Solid-state Anaerobic Digestion for Integrated Ethanol Production

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By

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Abstract

Anaerobic digestion (AD) is a biochemical process consisting of the microbiological conversion of organic materials for the purpose of generating biogas. Biogas is typically composed of 50-70% methane (CH_4) and 30-50% carbon dioxide (CO_2) with trace amounts of other compounds. Anaerobic digestion technology is a bioprocessing technology that has the potential to be integrated into an ethanol facility to further capture energy, in the form of CH_4, for use in a combined heat and power (CHP) generator or for integration into the natural gas pipeline grid after undergoing an upgrading process. The most simplistic design of an AD system is the solid-state digester (SSD) which is able to process very high solids content materials (greater than 15% solids). A SSD has the potential to be utilized as a manure management system in a beef cattle feedlot and it has the potential to integrate seamlessly into a combined ethanol-feedlot operation to capitalize on the eco-cluster concept in bioenergy production.

This thesis investigates the biogas and digestate composition seen from four material blends in a solid-state digester (SSD) system operated as a batch reactor. Wet distiller's grains (WDG) from a grain ethanol process and cattle manure were the substrates investigated. To assess the biogas composition the system was operated over a period of time to achieve a quasi steady state within the microbial population to maximize the CH_4 concentration. To assess the robustness of the microbial population within each substrate blend, the biogas concentrations were measured over three cycle periods where a portion of the used substrate was replaced with an equal amount of fresh substrate. The digestate

composition was analyzed at the end of each of the cycles and compared with the raw substrate to determine changes in solids and nutrient values.

The biogas production calculated in this study was 0.17, 0.21, 0.18 and 0.12 liters (L) per gram of volatile solids (VS) for the 100% WDG, 75% WDG and 25% manure, 25% WDG and 75% manure, and the 100% manure substrates (Group 1 through 4 respectively), averaged over all three digestion cycles. At the end of three cycles of digestion the biogas from Group 3 achieved a measured CH_4 concentration of 49% and the biogas from Group 4 achieved a CH_4 concentration of 59%. The substrate blends represented by Group 1 and 2 did not achieve a CH_4 concentration of significance. The duration for Group 3 and Group 4 to achieve the production of viable biogas (biogas with 50% CH_4 concentration or greater) was 100 and 90 days of operation respectively. Thus, it can be concluded that a SSD system start up duration would be between three and four months.

The gas data gathered in this research study indicates Group 3 established the most robust methanogenic culture as it had the lowest overall N_2 and CO_2 concentrations in the biogas, and the most consistent performance of CH_4 production during each cycle. The nutrient data gathered in this research supports the conclusion drawn from the gas data regarding the overall methanogenic performance of the substrate blends. The nutrient data for Group 3 maintained an average carbon to nitrogen (C:N) ratio of 25:1 over all three digestion cycles. The nitrogen, phosphorous, potassium and sulphur components of the manure fertilizer value were maintained throughout the digestion process, thus typical manure application rate calculations would be applicable when field applying digestate.

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List of Abbreviations

Abbreviations	Definition
AD	Anaerobic digestion
CDS	Condensed distillers solubles
CH_4_PC	$\%CH_4$
CO_2_PC	$\%CO_2$
DDG	Dry distillers grains
DDGS	Dry distillers grains with solubles
ECD	Electron capture detectors
GHG	Greenhouse gas
H_2_PC	$\%H_2$
JulDay	Julian Days
MSW	Municipal solid waste
N_2_PC	$\%N_2$
SSD	Solid-state digestion
TCD	Thermal conductivity detector
TS	Thin stillage
VS	Volatile solids
WDG	Wet distillers grains
WS	Whole stillage

1 Introduction

Biomass is defined in the American Heritage® Science Dictionary (2005) as "renewable organic materials, such as wood, agricultural crops or wastes, and municipal wastes, especially when used as a source of fuel or energy. Biomass can be burned directly or processed into biofuels such as ethanol and methane." Biomass-to-energy conversion technologies vary from the direct combustion of the raw product to chemical, mechanical and biological processes. The dominant biomass to energy conversion process employed in the world today is the combustion of biomass using raw wood, field and forest residues, or biomass process residues (Overend, 2002). However, due to the demand for increased environmental stewardship, greenhouse gas (GHG) reduction and improved energy efficiency, alternative biomass conversion technologies such as ethanol production, anaerobic digestion and gasification are being researched and implemented in some cases. Ethanol production using a corn based feedstock has become almost mainstream in the United States (US) with the objective to reduce the US dependence on foreign oil. According to Overend (2002), environmental technologies, especially those based on anaerobic digestion, are moving into commercialization following the large scale success of using landfill gas for power generation.

In recent history, Europe has put anaerobic digestion onto the bioenergy generation map as a process that harvests biogas from various organic feedstocks. The biogas produced is typically used for heat or electrical generation, while the effluent is typically land applied as a fertilizer supplement. The standard feedstock is commonly manure; however, other feedstock components such as excess grains and vegetables or wastes from intensive livestock operations (ILOs), abattoirs, value-added agriculture

industries (such as ethanol and biodiesel production) and food processing industries, as well as municipal solid wastes (MSW), can be used. In many cases the feedstock is a combination of different biowastes.

Anaerobic digestion (AD) is a biochemical process consisting of the microbiological conversion of organic materials, in the absence of oxygen, for the purpose of generating biogas. AD is a technology pioneered in waste water treatment facilities, but it is now being implemented in various agricultural enterprises such as ILOs involving dairy, hog and poultry production which have a waste stream with a higher solids content than that of wastewater (Neves, et al., 2005). Biogas, the desired product of an AD system, results from methanogenic bacteria metabolizing biodegradable materials in the anaerobic system. Biogas is typically composed of 50-70% methane (CH_4) and 30-50% carbon dioxide (CO_2) with trace amounts of other compounds, and can be used to generate heat and/or electricity.

While the core AD technology is well proven, there are various designs of anaerobic digesters for agricultural operations, which range from simple to sophisticated (Anozie et al., 2005). The digester designs can be classed into four types applicable for use with agricultural biomass. These digester types are the Solid-state Digestion (SSD) System, the Covered Lagoon Digester (CLD), the Stirred Tank Reactor (STR) that is either a continuous flow or a batch fed system, and the Plug Flow Reactor (PFR). Deublein and Steinhauser (2008) note that single stage AD systems have been installed most successfully in agriculture operations.

The type of reactor used within the AD process is dependant on the biomass, or substrate, fed into the system. Waste water facilities and ILOs typically implement a

liquid digester system, like a CLD or STR, due to the nature of the feedstock. However, industries with a more solid waste stream, such as cattle feedlots, are interested in digesters that can handle the waste streams in its natural state. The most simplistic design of an AD system is the SSD system which is able to process high solids content materials (greater than 15% solids) without agitation (Li et al, 2011). A SSD system has the potential to be utilized as a manure management system in a beef cattle feedlot where there is an abundance of feedstock in a solid form. As well, due to the potential to utilize wet distillers grains (WDG) from a grain ethanol facility as a feed supplement in beef cattle production, new ethanol facilities are being proposed coupled with beef cattle feedlots to maximize the revenue from each agri-venture. The inclusion of an AD system with an ethanol facility paired with a beef cattle feedlot is of interest, as a SSD system has the potential to integrate seamlessly into an ethanol and feedlot operation. Because SSD is typically a batch system, it involves the movement of solid material into either an earthen pit or fabricated container that can be designed to utilize equipment already being employed at the ethanol facility. As well, there is the potential to enhance the manure nutrient source to offer improved fertilizer value to local agricultural and horticultural operations.

The focus of this research study was to investigate the biogas and digestate composition from four material blends in a solid-state digestion (SSD) system operated as a batch reactor. Feedlot cattle manure (a blend of manure and bedding) and WDG from a grain ethanol process paired with a beef cattle feedlot were the substrates investigated. To assess the biogas composition the system was operated over a period of time to achieve a quasi steady state within the microbial population to maximize the CH_4 concentration in

the biogas. To assess the robustness of the microbial population within each substrate blend the biogas concentrations were measured over three batch cycle periods. At the end of the first cycle, a portion of the used substrate was replaced with an equal amount of fresh substrate. To determine the volume of biogas produced during each cycle the reduction in mass within each digester was measured and converted to gas volume. The digestate composition was analysed at the end of each of the three cycles and compared with that of the raw substrate to determine changes in solids and nutrient values and to assess the potential use of the digestate material as a supplemental fertilizer.

2 Production of Biogas from Agricultural Wastes

Biogas can be produced through the use of an anaerobic digestion (AD) system as a secondary wastewater treatment process (Neves, et al., 2005). However, over the last two decades, AD systems have been used in the management of biowastes from agriculture and agri-industries that produce waste with a higher solids content then wastewater (Neves, et al., 2005). Biogas production through AD in agriculture circles is generally synonymous with manure management. However, scientific advancements in AD technology have expanded the agricultural biomass available, which increases the potential biogas production levels. Crop residues from farming currently represent a large, unexploited source of energy in the form of biomass (Svensson, et al., 2006). In addition to biogas production, the availability of the nitrogen in the biomass is enhanced due to microbial metabolism and reduced emissions to the air and water (Svensson, et al., 2006). **Figure 1** depicts the range of biogas production potential of various biomass products available for use in anaerobic digestion processes in Canada. The data in **Figure 1** were adapted from the Government of Alberta Agriculture and Rural Development's website "Biogas Energy Potential in Alberta" (://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex11397#more).

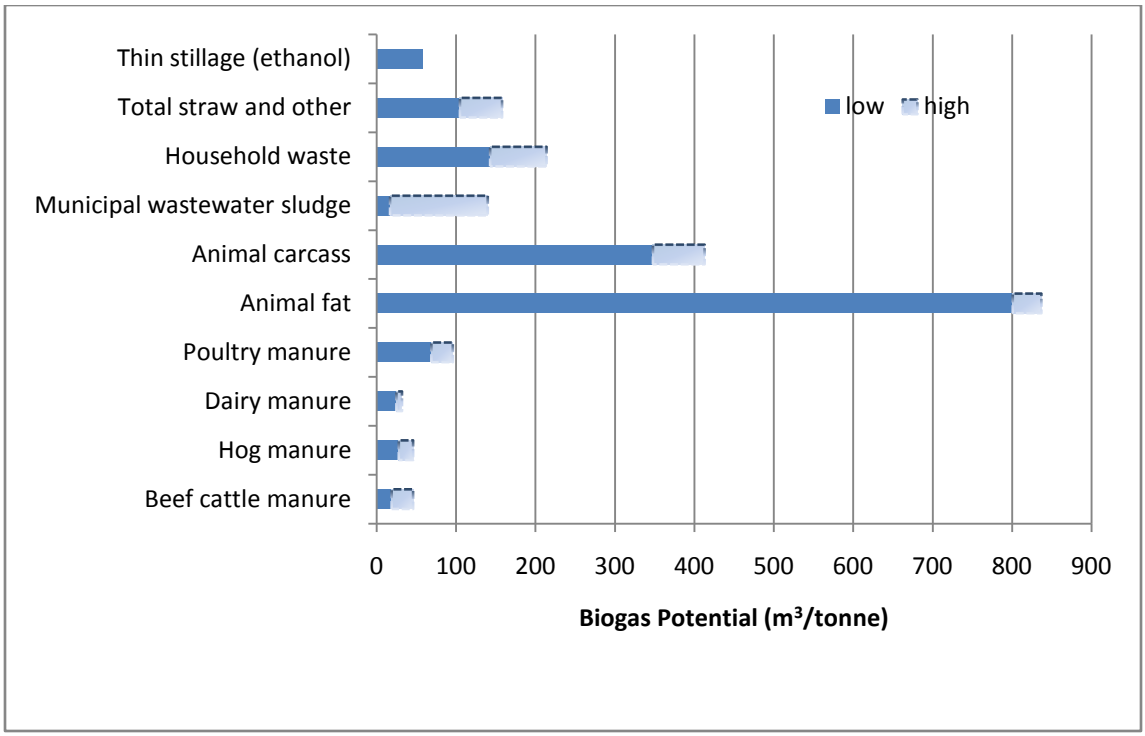


Figure 1. Biogas potential of organic residues

2.1 Canada's Green Advantage

Canada is home to approximately 0.5% of the world's population and responsibly manages 7.0% of the world's arable land mass and 10% of the world's forested area. (Barclay, 2007). There is an abundance of biomass from agriculture, forestry and food processing industries available in Canada with which to explore bioenergy production. Canadian energy requirements are currently being met using fossil fuels (natural gas, oil and coal), nuclear technology and a portion of renewable resources. **Figure 2** represents the contribution to Canadian energy requirements from each resource platform as of the 2007 calendar year.

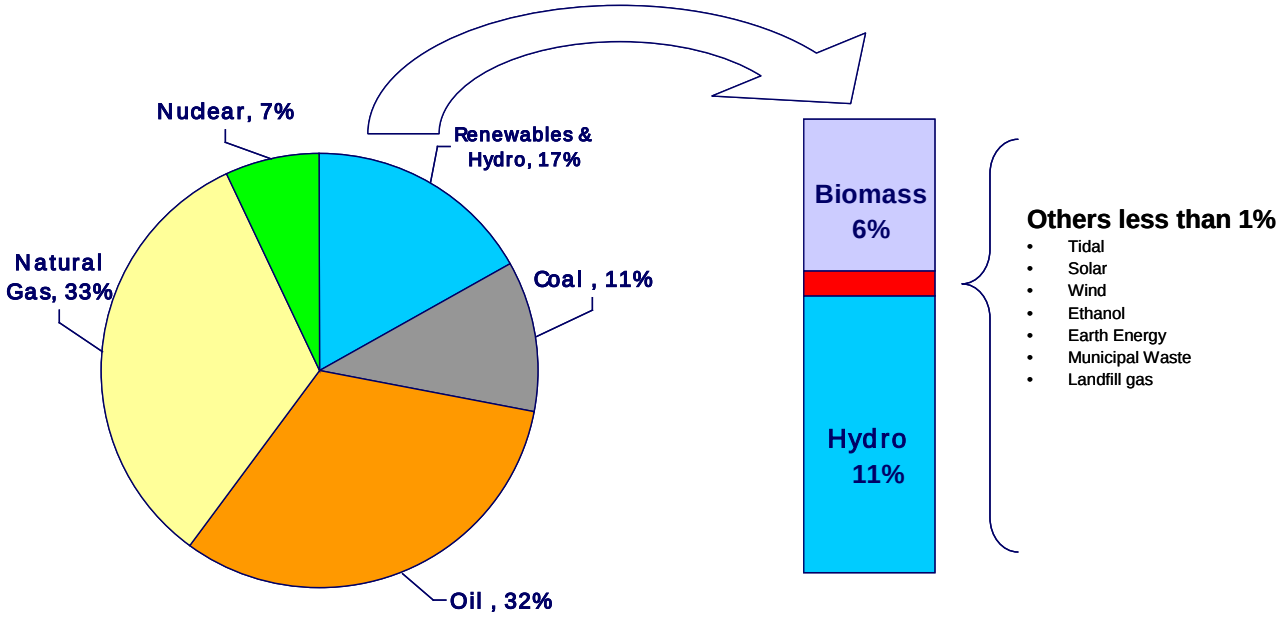


Figure 2. Canadian energy supply (Barclay, 2007, used with permission)

The figure above indicates that 6% of the current energy consumption of the Canadian population is provided by biomass. As a member of the Canadian Biomass Innovation Network (CBIN), Natural Resources Canada (NRCan) is actively promoting and involved in research pertaining to integrated biorefineries, biomass supply and logistics as well as biomass conversion technologies. One of the conversion technologies actively supported is that of anaerobic digestion. As of 2007 there were approximately 10 anaerobic digestion sites integrated, or proposed to be integrated, into farming operations in Canada. **Figure 3** illustrates the location of the 10 farm based anaerobic digester sites.

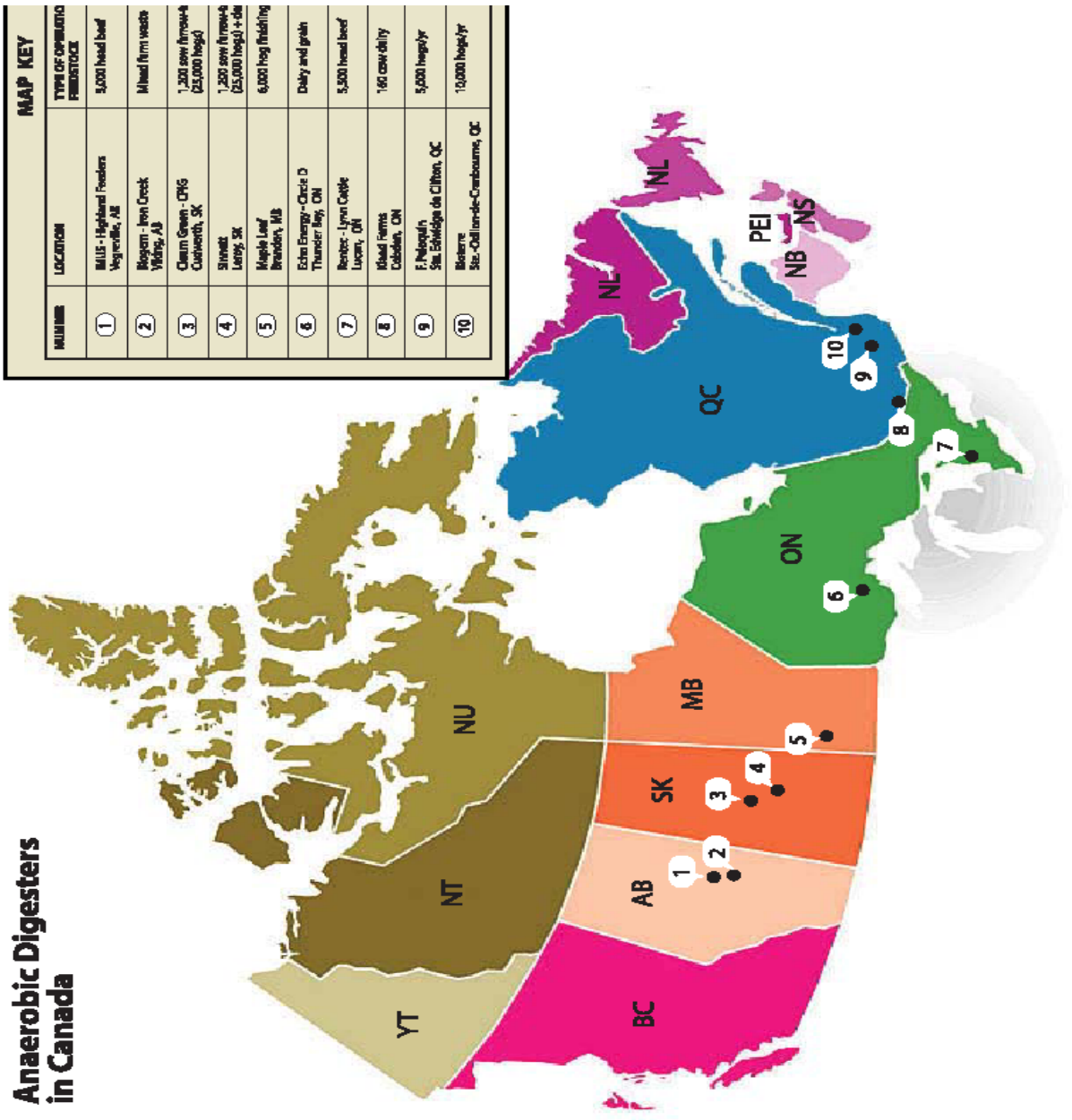


Figure 3. Farm based anaerobic digesters in Canada (Barclay, 2007 used with permission)

Overall there are approximately 100 sites in Canada using some form of anaerobic digestion technology to produce biogas. According to Barclay (2007), of the biogas production sites in Canada over 50 are situated on landfills, more than 25 have been integrated into municipal waste water treatment facilities, one site uses MSW to produce biogas, one production site has been integrated with a pulp and paper facility, and a number are with various food and beverage industries. For the farming community Natural Resources Canada acknowledges anaerobic digestion as an alternative manure management option for intensive livestock industries and considers it an exploratory option for the integration of renewable energy on farm in their April 2008 release of the following resource link .farm-energy.ca/IReF/. More than two thirds of the manure produced in Canada is solid manure (Statistics Canada, 2006), thus there is an abundant feedstock (greater than seven million tonnes of material) to explore solid-state anaerobic digestion as an alternative manure management option.

3 Literature Review

The literature reviewed defines anaerobic digestion (AD) and explores the AD research status of solid substrate materials including those materials of interest in this research report: solid beef cattle manure and WDG from an ethanol-feedlot operation. The literature reviewed also investigates the microbial potential of substrate materials used in solid-state AD.

3.1 What is Anaerobic Digestion?

Anaerobic digestion is a biochemical process consisting of the microbiological conversion of organic materials in a pH neutral and temperature controlled environment without oxygen, for generating methane. In comparison to an aerobic process, an anaerobic process is slow, usually involving a residence time of 30 to 60 days, and involves very complicated microbiology and biochemistry (Shuler and Kargi, 2002).

Temperature is a key process parameter in biogas production, and influences the design of an anaerobic digester using agricultural biomass. Biogas can be produced in temperature conditions that are psychrophilic (-10ºC to 30ºC), mesophilic (20ºC to 50ºC) and thermophilic (50ºC to 75ºC) (Parker et al. 2002, Shuler and Kargi 2002, Deublein and Steinhauser 2008). According to Parker et al. (2002), most anaerobic digesters are designed to operate in either the mesophilic or thermophilic temperature range to maximize biogas yield. The importance of system stability, hydraulic retention time of the substrate, solids retention time and pathogenic bacteria destruction will determine if a mesophilic or thermophilic process temperature is targeted. The process pH, operating pressure, and biogas quantity and quality are monitored, along with temperature, to maintain the stability of the system and to promote a healthy population of the methane

producing bacteria. Regardless of the operating temperature, the process of AD generates two main products. These products are digested organic material and biogas. The digested organic material, or effluent, is typically referred to as digestate and is comprised of solids that are applicable as a nutrient rich soil amendment material and liquor that has a high phosphorous and nitrogen value and can be applied as a nutrient rich liquid fertilizer. Biogas is a mixture of methane (CH_4) and carbon dioxide (CO_2) with trace amounts of other compounds, which can be used to generate heat and/or electricity. The typical biogas composition ranges, irrespective of substrate used in the AD process, are noted in **Table 1** (FAO, 1996, Demirbas and Ozturk, 2005, Deublein and Steinhauser, 2008).

Table 1. Typical composition of biogas

Substance	Formula	Percentage (%)
Methane	CH_4	50 – 80
Carbon Dioxide	CO_2	20 – 50
Hydrogen	H_2	5 – 10
Nitrogen	N_2	1 – 2
Water Vapour	H_2O	0.3
Hydrogen Sulphide	H_2S	Traces

The H_2S composition of the biogas can fluctuate between levels of less than 20 parts per million (ppm) to over 2,000 ppm depending on the stability of the AD system (Shuler and Kargi, 2002). The stability of the AD process is maintained by keeping the various stages of the digestion process in harmony. There are three key stages to the AD process: hydrolysis, fermentation and methanogenesis. Shuler and Kargi (2002), noted the major biological reaction steps involved in a typical AD process can be represented as follows (**Figure 4**):

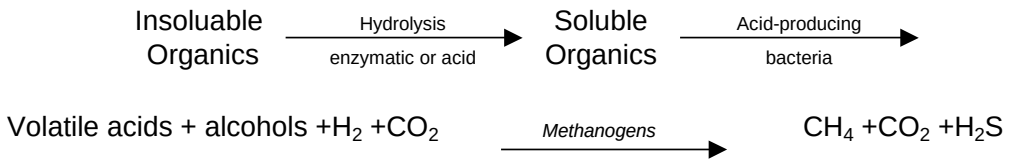


Figure 4. Typical AD process as noted by Shuler et al. (2002) (redrawn)

Li et al. (2011) represented the major biological reactions steps involved in anaerobic digestion as shown in **Figure 5**.

