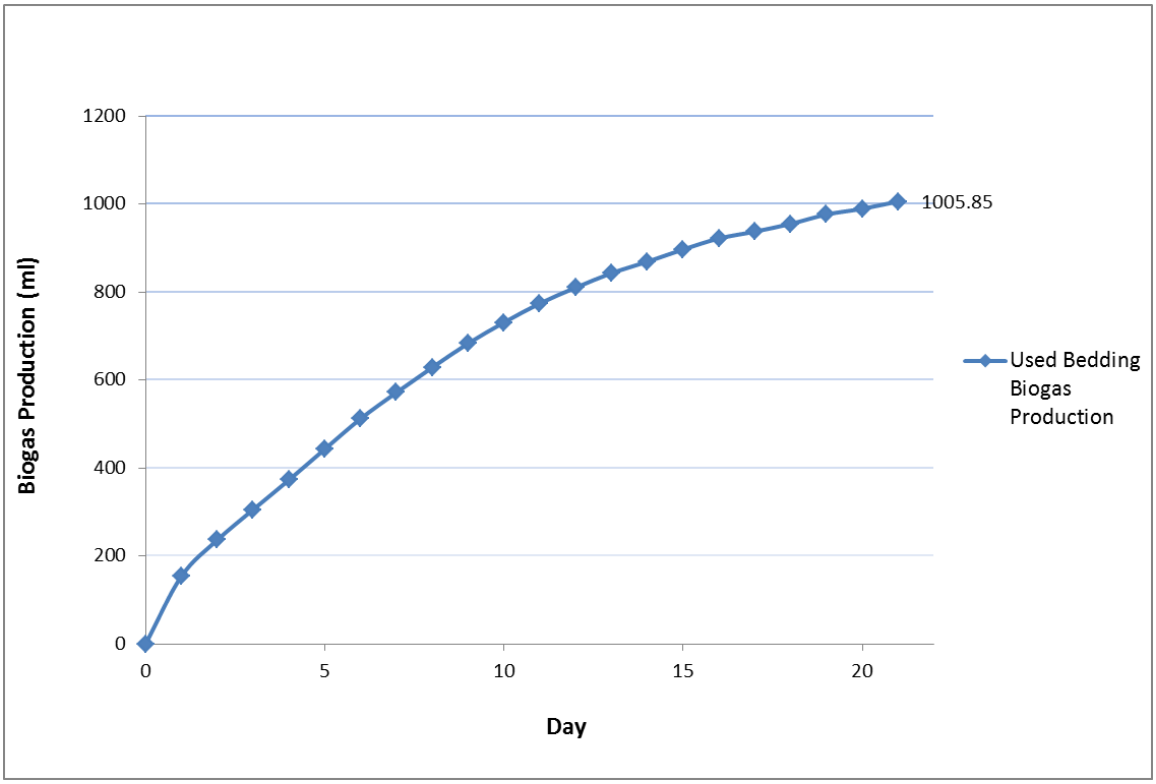

Figure 9. Biogas production from used cattle bedding measured using the AMPTS method, over a 21-day period. Total methane production was 1,005.85mL.

Manure scrape showed a low biogas production in comparison to other substrates. A total of 876mL of methane were produced over the 21 day experiment. Highest average of gas production for manure scrape peaked between day 0 and day 1 with 208.4mL of total methane produced. A gradual decrease in biogas production was observed throughout the 21 day experiment. There was still methane being produced at day 21. **(Figure 10)**

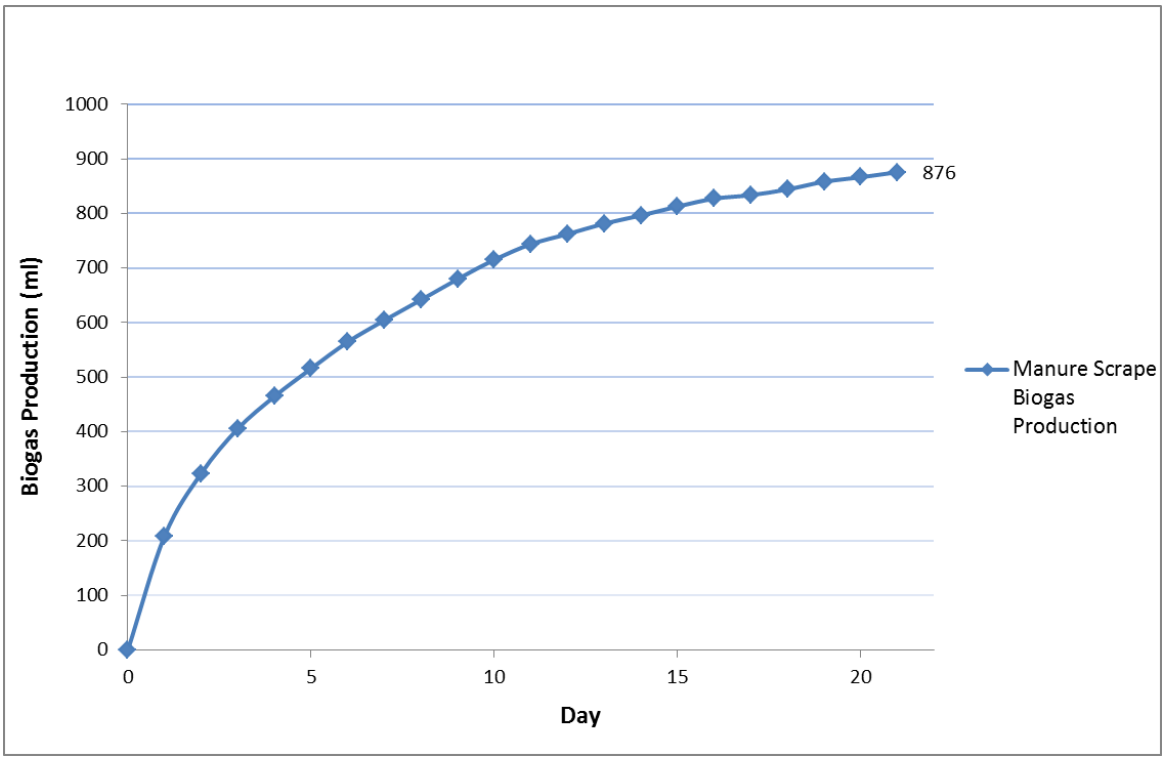

Figure 10. Biogas production from manure scrape measured using the AMPTS method, over a 21-day period. Total methane production was 876mL.

Parlor water produced a lower amount of biogas. A total of 278.95mL of methane were produced over the 21 day experiment. The highest average production peaked between day 0 and day 1 with 62.4mL produced. Methane produced gradually decreased over the 21 days, however methane production per day did not peak over the testing period, as seen in the figure. The trend line still shows a **(Figure 11)**

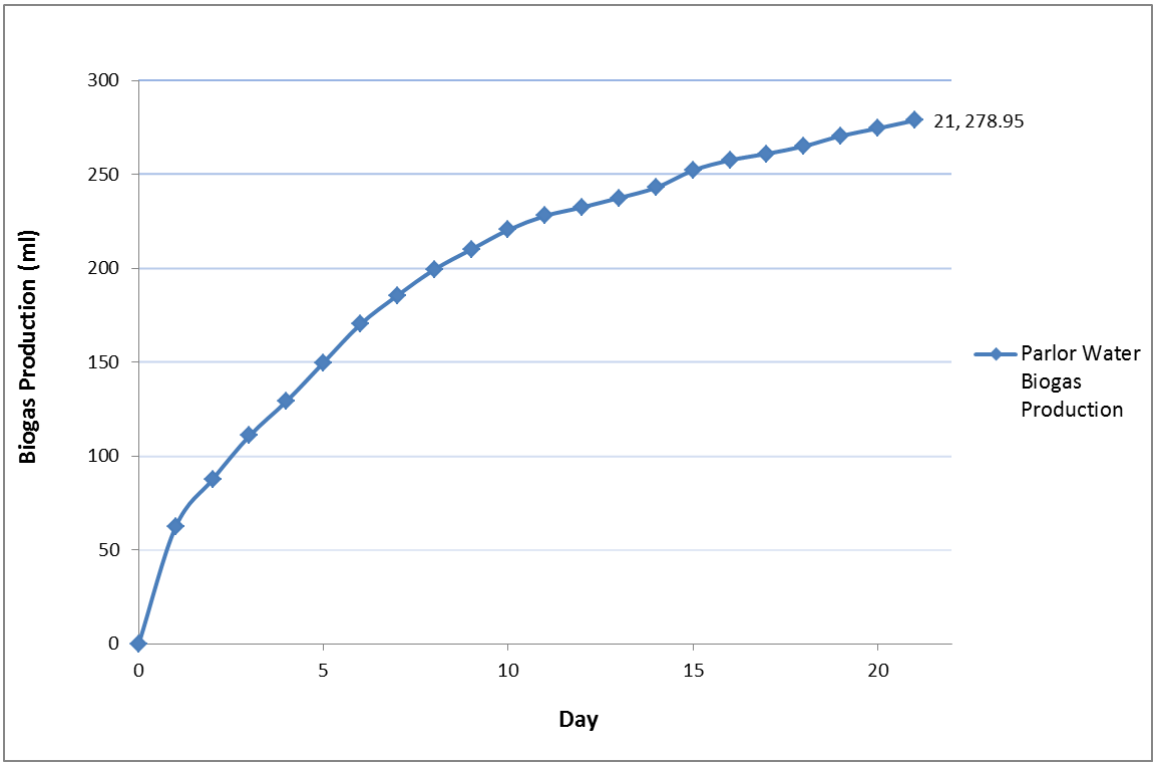

Figure 11. Biogas production from parlor water measured using the AMPTS method, over a 21-day period. Total methane production was 278.95mL.