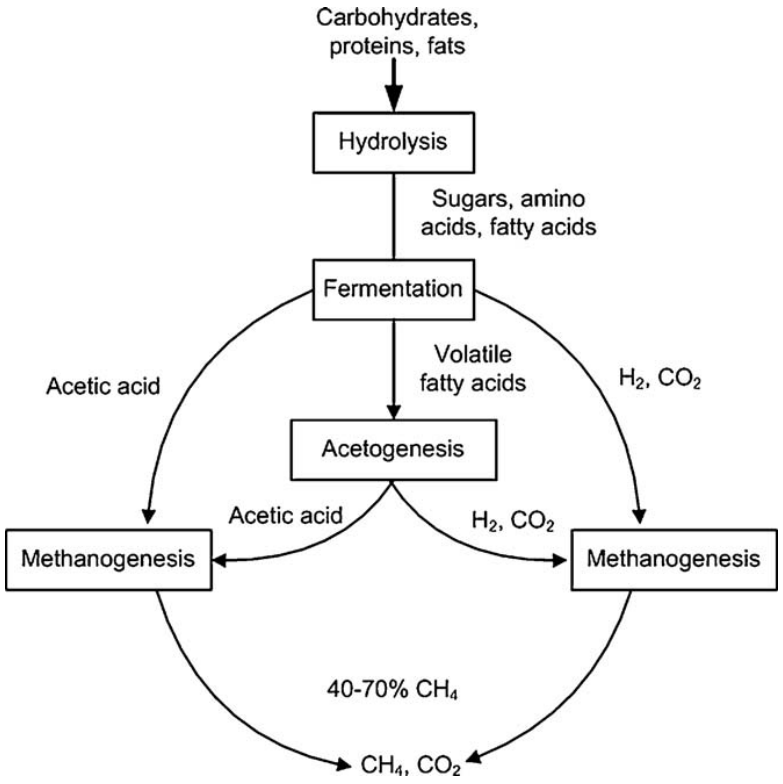


Figure 5. Process flow of the degradation of organic material through an AD (Li et al. 2011) (redrawn)

In the reaction sequences depicted above, the rate limiting step is either the hydrolysis of insolubles or methane production from volatile acids and is determined by the choice of substrate material. The hydrolysis stage of the digestion process can be completed

separately from the fermentation and methane production stages. However, in most anaerobic digesters designed for agricultural use, all three stages of the process are carried out in a single vessel known as single-stage AD.

3.1.1 Hydrolysis

Hydrolysis is also termed the solubilisation of insoluble organics and is the first step in the AD process (Shuler and Kargi, 2002). Organic material, like agricultural biomass, is converted from complex carbohydrates, fats and proteins into simple soluble compounds such as fatty acids, amino acids and monosaccharides that are used by bacteria during the fermentation process (Metcalf and Eddy, 2003). Hydrolysis is accomplished by acid or natural enzymes. The acids or enzymes break down the complex carbon chains in the organic biomass. Li et al. (2011), described hydrolysis as the process that utilizes extracellular enzymes to reduce complex organic polymers to simple soluble molecules. The simple soluble molecules were noted as amino acids, long chain fatty acids, and sugars, respectively. Hydrolysis is an essential part of the AD process because the complex organic material can not readily be digested by microorganisms without the completion of hydrolysis. Li et al. (2010) referred to hydrolysis as a critical rate limiting step in an AD process as this step determines the conversion efficiency of the biomass feedstock. Lakaniemi et al. (2010) investigated different hydrolysis processes in their research into the use of solid reed canary grass in the production of hydrogen and methane via an anaerobic digestion process to assess gas yield improvements. It was found that some process separation of the hydrolysis stage of digestion did improve the hydrogen and methane yields. According to Deublein and Steinhauser (2008) and Shuler and Kargi, (2002) operating a two stage digester allows for the first stage, hydrolysis, to

operate at a slightly lower pH (pH=4-6) than what is optimal for methane forming microorganisms (pH=6.7-7.8).

3.1.2 Fermentation

Fermentation is also referred to as a two stage process of acidogenesis and acetogenesis. This stage of digestion involves the formation of volatile fatty acids by fermentative bacteria using the hydrolysed organic compounds (Li et al., 2011). Anaerobic bacteria, known as acid producing organisms, metabolize the hydrolysed organic compounds to form volatile organic acids such as acetic, butyric, formic and propionic acids as well as short-chain fatty acids, H_2 and CO_2. The formation of alcohols during fermentation takes place to a lesser extent (Shuler et al., 2002). According to Li et al. (2010), the organic acids are further converted to acetate, H_2 and CO_2 using acetogenic bacteria. Fermentation reaction products such as volatile acids, H_2 and CO_2 are direct components of methane production during the final stage of anaerobic digestion (Metcalf and Eddy, 2003; Li et al., 2011).

3.1.3 Methanogenesis

According to Li et al. (2010), many AD studies focus on methanogenesis due to its sensitivity to feedback inhibition by acidic intermediates. Methanogenesis results in the formation of methane and is completed by a group of microorganisms known collectively as methanogens. These microorganisms convert the volatile acids and alcohols into CH_4 and CO_2, along with trace amounts of compounds like H_2S and water vapour (Metcalf and Eddy, 2003). Methanogenic bacteria are strictly anaerobic and can be comprised of *Methanobacterium* (non-spore-forming rods), *Methanobacillus* (spore-

forming rods), and *Methanococcus* and *Methanosarcina* (a cocci group) (Shuler and Kargi, 2002).

3.2 Current Research

Rosentrater et al. (2006) presented the potential of integrating an AD system into a corn based ethanol facility. It was found that AD technology for the production of methane was being managed successfully using a variety of organic feedstocks and food processing wastes, but this technology was not being brought into the ethanol industry. The objective of the study by Rosentrater et al. (2006) was to assess the potential of ethanol manufacturing co-product streams such as whole stillage (WS), thin stillage (TS), dry distillers grains (DDG), condensed distillers solubles (CDS), and dry distillers grains with solubles (DDGS), for use as feedstocks for AD. Wet distillers grains (WDG) were not used as a substrate for digestion in this study. Rosentrater et al. (2006) found that CDS was the most promising digestion feedstock producing 0.187 L CH_4/g sample; however, WS and TS produced acceptable levels of methane at 0.090 L and 0.066 L CH_4/g sample respectively.

The United States has seen exponential growth of the ethanol industry in the past five years in their mandated effort to reduce their dependence on foreign oil (Ileleji, 2010). With the growth in the ethanol industry there has naturally been an increase in the amount of ethanol byproducts. According to Rosentrater et al. (2006), these ethanol byproducts formulate a high protein organic feedstock that has the potential to contribute to value-added development within the ethanol facility and other sectors of bioprocessing. Morey et al. (2006), also noted that ethanol co-products from a dry grind ethanol facility, particularly DDGS, contain enough energy to provide for the electrical

and thermal requirements of the ethanol facility as well as generate additional revenue for the facility through the production of excess power. Morey et al. (2006), investigated biomass gasification and combustion technology focusing on the characterization of biomass from a dry grind ethanol facility using corn, as well as corn stover, and associated air emissions. At the time of the investigation conducted by Rosentrater et al. (2006), no studies had yet examined the feasibility of pairing an AD process with an ethanol production facility to utilize some of the ethanol co-products for the purpose of biogas energy generation.

The lack of investigation into an AD system integrated with an ethanol facility is primarily due to the nutrient value associated with WDG, DDG, and DDGS as animal feed. Cromwell et al. (1993) noted that DDGS has long been recognized as a valuable source of energy, protein, water-soluble vitamins and minerals for animals. Since the initial spark of the ethanol industry due to the rising cost of crude oil during the 1970's, there has been over two decades of research conducted on DDGS as a protein source for domestic animal production (Rosentrater et al. 2005). The climb in crude oil prices at the onset of the 21st century coupled with the environmental awareness associated with fossil fuel use and greenhouse gases has renewed interest in ethanol production as a green fuel. This renewed interest in ethanol has sparked an increase in associated ethanol co-product utilization research. The marketability of the co-product streams have the potential to significantly contribute to the profitability of the ethanol facility. As energy costs continue to rise the use of co-products like WDG, DDG, or DDGS as animal feeds becomes secondary to alternative uses, such as energy generation (Rosentrater et al. 2006).

An AD system is one biotechnology available to utilize ethanol co-products to provide alternative energy revenue or offset energy use as part of ethanol production. With an AD system there is the primary target product of biogas for the purpose of generating energy as well as the co-product of digested biomass that has nutrient value to agriculture and horticulture operations as a fertilizer supplement. As mentioned earlier, there are a number of different AD system designs that are available to complete the microbial conversion of the ethanol co-products to biogas; one example is the SSD. The SSD design is of particular interest if the ethanol facility has been paired with a beef cattle feedlot to maximize WDG usage and minimize the ethanol facilities cost of drying and transporting DDGS.

A SSD is a reactor designed for a substrate composition that ranges from a minimum of 15% to over 50% solids content by volume, and does not involve any mechanical agitation during the digestion process. Parker et al. (2002) conducted anaerobic digestion laboratory and field experiments using beef cattle manure from feedlots. The laboratory experiments were conducted using solids contents of 20, 30, 40 and 50% and a system temperature of 21^{o}C. The field experiments were conducted using solids contents of 40% and 50%. The laboratory experiments were conducted in 125 mL glass Erlenmeyer flasks where the volume of biogas was recorded over the duration of the 475-day project. It was observed that the 50% solids produced little biogas; whereas, the 20, 30 and 40% total solids blends saw gas productions of 0.18, 0.21 and 0.19 L per gram volatile solids (VS) respectively.

The design of the field reactors in the field experiments conducted by Parker et al. (2002) included two 90 m^3 unheated earthen pits lined on top and bottom with

geomembranes to capture the biogas. Biogas produced within these pits had a methane content of 52% to 60% and the biogas production was recorded for 300 days before it tapered off and eventually ceased after 450 days. The volume of biogas produced in the earthen reactors was reduced during winter months. Parker et al. (2002) recommended an operating temperature in the middle of the mesophilic temperature range for improved performance with a high solids anaerobic digester using cattle manure. Parker et al. (2002) also recommended that heat be provided to the digester during cooler months to ensure the target temperature and biogas production performance was maintained.

Demirbas (2006) investigated the potential for biogas production for manure and straw mixtures in slurry. The author focused on wheat straw wastes (WSW). The batch experiments were conducted using four 1800 mL bottle reactors. A 1200 mL mixture of substrate slurry and an inoculum of active digester slurry were used in each of the reactor blends and the system was maintained at a mesophilic temperature of 35^{o}C. The VS in the reactor blends were monitored and assessed based on four main components: lipid, protein, lignin and carbohydrate. Each substrate blend investigated was digested in triplicate. Demirbas (2006) noted that the VS in manure contained higher amounts of protein and lipid (23.2%) than wheat straw (5.8%); whereas, wheat straw contained higher amounts of carbohydrates and lignin (78.2%) than manure (75.1%). The C:N ratio of wheat straw was measured (approximately 90) as compared to the targeted C:N ratio of 25-35 for optimal anaerobic digestion. Therefore; nitrogen addition was required in this case to improve the anaerobic digestion of wheat straw. Deublein and Steinhauser (2008) report an acceptable C:N ratio range for anaerobic digestion of 16:1 – 25:1. Deublein and Steinhauser (2008) note that methane formation in the digestion process

requires little nutrients as there is little biomass created. They do note however that a C:N ratio too low can cause ammonia build-up which will inhibit methane production, and a C:N ratio too high results in a lack of nitrogen which inhibits the metabolism of the methane producing organisms. Dembiras (2006) documented methane concentrations in the biogas of between 73% and 79% in the runs over a 30-day run cycle, with the balance of the biogas being primarily CO_2. It was observed in this study that the parameters that had the greatest influence on biogas production were retention time, substrate, pH and total solids concentration in the slurry. Dembiras (2006) noted that approximately 80% to 85% of the biogas was produced in the first 15 to 18 days of a 30-day digestion period using a manure substrate. Dembiras (2006) concluded that the retention time could be designed accordingly to reflect the 80 to 85% biogas production time factor. Other factors that influenced the rate and amount of biogas produced were water/solids ratio, C:N ratio, mixing method and particle size.

Zhang et al. (2003), in their study of nutrient requirements of methane producing bacteria, found that if the substrate had an acceptable C:N ratio and sufficient sulphur to promote growth, the cell density of the methanogens and the methane production rate were only limited by the concentrations of Mg and Fe in the anaerobic system. Shuler and Kargi (2002) also noted that K^+, along with other metal ions (Na^+, Ca^{2+}, Mg^{2+}), can work to stimulate anaerobic microorganisms at low levels (< 100 g/L); however, they are known to be toxic to microorganisms in anaerobic digesters at high concentrations (>1000 mg/L). Shuler et al. (2002) explained that potassium (K^+) is a cation required by microorganisms in carbohydrate metabolism and is a cofactor for some enzymes. In

acting as a cofactor for some enzymes, K^+ works to speed the biochemical reactions with which it is involved.

In Germany, a company by the name of BEKON designed and built a pilot scale solid-state digester using municipal organic waste as the input substrate (Lutz, 2005). This digester was mesophilic in design, operated at a target temperature of 37^{o}C, and was comprised of four above ground concrete reactors that were heat traced. The reactors enabled a single stage digestion process producing a biogas containing 60% methane over a 28 to 35 day retention time. Due to the size of the SSD and the nature of the digestion process, percolation liquid was collected near the door of the reactors and pumped to a storage tank to be redistributed over the substrate material in the digester as a method of moving the microbial colony through the system. The transfer of the percolation liquid works to maximize the surface contact between the substrate material and the methanogenic bacteria (Luntz, 2005). The biogas continuously supplied a combined heat and power (CHP) generator that provides electricity to the local power grid and heat to the reactors.

BioFerm Energy Systems is another German company that is promoting SSD technology. The BioFerm system is a batch process where individual digesters are loaded with substrate material and operated on a 28-day cycle. The digesters are operated at a mesophilic temperature of 40^{o}C. Leachate liquid is collected and distributed over the biomass pile at regular intervals to transfer essential micronutrients and microorganisms to maximize biogas production (BioFerm, 2011).

3.3 Microbial Potential

The biochemistry and microbiology of anaerobic digestion (AD) are very complicated. There is virtually a microbial medley of bacterial cultures found within an AD process. These cultures support a symbiotic relationship to facilitate the required biochemical reactions to achieve the desired product of biogas. Nagamani and Ramassamy (1999) focused on the microbial diversity in AD systems designed around cattle manure and the microbial interactions within these systems. **Figure 6** represents the microbial interactions noted by Nagamani and Ramassamy (1999) with the various microbial populations found in the AD process being distinguished by Groups 1 through Groups 5.

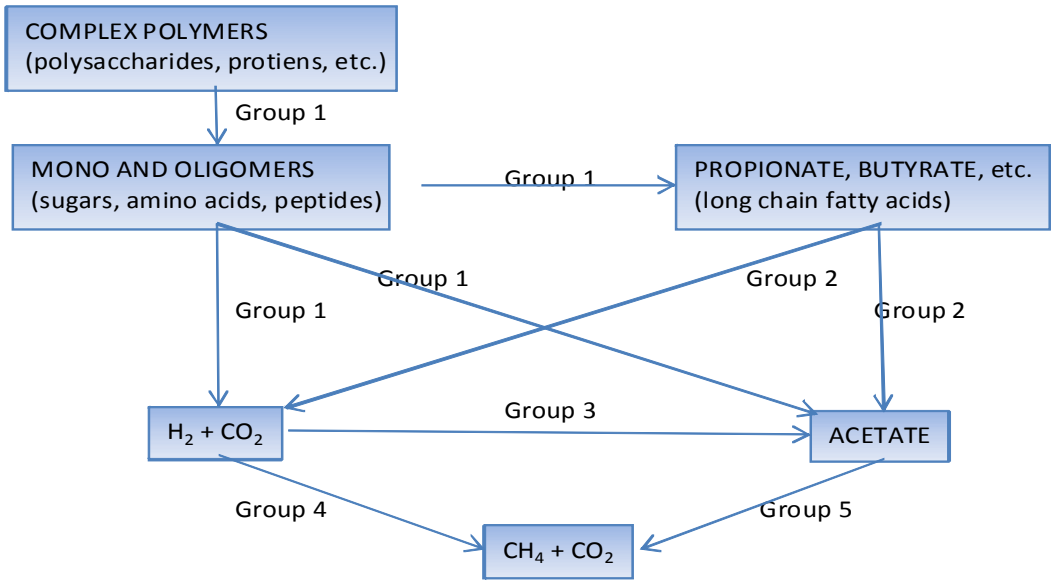


Figure 6. Biomass breakdown via microbial interactions in AD as noted by Nagamani and Ramassamy (1999) (redrawn)

The hydrolysis process of breaking down complex polymers into soluble products is conducted by Group 1 bacteria. Group 1 bacteria are fermentative bacteria producing

enzymes to breakdown the organic material. The microbial investigation conducted by Nagamani and Ramassamy (1999) found that over seventeen fermentative bacteria species have been reported to play an important role in the production of biogas. The dominant microorganism in the digester was found to be dependent on the choice of substrate. Deublein and Steinhauser (2008) note that the first two phases of degradation within the digestion process are dominated by the species *Clostridium, Ruminococcus*, *Eubacterium* and *Bacteroide*. A study conducted by Wang et al. (2009) supported the notion of microbial diversity within an anaerobic digestion system being dependant on substrate. Wang et al. (2009) investigated the bacterial communities within digesters containing grass silage, oat straw and sugar beet tops co-digested with cattle manure, and concluded that the bacterial communities within each of the three reactors differed. Nagamani and Ramassamy (1999) reported that *Ruminococcus flavefaciens, Eubacterium cellulosolvens, Clostridium cellulosolvens, Clostridium cellulovorans, Clostridium thermocellum, Bacteroides cellulosolvens* and *Acetivibrio cellulolyticus* were some of the predominant fermentative bacteria present in digesters fed with cattle manure. These are Gram-positive eubacteria strains that adhere to the substrate prior to going through extensive hydrolysis to promote the breakdown of the cellulose, hemicellulose and lignocellulosic material, as well as pectin, in organic biomass. These bacteria are commonly found in the digestive tract of ruminant herbivores, such as cattle, and are transferred into the AD process through the use of the cattle manure as an inoculant. According to Pettipher and Latham (1978) the degradation of the plant cell wall in rumen was dependent on the function of *Ruminococcus* and *Bacteroides* species, which are two of the species noted by Nagamani and Ramassamy (1999). Li et al. (2010) also noted

that cellulolytic bacteria such as *Cellulomonas*, *Clostridium*, *Bacillus*, *Thermomonospora*, *Ruminococus*, *Baceriodes*, *Erwinia*, A*cetovibrio*, *Microbispora* and *Streptomyces* also produce cellusases that hydrolyze cellulolytic biomass.

Alber et al. (2008) noted that *Ruminococcus flavefaciens* is an anaerobic bacterium that plays a key role in the degradation of plant cell walls by producing a highly organized multi-enzyme cellulosome complex. According to the research conducted by Noach et al. (2005), *Bacteroides cellulosolvens* also produce a multi-enzyme cellulosome complex in an anaerobic environment that assisted in the degradation of plant cell walls. Notenboom et al. (2001) found that members of the genus of *Clostridium cellulovorans* bind to non-crystalline cellulose materials. This genus represents a type of spore forming cellulolytic bacterium that is generally found in an anaerobic environment with a neutral pH and a slightly mesophilic temperature range.

Group 2 bacteria noted in **Figure 6** are hydrogen-producing acetogenic bacteria. The primary products resulting from the metabolism of Group 1 and 2 bacteria are H_2, CO_2 and acetate. These products are then used by Group 3, 4 and 5 bacteria in the production of methane. Group 3 bacteria are represented by hydrogen-oxidizing acetogenic bacteria. According to Nagamani and Ramassamy (1999), the Group 3 bacteria oxidize fatty acids longer than acetate into acetate and release CH_4. The description of acetogenic bacteria in Li et al. (2010) involved differentiating them from the bacteria involved with acidogenesis due to their ability to reduce CO_2 to acetate using a specialized enzymatic pathway.

Group 4 and 5 bacteria are represented by a group of microorganisms known collectively as methanogens. Methanogenic bacteria are obligate anaerobes that tend to

be Gram-positive and are classified as coccobacilli. They can be comprised of *Methanobacterium* (nonspore-forming rods), *Methanobacillus* (spore-forming rods), and *Methanococcus* and *Methanosarcina* (a cocci group) (Shuler and Kargi, 2002). Group 4 microorganisms, such as *Methanococcus*, get their energy by reducing CO_2 with H_2 to produce CH_4. Group 5 microorganisms, such as *Methanobacterium*, convert acetate and alcohols into CH_4 and CO_2. It was noted by Nagamani and Ramassamy (1999) that methanogens possess a very limited metabolic repertoire, using primarily volatile acids or single carbon compounds to facilitate their metabolic pathways.

The methanogens reported by Nagamani and Ramassamy (1999) were of the *Methanosarcina* species and *Methanobacterium* species. The *Methanobacterium* species utilize the acetate in the substrate for their growth and activity. According to Vavilin et al. (1998), the *Methanosarcina* species also use acetate yet have a lower affinity for it. These methanogens are obligate anaerobes that are dependent on substrate composition and temperature for the speed of their growth and development for the purpose of methane production. The study conducted by Vavilin et al. (1998) supports the slow growing aspect of these methanogenic bacteria in relation to temperature as they noted that the rate of methanogenesis sharply decreased when cattle manure was digested at temperatures less than 20^oC. However, after 2.5 years of acclimating at a temperature of 6^oC, an active methanogenic community was established. At temperatures in the mesophilic range, the methanogenic community can be established in a matter of weeks to months and in the thermophilic range the active microbial cultures are established in a matter of hours to days.

In order to successfully integrate AD with ethanol facilities, consideration needs to be made regarding the microbial potential within the WDG and how the WDG microbial population may inhibit or enhance the methanogenic microbial population targeted in AD. WDG material is discharged from the ethanol facility at a pH of between three and four, with the very real potential of containing residual yeasts from the ethanol fermentation process. Lyberg et al. (2008) investigated the biochemical and microbiological properties of WDG and noted a pH of 3.9. Rosentrater and Lehman (2008) looked at the physical and chemical properties of corn WDG. In their study they noted a shelf life of four to seven days, dependant on water content, before spoilage occurred due to mold growth.

A study conducted by Pedersen et al. (2003) looked into the microbial characterization of wheat WDG. The WDG was characterized by a low pH, high numbers of Lactobacilli, high concentrations of organic acids, high fibre content and a dry matter content of about 9.5%. When comparing WDG to wheat, WDG had three times the amount of ash, nitrogen and fibre as wheat, while having a negligible starch content. The objective of the study was to determine the *Lactobacillus* species present in wheat WDG and to investigate the probiotic potential of this alternative feedstock for beef and pork production. Pedersen et al. (2003) found that the micro flora of the WDG was primarily Lactobacilli with trace amounts of yeasts. The high numbers of Lactobacilli found within the WDG contributed to the low pH (approximately 3.6) of the material. Isolating microorganisms directly from the WDG samples taken found that *Lactobacillus amylolyticus* and then *Lactobacillus panis* were the most common species detected. Also detected in the material was lactic acid, with acetic acid and ethanol in lower

concentrations. To determine the probiotic potential of the Lactobacilli found within WDG, the study investigated the ability of the various species to survive passage into the gastrointestinal tract of an animal and then adhere to the mucus membranes of the small intestine. The investigation by Pedersen et al. (2003) focused on pigs as the animal digesting the WDG and it was found that some strains of Lactobacilli did adhere to the mucus within the gastrointestinal tract. Therefore, the authors concluded that the probiotic potential of the WDG warranted further investigation.

The significance of the WDG studies to this investigation is the recognition that WDG contain residual micro-flora from the ethanol process that may provide competition to the establishment of a methanogenic micro-culture within an AD system that would limit the amount of WDG that could be added to the digester. These studies also recognize that WDG have added value in their own right as a feed supplement, thus availability within an AD system integrated within an ethanol facility paired with a cattle feedlot would be limited to what is not favourable feed for the cattle because it had spoiled.

4 Objectives

A solid-state anaerobic digester has the potential to compliment the operation of an ethanol facility that has been paired with a cattle feedlot, either in terms of providing energy to offset current energy requirements or as an additional revenue source. Typically, if a feedlot is paired with an ethanol facility, it is to facilitate the utilization of the WDG and TS as cattle feed supplements as opposed to implementing a drying and separation process in the ethanol plant to manufacture DDGS. WDG have a storage duration of three to five days, thus if the cattle in the feedlot do not match the production capability of the ethanol facility, spoilage occurs. This spoiled WDG product could then be utilized in a SSD system. **Figure 7** illustrates the potential product and co-product stream interactions within an ethanol eco-cluster comprised of a grain ethanol facility, an AD system and a beef cattle feedlot.