Cyanobacterial biomass had the lowest biogas production of the feedstocks. A total of 224.4mL of methane were produced over the 21 day experiment. Highest average methane production peaked between day 0 and day 1, with a total of 43.85mL. Biogas production didn't decline to 0mL per day at any time, during the 21 days; there was still an upward trend in methane production at day 21. **(Figure 12)**

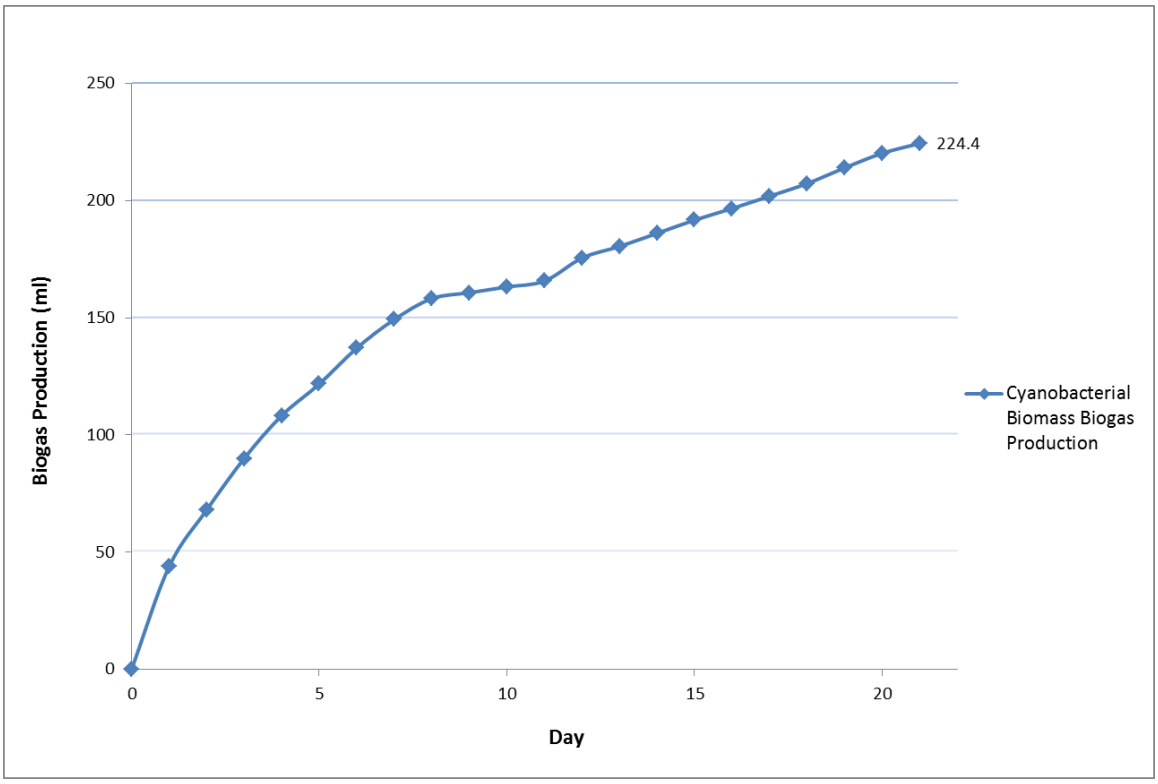

Figure 12. Biogas production from cyanobacterial biomass measured using the AMPTS method, over a 21-day period. Total methane production was 224.4mL.

Overall, data collected on biogas production for the ten feedstocks showed that organic matter rich substrates such as Smucker's jelly and lactose pellets produced a considerable amount of biogas, however, the expected correlation between high organic dry matter and high biogas production was not always observed. Hot dog casings had volatile solids of 39.16% (FM), and it was one of the feedstocks with higher total biogas production (3146.95ml). Cyanobacterial biomass, on the other hand, produced a low amount of total biogas (224.4ml) with a very low percentage of oDM present in the

sample. With this in mind, it is difficult to categorize a feedstock as better or worse for biogas production, cyanobacterial biomass had the lowest overall biogas production, however, it had the highest biogas production per gram of organic dry matter. The concept of biogas production per gram of organic dry matter is relevant, given that it promotes the use of liquid waste such as cyanobacterial biomass or parlor water, in combination with high solid waste, to reach the ideal dry matter balance in a dry biodigester (14).

Hot dog casings, a feedstock with low organic dry matter content (~39.16%), had relatively high biogas production, when compared to the amount of organic dry matter present in the sample. This feedstock would be expected to have low biogas production, given that the sample itself had the appearance of plastic with little hot dog remnants in it. On the other hand, feedstocks with high organic dry matter values such as fresh straw (89.01%) were expected to produce a relatively high amount of biogas, when compared to other substrates. However, this was not the case. Fresh straw produced a total of 2019.35ml of biogas over the course of the experiment, which places this feedstock in the bottom five biogas producing substrates.

It is important to note that between day 7 and 10 there was a decrease in biogas production, given that there was malfunctioning of equipment during the weekend. A water bath fuse burned out, which caused the temperature in the water bath to stay at room temperature for three days. This event was seen as a possible drawback for the AMPTS system, given that by being automated, there is a possibility of equipment

malfunctioning, and laboratory staff to not notice minor details of the system, whereas the DIN method requires readings of the eudiometers in a regular basis, thus equipment malfunctioning data errors can be diminished.

Deutsches Institut für Normung (DIN) Standard Method

This standardized method, using bio-reactors and eudiometers, has been used for many years for biomethane potential analysis. This method is characterized by manual readings of biogas production, over a 28 day period. However, with emerging technologies and expansion to medium and large scale farms around the world, there was a need for a time efficient and cheaper testing method. Previously tested feedstocks with the AMPTS method were then analyzed using the standard DIN method.

Average methane production from feedstocks ranged from 49% and 62%, of the total biogas sample; hot dog casings produced biogas with the lowest percentage of methane composition, while potato sludge produced biogas with the highest average methane composition. Median methane production for all substrates ranged from 58.9% and 65.7%. Parlor water produced biogas with the lowest average methane composition, while used bedding produced biogas with the highest average amount of methane **(Table 5).**

Table 5. Biogas composition from tested feedstocks (percent of total biogas). Mean methane percentages are shown in the second column. Median methane percentages are shown in the third column.

Feedstock Biogas Quality - DIN Method		
Substrate	**Mean CH4 (%)**	**Median CH4 (%)**
Erving paper sludge	57.66	62.66
Smuckers jelly	57.99	61.70
Lactose Pellets	59.38	62.80
Used bedding	58.99	65.70
Manure scrape	53.14	62.10
Potato sludge	62.81	65.30
Parlor water	55.97	58.90
Fresh straw	56.36	63.70
Cyanobacterial biomass	53.57	61.50
Hot dog casings	49.10	60.00

Both the mean and the median methane produced were used to calculate methane percentage, because methane production varied over the course of the experiment. Percent of methane in biogas, calculated using mean methane production, gave lower overall percentage of methane in biogas, while percentages calculated using the median methane production, gave higher percent of methane composition.

Twenty eight day DIN method biogas production analysis.

Feedstocks tested for BMP using the DIN method are presented from highest to lowest overall production (mL accumulated for the duration of the experiment). Data collected on daily biogas production for each feedstock, was averaged from the duplicate set of eudiometer and digestion jar system. Daily methane production for each feedstock was calculated using the median methane percentage, from the total biogas volume.

Hot dog casings produced the highest volume of methane over the course of the experiment, with a total of 3,916.54mL. Average biogas production per day, peaked at 395.51mL produced between day 10 and 11. Highest amount of biogas was produced between day 0 and day 17, with a total of 3,583.05mL of methane. The remainder biogas was produced in the last 11 days of the experiment. **(Figure 13)**

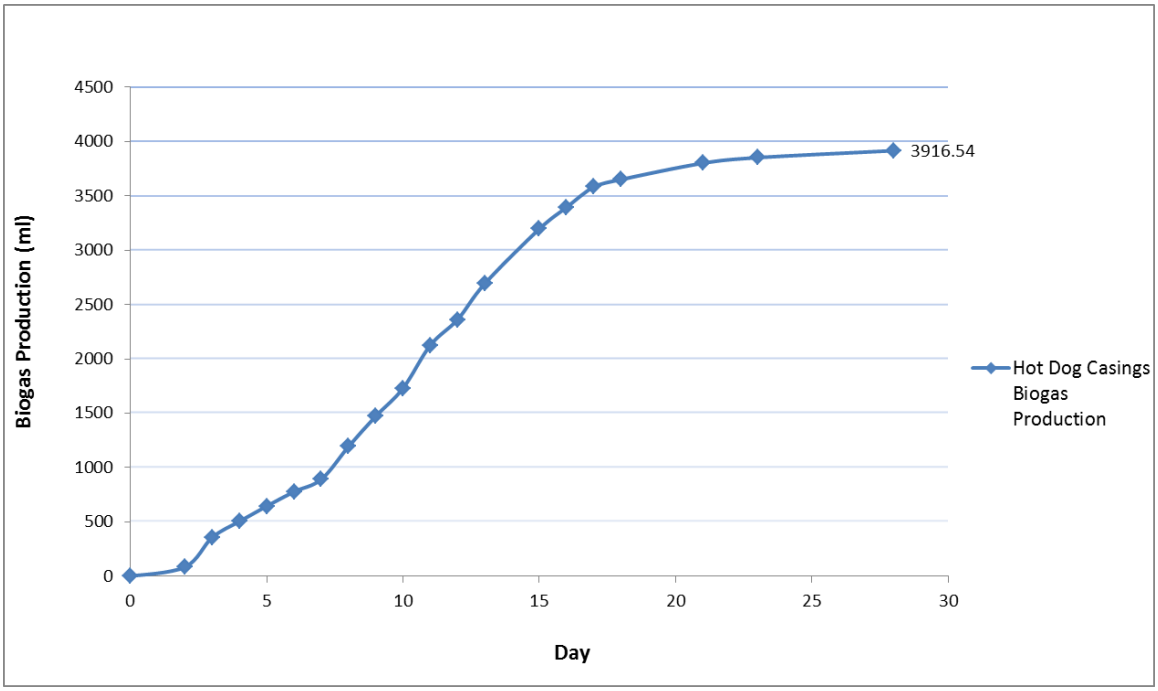

Figure 13. Methane production from hot dog casings measured using the DIN method, over a 28-day period. Total methane production was 3,916.54mL.