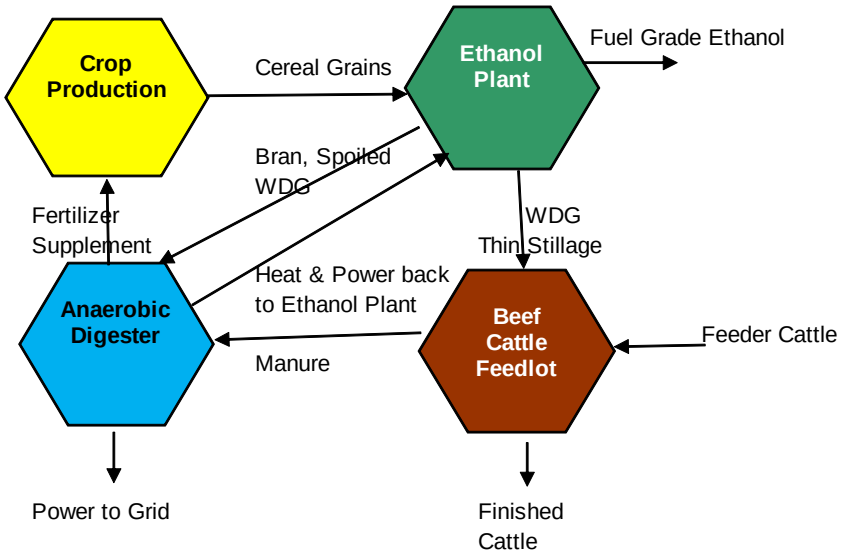


Figure 7. Product stream interactions within an ethanol eco-cluster

The primary objective of this research was to assess the performance of a bench scale SSD system using a WDG and manure feedstock mixture. The performance objective was to produce biogas with a composition of at least 50% CH_4 in one of the digesters containing WDG as well as in the 100% manure digester. A secondary objective was to determine a feedstock retention time in a solid-state AD system that could be integrated in a wheat based ethanol facility paired with a cattle feedlot. The final objective was to determine the nutrient value of the digester effluent (digestate) as a fertilizer supplement for agricultural producers within the vicinity of the ethanol and cattle feedlot operation.

5 Methodology

The research methodology, experimental design and techniques identified to achieve the objectives set in this project are similar to those used by Rosentrater et al. (2006), with the fundamental difference being that the reactor was designed for solid-state digestion of grain ethanol WDG and beef cattle feedlot manure (a blend of manure and bedding).

The solid-state reactor was represented using a two-litre (2 L) square container made of clear plastic. The sealing mechanism of the containers was an airlock seal using a rubber gasket. A total of 12 reactors were established, three reactors for each of four substrate blends. WDG and beef cattle feedlot manure were collected from an existing ethanol facility paired with a beef cattle feedlot, on the same day and in the quantities required for the duration of the research, then stored in an industrial cooler at 4^{o}C until required. Six control reactors were maintained, three were 100% WDG, with a buffer addition to maintain pH (sodium bicarbonate – $NaHCO_3$) and three were 100% feedlot manure. The remaining six reactors included three reactors containing 75% WDG and 25% manure by volume (with a buffer addition to ensure pH was maintained) and three reactors containing 75% manure with 25% WDG by volume, also with a buffer addition. **Table 2** lists the solid-state digestion substrate blends with their group allocation and their digester number.

Table 2. Solid-state digestion blend reference

Group	SSD Blend	Digester Number
1	100% WDG	1, 2 & 3
2	75% WDG / 25% Manure	4, 5 & 6
3	25% WDG / 75% Manure	7, 8 & 9
4	100% Manure	10, 11 & 12

Each reactor was filled ¾ full, leaving ¼ of the container volume for biogas and condensation accumulation. The initial weights of the reactors were recorded, then the reactors were placed in an incubator. **Figure 8** (a) through (d) illustrate the grouping of the digesters and the substrate blend within each digester group.

(a) Group 1

(b) Group 2

(c) Group 3

(d) Group 4

Figure 8. Research substrate groupings

Prior to placing each reactor in the incubator a sample of each substrate blend was collected for an initial analysis of total solids, total volatile solids and pH. The substrate blends were also analyzed for initial nutrient content including potassium, ammonia as nitrogen, nitrite and nitrate nitrogen, phosphorous, total nitrogen, total carbon and sulphur. After manual blending of the substrate material 250 mL glass containers were filled and sealed, then stored in an industrial cooler at $4^{o}C$ for up to 30 days until delivery was made to the Department of Soil Science at the University of Saskatchewan for analysis. Standard methods were used to analyse the substrate blends. Potassium chloride (KCl) extracts were used to determine the nitrite and nitrate in the substrate blends, and Kelowna extracts were used to determine the phosphorous and potassium in the substrates.

The incubator was fabricated from plywood and lined with 25 mm of insulation. The incubator dimensions required to accommodate the 12 reactors and the system heater were 1.25 m long by 0.51 m high by 0.60 m wide. The back plate of the incubator was 1.12 m high to facilitate the mounting of the pressure manometers and pressure release valves associated with each of the 12 reactors. The data collection equipment was mounted on top of the incubator, thus the door was a hinged front panel the full length of the incubator. Clear rubber tubing connected the reactor gas outlet with an individual pressure manometer and respective pressure release valve. The gas lines of the reactors were then connected to a single vent line to atmosphere after each pressure release valve. **Figure 9** illustrates the general arrangement of the 12 digesters within the incubator as well as the pressure manometers associated with each digester.

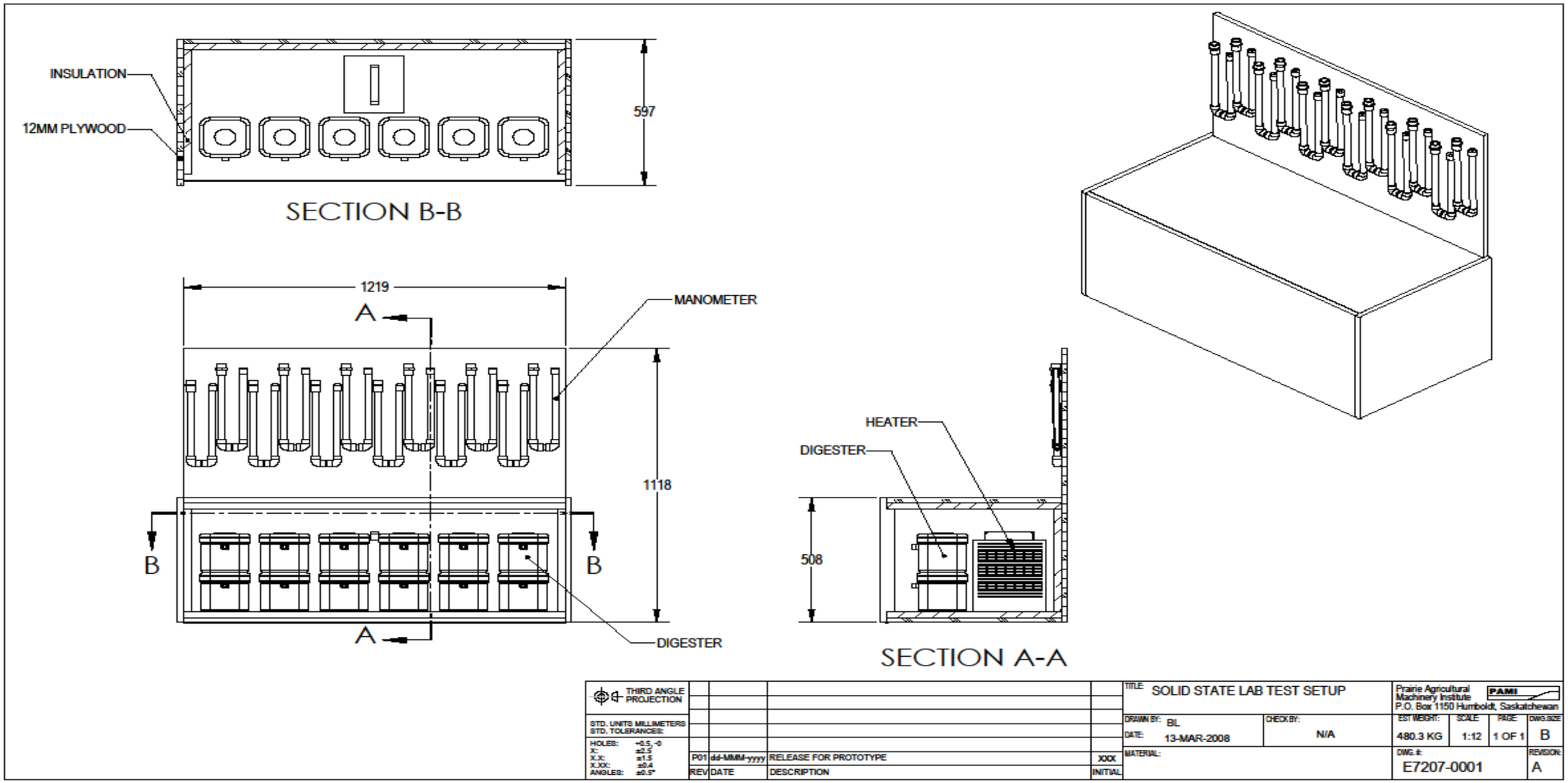


Figure 9. Bench-scale SSD general arrangement

A gas sample port was installed on each reactor gas line between the reactor outlet and the pressure manometer so isolated gas samples specific to each reactor could be taken. Each reactor was also equipped with a thermocouple connected to a data logger to monitor the internal temperature over time. Controlling the incubator temperature was accomplished using a temperature controller set to a specific set point and connected to the operation of the internal heater.

Once in the incubator, the data acquisition system was connected to the reactors. The data acquisition system was designed to measure the activation of pressure release valves associated with each individual reactor as well as the temperature of the individual reactors along with the temperature of the system incubator. Two dataTaker (DT800 and DT80) units were used to gather the pressure switch and temperature information during this investigation. One dataTaker recorded the internal temperature of each bench-scale digester at 15-minute intervals. The other dataTaker recorded the incubator temperature and each time any of the bench scale digesters reached the calibrated pressure of 51 mm of water. Each digesters gas outlet was piped through a pressure manometer and pressure release valve.

Each pressure manometer and pressure release valve was calibrated using a National Institute of Standards and Technology (NIST) calibration gas containing 50% CH_4 and 50% CO_2. The liquid employed in each manometer was silicone oil at a specific gravity of 0.98. Silicone oil was used to minimize the amount of CO_2 absorption in the manometer liquid and minimize the vaporization of the liquid over the duration of the investigation. The pressure relief system was set to release when 51 mm of water pressure was reached within the individual digesters, this release was then recorded. The

pressure dataTaker was set to record the pressure release of each digester and the incubator temperature every two seconds. The automated biogas measurement system was based on research conducted by Guwy (2004) by modifying a low-flow gas meter system.

Guwy (2004) discussed options for measuring the rate and volume of biogas produced from anaerobic biodegradability, which included the use of pressure manometers and low flow pressure valves to allow frequent releases of the headspace pressure. When a preset volume of liquid in the manometers was displaced, a fixed volume of gas was evacuated by opening of the solenoid valves. The solenoid valve opening was recorded using the data logger to facilitate the calculation of the volumetric flowrate over time.

The digesters were leak tested once loaded into the incubator. Positive pressure was applied to a locked incubator using compressed air and negative pressure was applied using suction. The fluid level within the manometer associated with the individual reactors was monitored during the pressure application. If the manometer level did not move during the pressure application the reactors were determined to be sealed. **Figure 10** shows the incubator at the onset of the research investigation with the 12 digesters loaded with their respective substrate blends. The incubator was equipped to maintain a temperature set point of 38^{o}C to promote mesophilic microbial development and maximize biogas production. The heater inside the incubator was connected to a temperature controller set at 38^{o}C. A thermocouple within the incubator was connected to the same data recorder monitoring the individual pressure relief valves on each reactor to track the temperature fluctuations within the incubator box.

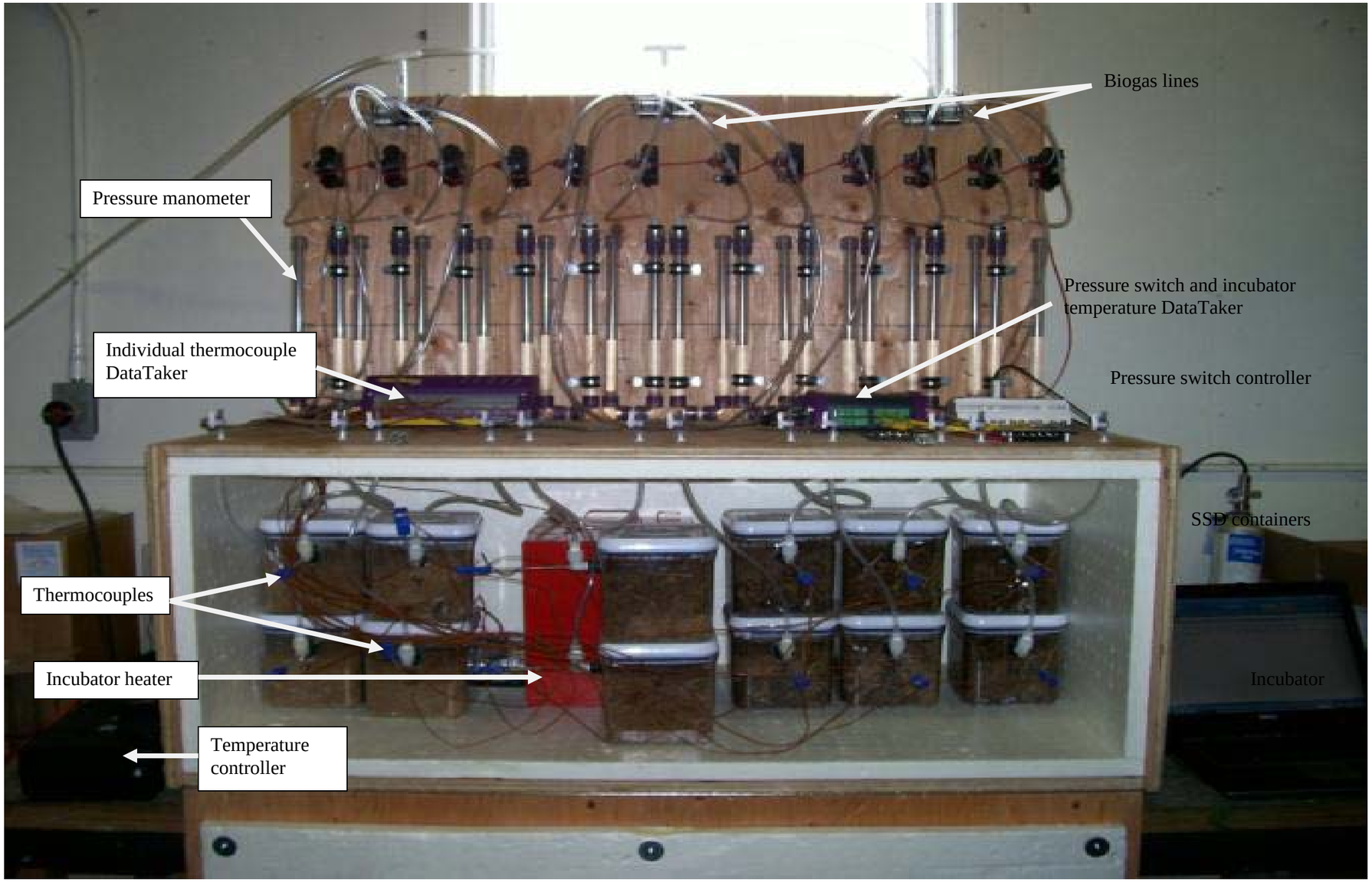


Figure 10. Research incubator loaded with digesters

The quality of the biogas was measured using a gas chromatograph (GC) to detect methane (CH_4), carbon dioxide (CO_2), hydrogen (H_2), hydrogen sulphide (H_2S), oxygen (O_2) and nitrogen (N_2) gas present in the individual samples. One reactor in each of the four groups represented was sampled every weekday during the study. It was assumed that the gas composition within the reactors in the same grouping would be similar. The same reactor was not sampled each time, the sampling was randomized between the three reactors within each of the four substrate blends. The gas sample was retrieved by integrating a syringe port in the gas lines connected to each reactor. Connecting a syringe to the syringe port allowed a gas sample to be drawn and deposited in a GC vial. Each GC vial contained a desiccant and was filled to the point of pressurization to prevent sample leakage. Gas samples were delivered to the University of Saskatchewan, Department of Soil Science where the samples were analysed by gas chromatography. The gas samples were stored in an industrial cooler at 4^{o}C, for up to 30 days, before delivery to the University of Saskatchewan.

A Varian CP-3800 GC was used to detect CH_4 in the samples using a FID (flame ionization detector). The column used was a Porapak Q8 that was 3.66 m in length, 3.2 mm diameter, with a 2 mm film thickness. The hydrogen and air flow listed under the FID information on the specification sheet in **Appendix I** is just fuel for the flame in the detector. The detection limit for methane was 360 parts per billion (ppb). The detector used for CO_2 analysis was a Varian Micro-GC CP-2003. CO_2 was identified using a TCD (thermal conductivity detector). The column used was a Poraplot U 10 meters in length with a 0.32 mm inner diameter. The detection limit for CO_2 was 80 parts per million (ppm). The detector used for H_2S was a Varian CP-3800 GC. H_2S was detected with one of two ECD (electron capture detectors). The columns used were Poraplot Q coated plot fused silica 10 m in length x 0.32 mm in diameter, with a

0.32 um film thickness. The two columns were the same, however they used different parameters because of slight differences in the way they were made. Oxygen (O_2), nitrogen (N_2) and hydrogen (H_2) were identified using a TCD and a molecular sieve column 10 m in length. The carrier gas for all detectors was helium, and the make-up air for the ECD was argon with 5% CH_4, also called P5.

The initial cycle duration of this research was determined by operating the SSD system until a noticeable drop was observed in gas production from each of the blended units. The gas production drop was determined by referencing the frequency of the pressure data gathered for each reactor using the dataTaker. It was assumed that the timeframe when the gas production decreased indicated the maximum operating curve for the establishment of the methanogenic microbial colony in the reactors. A gas production decrease would be represented by a decrease in activation of the pressure release valves set on each digester as part of the pressure monitoring system. After observing the gas production decline, the reactors were then replenished with fresh substrate matching the blend already existing in each digester.

Fifty percent of the digested substrate, by weight, was removed from each reactor and replaced with the same amount of fresh substrate. Manual mixing of the fresh substrate and the digested substrate occurred during the addition process then the containers were sealed and placed back into the incubator to begin the next cycle of digestion. The purpose of removing only 50% of the digested substrate was to leave a portion of the established microbial colony in the digester as an inoculum to the fresh substrate material to see if quality biogas was reached in a timelier manner than if one used fresh substrate for each cycle. Digestate analysis testing, the same as that listed for the substrate analysis, was completed on the digested material at the end of each cycle to allow the comparison of the nutrient values before and after digestion. At the end

of the second digestion cycle, 50% of the digested substrate was again removed and replenished with fresh substrate. A total of three operating cycles were completed on the entire system. Digestate was removed at the end of the third cycle to complete a final nutrient analysis.

Table 3 represents the SSD experimentation matrix described above.

Table 3. SSD experimentation matrix

Cycle	Group 1	Group 2	Group 3	Group 4
1	100% WDG	75%WDG / 25%Manure	75%Manure / 25%WDG	100% Manure
2	50% Cycle 1 + 100% WDG	50% Cycle 1 + 75%WDG / 25%Manure	50% Cycle 1 + 25%WDG / 75%Manure	50% Cycle 1 + 100% Manure
3	50% Cycle 2 + 100% WDG	50% Cycle 2 + 75%WDG / 25%Manure	50% Cycle 2 + 25%WDG / 75%Manure	50% Cycle 2 + 100% Manure

The overall evaluation timeline was estimated to be between 120 and 180 days with the final blend in the test reactors theoretically containing 25% of the original input material. The time estimate was based on the BEKON and BioFerm operational durations of existing solid-state digestion facilities. (Lutz 2005, BioFerm 2011) The exact duration of the study was to be determined by the pressure indication associated with the biogas production in the first cycle. When the biogas was observed to decrease over time in the first cycle, the day in the cycle that the decrease was observed was used as the duration of the subsequent cycles.

6 Results

The initial data inspection involves a basic mass balance associated with the mass reduction measured during the three digestion cycles to determine the volume of biogas produced during each cycle, recorded pH changes, and temperature observations. Subsequent data inspection includes gas and nutrient analysis of the entire investigation and of each individual cycle of digestion.

6.1 Percent Reduction in Mass

Material weights were taken for each of the 12 digesters at the beginning and end of the three observation cycles. **Table 4** outlines the mass reduction percentage measured for each of the digesters. This mass reduction was taken to represent the microbial activity and the potential biogas production during the observed cycle.

Table 4. Mass reduction over each observed cycle

Group No.	Digester No.	Cycle 1 Reduction (%)	Cycle 2 Reduction (%)	Cycle 3 Reduction (%)
1	1	4.17	2.14	2.30
	2	4.44	1.91	2.56
	3	4.40	2.13	2.63
2	4	5.40	2.36	2.44
	5	4.91	2.38	2.56
	6	5.53	2.09	2.41
3	7	4.29	2.89	2.82
	8	4.37	2.60	3.10
	9	4.26	2.70	2.80
4	10	2.44	1.77	1.89
	11	3.24	3.64	3.60
	12	4.61	2.03	3.65

Digesters No. 1 through No. 3 collectively represent Group 1 in this investigation, which indicates a substrate blend of 100% WDG. Digesters No. 4 through No. 6 represent a substrate

blend of 75% WDG and 25% beef cattle manure and they collectively make up Group 2 in this investigation. Group 3 encompasses digesters No. 7 through No. 9, collectively representing the substrate blend of 75% manure and 25% WDG. Digesters No. 10 through No. 12 represent a 100% manure substrate blend as Group 4. Grouping the digesters into their substrate blends and averaging the mass reduction percentage results in **Table 5**.

Table 5. Group average mass reduction over each observed cycle

Group	Cycle 1	Cycle 2	Cycle 3
	(% reduction)	(% reduction)	(% reduction)
1	4.33±0.15	2.06±0.13	2.50±0.17
2	5.28±0.32	2.28±0.17	2.47±0.08
3	4.31±0.05	2.73±0.15	2.90±0.17
4	3.43±1.10	2.48±1.02	3.05±1.00

The mass reduction was largest in the first cycle as this initial cycle was 45 days in duration, 15 days longer than the subsequent cycles; as well, the subsequent cycles also included some digested material in each reactor. The initial cycle was longer to observe a decrease in biogas production, based on the activation of the pressure release valve, and to ensure the establishment of a robust microbial culture of methane producing bacteria.

Group 4 exhibited a lot of variability between each respective digester in the mass reduction observed as the manure did contain residue bedding material in the samples which naturally introduced variability in the substrate. The Group 4 substrate material already contains the correct microbial populations to support methane production as it’s a material that has already undergone a digestion process within the cattle. The nature of the Group 4 substrate offers limited fresh feed to break down for further digestion and the nature of the digestion process in this investigation appears to have influenced the effectiveness of the microbial movement within the substrate to convert the complex carbon chains into biogas.

6.2 Volume Calculation

Taking direction from Guwy (2004), a method of measuring the rate and volume of biogas produced by anaerobic biodegradation was implemented, which included the use of pressure manometers and low-flow pressure valves to allow frequent releases of the headspace pressure. The goal was to measure the daily biogas production which could then be cumulated over the duration of the cycle to determine the total volume of biogas, in litres (L), produced per gram of volatile solids (gVS) in the substrate. This calculation would then be cross-referenced with the mass reduction noted in each reactor from the start to the end of the cycle. However, interference with barometric pressure observed in the pressure manometers connected to each reactor, and the large amount of headspace in each reactor compromised the confidence in the daily volume measurement. The volume calculation used to determine the biogas production during each cycle was based on the assumption that the biogas behaved as an ideal gas. The reactor pressure was known to be set at 51mm of water, the average temperature over the cycle duration for the individual reactors was known, as was the temperature of the overall incubator, and the mass reduction in each reactor was determined. The measured volumetric biogas composition for each reactor averaged over the entire cycle was used to determine the mass fraction of the composition components. This result was then used to determine the volume of biogas produced in each reactor, over each digestion cycle, at standard pressure (1 atmosphere) and temperature (0^{o}C). **Table 6** notes the average volume of biogas produced for each reactor group.

Table 6. Group average biogas volume production over each observed cycle

Group	Cycle 1 L/gVS	Cycle 2 L/gVS	Cycle 3 L/gVS
1	0.18	0.17	0.17
2	0.24	0.19	0.21
3	0.24	0.15	0.17
4*	0.13	0.10	0.13

*One of the three digesters in this group did not perform as the other two, if this outlier was removed, the average biogas production of the remaining two in this group over the three cycles were 0.15,0.11, and 0.17 L/gVS respectively.

Appendix III details the volume calculations made in this investigation.

6.3 pH

Table 7 denotes the pH values of the individual digesters throughout the course of the investigation. All of the reactors began the investigation within the optimal pH range (6.7-7.8) noted by Deublein and Steinhauser (2008) and Schuler and Kargi (2002) to promote methane forming microorganisms. At the end of Cycle 1 each reactor has become more basic which can be attributed to the denitrification activities ongoing in the initial hydrolysis stage of digestion.

Table 7. pH values of the substrate blends during the investigation

Group No.	Digester No.	Initial pH	Cycle 1 pH	Cycle 2 pH	Cycle 3 pH
1	1	6.98	8.72	9.97	9.14
	2	7.29	8.69	9.01	8.86
	3	7.46	8.72	9.52	9.02
2	4	6.93	8.77	8.30	8.73
	5	7.36	8.33	8.58	8.88
	6	7.18	8.26	8.60	8.87
3	7	7.39	8.47	8.39	8.51
	8	7.34	8.55	8.41	7.62
	9	7.03	8.43	8.5	8.33
4	10	7.35	8.27	6.85	4.22
	11	7.47	8.31	7.57	8.21
	12	7.38	8.39	7.19	7.74

Schuler and Kargi (2002) noted that the denitrification bacteria prefer a pH of 6.5-7.0, which is very similar to that of the methanogen bacteria required for CH_4 production in anaerobic

digestion. However, Deublein and Steinhauser (2008) note that a rising pH value is naturally controlled through two separate buffering systems. One buffering system is the ammonia-ammonium reversible reactions, which swings around a pH value of 10. The other is the CO_2, hydrogen carbonate (HCO_3^-), and carbonate (CO_3^{2-}) buffering reactions which swings around a pH of 6.5. Deublein and Steinhauser (2008) also note that a pH value greater than 10 will lead to irreversible loss in bacteria activity. All reactors in Group 1 at the end of Cycle 2 are approaching the pH 10 limit.

Reactor No. 10 in Group 4 shows a very low pH at the end of Cycle 3 which can be attributed to the reactor getting stuck in the acidogenesis phase of digestion. According to Deublein and Steinhauser (2008) the pH falling below 6.5 can promote increased organic acid production by the bacteria.

Table 8 denotes the average pH values of the substrate groups at the outset of the investigation and then again at the end of each cycle.

Table 8. Group average pH over each observed cycle

Group	Initial pH	Cycle 1 pH	Cycle 2 pH	Cycle 3 pH
1	7.24±0.24	8.71±0.02	9.50±0.48	9.01±0.14
2	7.16±0.22	8.45±0.28	8.49±0.18	8.83±0.08
3	7.25±0.20	8.48±0.06	8.49±0.06	8.15±0.47
4	7.40±0.06	8.32±0.06	7.20±0.36	6.72*±2.18

*One of the three digesters in this group had a pH of 4.22, if this outlier was removed; the average pH of the remaining two in this group was 7.98.

It was observed that each digester became more alkaline during the initial 45 day cycle. This observation was assumed to be due to digestion process as the microorganisms established an environment conducive to methane production along with the methanogen bacteria culture. By the end of Cycle 2, Group 3 and Group 4 substrate blends stabilized within an acceptable pH range for methane production. The pH stabilization of Group 3 and Group 4 may be due to the

fact that these groups contained a higher manure percentage than did Group 1 and Group 2, and as such had a higher initial methanogen bacterium culture enter the digester naturally along with the manure.

6.4 Temperature

The incubator temperature was found to operate one to three degrees below the internal temperatures of the individual reactors which is within the standard deviation of the incubator temperature data. Therefore; the temperature used to determine the gas volume was the incubator temperature recorded as more data points were gathered on the incubator temperature performance than on the individual reactors. Over each observation cycle, the average incubator temperature is reported in **Table 9**.

Table 9. Average incubator temperature over each cycle

Cycle	Incubator Temp (^{o}C)
1	38.82±1.2
2	36.58±3.4
3	29.02±0.7

The targeted mesophilic temperature for an AD system is between 35 and 40^{o}C, however mesophilic bacteria have a temperature performance range of 20^{o}C to 50^{o}C (Parker, et al. 2002, Shuler and Kargi 2002, Deublein and Steinhauser 2008). Two of the three cycles operated within the targeted temperature range. Cycle 3 operated into October and it appears the heater in the incubator was not able to maintain the temperature set point as cooler atmospheric conditions were experienced despite being inside a building regulated at 15^{o}C.

6.5 Statistical Inspection

The modeling program used to inspect the data gathered was called “R Project (2009)”. The biogas data and nutrient data were inspected separately, observations noted and microbial inferences drawn. The biogas and nutrient data were initially looked at as a group over the entire investigation, then the data was broken into each digestion cycle within each respective substrate group. The first stage of statistically inspecting the data involved generating a pairs plot from the data frame entered into the statistical modeling program. This plot takes every variable in the data frame and checks for subtle dependencies by plotting every variable on the *x* axis against every variable on the *y* axis. When reading a pairs plot the row the variable label occurs on indicates that variable is plotted on every *y* axis in that row. The column the variable label occurs on indicates that variable is plotted on every *x* axis in that column. Thus by referencing the different columns and rows each variable is plotted two ways against every other variable of interest.

Scatterplots and boxplots were then use to further differentiate the data gathered in this investigation. These plots are used to represent two variable, the response variable on the *y*-axis and the explanatory variable on the *x*-axis. Scatterplots were used to represent the biogas quality components as compared to time. In these plots the response variable is the biogas component and the explanatory variable was the duration. Boxplots were generated to differentiate the biogas components and nutrient performance with respect to the substrate blend. The substrate blend is classed as a categorical explanatory variable and is a factor used to explain differences in biogas quality measurements and changes in nutrient values over each digestion cycle. The horizontal line in each box plot represents the median of the data plotted. The bottom and top of the box represent the 25^{th} and 75^{th} percentiles respectively, also known as the first and third

quartiles. The vertical dashed lines are the 'whiskers' and they represent the minimum and maximum data point or 1.5 times the interquartile range, whichever is smaller. Values that are above or below and whiskers are noted as outliers and plotted individually. These plots indicate the skewness of the data as well as illustrate the location and spread of the data. They are also excellent plots for spotting errors in the data when extreme outliers are present.

6.5.1 Biogas Data

Figure 40, in **Appendix II**, illustrates a pairs plot of the biogas composition and how that composition changed as time passed. Time was recorded in Julian Days (JulDay) beginning June 29, 2008 and extending through all three cycles to encompass October 9, 2008. The gas concentrations were recorded as percentages (%).

Focusing on the bottom row there appears to be a strong negative linear relationship between both percent (%) CO_2 (CO_2_PC) and %CH_4 (CH_4_PC) with %N_2 (N_2_PC) as illustrated in **Figure 41**, in **Appendix II**. However, focusing on the row for %CH_4 the trend with %N_2 shows a strong negative non-linear relationship. In the row with JulDay there appears to be a negative correlation between JulDay and both %CO_2 and %H_2 as well as a positive correlation between JulDay and %CH_4. This information confirms that %CH_4 is the response variable of interest in biogas production but it also indicates that the influence of %CO_2 and %N_2 should be considered as the data are further evaluated. The remaining gases were assumed to have little to no impact on the response variable.

Figure 11 represents the three gas concentrations of interest against the entire time frame of the study.

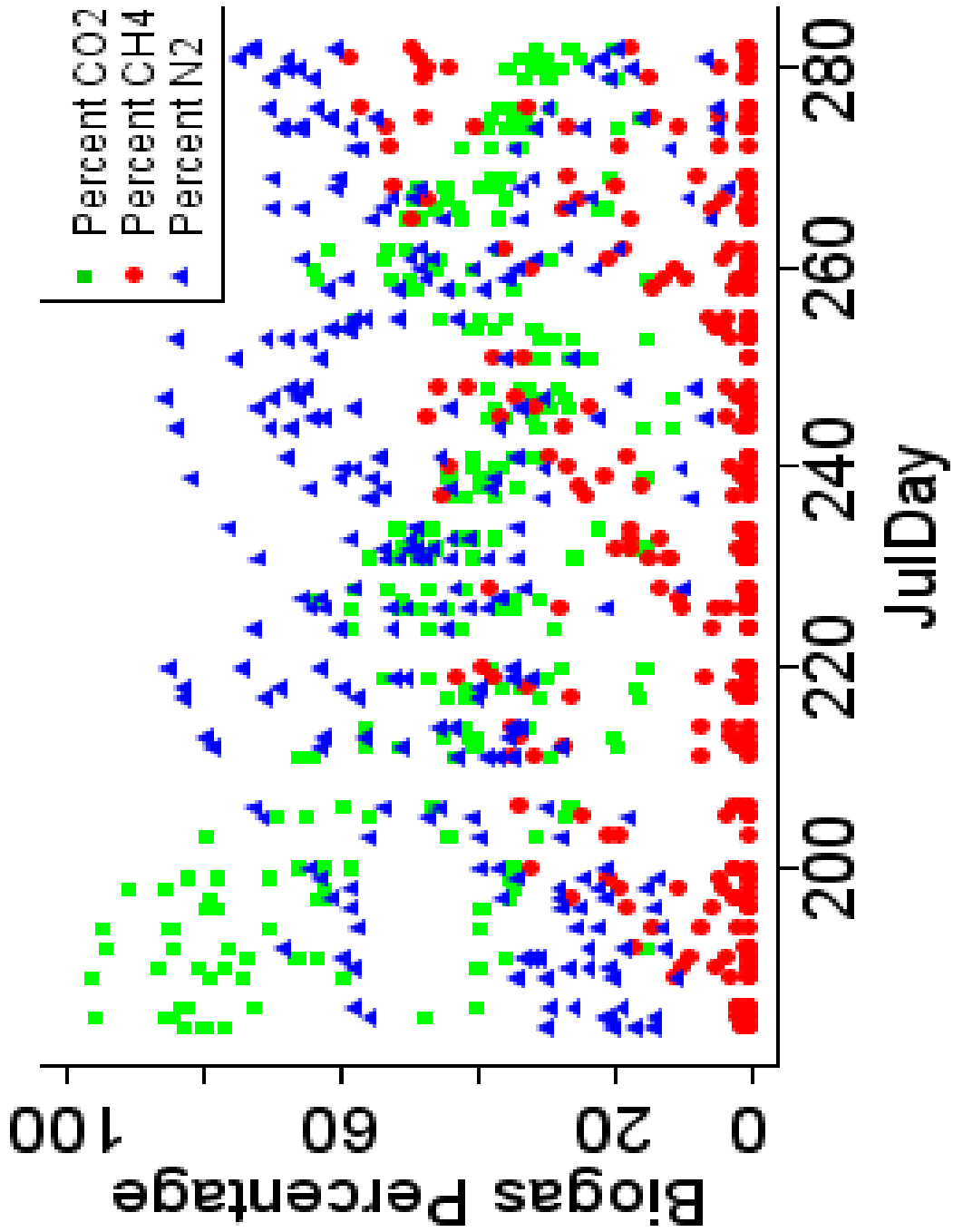


Figure 11. Biogas percentage achieved vs. study time cited in Julian Days

The plot in **Figure 11** represents all of the biogas produced over the entire experiment by all of the substrate blends. Illustrated in **Figure 11** are three distinct positive peaks in both $\%N_2$ and $\%CH_4$ and three distinct negative peaks in $\%CO_2$. These peaks appear to coincide with the duration of the three respective cycles however further inspection of the data is required to determine the substrate blends responsible for the peaks.

The substrate blends, in terms of percentage of WDG or cattle manure, can be classed as categorical factors with four levels. Therefore, the individual gas concentrations for $\%CH_4$, $\%CO_2$, and $\%N_2$ were represented in box plots against the respective substrate blend. **Figure 12** represents the $\%CH_4$ as a function of the four substrate blends using either WDG or manure as the distinguishing factor. The two box plots are mirror opposites of each other; as such, it can be concluded that WDG or manure composition can be used to distinguish the substrate blends.

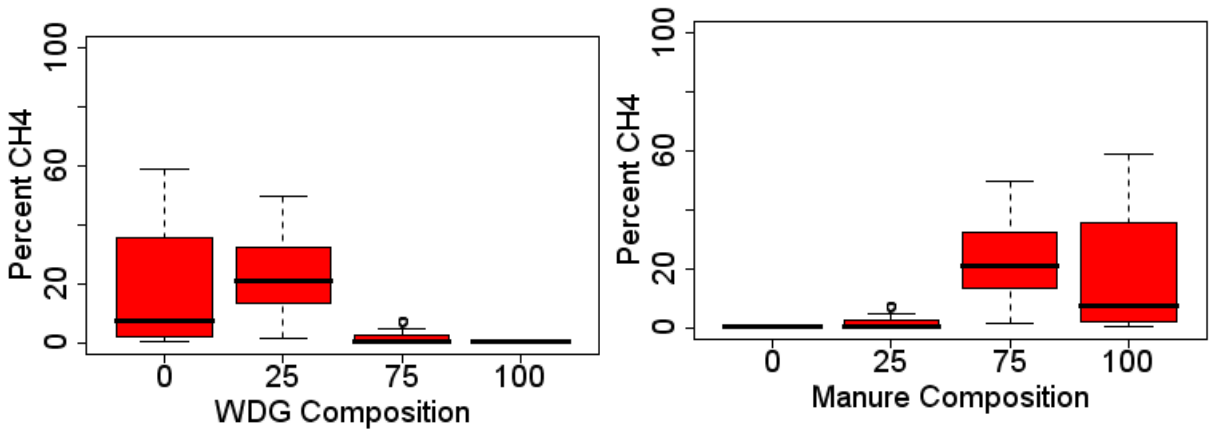


Figure 12. (a) $\%CH_4$ vs. WDG composition (%) **(b)** $\%CH_4$ vs. manure composition (%)

The box plots in **Figure 12** appear to show that CH_4 concentration was the most consistent in the Group 3 substrate blend containing 75% manure with a median CH_4 content of approximately 20% and a maximum value of approximately 55%. The Group 4 blend (100%

manure) resulted in a median of less than 10% but a maximum CH_4 content of just under 60%. Parker et al., (2002) reported the digestion of beef cattle manure producing a biogas with a methane content of 52% to 60%. A single outlier is seen in the Group 2 blend of 25% manure.

Figure 13 and **14** represent the $\%CO_2$ and $\%N_2$ respectively, referenced against the four substrate blends using either WDG or manure as the distinguishing factor.

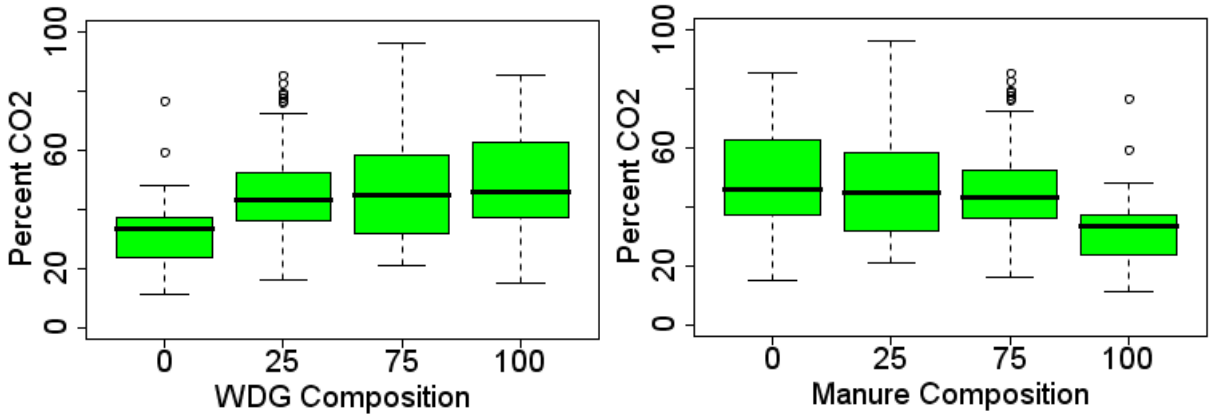


Figure 13. (a) $\%CO_2$ vs. WDG composition (%) **(b)** $\%CO_2$ vs. manure composition (%)

The CO_2 concentration appears to be the lowest in the manure control in **Figure 13** and it is relatively consistent across the other blends with a median of approximately 45%. **Figure 13** also notes outliers in both Group 3 and Group 4.

In **Figure 14** the N_2 concentration appears to be the lowest in the Group 3 substrate blend containing 75% manure and with a median of approximately 30%.

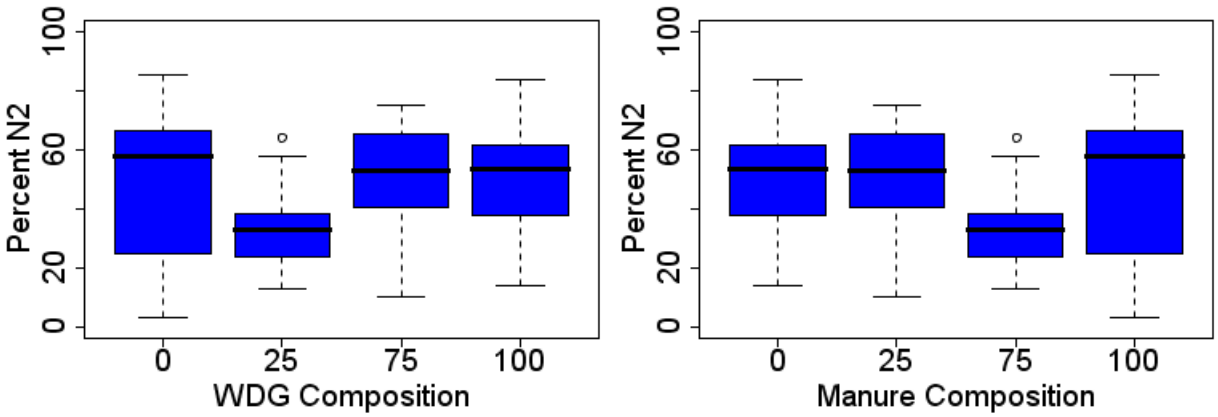


Figure 14. (a) $\%N_2$ vs. WDG composition (%) **(b)** $\%N_2$ vs. manure composition (%)

Across the other blends $\%N_2$ has a relatively consistent median of between 50% and 55%. A single N_2 outlier is seen in the 25% WDG blend in Group 3. According to Deublein and Steinhauser (2008) the N_2 composition in biogas by volume is between zero and five percent, with some substrate dependent performance increasing it to over 15% by volume. Recording such a high N_2 composition in the biogas is indicative of either an air leak in the system (however O_2 levels do not correspond), an air leak into the sample vial during storage prior to processing, or anaerobic denitrification.

The box plots illustrated in **Figure 12** through **14** are used to summarize the information gathered over the entire investigation. A total of 268 data points were used to generate the box plots illustrating the entire investigation. Missing from all of the box plots illustrated in **Figure 12** through **14** is the ability to distinguish the gas performance per cycle. The gas performance per cycle is illustrated in the following sections.

Cycle 1 Biogas Data Inspection

Figure 15 and **16** illustrate the biogas percentage of the three gases of interest against the time denoted as Cycle 1 in Julian days plotted as per the individual substrate blends. These figures do not isolate the individual reactors for each substrate blend rather the reactors are observed in their respective groupings.

Figure 15 illustrates a negative linear relationship in % CO_2 in the biogas for the duration of Cycle 1 in the Group 1 and Group 2 substrate blends containing 100% and 75% WDG respectively. A positive linear relationship in the %N_2 in the biogas for the duration of Cycle 1 is seen in **Figure 15**. This positive linear relationship is indicative of the anaerobic denitrification suspected. According to Schuler and Kargi (2002) N_2 is an end product of some of the possible microbial reactions occurring during the denitrification process.

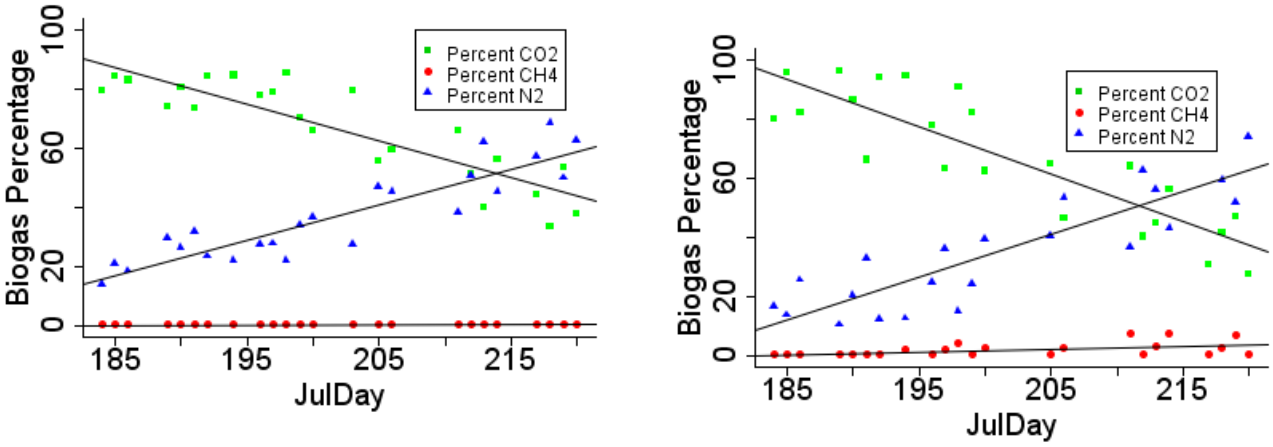


Figure 15. (a) Cycle 1 biogas percentage in Group 1

(b) Cycle 1 biogas percentage in Group 2

It is noted that very little CH_4 is produced in the biogas in the Group 1 and Group 2 substrate blends during Cycle 1. The lack of CH_4 production in Group 1 may be due to the fact

that this group contains no manure, thus it has no initial methanogenic bacterial population to act as an inoculum. Rosentrater et al. (2006) utilized sludge from an operating anaerobic digester to inoculate each ethanol by-product observed in his investigation to promote methane production. Group 2 contains 25% manure so it does have an initial methanogen culture inoculum. The lack of CH_4 production in Group 2 may be due to the bacterial culture the WDG brings to the substrate blend overpowering that of the manure. Pederson et al. (2003) noted that WDG was primarily Lactobacilli, which is not a methane producing bacteria, nor is it a species referenced as active in any of the stages of anaerobic digestion.

The CH_4 concentration is on a steady rise in the Group 3 substrate blend as seen in **Figure 16 (a)** and in at least one reactor in the Group 4 substrate blend in **Figure 16 (b)**.

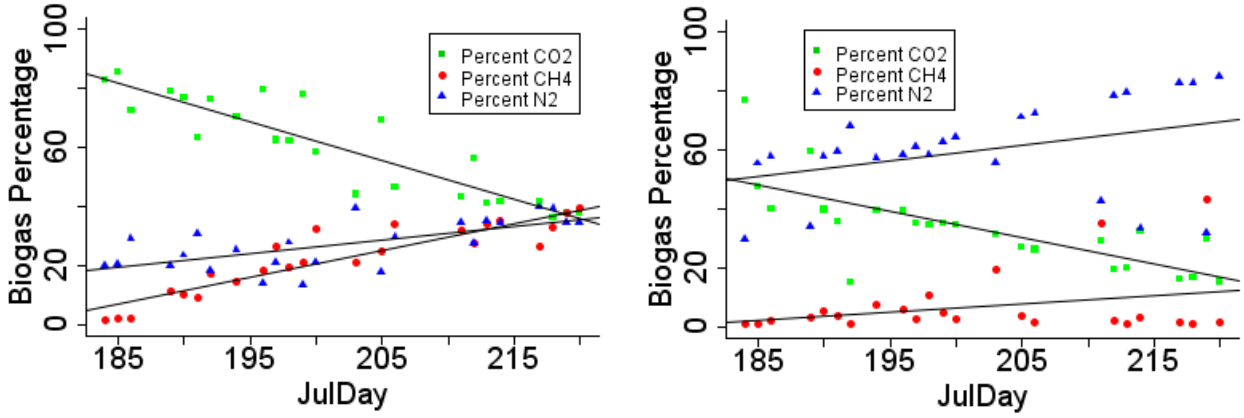


Figure 16. (a) Cycle 1 biogas percentage in Group 3 **(b)** Cycle 1 biogas percentage in Group 4

Like **Figure 15**, **Figure 16** illustrates a negative linear relationship in $\%CO_2$ in the biogas for the duration of Cycle 1 in the Group 3 and Group 4 substrate blends containing 75% and 100% beef cattle manure respectively, and a positive linear relationship in $\%\ N_2$. As the CH_4 concentration was increasing over the observation cycle the CO_2 concentration was decreasing as

expected; however the N_2 concentration was increasing. Group 3 CH_4 concentrations exceeded CO_2 after approximately 35-days of digestion in the initial observations cycle. Group 4 CH_4 concentrations exceeded the CO_2 after approximately 25-days of digestion. The Cycle 1 biogas composition is illustrated as individual box plots in **Figure 17, 18** and **19**.