Potato sludge produced the second highest volume of methane over the course of the experiment, with a total of 3,380.02mL. Average biogas production per day, peaked at 687.54mL produced between day 3 and 4. The highest amount of biogas was produced between day 0 and day 9, with a total of 3,140.67mL of methane. The remainder biogas was produced during the last 19 days of the experiment. **(Figure 14)**

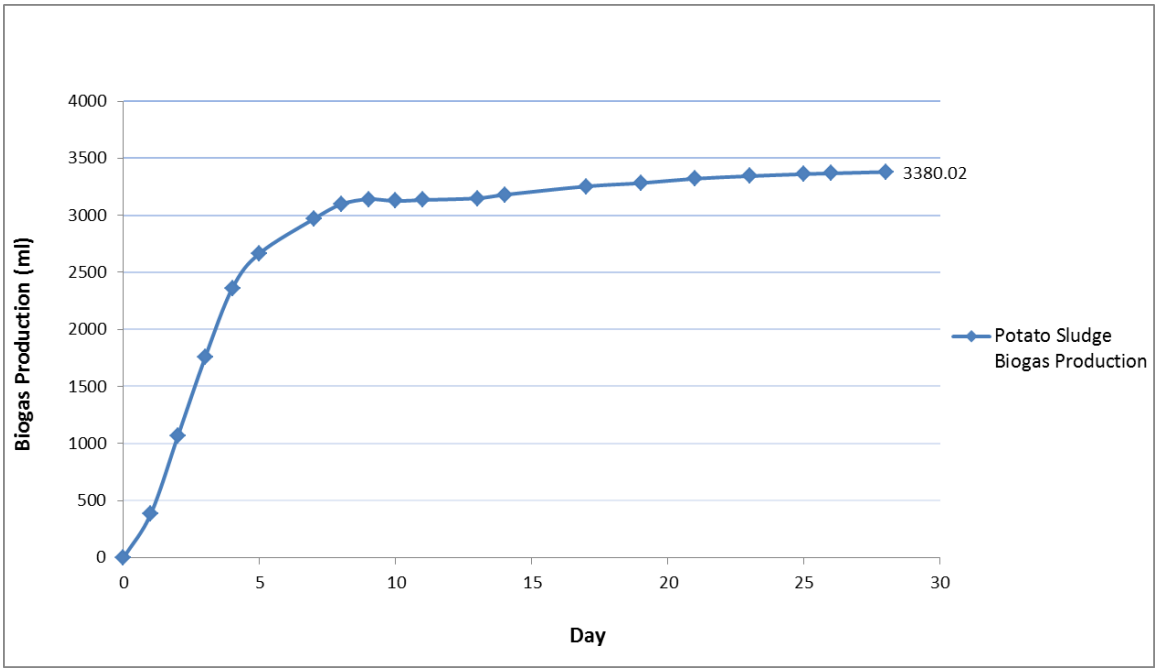

Figure 14. Methane production from potato sludge measured using the DIN method, over a 28-day period. Total methane production was 3,380.02mL.

Smucker's ® jelly produced the third highest volume of methane over the course of the experiment, with a total of 3,466.42mL. Average biogas production per day, peaked at 902.27mL produced between day 0 and day 1. The highest amount of biogas was produced between day 0 and day 8, with a total of 3,295.20mL of methane. The remainder biogas was produced in the last 20 days of the experiment. **(Figure 15)**

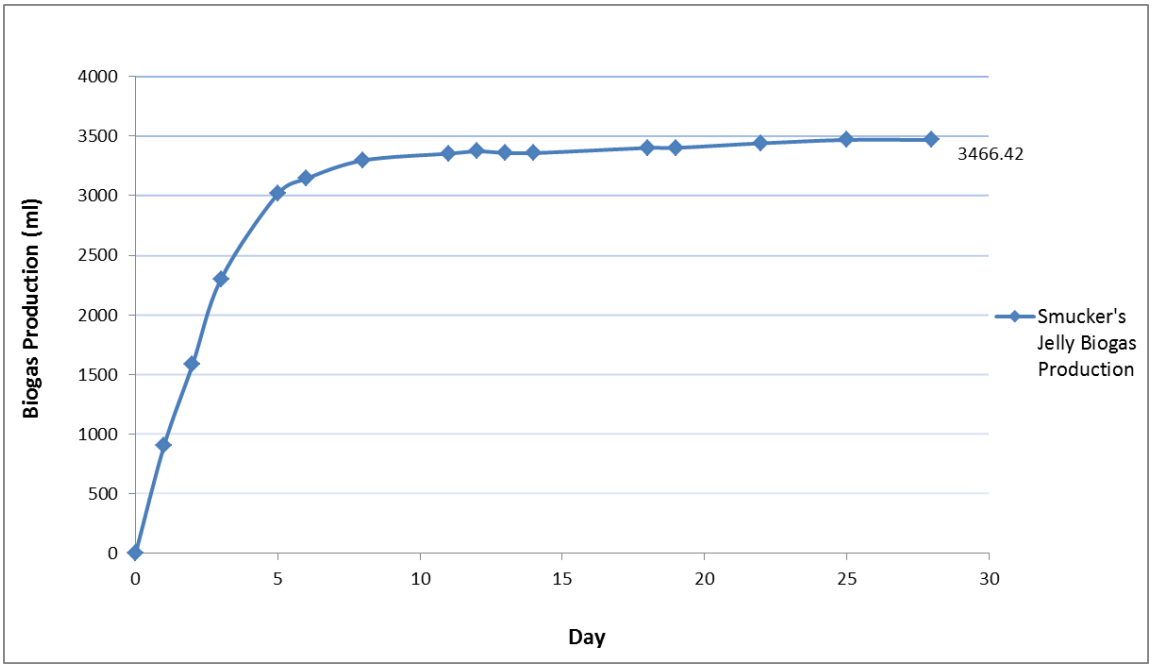

Figure 15. Methane production from Smucker's jelly measured using the DIN method, over a 28-day period. Total methane production was 3,466.42mL.

Lactose pellets produced a moderate volume of methane over the course of the experiment, with a total of 2,724.35mL. Average biogas production per day, peaked at 751.10mL produced between day 6 and 7. Highest amount of biogas was produced between day 0 and day 8, with a total of 2,608.40mL. The remainder biogas was produced in the last 20 days of the experiment. **(Figure 16)**

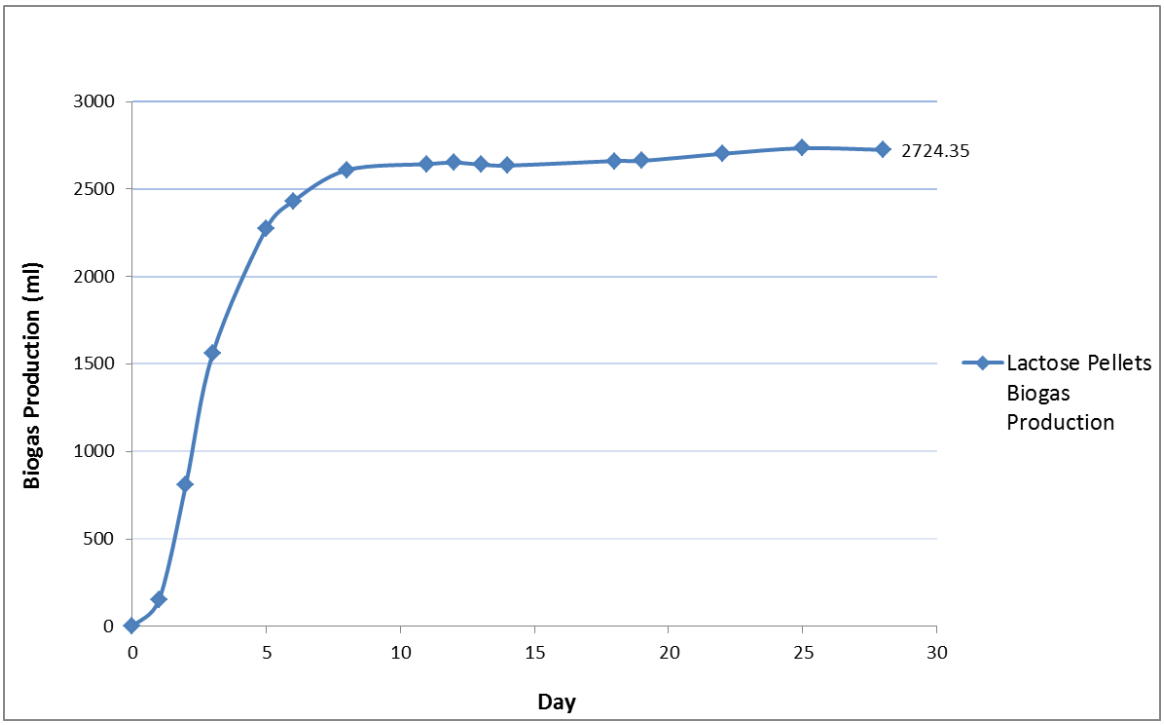

Figure 16. Methane production from lactose pellets measured using the DIN method, over a 28-day period. Total methane production was 2,724.35mL.