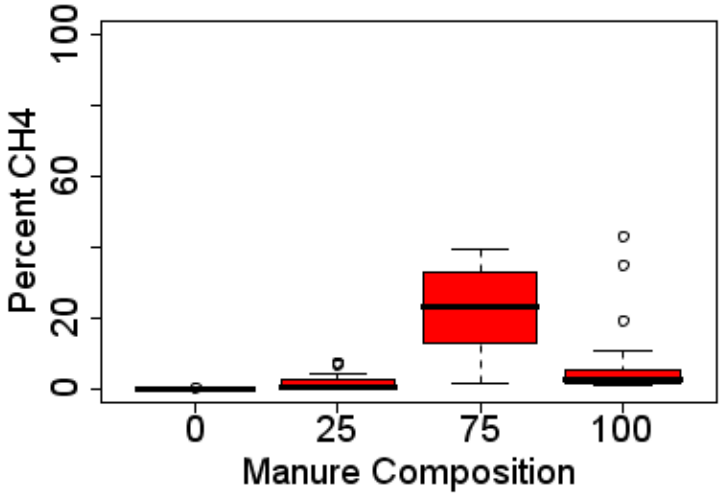


Figure 17. Cycle 1 CH_4 box plot

Figure 17 shows that CH_4 concentration was the most consistent in the Group 3 substrate blend containing 75% manure with a median CH_4 content of approximately 25% and a maximum value of just under 40%. No outliers are illustrated in the Group 3 plot indicating all three reactors in the group were performing in a similar manner. Outliers are seen in both the Group 2 and Group 4 (25% and 100% manure) substrate blends indicating one of the three reactors in each group was not producing a biogas equivalent to the other two. The Group 4 outliers indicate one reactor was producing biogas with a CH_4 content of just over 40%. The plot of Group 1

shows that none of the three reactors produced any CH_4 of record. However, **Figure 18** shows that each reactor group recorded CO_2 production in Cycle 1.

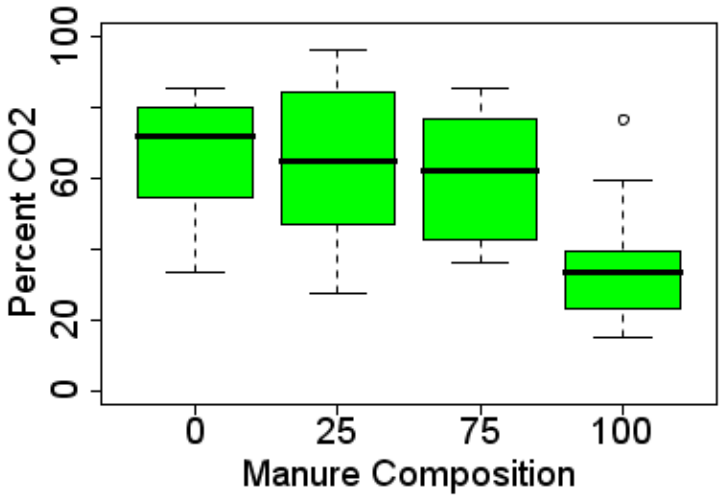


Figure 18. Cycle 1 CO_2 box plot

Figure 18 illustrates a very consistent CO_2 concentration across three of the four reactor groups. The 100% manure substrate blend (Group 4) has a noticeably lower median, at approximately 30%, than the other three reactor groups which show a median CO_2 concentration of between 65% and 70%. Group 4 does show one outlier at approximately 80% CO_2 in **Figure 18**. One outlier in Group 4 is also shown in **Figure 19**.

Figure 19 represents the N_2 concentrations seen in each substrate blend. Three of the four reactor groups show a median ranging between 25% and 35%; whereas, one group shows a median of approximately 60%. As with the CO_2 concentrations, the reactor group that stands out is Group 4.

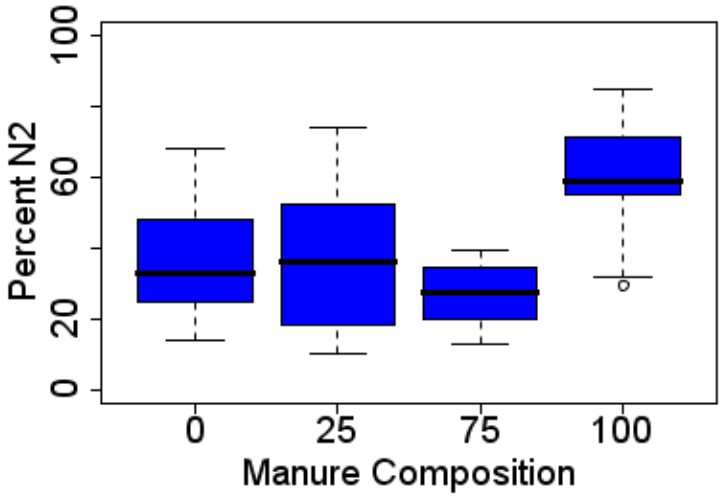


Figure 19. Cycle 1 N_2 box plot

A total of 96 data points were used to generate the box plots that represent Cycle 1 biogas activity in the reactors which equates to 24 data points for each reactor group.

Cycle 2 Biogas Data Inspection

Figure 20 and **21** illustrate the biogas trends seen during Cycle 2 for each respective substrate blend. The data trends show that each reactor in Groups 1, 2 and 3 had biogas present comparable to the other two reactors in the respective group. Group 4 however, had one reactor producing a biogas that was not comparable to the other two in the group. Groups 1 and 2, 100% and 75% WDG substrate blends respectively, in **Figure 20 (a)** and **(b)** shows the relationship between N_2 and CO_2 concentrations in the biogas for the duration of Cycle 2. As the CO_2 concentration decreased the N_2 concentration increased in the Groups 1 and 2 observations in Cycle 2. As with Cycle 1, very little CH_4 was present in the biogas in these two blends during Cycle 2.

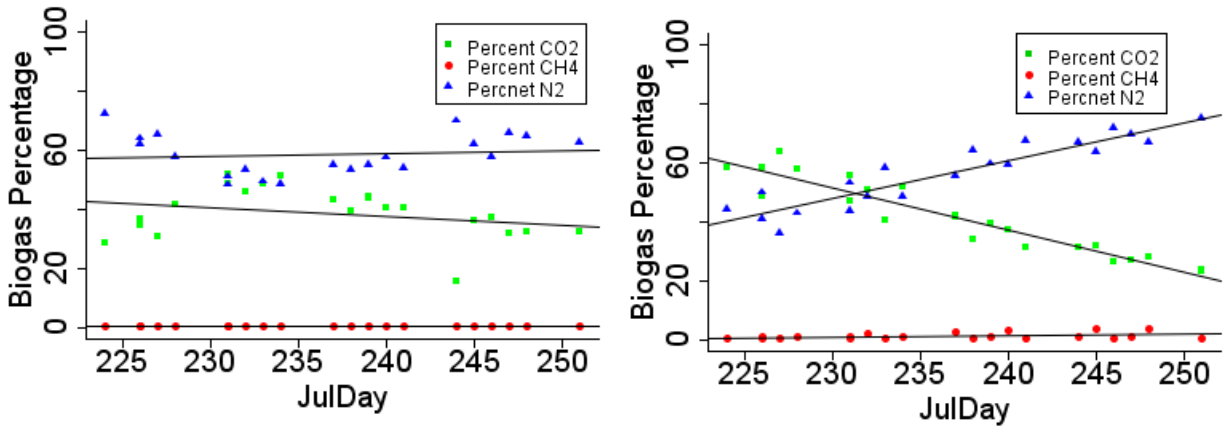


Figure 20. (a) Cycle 2 biogas percentage in Group 1 **(b)** Cycle 2 biogas percentage in Group 2

In **Figure 21 (a)** and **(b)** the $\%N_2$ and $\%CO_2$ data show a strong negative relationship during Cycle 2. The CH_4 concentration exhibits a steady increase in **Figure 21 (a)** and in two of the three reactors of the 100% manure substrate blend in **Figure 21 (b)**.

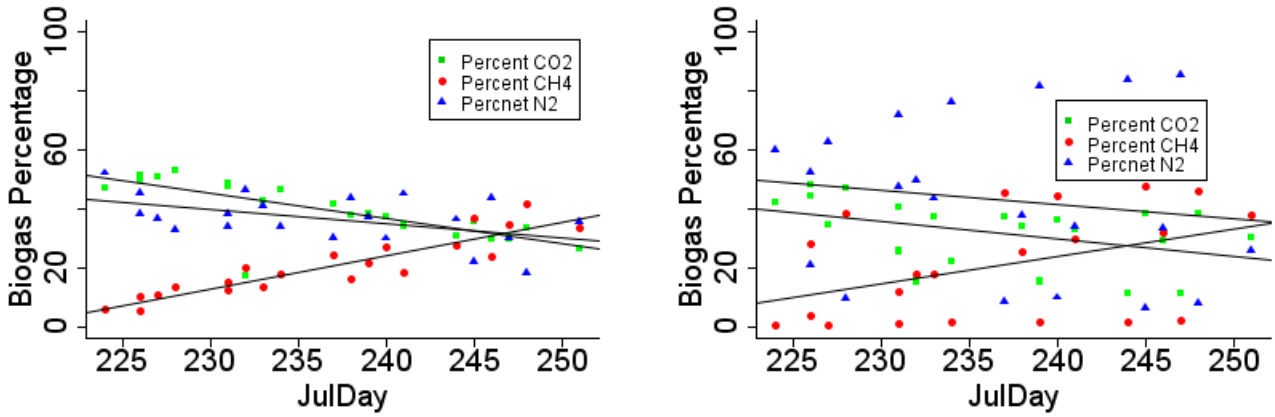


Figure 21. (a) Cycle 2 biogas percentage in Group 3 **(b)** Cycle 2 biogas percentage in Group 4

It is noted in **Figure 21 (a)** and **(b)** that the concentration of CH_4 exceeded that of CO_2 after 20 days for Group 3 and after 15 days for two of the three reactors in Group 4 during Cycle 2. The Cycle 2 biogas composition illustrated as individual box plots result in **Figure 22**, **23** and **24**. As in Cycle 1, Group 3 (75% manure) appears to be the most consistent substrate blend producing CH_4 in the biogas in Cycle 2.

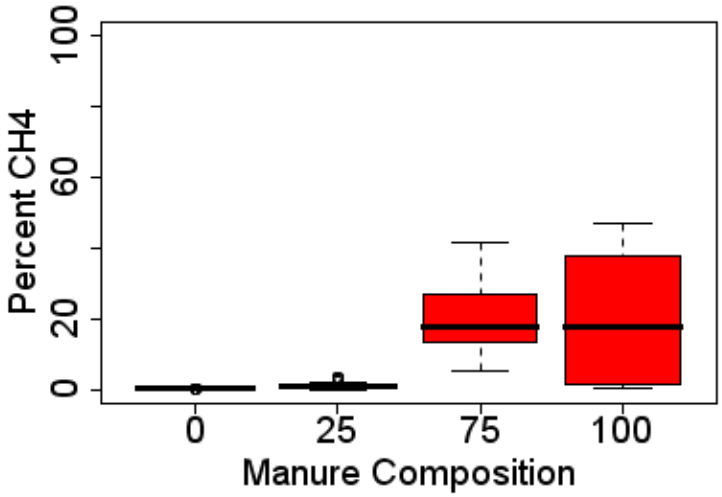


Figure 22. Cycle 2 CH_4 box plot

Figure 22 shows that the Group 3 median is approximately 20%; however, a maximum value of just over 40% is seen in the whiskers. Both Groups 1 and 2 show no CH_4 present in Cycle 2; whereas, Group 2 had CH_4 present during Cycle 1. Due to 75% of the substrate blend in Group 2 being WDG it is possible the microbial content in the WDG negated the establishment of a viable methanogenic bacteria colony from the 25% manure content in this group. The 100% manure blend (Group 4) in **Figure 22** shows a very wide first and third quartile range, with a median equivalent to Group 3 (approximately 20%) and a maximum CH_4 value of almost 50%. The significant skewness was due to one of the three reactors in Group 4 having little CH_4 present in the biogas during Cycle 2.

The CO_2 concentration in Cycle 2 appears to have stabilized across all four substrate groups when compared to Cycle 1, as illustrated in **Figure 23**.

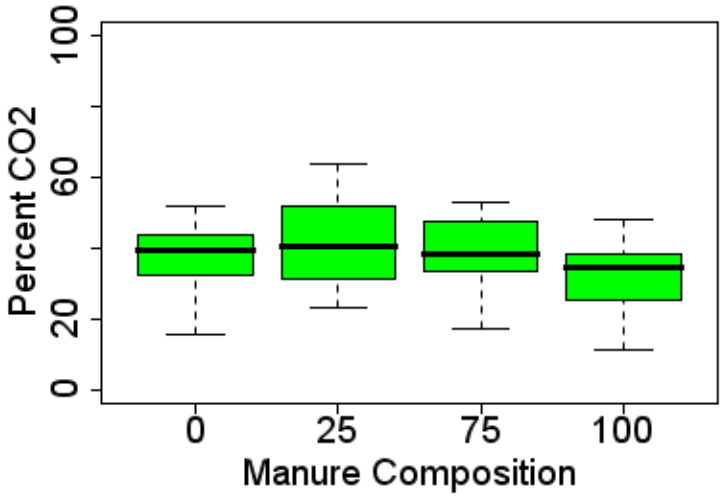


Figure 23. Cycle 2 CO_2 box plot

Figure 23 shows a median of approximately 40% for all substrate groups with no outliers in any of the groups. The N_2 concentration has stabilized in three of the four substrate groups as shown in **Figure 24** . It appears that the 100% manure (Group 4) substrate has one reactor producing N_2 as part of the microbial development within that reactor. The concentrations of N_2 measured within each reactor do not coincide with air leakage into the reactor, thus the process of anaerobic denitrification would explain the excess N_2 within one of the Group 4 reactors.

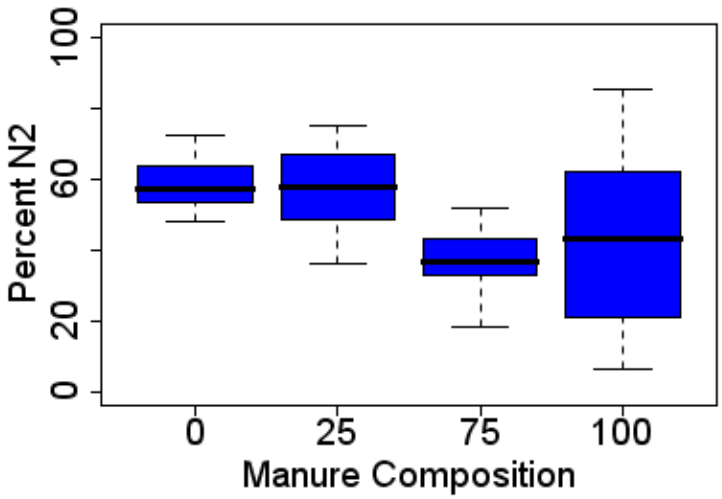


Figure 24. Cycle 2 N_2 box plot

A total of 84 data points were used to generate the box plots that represent Cycle 2 biogas activity in the reactors which equates to 21 data points for each reactor group.

Cycle 3 Biogas Data Inspection

The biogas trends seen during Cycle 3 for each respective substrate blend are illustrated in **Figure 25** and **26**. **Figure 25 (a)** and **(b)** maintain the positive and negative correlations in $\%N_2$ and $\%CO_2$ respectively for the duration of Cycle 3, as well as show that very little CH_4 is produced in the biogas in the Group 1 or Group 2 substrate blends during this final cycle.

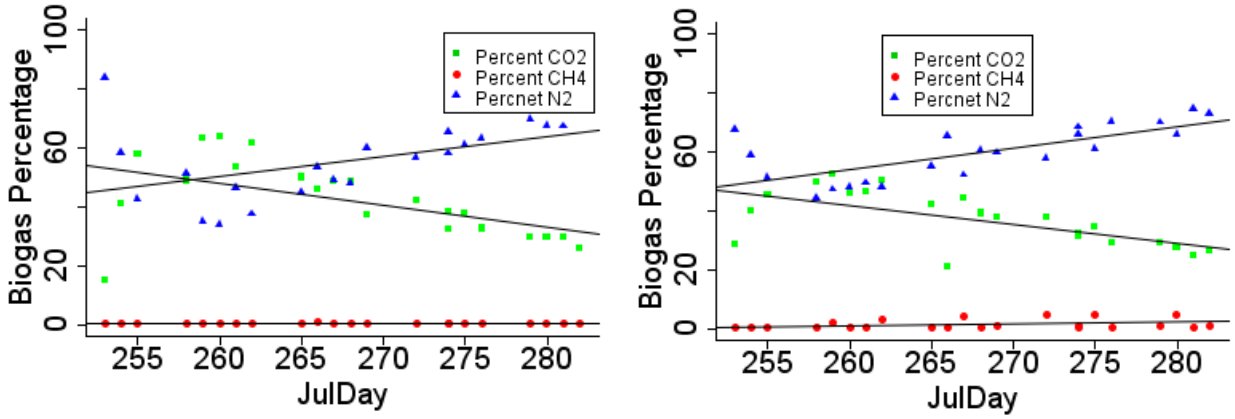


Figure 25. (a) Cycle 3 biogas percentage in Group 1 **(b)** Cycle 3 biogas percentage in Group 2

Figure 26 (a) and **(b)** show a strong negative relationship in both $\%N_2$ and $\%CO_2$ during Cycle 3. CH_4 concentration is on a steady rise in Group 3 as shown in **Figure 26 (a)** and in two of the three replicates of Group 4 in **Figure 26 (b)**. Group 3 was observed to perform the same in Cycle 3 as Cycle 2, where the CH_4 concentrations exceeded CO_2 after 20 days of operation. Group 4 improved upon its Cycle 2 results during Cycle 3 as the CH_4 concentrations exceeded CO_2 after only 10 days of digestion in two of the three reactors.

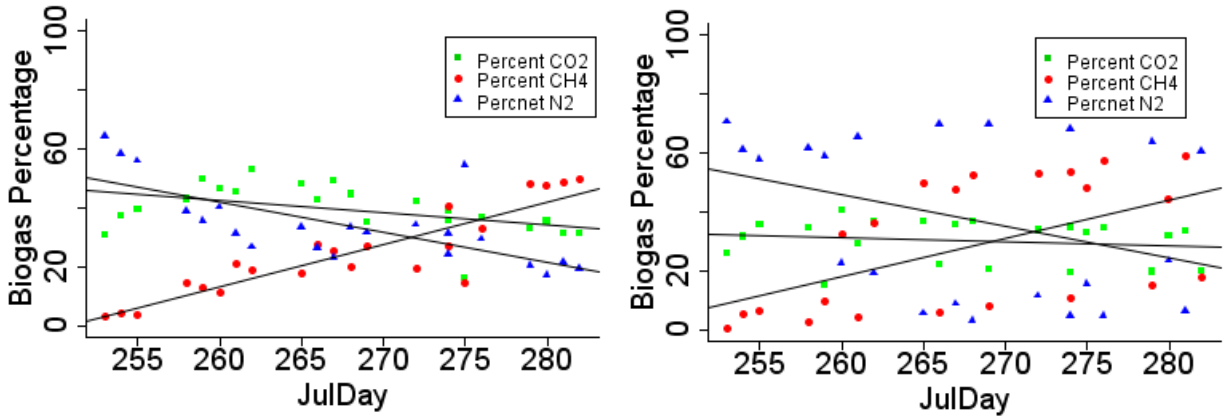


Figure 26. (a) Cycle 3 biogas percentage in Group 3 **(b)** Cycle 3 biogas percentage in Group 4

The Cycle 3 biogas composition illustrated as individual box plots result in **Figure 27**, **28** and **29**.

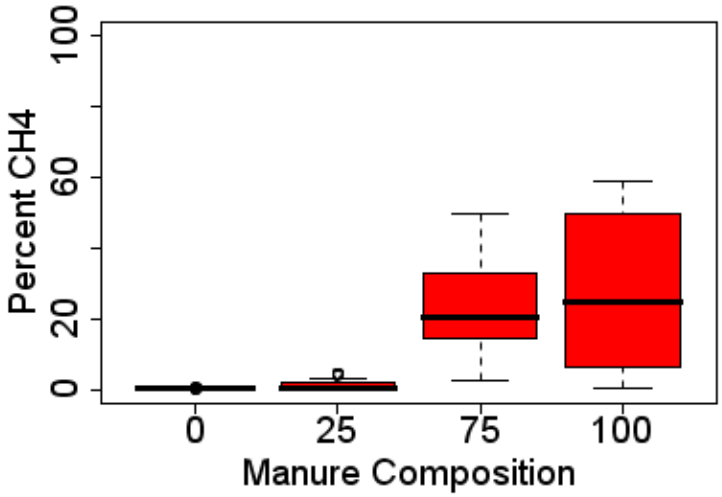


Figure 27. Cycle 3 CH_4 box plot

Figure 27 shows little to no CH_4 was produced in the 0% and 25% manure substrate blends; however, up to 50% and nearly 60% CH_4 concentrations are seen in the 75% and 100% manure blends respectively. As in Cycles 1 and 2, Group 3 (75% manure) appears to be the most consistent substrate blend producing CH_4 in the biogas in Cycle 3 due to the limited skewness of the data. The biogas CO_2 concentration, illustrated in **Figure 28**, appears to be stabilizing around the 50% range in each substrate blend.

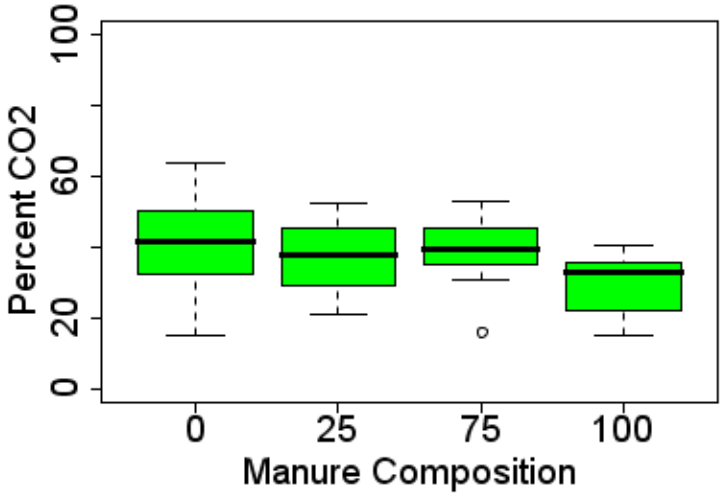


Figure 28. Cycle 3 CO_2 box plot

The N_2 concentration in the biogas represents a significant percentage in each substrate blend (**Figure 29**). The 75% manure substrate blend (Group 3) reported the least amount of N_2 production in Cycle 3. One outlier was seen in the Group 3 plot. Group 4 continued to have one of the three reactors demonstrate a high concentration of N_2 in the biogas captured. A total of 88 data points were used to generate the box plots that represent Cycle 3 biogas activity in the reactors which equates to 22 data points for each reactor group.

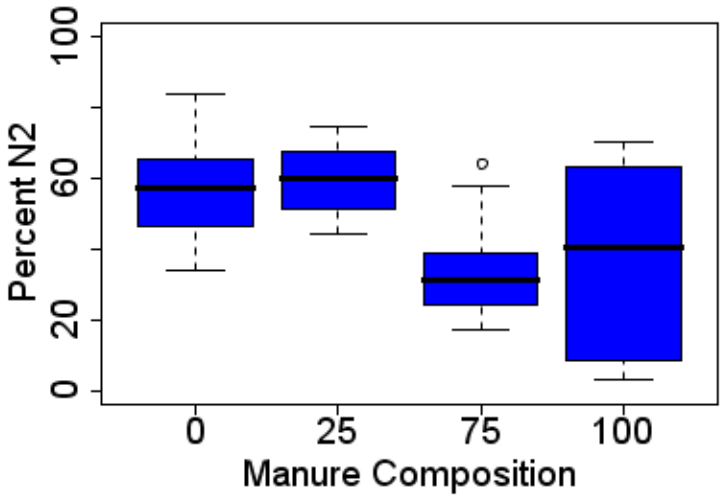


Figure 29. Cycle 3 N_2 box plot

The continued presence of N_2 in the reactors indicates an issue with the anaerobic environment. All reactors were leak tested under both positive and negative pressure to ensure a tight seal at the onset of each cycle. However, there was air captured within the headspace of the reactors when the reactor weights were measured, the digestate sample removed and new substrate material added. There is also the possibility of anaerobic denitrification occurring as the conditions required for this type of denitrification are similar to those required to promote methanogenic bacteria activity. Denitrification is a microbial nitrate reduction process. According to Schuler and Kargi (2002) the denitrification bacteria prefer a pH of 6.5-7.0 and a temperature range of 20^{o}C to 30^{o}C, which is very similar to that of the methanogen bacteria required for CH_4 production in anaerobic digestion

6.5.2 Biogas Statistical Model Identification

In the final days of the third cycle the biogas in Groups 3 and 4 (75% and 100% manure respectively) consisted primarily of CH_4 as its concentration was higher than 50% (**Figure 26 (a)** and **(b)**). The results described in the biogas assessment appear to fit statistically a linear mixed effects model complete with temporal pseudoreplication and nesting. The response variable of interest is the biogas production, specifically the concentration of CH_4; as such, the other gas compositions noted can be viewed as random effects influencing the variance of CH_4 concentration. The other gas concentrations could be viewed as response variables; however, as CH_4 is the component within the biogas that gives the biogas value it is viewed as the primary response variable. Lattice plots like those in **Figure 30** can be used to assess a mixed-effect model.

Figure 30 illustrates separate time series lattice plots for each of the individual substrate blends referencing each of the gases tested during the study. The $\%CH_4$ lattice plot clearly illustrates the upward trend in gas composition during each cycle at a 25% WDG substrate blend. An upward trend of $\%CH_4$ is implied in the 0% WDG substrate blend also. The lattice plot for $\%CO_2$ clearly illustrates the downward trend in gas composition during each cycle in all of the substrate blends. The $\%N_2$ lattice plot illustrates the upward trend in gas composition during each cycle at a 75% and 100% WDG substrate blends; however, there is a downward trend in the 25% and 0% WDG substrate blends. The downward trends in the 25% and 0% WDG substrate blends of $\%CO_2$ and $\%N_2$ coincide with the upward trend of $\%CH_4$ in these blends. As such, there is an influence of CO_2 and N_2 concentration on CH_4 concentration, as such these gas need to be evaluated together.

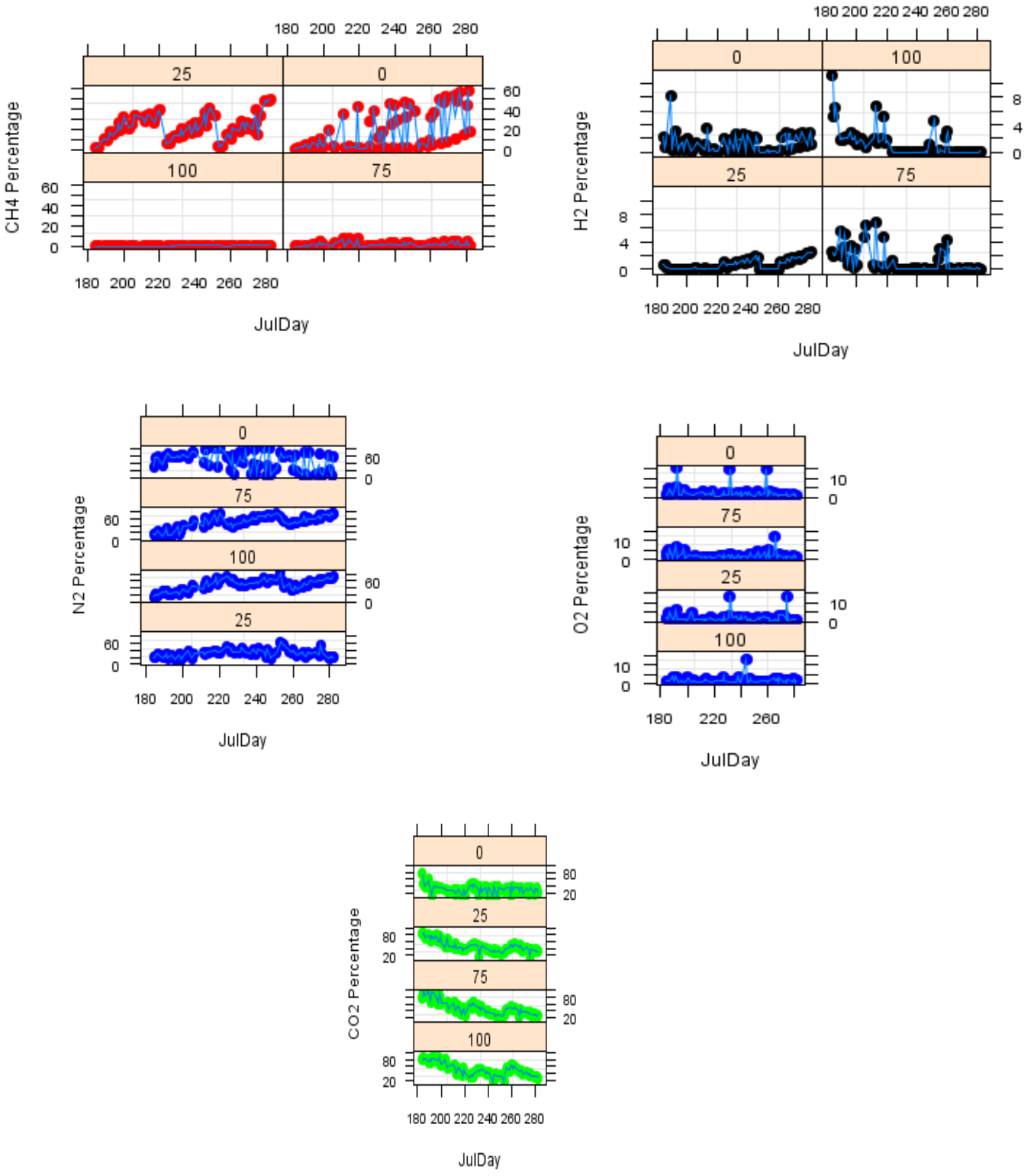


Figure 30. Mixed-effect model lattice plots of biogas composition based on WDG

The trends noted in the mixed effect model lattice plots illustrate that the biogas composition is changing with time. The CH_4 concentration in the biogas is increasing and the CO_2 concentration is decreasing. These plots are also indicating the N_2 concentration is significant; however, its significance is decreasing as time passes.

The fixed effect in this study was the substrate blends, and as such the influence these blends have on the response variable (CH_4 production) and the explanatory variables (remaining biogas composition). Nesting was determined by the biogas composition being nested within the substrate blends, which were nested within the cycle number.

6.5.3 Linear Mixed-Effect Model Criticism

The interactions in this study were analysed using a linear mixed-effects model. The initial model investigated was a maximal model taking into account all potential interactions of the gas composition with the substrate blends in relation to the response variable, $\%CH_4$. The Akaikes Information Criterion (AIC) for this maximal model was determined to be 1368. AIC is a penalized log-likelihood and its a measure of fit of a model (R Project, 2009). Simplifying the maximal model and removing the interactions involving $\%H_2S$ results in an AIC of 1355. Adjusting the model and removing the three way interactions results in an AIC of 1379. The lower the AIC from the maximal model the better the fit of the model (R Project, 2009). Overall, five separate models were investigated resulting in **Table 10** noting the degrees of freedom and AIC for each model.

Table 10. Linear mixed-effects model ANOVA summary

Model Number	Degrees of Freedom	AIC	p-value
Maximal	71	1368	
1	39	1355	0.0165
2	23	1361	0.0016
3	21	1464	0.0001
4	19	1379	<0.0001

The statistical model determined to best fit the response and explanatory variables in this solid-state anaerobic digestion study is the following:

*Model2<-lme(CH4_PC~CO2_PC*N2_PC*O2_PC*WDG_Comp,random=*

~JulDay|Cycle_No/WDG_Comp,method="ML")

The model above is represented in R Project code and it references the $\%CH_4$ (response variable) in an analysis of covariance against the explanatory variables of $\%CO_2$, $\%N_2$, $\%O_2$ and WDG composition, with time (JulDay), cycle number and WDG composition as independent considerations. This model was chosen as the degrees of freedom within the model are representative of those in the investigation, the overall interactions in the model are significant as noted by the p-value and the AIC is lower than that of the maximal model. Therefore, this model provides explanatory power for the interactions noted despite the reduced degrees of freedom. This model was also chosen because based on the biology of the system the influence between $\%CO_2$, $\%N_2$, and $\%O_2$ on the $\%CH_4$ achieved by the microbial population in the respective substrate blends will be significant. This significance was seen because CO_2, N_2, and O_2 are elements found in primary metabolic processes, whereas H_2, and H_2S result from secondary metabolic processes. Some of the significant interactions between the gas composition and substrate blend within this model are noted in **Table 11**.

Table 11. Significant interactions noted in the linear mixed-effects model

Interaction	p-value
CO2_PC:O2_PC	0.0066
N2_PC:O2_PC	0.0212
CO2_PC:O2_PC:WDG_Comp	0.0036
N2_PC:O2_PC:WDG_Comp	0.0078

To check the validity of the model plots of residuals, response variable and errors with respect to fitted values were required. A correctly fit model should have residuals about zero showing no discernable pattern. **Figure 31** illustrates the plot of residuals with respect to fitted values for the model chosen for this study. The plot shows that the residuals fit tightly around zero with the exception of one outlier. Ignoring the outlier the residuals are randomized about zero along the entire fitted value axis. There is no discernable pattern in the residuals depicted in **Figure 31**.

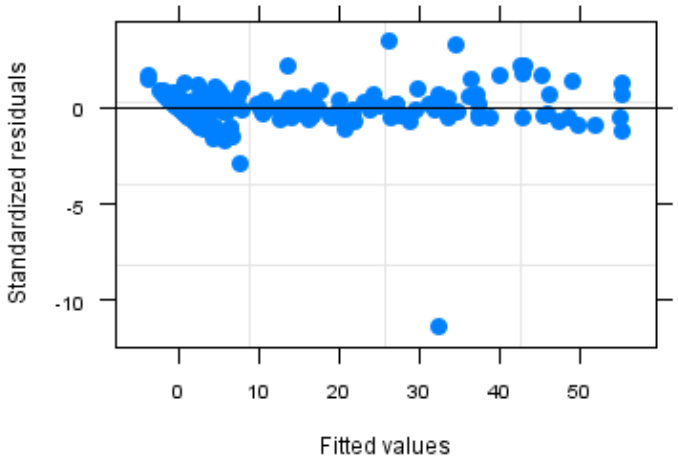


Figure 31. Linear Mixed-Effects model of residuals

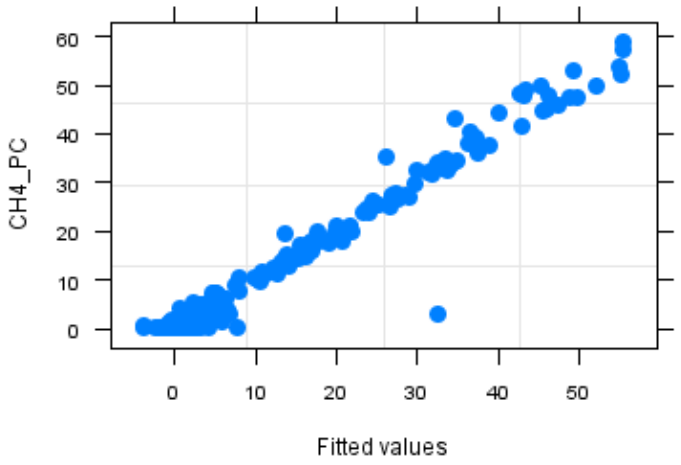


Figure 32. Linear Mixed-Effects model of $\%CH_4$ vs. fitted values

The plot of response variable $\%CH_4$ with respect to the fitted values generated by the best fit linear mixed-effects model is illustrated in **Figure 32**. This plot clearly illustrates a linear relationship of the response variable to the fitted values even with the presence of a single outlier.

A plot of errors using the chosen model for this study is depicted in **Figure 33**. The plot of errors appears to be normally distributed in all four substrate blends referenced by WDG composition. In the block that denotes the 25% WDG / 75% cattle manure blend (bottom left-hand quadrant of the figure) there is one outlier in the errors. This outlier may skew the normal distribution of this blend.

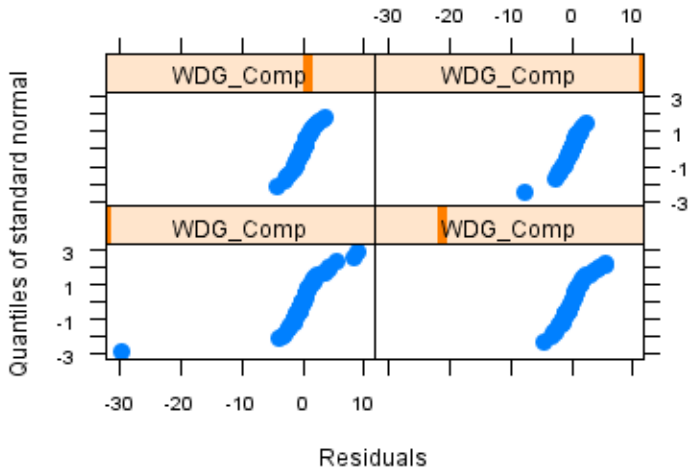


Figure 33. Linear Mixed-Effects model plot of errors

The model of choice supports the inverse relationship noted in the data inspection between $\%CH_4$ and both $\%CO_2$ and %N2, and indicates a significant interaction with $\%O_2$ despite the average O_2 concentration being just over 2% throughout the cycles. To improve upon the linear mixed model chosen the outlier indicated in the model validation plots could be determined and removed. As well, rather than using all of the data generated in the study, the data could be sorted by reactors within each substrate blend achieving the upward trend in CH_4 concentration. Sorting the data in this manner implies using only those reactors within each substrate blend that achieved productive methane producing microbial populations.

6.5.4 Digestate Data

As with the biogas data, results of the digestate data are represented using pairs plots of key data points. The pairs plots give an overview of the data gathered and illustrate areas where further investigation is warranted. When inspecting the key variables in the digestate data, the inspection is conducted over the entire investigation rather than looking at the data on a per cycle basis. The key variables are not looked at on a per cycle basis as there is only one data point per SSD unit collected at the end of each cycle, giving a total of four data points per reactor, 12 data points per reactor grouping.

Figure 42, **43** and **44**, in **Appendix II** represent pairs plot groupings of nutrient data as compared to the reactor "rep" number indicated on the SSD unit. The numbers used were one through twelve with the four substrate blends making up four groups of three. The row and column representing the reactor numbers are both number one. As noted in the biogas data inspection the reactors numbered one through three represent the 100% WDG control, four through six indicate the 75% WDG blend, seven through nine show the 75% manure blend, and ten through twelve indicate the 100% manure control.

Figure 42 illustrates an upward trend in the percentage of total carbon and total sulphur content as the manure in the substrate blends increase. The total nitrogen percentage and C:N ratio appear to peak in reactors seven, eight and nine which are the 75% manure substrate blend. **Figure 43** illustrates the relationship between the substrate reactors and the nitrite (NO_3^-), ammonium (NH_4^+), phosphorous (PO_4^{-3}) and potassium (K^+) contents. There is a distinctive upward trend in K as the manure content increases in the substrate blends. **Figure 44** illustrates the moisture content, total solids (TS), total volatile solids (TVS) and total fixed solids (TFS) in the solid material samples as

percentages (%). Of note is the upward trend in TVS as the manure content of the substrate material increases.

Based on the results shown in the pairs plots, a key variable to investigate is the C:N ratio of the various substrate blends. The C:N ratio was calculated based on total C and total N found within the substrate blend. **Figure 34 (a)** and **(b)** illustrate the C:N ratio measured over the entire research investigation of the various substrate blends. The figures are mirror opposites of each other as they reference the WDG versus manure composition of the substrate.

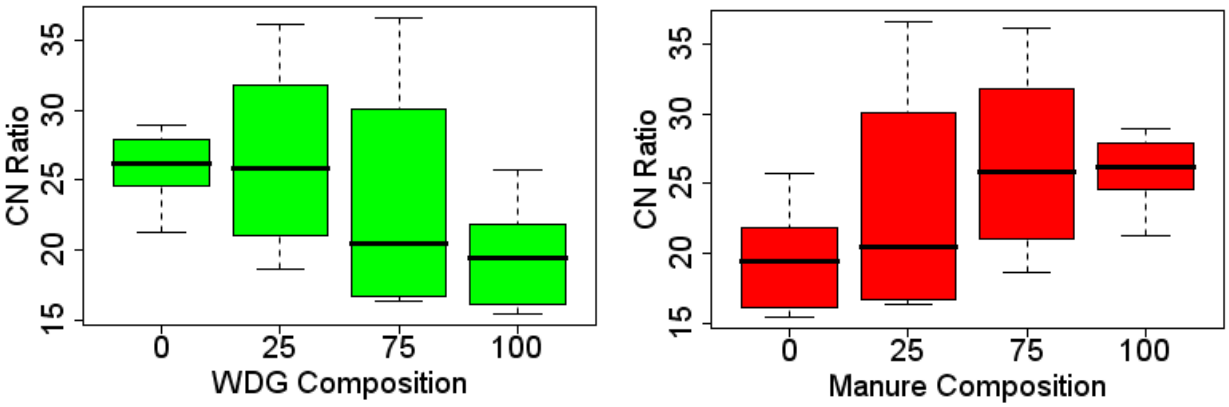


Figure 34. **(a)** C:N ratio vs. WDG composition **(b)** C:N ratio vs. manure composition

Both Groups 3 and 4 (75% and 100% manure substrate blends respectively) in **Figure 34 (b)** have medians of approximately 25:1, with the 100% manure blend having a very little skewness in the data around the median. **Table 12** notes the C:N ratios measured during this investigation. Groups 3 and 4 had an average C:N ratio of 25:1 over all three observation cycles. Groups 1 and 2 had an average C:N ratio less than 25:1. C:N ratio appears to peak and stabilize in Group 3.

Table 12. Average C:N ratios measured over each observation cycle.

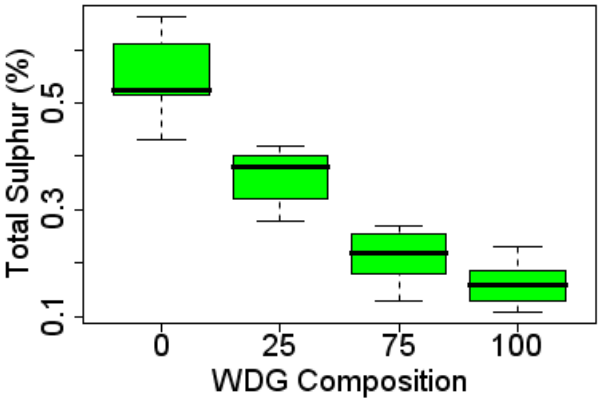
Group	Initial C:N ratio	Cycle 1 C:N ratio	Cycle 2 C:N ratio	Cycle 3 C:N ratio
1	16.1:1	17.4:1	21.0:1	23.4:1
2	16.3:1	29.8:1	25.8:1	21.5:1
3	18.6:1	34.1:1	24.8:1	27.8:1
4	24.6:1	28.4:1	23.9:1	26.6:1

Table 13 presents the sulphur (S) content as a percentage measured during this investigation at the conclusion of each observation cycle.

Table 13. Average percent sulphur (S) measured over each cycle

Group	Initial S (%)	Cycle 1 S (%)	Cycle 2 S (%)	Cycle 3 S (%)
1	0.11	0.19	0.16	0.18
2	0.27	0.21	0.15	0.23
3	0.40	0.31	0.38	0.37
4	0.52	0.47	0.60	0.60

Table 13 illustrates an upward trend in the total sulphur numbers as the manure in the substrate blends increase. Mg and Fe were not measured in this research study; however, the low C:N ratio and low sulphur content in the Group 1 and 2 substrate blends correlate with the low concentration of CH_4 observed. **Figure 35 (a)** and **(b)** illustrate that the Group 3 and 4 substrate blends had the highest percentage of sulphur of the four blends. This sulphur content correlates to a stepwise change in the percentage of manure within the substrate blend. In **Figure 35** Group 1 contains no manure and had the lowest sulphur content, whereas Group 4 was 100% manure and had the highest sulphur content.



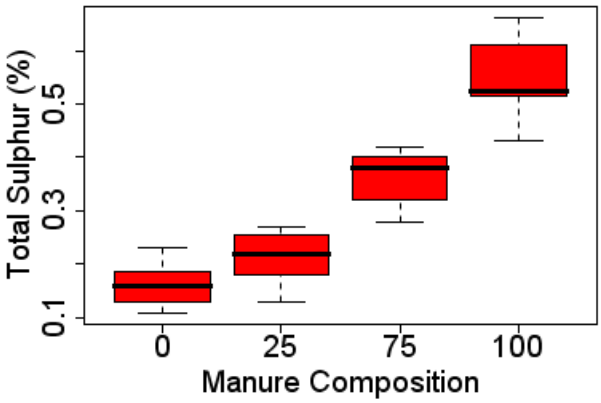
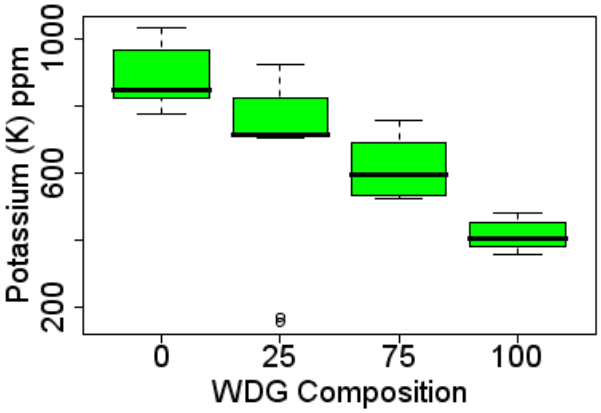


Figure 35. **(a)** Total S vs. WDG composition **(b)** Total S vs. manure composition

Like sulphur, potassium (K^+) was another nutrient that varied significantly with substrate blend. **Figure 36 (a)** and **(b)** shows that Group 3 and 4, the 75% and 100% manure substrate blends respectively, had the highest K^+ measurement of the four blends.



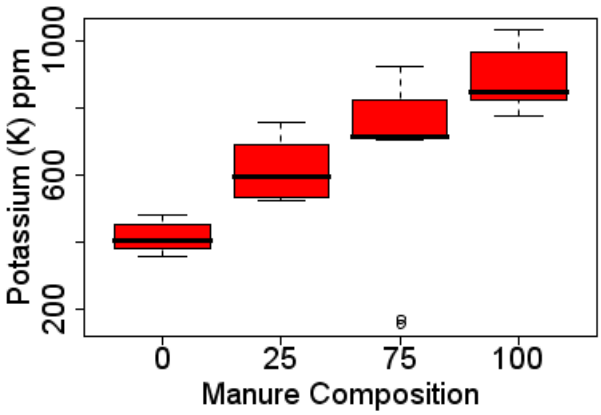


Figure 36. **(a)** K^+ vs. WDG composition **(b)** K^+ vs. manure composition

In the Groups 2 and 3 substrate blends in this study, the phosphorous content (PO_4-P) increased significantly after the digestion process (**Appendix III**). **Figure 37**

illustrates the total phosphorous content measurements for each Group over each of the three digestion cycles.

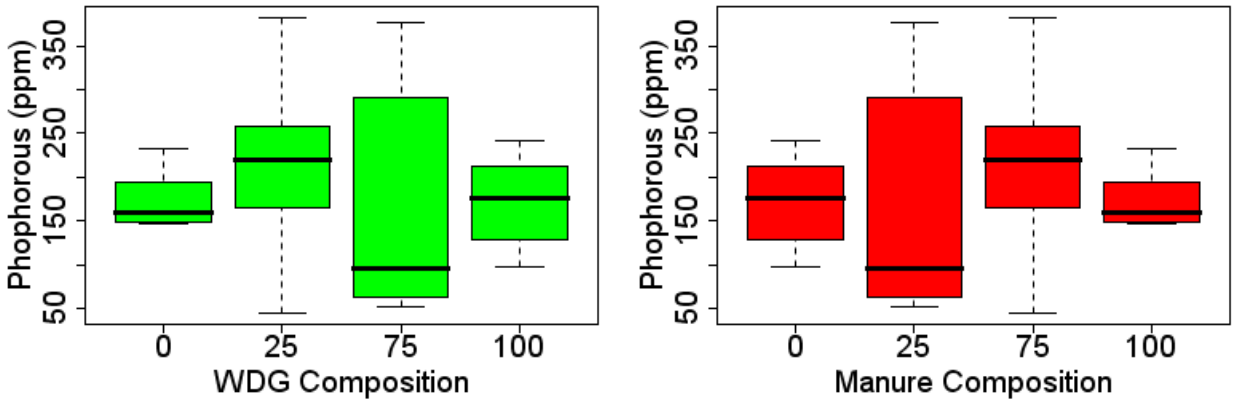


Figure 37. **(a)** PO_4^{-3} vs. WDG composition **(b)** PO_4^{-3} vs. manure composition

Figure 38 (a) and **(b)** show a high percentage of total volatile solids (TVS) in the 100% manure mixture plus a single outliner. The Group 3 substrate blend (75% manure mixture) had a median %TVS of approximately 60%; however, the first and third quartile data ranged between 45% and 65%.