Fresh straw produced a moderate volume of methane over the course of the experiment, with a total of 1,545.91mL. Average biogas production per day, peaked at 171.47mL produced between day 0 and day 1. Highest amount of biogas was produced between day 0 and day 19, with a total of 1,438.65mL. The remainder biogas was produced in the last 9 days of the experiment. **(Figure 17)**

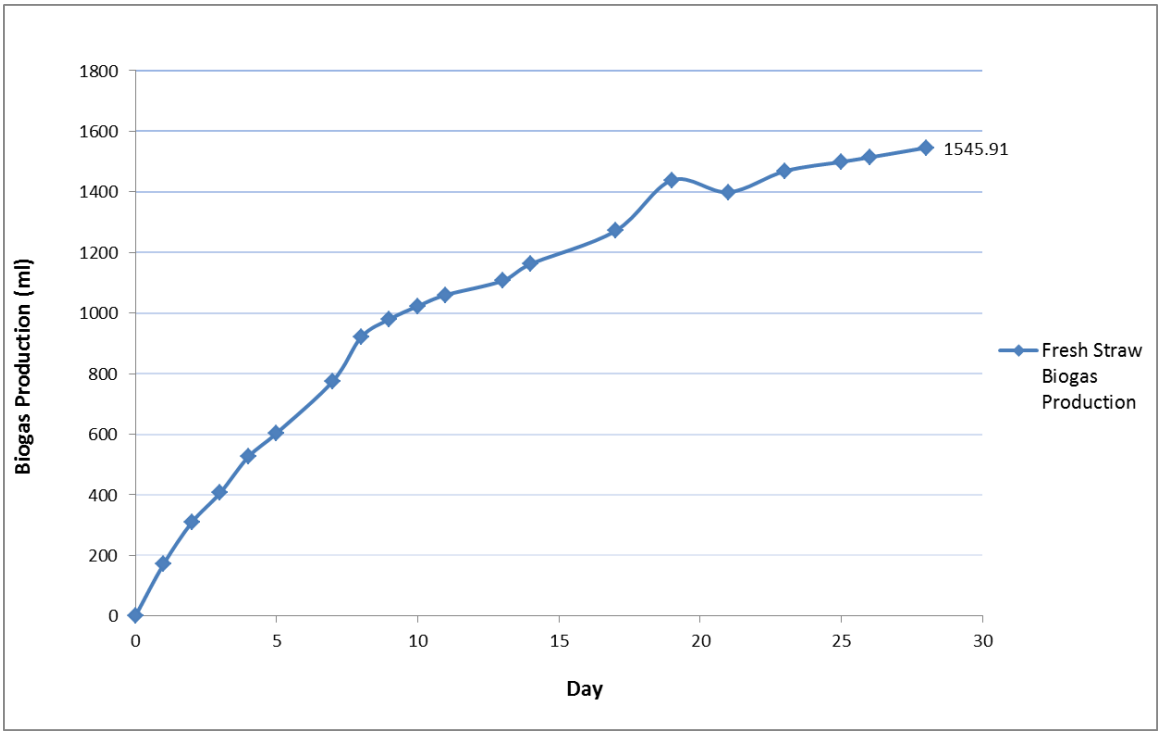

Figure 17. Methane production from fresh straw measured using the DIN method, over a 28-day period. Total methane production was 1,545.91mL.

Used bedding produced a moderate volume of methane over the course of the experiment, with a total of 1,150.46mL. Average biogas production per day, peaked at 150.54mL produced between day 2 and 3. Highest amount of biogas was produced between day 0 and day 26, with a total of 1,141.39mL. The remainder biogas was produced in the last 2 days of the experiment. **(Figure 18)**

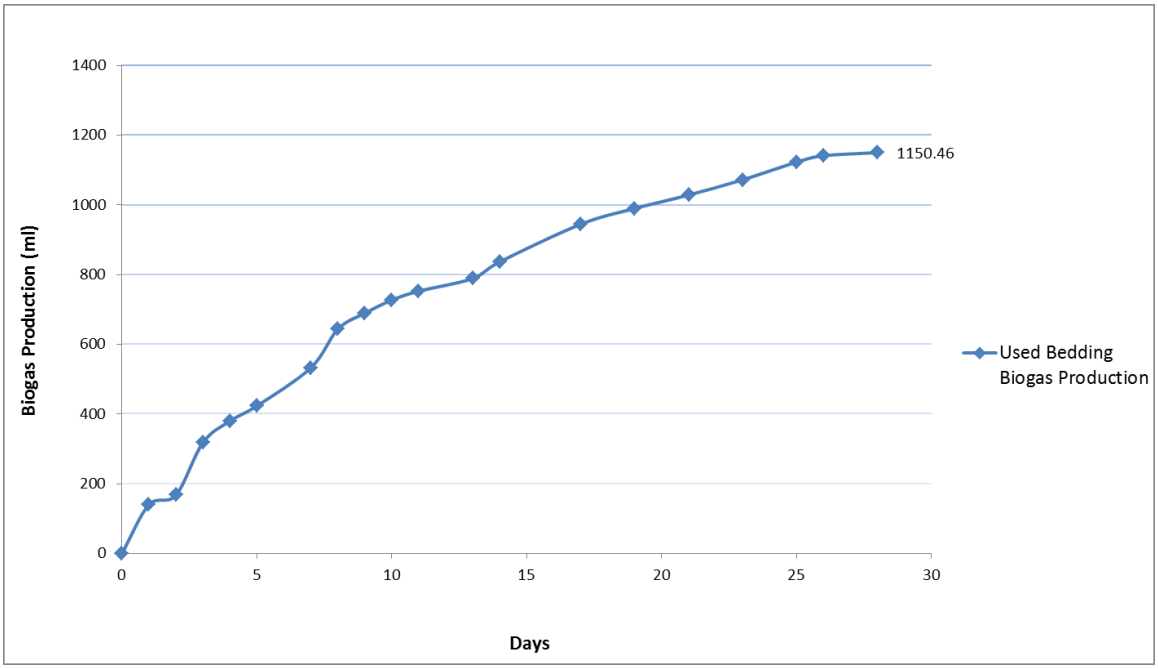

Figure 18. Biogas production from used bedding measured using the DIN method, over a 28-day period. Total methane production was 1,150.46mL.

Manure scrape produced a low volume of methane over the course of the experiment, with a total of 1,080.65mL. Average biogas production per day, peaked at 181.85mL produced between day 1 and 2. Highest amount of biogas was produced between day 0 and day 11, with a total of 890.56mL. The remainder biogas was produced in the last 17 days of the experiment. **(Figure 19)**

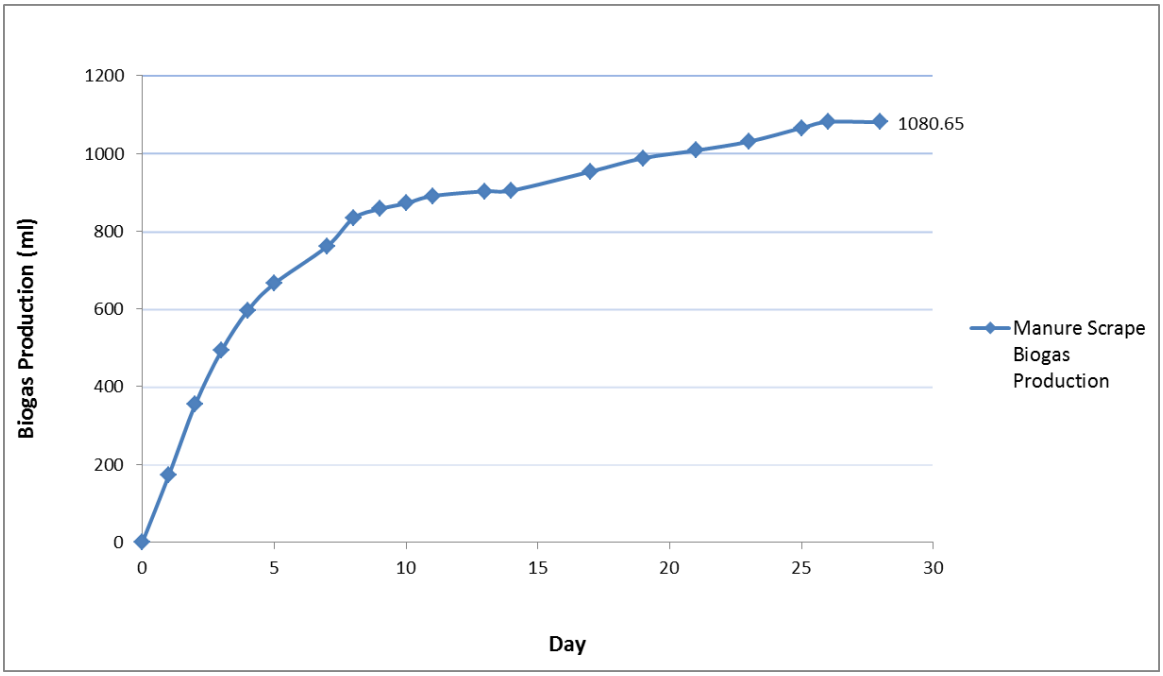

Figure 19. Methane production from manure scrape measured using the DIN method, over a 28 day period. Total methane production was 1,080.65mL.

Paper sludge produced a low volume of methane over the course of the experiment, with a total of 920mL. Average biogas production per day, peaked at 166.86mL produced between day 4 and 5. Highest amount of biogas was produced between day 0 and day 14, with a total of 884.55mL. The remainder biogas was produced in the last 14 days of the experiment. **(Figure 20)**

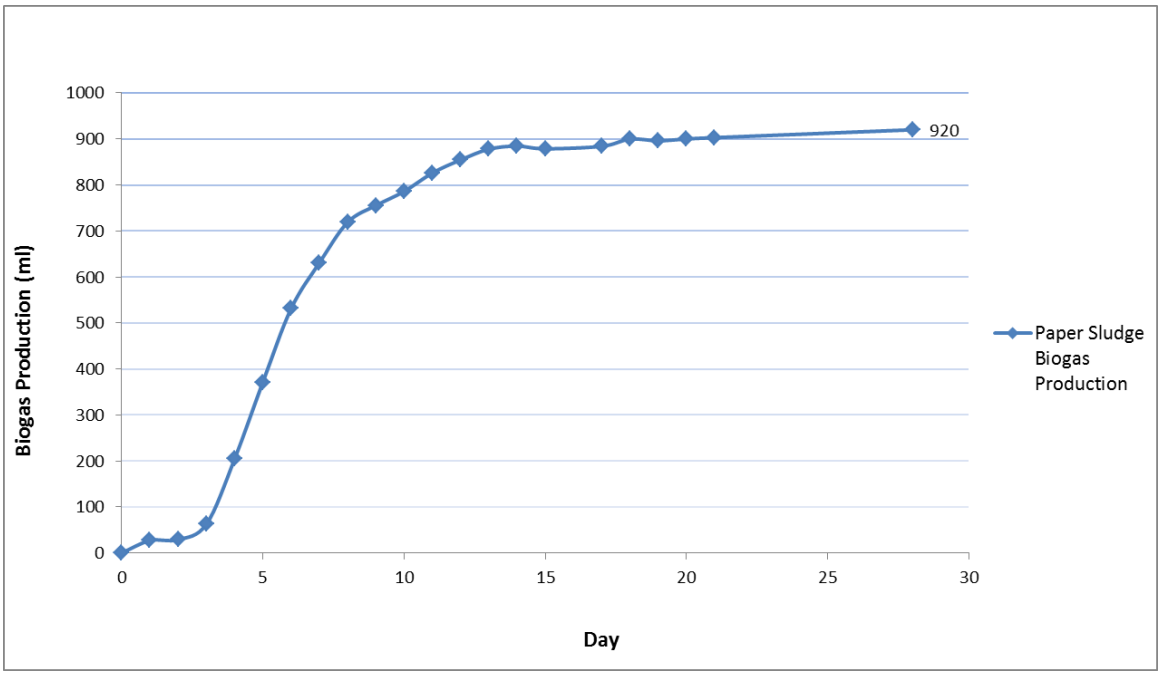

Figure 20. Methane production from paper sludge measured using the DIN method, over a 28-day period. Total methane production was 920mL.

Cyanobacterial biomass produced a low volume of methane over the course of the experiment, with a total of 64.32mL. Average biogas production per day, peaked at 58.18mL produced between day 1 and 2. Highest amount of biogas was produced between day 0 and day 2, with a total of 116.15mL. The remainder days shows a variable decline in biogas production. Normalized data with the negative control as a baseline in biogas production showed that cyanobacterial biomass had a drastic decline in biogas production after day 2. Spikes in biogas production were observed between days 5 and day 28. **(Figure 21)**

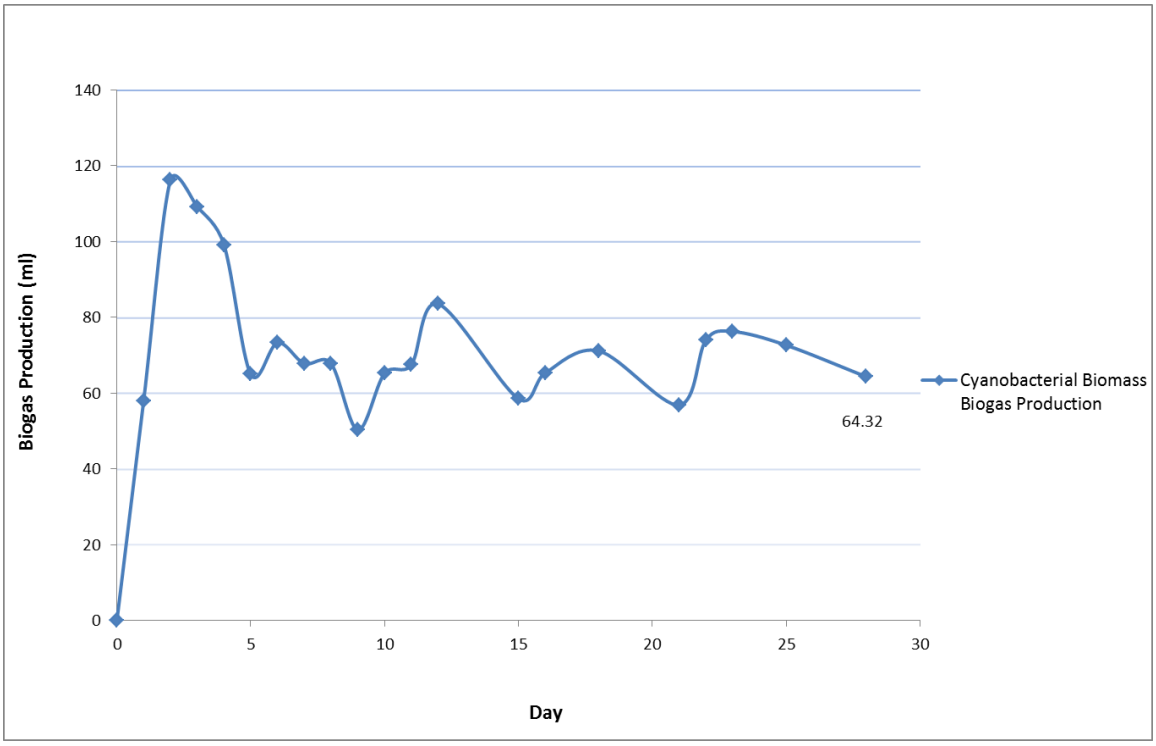

Figure 21. Methane production from cyanobacterial biomass measured using the DIN method, over a 28-day period. Total methane was 64.32mL.

Parlor water produced a low volume of methane over the course of the experiment, with a total of 248.92mL. Average biogas production per day, peaked at 73.68mL produced between day 0 and 1. Highest amount of biogas was produced between day 0 and day 9, with a total of 263.59mL. The remainder biogas was produced in the last 19 days of the experiment. **(Figure 22)**

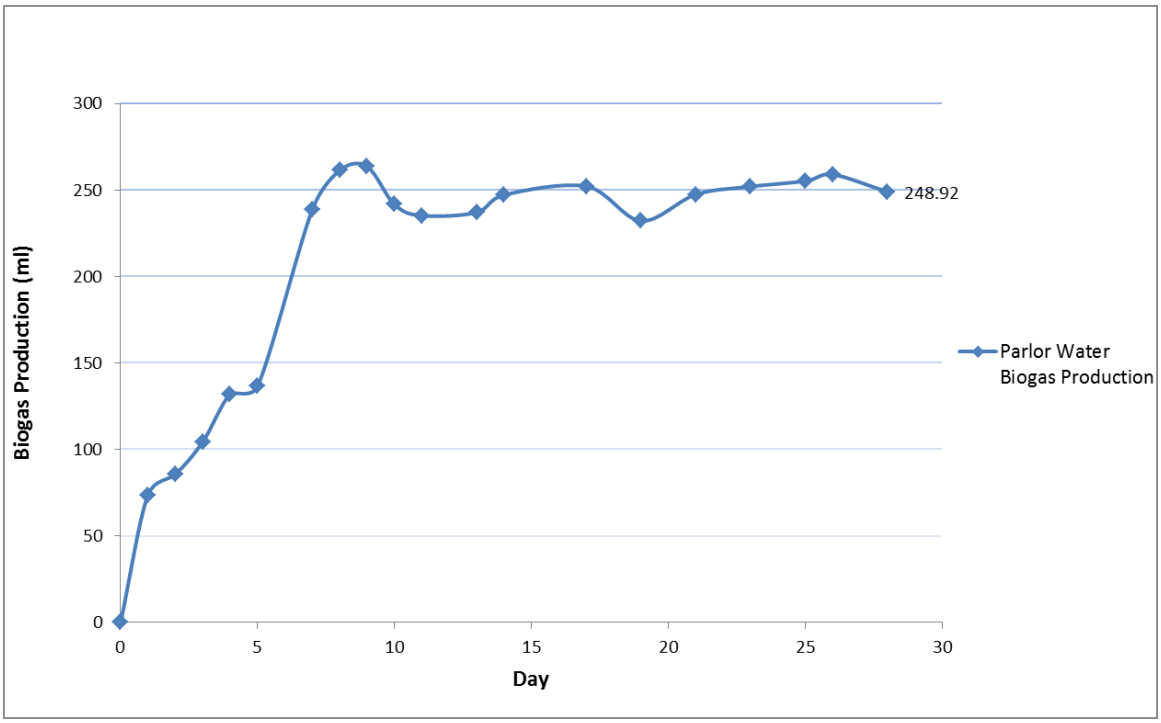

Figure 22. Methane production from parlor water measured using the DIN method, over a 28-day period. Total methane production was 248.92mL.