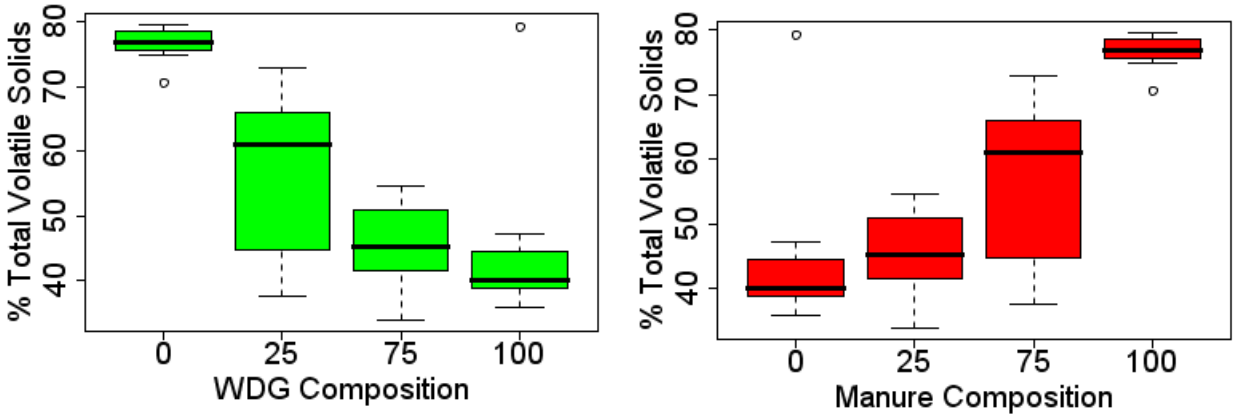


Figure 38. **(a)** TVS vs. WDG composition **(b)** TVS vs. manure composition

The potassium (K^+) content and the volatile solids (VS) content were seen to increase steadily as the manure content increased in the substrate blends. The four substrate groups had an average solids content between 20% and 25%. However, **Table** 14 shows that significantly higher volatile solids contents were seen in the substrate blends with higher manure content, Groups 3 and 4.

Table 14. Average total solids and total VS per substrate group

Group	Total Solids (%)	Total VS (%)
1	24.2	45.0
2	21.6	44.1
3	20.1	60.8
4	24.9	76.5

The nitrogen content (NO_3-N and NH_4-N) did not follow a consistent pattern during the digestion process so it is not represented graphically. The solids content and remaining nutrients assessed in the digestate material are noted in

Table 15. Solids and nutrient values for each Group over each observation cycle

Initial	Group 1	Group 2	Group 3	Group 4
Total N (%)	1.64	2.19	2.22	1.78
NO_3 –N (ppm)	1.27	1.39	1.07	0.53
NH_4 –N (ppm)	24.3	8.5	29.0	15.6
PO_4 –P (ppm)	97.4	51.0	164.8	147.7
K (ppm)	480.5	756.3	708.8	822.0
TS (%)	23.0	19.5	23.9	28.3
VS (%)	38.8	51.0	37.5	78.5
Cycle 1				
Total N (%)	2.19	1.41	1.26	1.58
NO_3 –N (ppm)	0.82	0.91	0.50	1.13
NH_4 –N (ppm)	4.7	15.4	109.0	6.0
PO_4 –P (ppm)	172.3	248.6	321.3	180.2
K (ppm)	380.4	551.3	723.2	934.2
TS (%)	28.5	27.1	20.9	23.4
VS (%)	55.2	48.5	57.1	77.1
Cycle 2				
Total N (%)	1.82	1.51	1.64	1.84
NO_3 –N (ppm)	1.09	1.59	0.85	0.35
NH_4 –N (ppm)	3.1	4.6	78.1	166.6
PO_4 –P (ppm)	224.5	156.1	170.0	162.3
K (ppm)	397.3	566.1	636.0	925.6
TS (%)	21.2	19.8	18.8	25.1
VS (%)	38.7	40.0	69.4	75.6
Cycle 3				
Total N (%)	1.54	1.91	1.46	1.64
NO_3 –N (ppm)	0.65	0.91	0.95	1.09
NH_4 –N (ppm)	74.4	4.1	5.4	13.2
PO_4 –P (ppm)	188.6	189.7	164.0	205.6
K (ppm)	401.2	588.0	625.2	854.5
TS (%)	23.4	18.6	19.2	24.9
VS (%)	43.2	41.6	63.6	76.2

7 Discussion

This research monitored the performance of a bench-scale SSD system using a wet distillers grains (WDG) and cattle feedlot manure mixture. The objectives of this research were

- To assess the performance of a bench scale SSD system using a WDG and manure feedstock mixture by producing a biogas with a composition of at least 50% CH_4 within one of the digesters containing WDG as well as the 100% manure digester.
- To determine a feedstock retention time within a solid-state AD system that could be integrated in a wheat-based ethanol facility paired with a cattle feedlot.
- To determine the nutrient value of the digester effluent (digestate) as a fertilizer supplement for agricultural producers within the vicinity of the ethanol and cattle feedlot operation.

The performance objective of this research was to produce biogas with a composition of at least 50% CH_4 within one of the digesters containing WDG as well as the 100% manure digester. At the end of three cycles of digestion, the biogas within the substrate blend containing 25% WDG and 75% manure achieved a measured CH_4 concentration of 49% and the biogas within the 100% manure substrate achieved a 59% concentration of CH_4. The study conducted by Parker et al. (2002), reported biogas containing 52% to 60% methane using a dry substrate of 100% beef cattle manure from feedlots and a solids content range of 20% through to 50%. It was noted in the Parker et al. (2002) study that the substrate with 50% solids content produced little biogas. The

solids content recorded in this SSD study ranged between 20% and 25% across all four substrate blends and produced biogas containing a similar methane concentration as the Parker et al. (2002) study using a comparable substrate.

The Parker et al. (2002) study converted the biogas production to litres (L) per gram of volatile solids (VS) and saw gas productions of 0.18, 0.21 and 0.19 L per gram VS within the 20%, 30% and 40% total solids substrate blends, respectively. The biogas production calculated in this study was determined to be 0.17, 0.21, 0.18 and 0.12 L per gram VS within substrate Groups 1 through 4 respectively, averaged over all three digestion cycles. However, Cycle 3 was the cycle where the CH_4 content within two of the four substrate groups (75% and 100% manure) was measured at an acceptable biogas level so the production level during this cycle is the most significant. Both Groups 3 and 4 (75% and 100% manure) substrate blends had a production level of 0.17 L per gram (VS) during Cycle 3.

An improvement on the biogas production quantity and quality may be seen by incorporating a leachate recirculation technology in a batch SSD system. The leachate is used to move the microbial colony through the substrate material, as such increasing the surface area interactions between the methanogenic bacteria and the volatile solids available within the substrate.

The goal of this study was to measure the daily biogas production which could then be cumulated over the duration of the digestion cycle to determine the total volume of biogas, in litres (L), produced per gram of volatile solids (gVS) in the substrate. However, interference with barometric pressure observed in the pressure manometers connected to each reactor, and the large amount of headspace in each reactor

compromised the confidence in the daily volume measurement. The volume calculation used to determine the biogas production during each cycle was based on the assumption that the biogas behaved as an ideal gas and the mass reduction noted in each reactor from the start to the end of the cycle was used to determine the mass of the gas produced. The measured volumetric biogas composition for each reactor averaged over the entire cycle was used to determine the mass fraction of the components. This result was then used to determine the volume of biogas produced in each reactor, over each digestion cycle, at standard pressure (1 atmosphere) and temperature (0^{o}C). Should this study be repeated, an alternate method of measuring the daily biogas production of each substrate is suggested. A similar method could be used; however, a smaller headspace in the reactors is suggested along with a method of compensating for barometric pressure influences.

When gathering the biogas samples during this investigation it was assumed that the gas composition within the reactors in the same grouping would be similar. The same reactor was not sampled each time, rather the sampling was randomized between the three reactors within each of the four substrate blends. At the onset of the investigation, this seemed like a valid assumption as the same material was placed in the three reactors making up each of the four substrate groups. Upon investigating the data this assumption was proven valid in three of the four substrate blends, as the biogas composition was similar within each of the three reactors in these blends during every stage of the investigation. In the 100% manure substrate one reactor did not perform as expected thus negating the assumption noted above. However, if the data gathered from this one reactor is removed from the investigation then the assumption holds. Measuring the microbial composition of the substrate blends before, during and after digestion throughout the

study would have added key information to validate the assumption that like blended reactors would perform in the same manner; however, investigating the microbial culture development in detail was outside the scope of this investigation.

The composition of the gas produced by both Groups 1 and 2 was less than 5% CH_4 by volume over all three cycles. It is suspected that due to the amount of WDG in these groups and the corresponding microbial population in the WDG, the methanogenic microbial colony was not able to establish itself despite the mesophilic, anaerobic atmosphere maintained in the reactors.

The biogas produced by Groups 3 and 4 substrate blends in the SSD system was primarily CH_4 and CO_2 with N_2 making up the majority of the remainder in the latter stages of each observation cycle. Based on the theoretical microbiology of the system, and the statistical modeling, the influence of CO_2 and N_2 on the CH_4 percentage achieved by the microbial population in the respective substrate blend was significant. This significance was most likely observed because CO_2 and N_2 are elements found in primary metabolic processes, whereas H_2 and H_2S result from secondary metabolic processes, as such the CO_2 and N_2 had more influence on the production of CH_4 than H_2 and H_2S production (Nagamani and Ramassamy 1999, Shuler and Kargi 2002, Deublein and Steinhauser 2008). Based on the amount of N_2 seen in Groups 1 and 2 substrate blends it appears a denitrification process was taking place. Denitrification is a microbial nitrate reduction process. According to Schuler and Kargi (2002) there are two types of nitrate reduction that are possible under anaerobic conditions, namely assimilatory reduction and dissimilatory reduction. Due to the amount of N_2 recorded in the biogas from Groups 1 and 2 the nitrate reduction process most probable was dissimilatory reduction as N_2 was

the end product of the microbial reactions. Schuler and Kargi (2002) illustrated the dissimilatory reduction denitrification process as shown in **Figure 39**.

Figure 39. Dissimilatory reduction denitrification process (redrawn)

Without determining the carbon source used in the denitrification process it cannot be said with certainty that this process was in fact the cause of the excess nitrogen measured in the reactors. However, the correlation between the variation in the NO_3-N measurement in the reactors over the three digestion cycles and the variation in the N_2 detected supports a denitrification theory. In addition, Schuler and Kargi (2002) noted that the microbes that act as denitrifiers include species of the genera *Pseudomonas*, *Acaligenes, Arthobacter* and *Corynebactor*. The denitrification bacteria prefer a pH of 6.5-7.0 and a temperature range of 20^{o}C to 30^{o}C, which is very similar to that of the methanogenic bacteria required for CH_4 production in anaerobic digestion.

One reactor within the three that made up Group 4 (100% manure) also had an excess amount of N_2 reported in the biogas analysis. As the digester seals were all tested at the outset of each cycle, it was determined that the excess N_2 was not due to an air leak in the system.

A second objective of this research was to determine a feedstock retention time within a solid-state AD system that could be integrated into a wheat-based ethanol facility paired with a cattle feedlot. It was observed that the duration for each of Groups 3 and 4 to achieve the production of viable biogas, that is biogas with approximately 50% CH_4, was 100 and 90 days of operation respectively. During the

initial cycle, Group 3 CH_4 concentrations exceeded CO_2 after approximately 35 days of digestion. Group 4 CH_4 concentrations exceeded CO_2 after approximately 25 days of digestion. The CH_4 concentrations exceeded CO_2 after 20 days for Group 3 and 15 days for Group 4 during Cycle 2. Group 4 improved upon its Cycle 2 results during Cycle 3 as the CH_4 concentrations exceeded CO_2 after only 10 days of digestion. There was steady improvement in the ratio of CH_4 to CO_2 in the biogas with each subsequent cycle. However, it was 90 to 100 days of operation before the concentration of CH_4 in the biogas was viable for energy production. Deublein and Steinhauser (2008) suggest a typical start-up time for an AD system is two to four months, depending on substrate blend utilized and the use of an inoculant. The results of this study indicated a substrate blend of 75% manure with 25% WDG will take between three and four months to produce a viable biogas for use within the ethanol facility. The introduction of a leachate recirculation system such as those used by BEKON and BioFerm may have improved upon the duration it took to reach a CH_4 concentration viable for energy production.

The final objective was to determine the nutrient value of the digester effluent (digestate) to maximize the digestion process and for use as a fertilizer supplement for agricultural producers within the vicinity of the ethanol and cattle feedlot operation. In an AD process, Nagamani and Ramasamy (1999) suggested that a C:N ratio of 25-30:1 in the substrate was optimal to promote biogas production. Demirbas (2006) also noted a target C:N ratio of 25-35:1 for optimal anaerobic digestion. The data gathered in this research showed that both Groups 3 and 4 substrate blends had C:N ratio medians of approximately 25:1. Zhang et al. (2003), in their study of the nutrient requirements of methane producing bacteria found that if the substrate has an acceptable C:N ratio, as

well as sufficient sulphur to promote growth, that the cell density of the methanogens and the methane production rate were only limited by the concentrations of Mg and Fe in the anaerobic system. Mg and Fe were not measured in this research study; however, the low C:N ratio and low sulphur content in Groups 1 and 2 correlate with the low concentration of CH_4 observed. The Groups 3 and 4 substrate blends, 75% and 100% manure respectively, had the highest percentage of sulphur of the four blends. Sulphur is a macronutrient required by microorganisms to complete catabolic pathways. That higher percentage of sulphur coupled with the significant C:N ratio seen in Groups 3 and 4 support the higher CH_4 concentration seen in these substrate blends in all three cycles of the investigation. The higher C:N ratio and sulphur content in the 75% and 100% manure substrate blends support an established methanogenic culture. It can be inferred in Groups 3 and 4 that the limiting factors to improved methanogenesis could have been the Mg and Fe concentrations.

Overall, the nutrient value of the manure as a soil conditioner was maintained throughout the digestion process. Comparing the initial substrate analysis to the digestate results of each cycle there was a total solids reduction of 3% to 5% in both the 75% and 100% manure substrate blends. This total solids reduction equated to a total VS reduction of 1% to 3% in the 100% manure substrate. The 75% manure substrate saw a total VS increase from the initial substrate analysis as compared to the digestate results at the end of each cycle. The total VS increase seen was between 20% and 30%, which could be attributed to the microbial population breaking down the WDG in this Group 3 substrate blend into a more usable form.

The nitrogen, phosphorous, potassium, and sulphur components of the manure fertilizer value were maintained throughout the digestion process, thus typical manure application rate calculations would be applicable. Nitrogen content is typically the basis of manure application rates. The nitrogen requirement is determined after performing a soil test for nutrient requirements and knowing the crop that is to be grown on the land. This same application assessment can be done with digested manure.

8 Conclusion and Recommendations

The overall question to answer in this study was whether an acceptable concentration of CH_4 could be achieved in a SSD system, and over what cycle period, using specific substrate blends of WDG and cattle manure. This overall question indirectly answered the questions of whether or not healthy methane producing microbial populations could be established within each substrate blend.

The data gathered during this study showed CH_4 production in three of the four substrate blends. Two of the substrate blends, represented by Groups 3 and 4, resulted in significant CH_4 concentrations in the biogas produced. At the end of three cycles of digestion the biogas from the substrate blend containing 25% WDG and 75% manure (Group 3) achieved a measured CH_4 concentration of 49% and the biogas from the 100% manure substrate (Group 4) achieved a 59% concentration of CH_4. For each substrate blend where CH_4 concentrations were on the rise both CO_2 and N_2 concentrations were declining. This inverse correlation implied a strong statistical interaction between the concentration of these two gases, the substrate blend and the CH_4 concentrations achieved.

The gas data gathered in this research study indicated Group 3, of the Groups containing WDG, had the most robust methanogenic culture established as it has the lowest overall N_2 and CO_2 concentration detected in the biogas, and the most consistent performance of CH_4 production during each cycle. Group 4 also had a very robust methanogenic bacteria culture establish; however, as the investigation pertained to the integration of an AD system within an ethanol facility paired with a cattle feedlot, the potential use of spoiled WDG in the AD system was more relevant than 100% manure.

The total solids and VS in the Group 3 substrate indicate this 75% manure blend warrants further investigation as there appears to be the potential for continued improvement in the CH_4 concentration in the biogas. This potential for continued improvement was indicative in the amount of VS still available for CH_4 production.

This initial investigation provided some answers to the question involving the interactions within a SSD system affecting CH_4 production and indicated that the most effective substrate blend to maximize CH_4 production was Group 3, a 25% WDG, 75% cattle manure blend. The duration for each of Group 3 and Group 4 to achieve the production of viable biogas; that is biogas with approximately 50% CH_4, was 100 and 90 days of operation respectively. Thus, it can be concluded that a SSD system start up duration would be between three and four months in duration using one of these two substrate blends.

The investigation conducted on the nutrient data gathered in this research supports the conclusion drawn from the gas data regarding the overall methanogenic performance of the substrate blends. The nutrient data supports a Group 3 substrate blend as the blend of choice to provide for the methanogenic activity of the microorganisms within the solid-state anaerobic digestion process paired with a grain ethanol facility. In fact, the total solids and VS in the Group 3 substrate indicated this 75% manure blend warrants further investigation as a total VS increase is seen when comparing the initial substrate nutrient analysis with that after each cycle. This VS increase indicates the potential for continued improvement in the CH_4 concentration in the biogas.

The nitrogen, phosphorous, potassium and sulphur components of the manure fertilizer value were maintained throughout the digestion process of this investigation.

Thus, typical manure application rate calculations are applicable when field applying digestate. Nitrogen content is typically the basis of manure application rates; as such, it would also be the basis for digestate application. The nitrogen requirement is determined after performing a soil test for nutrient requirements and knowing the crop that is to be grown on the land.

Further research involving refining the substrate blend of WDG and manure to contain between 0% and 25% WDG and assessing the biogas quality and quantity of each blend is recommended. It is also recommended to investigate the cycle duration required to achieve a viable biogas concentration using leachate recirculation technology in a SSD system. As well, further research into the anaerobic performance of the selected substrates should include full sampling from each reactor in the investigation on a daily basis for the duration of the first digestion cycle to determine the biogas performance in each reactor before making the assumption that each reactor in the substrate group will perform in the same manner. Also, measuring the microbial composition of the substrate blends before, during and after digestion throughout the study will add key information to validate the assumption that like-blended reactors perform in the same manner.

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Appendix I: Gas Chromatograph Specifications

Description	Specification	
ECD's		
Component	front	back
Injector temp ©	70	70
Split ratio	5	5
Pressure (psi)	12.5	20
Column flow (ml/min)	7.9	14.4
Total flow (ml/min)	53.5	96.4
Linear Velocity (cm/sec)	117	177
Oven temp ©	35	35
Detector temp ©	370	370
Make-up flow (ml/min)	10	12
FID		
injector ©	70	
Split ratio	0	
Pressure (psi)	varies as temp changes	
Column flow (ml/min)	40	
Total flow (ml/min)	163	
Linear Velocity (cm/sec)	69	
Oven temp ©	50 - 200 ramp	
Detector temp ©	200	
Make-up flow (ml/min)	0	
Hydrogen flow (ml/min)	28	
Air flow (ml/min)	280	
Micro-gc		
column temp ©	100	
sensitivity	Hi	
injection time (ms)	150	
injector temp ©	110	
sample time (s)	40	
stabilizing time (s)	2	
initial pressure (kPa)	100	
final pressure (kPa)	100	