Overall, data collected on biogas production for the 10 feedstocks showed that organic matter rich substrates such as Smucker's jelly and lactose pellets produced a considerable amount of biogas. However, the expected correlation between trend of organic dry matter and high biogas production was not observed. Potato sludge and hot dog casings had an oDM (%FM) of 20.8% and 39.16% (%FM) respectively, however, they both had high biogas production in comparison to other feedstocks. This feedstock also had slow biogas production over the first 7 days, and production rapidly increased at day 7, until day 17. Cyanobacterial biomass and parlor water consistently produced lower

amount of biogas over the 28 days, which was expected as both feedstocks had low oDM percentage.

One of the main concerns when utilizing the eudiometers to analyze biogas potential was the use of only two eudiometer set ups for each feedstock, rather than the normal triplicate used for the AMPTS system. These readings were precise, close enough that a small standard deviation was observed. Some feedstocks showed Variation between duplicate reads, which lasted the length of the experiment. While these readings might not greatly affect the overall methane production values in a large scale, the median values used to calculate the methane total production might be affected, which in turn can affect the statistical analysis comparison between the DIN and AMPTS method. An example of this is with the feedstock "fresh straw", in which there were gas quality readings with variation between duplicates greater than 5%; these readings were noticed during the readings of the first 4 days.

Biomethane Potential Analysis Summary

Following 21-day biogas production analysis for the ten individual feedstocks using the AMPTS method, statistical analysis of the data was performed for the feedstocks. Data on biogas production at day 21 was used for comparative analysis, as suggested by the manufacturer, between the AMPTS and DIN methods. The biogas production comparison was done from day 0 to day 21, to gain insight into the possible similarities or differences between the two methods.

AMPTS summary.

Biogas production analysis for the feedstocks using the AMPTS method determined that the maximum methane production occurred with Smucker's jelly, lactose pellets and manure scrape; Smucker's jelly had the most dramatic biogas production in the initial stages of the experiment. Parlor water, cyanobacterial biomass and manure scrape had the lowest biogas production throughout the experiment. There was a stage of minimal methane production during the experiment for most substrates, between day 8 and 11; growth peaked and stabilized during these days, but then production continued at its normal rate following day 11 **(Figure 23)**.

Most substrates achieved or were close to achieving maximum biogas production, right before declining on production, by the end of the experiment; lactose pellets, paper sludge, hot dog casings, potato sludge, used bedding, manure scrape, cyanobacterial biomass and parlor water achieved or nearly achieved this in the 21 day time-span of the experiment. Smucker's jelly and lactose pellets displayed biogas production at day 21. In summary, eight out of ten feedstocks reached maximum biogas production over the 21 days. The remaining two feedstocks were close to reaching maximum biogas production (additional 2-3 day estimate), as suggested by the amount of biogas production at day 21, and as suggested by the trend line **(Figure 23)**.

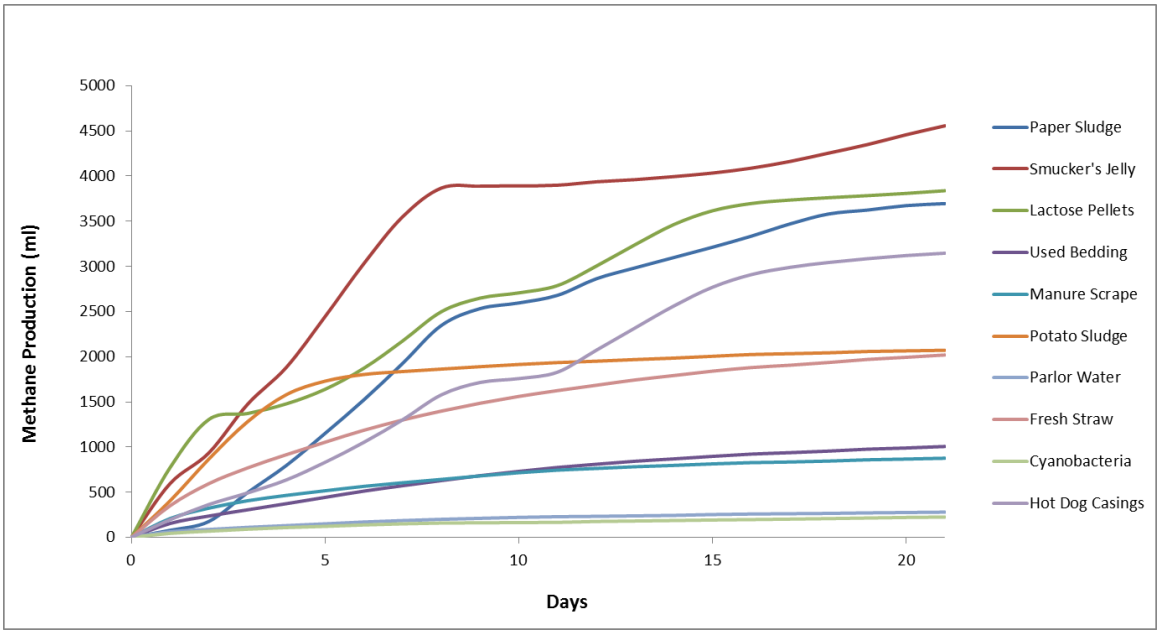

Figure 23. Biogas production analysis of ten feedstocks, using the AMPTS method, over a 21 day period.

DIN summary.

Biogas production from the ten feedstocks analyzed using the DIN method yielded a methane maximum production from hot dog casings, Smucker's jelly and potato sludge; Smucker's jelly had the highest amount of biogas production per day, during the initial stages of the experiment. Parlor water, cyanobacterial biomass and used bedding had the lowest biogas production throughout the experiment. Substrates with the highest amount of biogas produced, were characterized by high methane production during the initial stages of the experiment, between day 5 and 6. Hot dog casings however, had a low biogas daily production until day 7, following by a dramatic increase

until day 16 of the experiment, and nearly reaching maximum biogas production at day 21 **(Figure 24)**.

All substrates, except hot dog casings, achieved maximum biogas production by day 21. Smucker's jelly, lactose pellets, potato sludge, cyanobacterial biomass and parlor water's biogas production peaked by day 7. Fresh straw, manure scrape, paper sludge and used bedding's biogas production peaked by day 8 of the experiment. The hot dog casings feedstock appeared to be close to reaching maximum biogas production by day 21 (additional 1-2 day estimate). In summary, nine out of ten feedstocks reached maximum biogas production over the first 21 days of the experiment **(Figure 24)**.

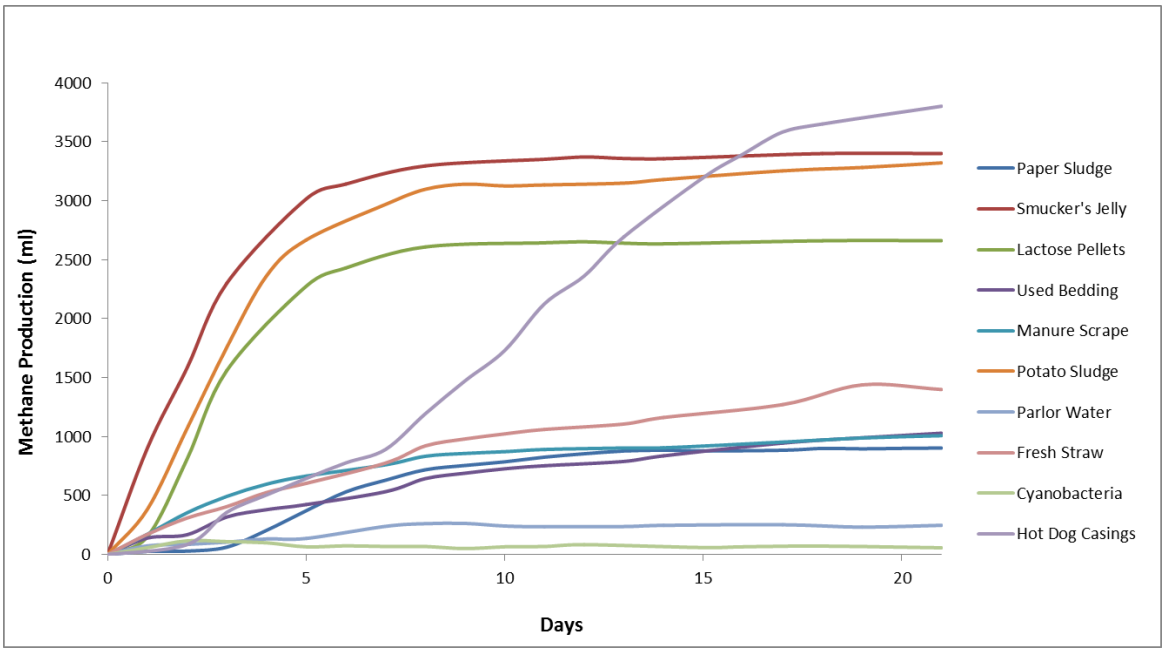

Figure 24. Biogas production analysis of ten feedstocks, using the DIN method, over a 21 day period.

Comparison of Methods

AMPTS vs DIN method analysis.

Measured biogas production from the ten different substrates. The first comparison done was based on the total biogas production. Feedstocks were ranked from 1-10, with 1 being the top biogas producer with the highest total biogas production, and 10 being the substrate with lowest biogas production. Ranking was done for both the DIN and the AMPTS methods **(Table 6).**

Table 6. Ranking of overall biogas production from the ten different substrates. The DIN method is shown in the second column, and the AMPTS method in the third column.

Substrate	DIN method	AMPTS method
Hot dog casings	1	4
Potato sludge	2	5
Smuckers jelly	3	1
Lactose Pellets	4	2
Fresh straw	5	6
Used bedding	6	7
Erving paper sludge	7	3
Manure scrape	8	8
Cyanobacterial biomass	9	9
Parlor water	10	10

Biogas production data was categorized based on three parameters; total methane produced by each feedstock **(Figure 25)**, methane production based on grams of fresh

matter added to the bioreactors (L/Kg FM), and methane production based on grams of organic dry matter present in the added sample (L/Kg oDM). This 3 step method summary was done for the DIN method at 28 days, and the AMPTS method at both 21 and 28 days.

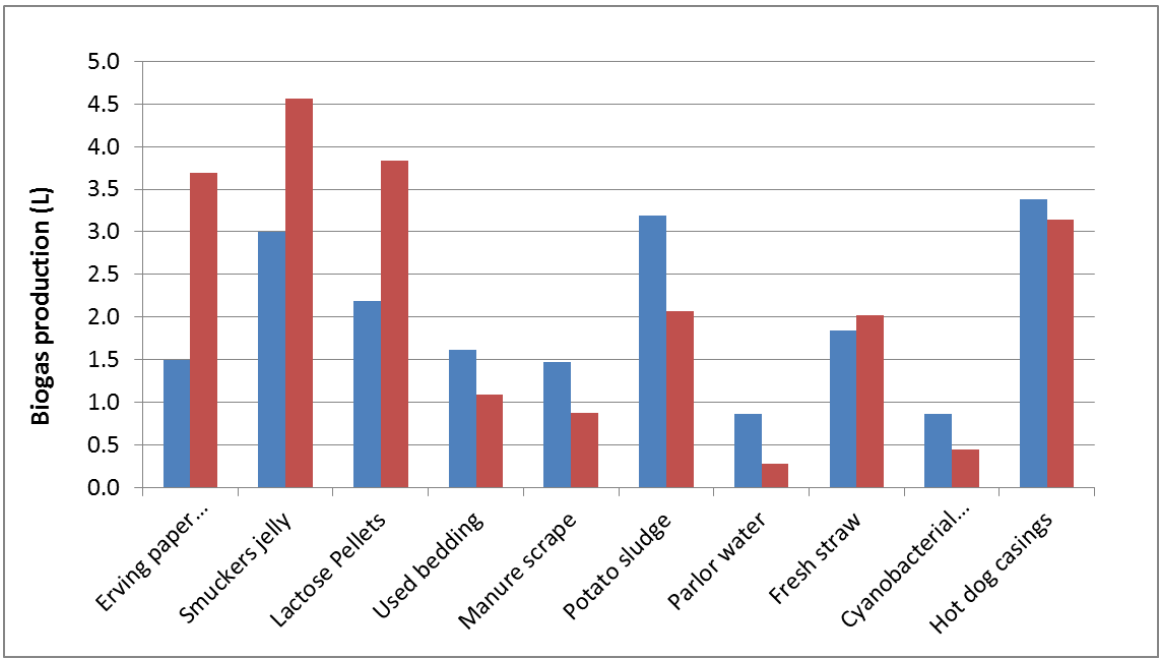

Figure 25. Biogas production (L) of ten feedstocks, using the DIN (in blue) and AMPTS (in red) methods.

DIN method – 28 day biomethane potential summary.

Biomethane potential summary for the DIN method was done at day 28. A summary on total methane produced (column 2), production per gram of fresh matter (column 3), and production per gram of organic of dry matter (column 4) was compiled

(Table 7). The biomethane potential analysis of the 10 substrates showed hot dog casings, potato sludge and Smucker's jelly as the highest total biomethane (L) producers. Parlor water, cyanobacterial biomass and manure scrape produced the lowest amount of methane (L) of the substrates. Methane production based on the amount of fresh matter added to the bioreactors provided a glimpse into the feedstock's with the highest "profitability", by providing higher biomethane production from smaller amounts of fresh sample. These data showed that Smucker's jelly and lactose pellets had the highest biomethane yields/ kilogram of fresh matter. Cyanobacterial biomass, parlor water and manure scrape produced low biomethane (L) yield/kg of fresh matter. Methane production/kg of organic dry matter was highest for parlor water (6586.23 L/Kg oDM), cyanobacterial biomass, potato sludge and manure scrape, while fresh straw produced the lowest amount of biogas production/kg of oDM (20.65 Kg/L oDM) **(Table 7)**.

Table 7. 28-day biomethane potential summary for the DIN method. Total methane production is shown in red, Methane production based on fresh matter is shown in blue, and methane production based on organic dry matter is shown in green.

Eudiometers (DIN) - 28 Day Analysis			
Feedstock	**CH4 prod (total L)**	**CH4 prod (L/Kg FM)**	**CH4 prod (L/Kg) oDM)**
Cyanobacterial biomass	0.87	8.62	298.38
Erving paper sludge	1.49	121.11	77.89
Fresh straw	1.84	237.08	20.65
Hot dog casings	3.39	232.52	85.36
Lactose Pellets	2.19	339.18	29.00
Manure scrape	1.47	46.07	142.22
Parlor water	0.86	8.60	6586.24
Potato sludge	3.19	134.13	153.31
Smuckers jelly	3.00	361.77	47.68
Used bedding	1.61	73.90	74.13

AMPTS method – 21 day biomethane potential summary.

Biomethane potential summary for the AMPTS method was done at day 21. A summary of total methane produced (column 2), production per gram of fresh matter (column 3), and production per gram of organic of dry matter (column 4) were compiled **(Table 8)**. The biomethane potential analysis for the ten substrates revealed hot dog casings, paper sludge, Smucker's jelly and lactose pellets as the highest total biomethane (L) producers. Parlor water, cyanobacterial biomass and manure scrape produced the lowest amount of methane (L) of the substrates. Smucker's jelly, lactose pellets and fresh straw were the feedstocks with highest amount of biomethane yield/kg fresh matter.

Cyanobacterial biomass and parlor water produced low biomethane (L) yield/kg fresh matter. Methane production/kg organic dry matter was higher for parlor water (2137.55 L/Kg oDM), cyanobacterial biomass and paper sludge, while fresh straw had the lowest amount of biogas production/kg oDM (22.69 Kg/L oDM) **(Table 8)**.

Table 8. 21-day biomethane production potential summary using the AMPTS method. Total methane production is shown in red, methane production based on fresh matter is shown in blue, and methane production based on organic dry matter is shown in green.

AMPTS - 21 Day Analysis			
Feedstock	CH4 prod (total L)	CH4 prod (L/Kg FM)	CH4 prod (L/Kg) oDM
Cyanobacterial biomass	0.45	4.49	154.23
Erving paper sludge	3.70	48.60	192.76
Fresh straw	2.02	104.90	22.69
Hot dog casings	3.15	77.45	79.35
Lactose Pellets	3.84	172.52	50.24
Manure scrape	0.88	31.65	84.56
Parlor water	0.28	2.79	2137.55
Potato sludge	2.07	29.12	99.57
Smuckers jelly	4.56	173.44	72.38
Used bedding	1.09	52.91	49.98

AMPTS method – 28 day biomethane potential summary.

Biomethane potential summary for the AMPTS method aslo was conducted at day 28, to better compare with the DIN methods. A summary of total methane produced

(column 2), production per gram of fresh matter (column 3), and production per gram of organic of dry matter (column 4) were compiled **(Table 9)**. The biomethane potential analysis of the ten substrates showed paper sludge, Smucker's jelly, and lactose pellets as the highest total biomethane (L) producers. Parlor water, cyanobacterial biomass and manure scrape produced the lowest amount of methane (L) of the substrates. Smucker's jelly, lactose pellets and hot dog casings were the feedstocks with highest amount of biomethane yield/kg fresh matter. Cyanobacterial biomass, parlor water, and manure scrape showed low biomethane (L) yield/kg fresh matter. Methane production/kg organic dry matter showed higher amounts of biogas production by parlor water (2303.45 L/kg oDM), potato sludge and paper sludge, while fresh straw showed the lowest amount of biogas production/kg oDM (24.12 Kg/L oDM) **(Table 9)**.

Table 9. 28-day biomethane potential summary for the AMPTS method. Total methane production is shown in red, Methane production based on fresh matter is shown in blue, and methane production based on organic dry matter is shown in green.

AMPTS - 28 Day Analysis			
Feedstock	CH4 prod (total L)	CH4 prod (L/Kg FM)	CH4 prod (L/Kg) oDM)
Cyanobacterial biomass	0.23	7.03	80.58
Erving paper sludge	3.76	49.43	196.03
Fresh straw	2.15	124.70	24.12
Hot dog casings	3.21	183.36	80.96
Lactose Pellets	4.06	203.84	53.09
Manure scrape	0.92	33.34	89.09
Parlor water	0.30	12.17	2303.45
Potato sludge	2.10	99.73	100.90
Smuckers jelly	4.70	253.79	74.68
Used bedding	1.08	52.60	49.69

According to the presented data, one would have expected low biogas producing feedstocks such as parlor water to have very low methane production per gram of organic dry matter. The production resulted at 6,586.24L of methane per kg of organic dry matter. These results seem quite large, though given that the total methane production of this feedstock was 0.87L, with such low amount of organic dry matter, the results do agree with the amount of substrate added. In theory, these low organic dry matter content feedstocks are the most productive, by being liquid, thus allowing co-digestion, and producing a sizeable amount of methane, compared to some of the solid feedstocks. Fresh straw produced a total of 1.84L of methane using the DIN method, which is a sizeable

amount of methane when compared to some of the lower producing feedstocks. However the analysis provided data regarding the low methane production per kilogram of organic dry matter, at 20.64L, which is the lowest of all feedstocks. One of the discrepancies found among the summarized data was between AMPTS 21 and AMPTS 28 day analysis. Nine out of the ten substrates had very comparable methane production for both dates. However cyanobacterial biomass had a methane production of 154.22L per kilogram of oDM at day 21, but only 80.58L per kilogram of oDM at day 28.

Statistical analysis was performed to compare the summarized data on biomethane production using both the AMPTS and DIN methods; statistical analysis was performed using the Excel data analysis software, and "R" statistics software. The first step of this comparison consisted of comparing each feedstock's raw biogas production over the period of the experiment. Data from feedstock's biogas production between the AMPTS and DIN method was compared using a two-tail T-test for all feedstocks. The two-tailed T-test suggested that seven out of 10 feedstocks' data was not statistically different. At the start of the experiment, it was not known what the results were going to be, in terms of the feedstock relationship to each other. However this data was very promising, in terms of proving the stated hypothesis.

One of the feedstocks that showed to be non-equal, between the AMPTS and DIN methods biogas analysis, with the two-tailed T-test was cyanobacterial biomass ($p = 0.0002$). Even the data regarding methane production per gram of oDM was certainly different between methods. This could be attributed to mistakes made during the

experiment, which could have affected the overall methane production of this substrate; during BMP analysis using the DIN method, some of the H_2S based medium from the eudiometers leaked into the fermentation jar during biogas quantification, which could have lowered biogas production in that specific bio-reactor, thus decreasing the average biogas quantity, and possibly affecting both the BMP summary and statistical analysis for this substrate. This can also be observed more directly in the graph for cyanobacterial biomass in the results section for the DIN method. The graph shows a great deal of variability in biogas production over the 28 days, which suggests that there was a factor that could have altered biogas production for this substrate.

The other two substrates that were not statistically similar when comparing the DIN and AMPTS methods were fresh straw ($p=0.02$) and paper sludge ($p=0.00006$). The BMP analysis for paper sludge was conducted appropriately without anything that we thought could have gone wrong, thus the results for these substrates were used to determine the validity of the hypothesis. Testing for fresh straw was done appropriately as well, and no complications were encountered during the experiment. The testing period between DIN and AMPTS methods, was of 3 months for this substrate, thus we concluded that there was a possibility of the fresh straw soaking up moisture from the refrigerator, even though it was in a re-sealable bag. This could have allowed for discrepancies in the DM% and oDM% at the time of loading the substrate into the fermentation jars. However this couldn't be tested, given that the sample was all used between feedstock analysis and DIN and AMPTS fermentation jars loading. With this in mind, confirmation of the hypothesis was done using all feedstocks but cyanobacterial

biomass. This brought us to 7 out of 9 feedstocks having statistically similar data for raw biogas production **(Table 10)**.

Table 10. Two-tailed T-test analysis of tested feedstocks, for biogas production between DIN and AMPTS methods. p-value is shown in the right column.

Feedstock	Two-tailed T-test (p-value)
Erving paper sludge	< 0.01
Smuckers jelly	0.74
Lactose Pellets	0.51
Used bedding	0.92
Manure scrape	0.26
Potato sludge	0.08
Parlor water	0.81
Fresh straw	0.02
Hot dog casings	0.81

In summary, seven out of ten feedstock's data was statistically similar, when analyzed with both DIN and AMPTS methods ($p > 0.05$); these feedstocks were Smucker's jelly, lactose pellets, used bedding, manure scrape, parlor water, potato sludge, hot dog casings. The two-tailed T-test also provided data on feedstock's that were statistically different ($p > 0.05$); these feedstocks were paper sludge, cyanobacterial biomass and fresh straw.

Further statistical analysis was performed on the compiled data from the BMP summary. The first step of this comparison was assessing whether or not there was a difference between the data for the AMPTS analysis at 21 and 28 days. A paired T-test

was done for the methane production/kg oDM at both time points using the AMPTS method. Methane production/kg oDM at day 21 and 28 days was statistically similar ($p=0.97$). This suggests that it is unnecessary to test feedstocks for 28 days, using the AMPTS method.

After confirmation that the AMPTS method did not have to run for 28 days, we compared methane production determined by the AMPTS method at 21 days and the DIN method at 28 days. There was no significant difference between the determined methane production for any of the feedstocks using the AMPTS and the DIN methods ($p=0.83$). These data suggests that the AMPTS system can be used for BMP analysis, and is comparable to the DIN method. The AMPTS method is valuable because it reduces testing costs and testing time by a half. Problems with the method can arise if malfunctioning of equipment occurs and the system is not monitored as closely as required for the DIN system. As mentioned in this study, problems also arose with DIN method equipment. Statistical comparison on biogas production gave us support for the validity of the AMPTS method for BMP analysis, however we were not sure if there was a possibility that the methods were not statistically similar, given that only 7 out of 9 feedstocks were considered statistically similar, and testing for fresh straw could have been compromised by testing practices; this suggesting that possibly a 8 out of 9 feedstock biogas similarity could have been achieved. Finally, a paired T-test confirmed the validity of the previously tested data, which suggests that the biogas data compiled with the AMPTS method is not statistically different to that of the DIN method.

SUMMARY

The main objective of this study was to assess the viability of the AMPTS system as a method for BMP analysis. Analysis of biogas production of ten feedstocks for the DIN and AMPTS methods was performed. Feedstock's BMP analysis using the DIN method was done for a 6 week period; 2 weeks of percolate incubation and 4 weeks of feedstock biogas analysis. Feedstock's BMP analysis using the AMPTS method was done for a 3 week period. Results in biogas production were analyzed, and data on biogas production per FM, DM and oDM were calculated. Summarized data suggested that low oDM feedstocks such as parlor water had low overall biogas production, but higher biogas production potential/kg oDM. On the other hand high oDM feedstocks such as Smucker's jelly and hot lactose pellets, had high overall biogas production, but lowed biogas production potential/kg oDM. Statistical comparison was performed, to find data that could support the hypothesis or not. two tailed T-tests were performed for the methane production data, using the DIN and AMPTS method, for the analysis of ten feedstocks. Cyanobacterial biomass data was not used for this statistical analysis, since data could have been compromised by laboratory analysis complications. Seven out of nine feedstocks showed no statistical difference in the biogas production between the DIN and AMPTS methods. However these results were not substantial enough to validate the AMPTS method for BMP analysis.

A paired T-test was performed on the biogas production/kg oDM data for the feedstocks. This comparison suggested that there was no difference in biogas production between the AMPTS method and the DIN method. This was relevant because it suggested that the AMPTS method is a valid method for BMP analysis, in comparison to the DIN method, which supports the stated hypothesis.

CONCLUSIONS

This project aimed to determine the viability of the AMPTS system as a method for BMP analysis, by comparing it to the standard DIN method. This was done by assessing biogas production of 10 different feedstocks with both methods, and comparing both raw data and overall methane production. Statistical analysis was used to analyze biogas production for the ten feedstocks between methods, to determine whether they were statistically similar or not. Raw data comparisons suggested that seven out of nine viable substrates were statistically similar. This suggested that there was a possibility of data supporting the initial hypothesis. Further statistical analysis was performed to further test the hypothesis. A paired T-test led to the conclusion that there was not any statistically significant difference between the DIN method at 28 days and the AMPTS method at 21 days, thus supporting the hypothesis, and validating the AMPTS method as a viable method for BMP analysis.

FUTURE PROJECTS

As a result of this project a continued approach should be to implement and develop methods that could optimize the process of biodigestion at the University of Wisconsin Oshkosh. Future projects should address the following:

- Testing of high solid feedstocks for method comparison of biogas production between the DIN and AMPTS method.
- Elimination of two-week incubation period for the DIN method. This will result in no incubation period for the percolate. Biogas production comparison between DIN and AMPTS methods to follow.
- Analysis of feedstock composition, using total organic carbon (TOC) and biochemical oxygen demand (BOD) analyses, with the aim of better understanding the role of feedstock composition in biogas production.
- Tracking of microbial species in percolate sludge, which could aid in assessing optimal cycles in the biodigester, thus lowering the time used for the biodigestion process in the anaerobic chamber.
- Genetic manipulation of both fermentative bacteria and methanogens, to increase methane yield in the biodigester.

- Assessment on the treatment of feedstock, to increase biogas production. This could be done by comparing fresh feedstock vs feedstock that has started decomposing for a few days. This could provide data on the importance of using feedstocks for biodigestion in a timely manner.

APPENDIX

BMP Equipment

Automated Methane Potential Testing System

Rubber stopper with 2 metal tubes

Plastic screw cap with hole (100 ml)

Bottle holder

Stirrer

Screw motor cap

Thermostatic water bath

Gas measuring device

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