Appendix II: Pairs Plots of Biogas and Digestate Data

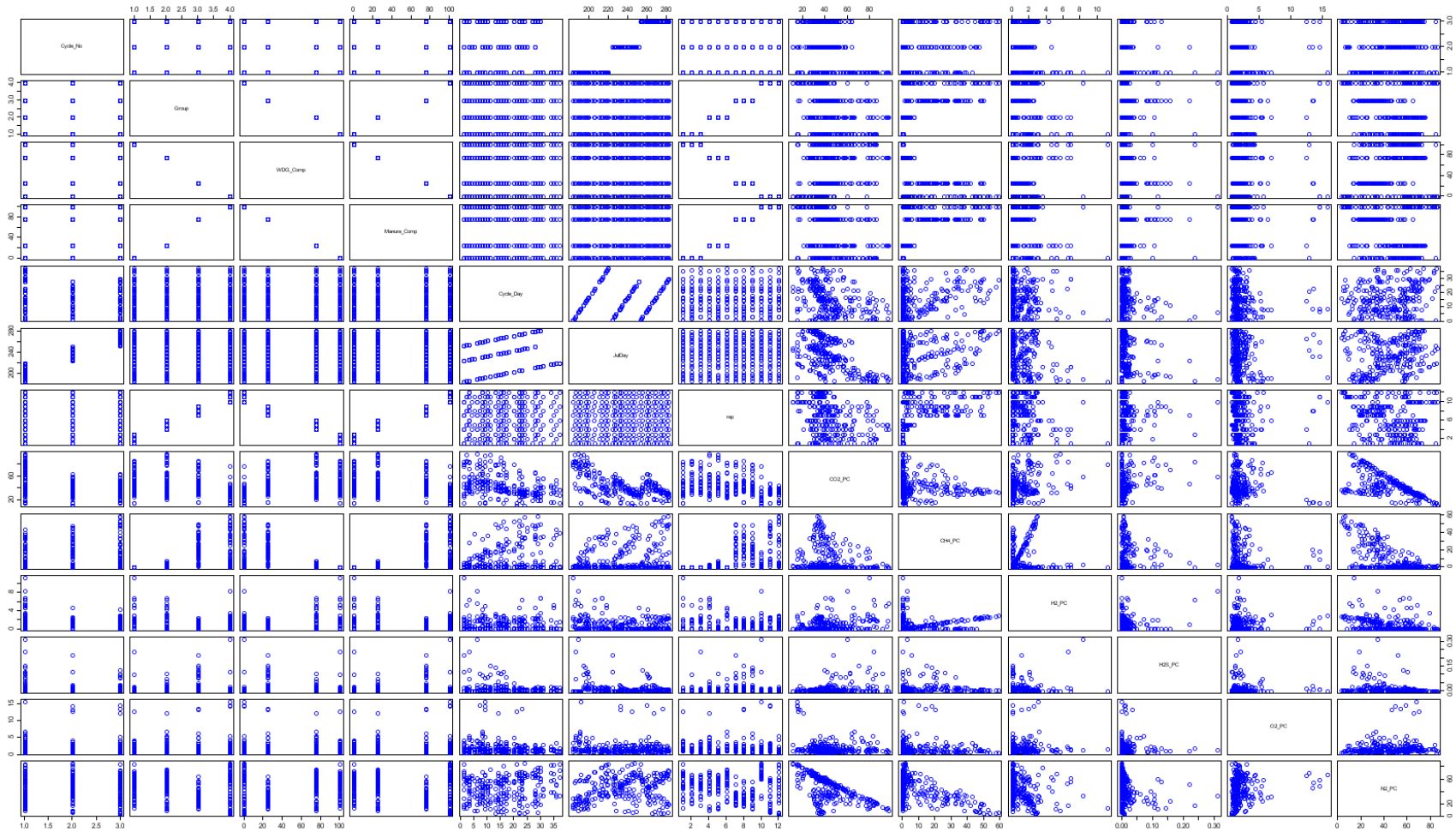

Figure 40. Pairs plot of biogas production concentrations achieved in the study

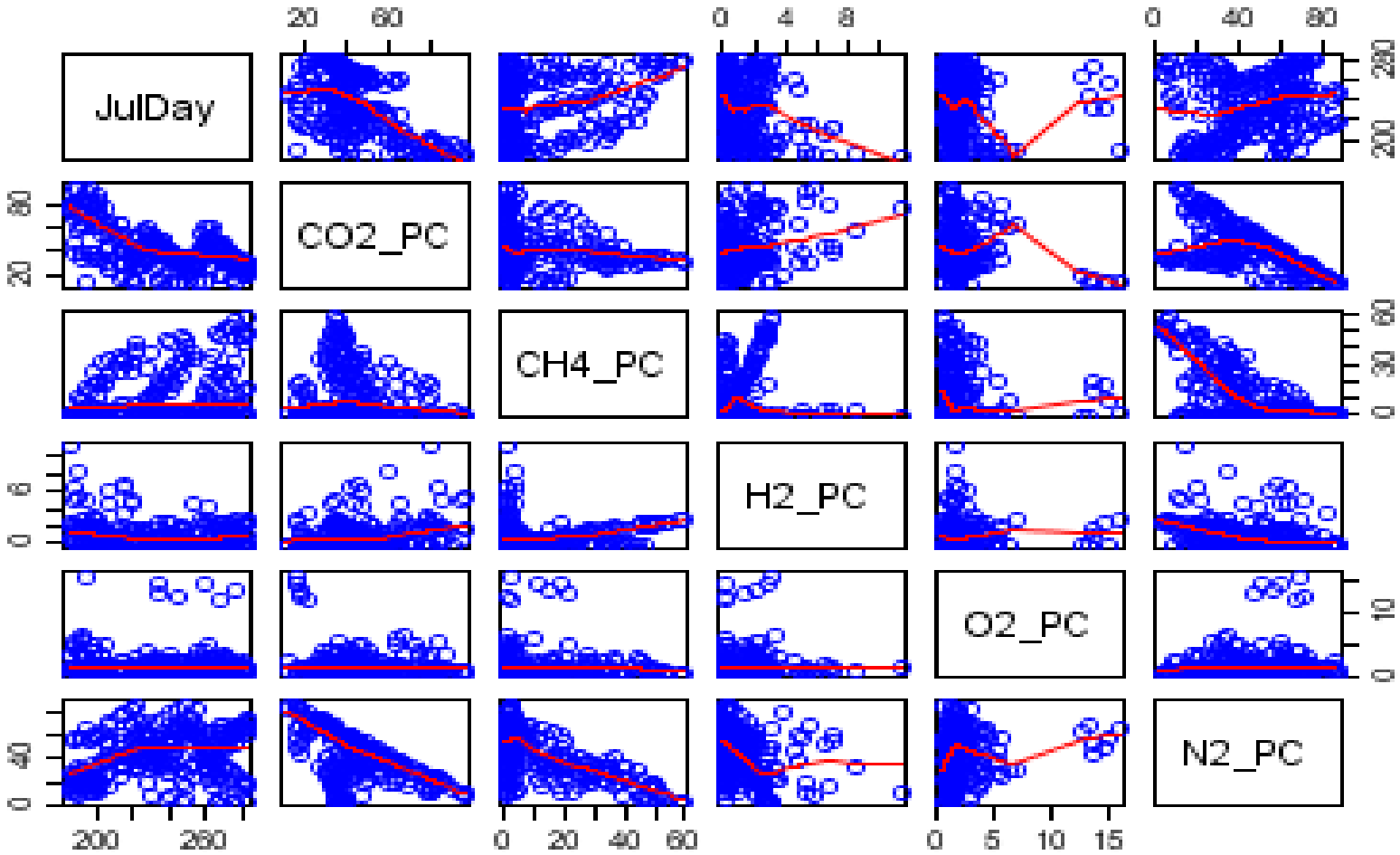

Figure 41. Pairs plot of key solid-state digestion biogas components

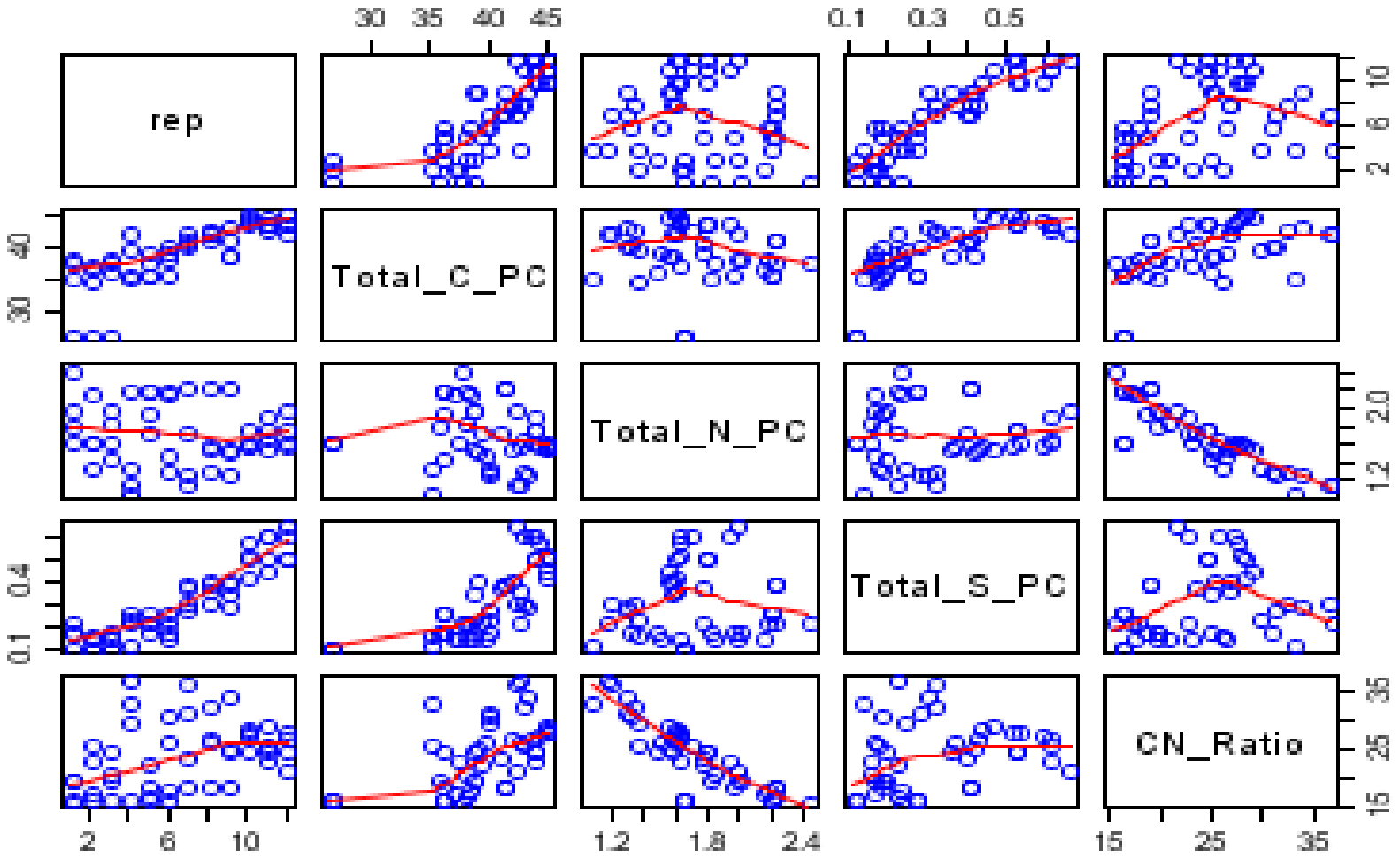

Figure 42. Pairs plot of total C, N and S in (%) plus C:N ratio in the digestate

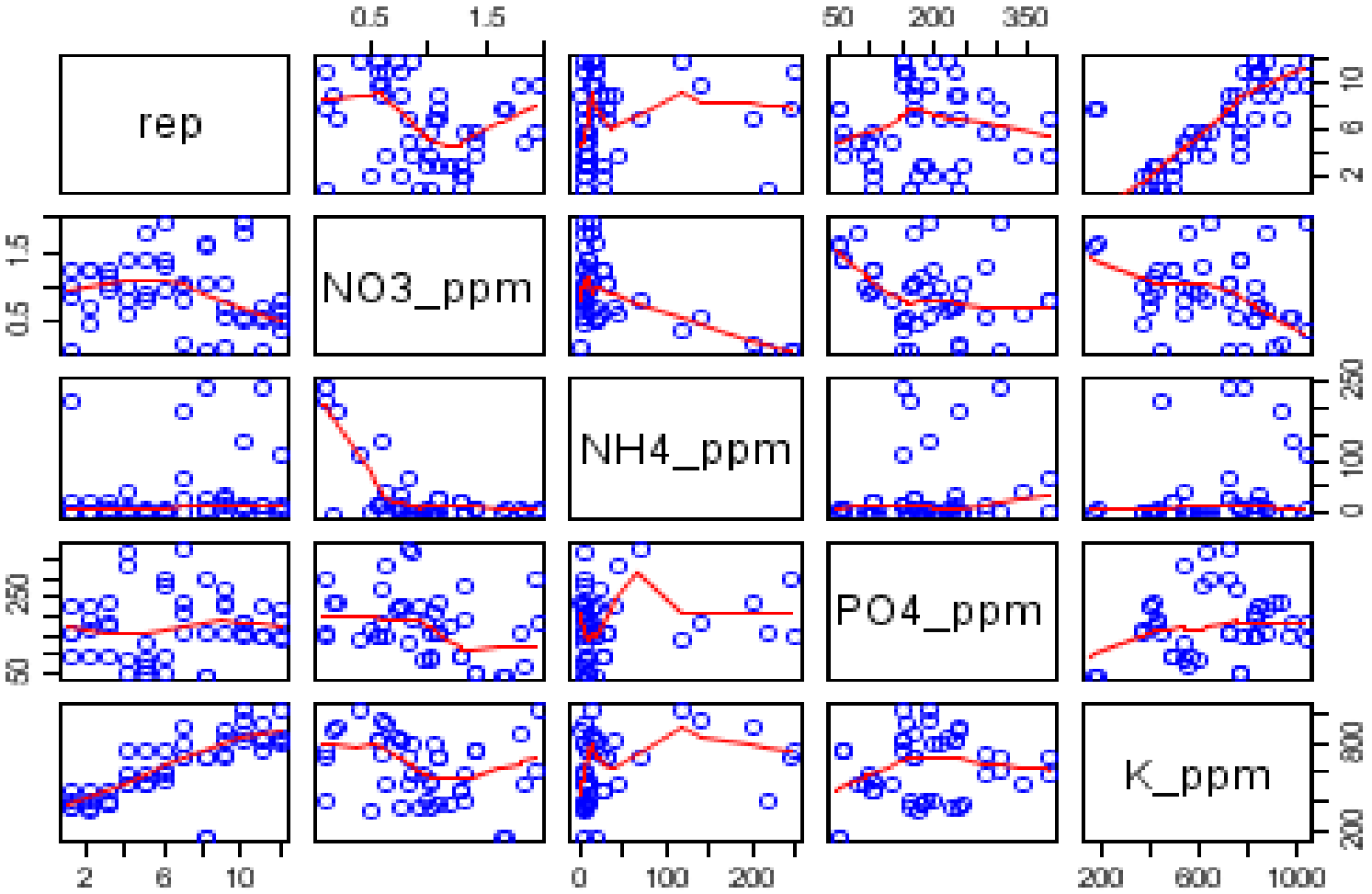

Figure 43. Pairs plot of NO_3, NH_4, PO_4 and K (ppm) in the digestate

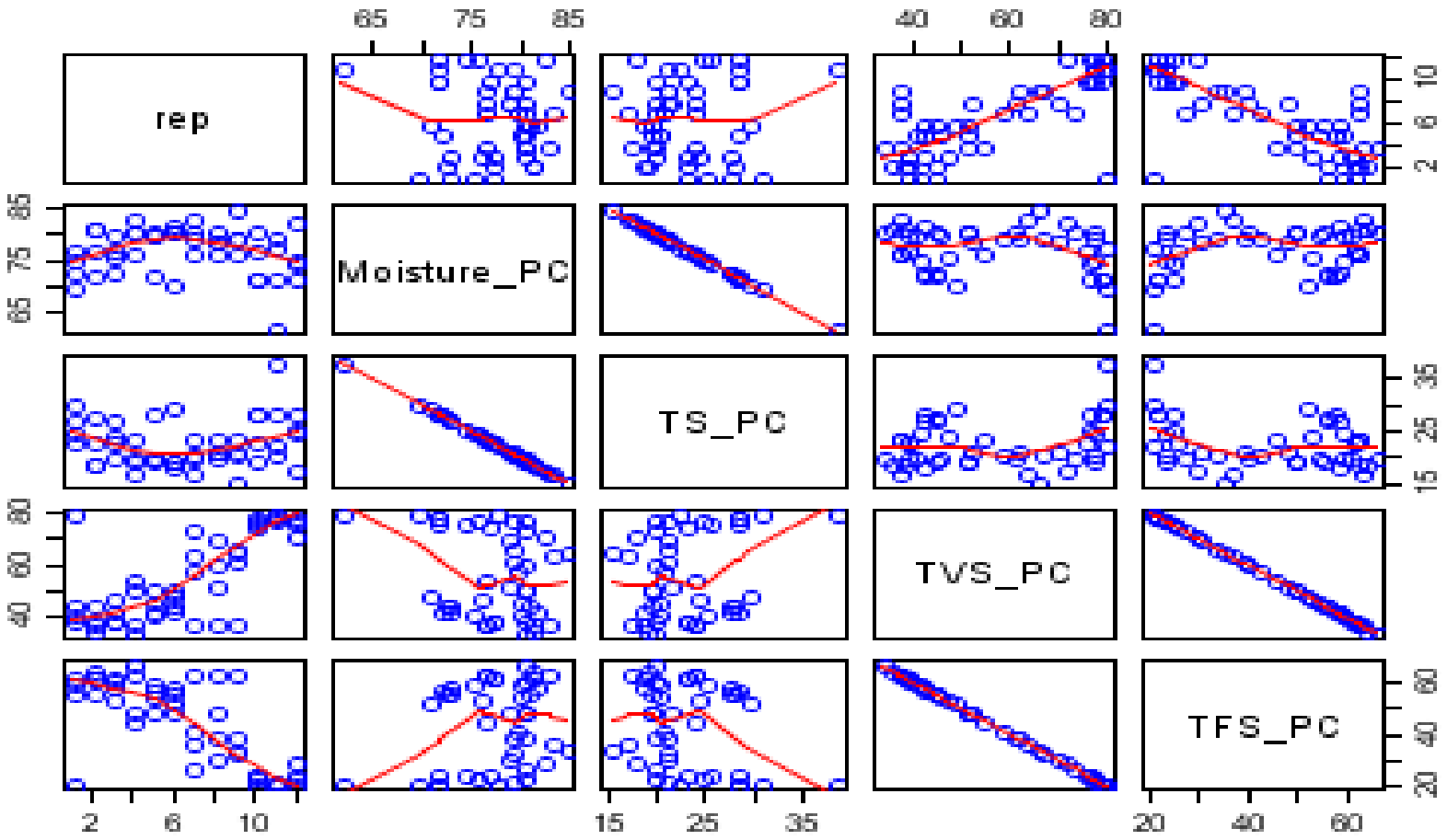

Figure 44. Pairs plot of moisture, TS, TVS and TFS in the digestate

Appendix III: Raw Research Data

Solid State Digestion Gas Analysis

JulDay = Julian Day

rep = digester container number

ID = Julian Day and container number reference

Mean Total Recovery (%) = 102.99

sd = 5.13

Cycle 1 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
184	01	184-01	79.54	0.00	11.26	<0.0001	1.59	13.78	*106.17*
184	04	184-04	80.08	0.00	2.54	<0.0001	3.51	16.57	*102.70*
184	07	184-07	83.07	1.37	0.64	<0.0001	1.97	19.71	*106.76*
184	10	184-10	76.70	0.62	2.26	<0.0001	0.83	29.40	*109.82*
185	02	185-02	84.30	0.00	5.23	<0.0001	1.61	20.64	*111.78*
185	05	185-05	95.78	0.02	1.91	<0.0001	1.19	13.43	*112.33*
185	08	185-08	85.36	2.08	0.55	<0.0001	1.57	20.29	*109.85*
185	11	185-11	47.75	0.85	0.61	<0.0001	1.74	55.22	*106.17*
186	03	186-03	83.11	0.00	6.52	0.2355	1.93	18.29	*110.09*
186	06	186-06	82.50	0.00	2.24	0.0711	5.48	25.37	*115.67*
186	09	186-09	72.33	2.15	0.33	<0.0001	5.09	28.91	*108.79*
186	12	186-12	40.16	1.66	1.37	<0.0001	4.57	57.83	*105.58*
189	01	189-01	74.24	0.00	1.88	<0.0001	4.09	29.36	*109.57*
189	04	189-04	96.51	0.04	5.59	<0.0001	0.91	10.18	*113.24*
189	07	189-07	79.11	11.05	0.07	<0.0001	1.39	19.66	*111.28*
189	10	189-10	59.57	2.83	8.32	0.3099	1.49	33.98	*106.51*
190	02	190-02	80.47	0.00	2.11	<0.0001	1.79	26.13	*110.50*
190	05	190-05	86.47	0.34	1.88	<0.0001	3.71	20.41	*112.82*
190	08	190-08	76.81	9.86	0.08	<0.0001	1.48	23.25	*111.47*
190	11	190-11	39.67	4.88	0.29	<0.0001	3.24	57.72	*105.80*
191	03	191-03	73.51	0.00	1.86	<0.0001	3.94	31.63	*110.94*
191	06	191-06	66.30	0.01	3.16	<0.0001	6.83	32.53	*108.83*
191	09	191-09	63.40	8.94	0.05	<0.0001	6.21	30.66	*109.27*
191	12	191-12	35.47	3.76	2.02	<0.0001	5.52	59.24	*106.01*
192	01	192-01	84.43	0.00	2.13	<0.0001	1.52	23.20	*111.28*
192	04	192-04	94.48	0.35	5.13	<0.0001	0.99	12.26	*113.21*
192	07	192-07	76.49	17.00	0.05	<0.0001	1.14	18.21	*112.88*
192	10	192-10	14.96	0.92	3.00	<0.0001	15.74	67.95	*102.56*
194	02	194 - 02	84.76	0.00	2.02	0.0995	1.42	21.97	*110.26*
194	05	194 - 05	94.92	1.87	0.66	0.0253	1.31	12.36	*111.14*
194	08	194 - 08	70.34	14.50	0.04	0.1555	1.16	24.97	*111.17*
194	11	194 - 11	39.45	7.19	0.35	0.0167	1.38	57.25	*105.64*
196	03	196 - 03	78.08	0.00	2.96	0.0153	1.56	27.30	*109.92*
196	06	196 - 06	78.14	0.01	3.38	0.0371	3.25	24.54	*109.36*
196	09	196 - 09	79.51	17.93	0.05	0.1429	1.10	13.49	*112.22*
196	12	196 - 12	39.15	5.48	1.30	0.0126	1.55	58.07	*105.57*
197	01	197 - 01	79.04	0.00	1.76	0.0215	1.22	27.53	*109.57*
197	04	197 - 04	63.40	1.64	1.30	0.0581	5.15	36.05	*107.59*
197	07	197 - 07	62.50	26.08	0.02	0.1083	1.63	20.98	*111.32*
197	10	197 - 10	35.12	2.37	1.52	0.0144	4.03	61.07	*104.12*
198	02	198 - 02	85.72	0.00	1.84	0.0270	1.04	21.87	*110.50*
198	05	198 - 05	91.20	4.02	0.16	0.1032	1.26	14.99	*111.74*
198	08	198 - 08	62.31	19.51	0.03	0.1054	1.12	27.59	*110.66*
198	11	198 - 11	34.29	10.46	0.28	0.0111	2.35	58.17	*105.57*

Solid State Digestion Gas Analysis

Cycle 1 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
199	03	199 - 03	70.30	0.01	2.39	0.0108	1.98	33.72	*108.41*
199	06	199 - 06	82.21	0.12	3.06	0.0302	1.26	24.08	*110.76*
199	09	199 - 09	77.82	21.03	0.04	0.1168	0.73	12.95	*112.70*
199	12	199 - 12	34.88	4.75	1.33	0.0107	1.78	62.57	*105.33*
200	01	200 - 01	66.12	0.01	1.15	0.0208	3.72	36.54	*107.57*
200	04	200 - 04	62.50	2.38	0.64	0.0400	2.46	39.38	*107.40*
200	07	200 - 07	58.20	32.46	0.02	0.0848	0.98	20.77	*112.53*
200	10	200 - 10	34.60	2.23	1.95	0.0122	1.65	64.30	*104.74*
203	02	203 - 02	79.81	0.01	1.97	0.0227	0.84	27.19	*109.84*
203	05	203 - 05				missing tube			
203	08	203 - 08	44.00	20.78	0.01	0.0473	5.01	39.24	*109.09*
203	11	203 - 11	31.42	19.32	0.13	0.0086	1.55	55.34	*107.76*
205	03	205 - 03	55.78	0.01	1.42	<0.0001	2.00	46.75	*105.97*
205	06	205 - 06	65.01	0.02	4.68	0.0013	1.47	40.46	*111.64*
205	09	205 - 09	69.12	24.65	0.24	0.0206	0.77	17.53	*112.32*
205	12	205 - 12	26.92	3.44	0.98	0.0020	0.96	71.01	*103.31*
206	01	206 - 01	59.68	0.01	0.61	<0.0001	1.33	45.13	*106.76*
206	04	206 - 04	46.61	2.65	6.45	0.0184	2.49	53.21	*111.43*
206	07	206 - 07	46.30	34.13	0.12	0.0192	1.29	29.68	*111.53*
206	10	206 - 10	26.07	1.38	1.38	0.0029	1.62	72.30	*102.75*
211	02	211 - 02	66.17	0.01	1.78	0.0070	1.12	38.35	*107.45*
211	05	211 - 05	64.10	7.34	0.68	0.0146	0.76	36.61	*109.50*
211	08	211 - 08	43.44	31.84	0.11	0.0208	1.09	34.48	*110.99*
211	11	211 - 11	29.06	35.25	0.26	0.0040	2.89	42.35	*109.81*
212	03	212 - 03	51.20	0.02	2.10	0.0008	1.60	50.63	*105.55*
212	06	212 - 06	40.25	0.03	0.24	0.0071	1.56	62.51	*104.60*
212	09	212 - 09	56.14	27.44	0.16	0.0233	0.87	27.34	*111.98*
212	12	212 - 12	19.14	2.13	1.04	0.0007	1.75	78.23	*102.29*
213	01	213 - 01	40.05	0.02	6.83	<0.0001	1.77	61.90	*110.57*
213	04	213 - 04	44.64	2.83	6.90	0.0164	1.43	55.98	*111.81*
213	07	213 - 07	41.00	33.85	0.08	0.0203	1.10	35.00	*111.05*
213	10	213 - 10	19.85	0.85	3.60	<0.0001	1.78	79.30	*105.38*
214	02	214 - 02	56.23	0.01	1.42	0.0044	2.89	45.31	*105.87*
214	05	214 - 05	56.39	7.09	0.44	0.0128	1.20	42.99	*108.14*
214	08	214 - 08	41.63	34.89	0.10	0.0201	0.91	34.18	*111.73*
214	11	214 - 11	32.14	2.87	0.24	0.0036	1.50	33.48	*70.24*
217	03	217 - 03	44.44	0.04	1.94	<0.0001	1.43	56.98	*104.82*
217	06	217 - 06	30.61	0.03	0.13	0.0030	1.37	70.82	*102.96*
217	09	217 - 09	41.30	26.41	0.08	0.0240	2.39	39.47	*109.67*
217	12	217 - 12	15.87	1.57	0.54	0.0001	1.68	82.70	*102.37*
218	01	218 - 01	33.29	0.03	5.24	<0.0001	1.38	68.37	*108.31*
218	04	218 - 04	41.54	2.67	4.76	0.0147	1.11	59.23	*109.33*
218	07	218 - 07	35.95	32.98	0.07	0.0217	1.67	39.35	*110.05*
218	10	218 - 10	16.70	0.60	0.11	<0.0001	1.53	82.62	*101.55*
219	02	219 - 02	53.57	0.02	1.42	0.0032	1.24	49.85	*106.11*
219	05	219 - 05	47.15	6.94	0.37	0.0086	0.82	51.58	*106.86*
219	08	219 - 08	38.00	37.78	0.06	0.0204	1.04	34.51	*111.41*
219	11	219 - 11	29.69	42.97	0.15	0.0055	3.35	31.84	*108.00*

Cycle 1 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
220	03	220 - 03	37.75	0.03	1.73	0.0000	1.80	62.52	*103.82*
220	06	220 - 06	27.59	0.03	0.10	0.0031	1.20	73.95	*102.87*
220	09	220 - 09	37.61	39.15	0.08	0.0207	0.86	34.14	*111.85*
220	12	220 - 12	15.20	1.27	0.17	<0.0001	0.59	85.06	*102.29*
224	01	224 - 01	28.74	0.00	0.03	<0.0001	1.50	72.45	*102.73*
224	04	224 - 04	58.48	0.06	1.37	0.0215	2.42	43.98	*106.34*
224	07	224 - 07	47.01	5.45	1.07	0.2174	1.26	51.98	*106.98*
224	10	224 - 10	41.95	0.10	1.98	0.1154	0.55	59.92	*104.61*
Cycle 2 - Sample JulDay	**rep**	**ID**	**CO_2 (%)**	**CH_4 (%)**	**H_2 (%)**	**H_2S (%)**	**O_2 (%)**	**N_2 (%)**	***Total (%)***
226	02	226 - 02	36.88	0.00	0.00	<0.0001	1.95	61.87	*100.71*
226	03	226 - 03	34.82	0.00	0.00	<0.0001	1.63	63.95	*100.40*
226	05	226 - 05	58.39	0.65	0.02	<0.0001	0.58	40.71	*100.34*
226	06	226 - 06	48.92	0.01	0.00	<0.0001	1.18	49.80	*99.92*
226	08	226 - 08	51.54	10.00	0.60	<0.0001	0.75	38.20	*101.09*
226	09	226 - 09	49.72	5.38	0.32	<0.0001	0.80	45.29	*101.51*
226	11	226 - 11	47.89	27.83	1.57	<0.0001	0.96	20.79	*99.04*
226	12	226 - 12	44.28	3.36	0.20	<0.0001	0.68	52.34	*100.87*
227	01	227 - 01	30.86	0.34	0.00	<0.0001	4.17	65.42	*100.79*
227	04	227 - 04	63.85	0.46	0.01	<0.0001	2.30	36.02	*102.64*
227	07	227 - 07	50.92	10.36	0.62	<0.0001	2.59	36.55	*101.04*
227	10	227 - 10	34.56	0.49	0.01	<0.0001	2.50	62.43	*99.99*
228	02	228 - 02	41.65	0.00	0.00	<0.0001	1.55	57.93	*101.13*
228	05	228 - 05	57.75	1.03	0.03	<0.0001	0.86	42.77	*102.44*
228	08	228 - 08	53.03	13.46	0.64	<0.0001	0.85	32.77	*100.76*
228	11	228 - 11	47.01	38.04	1.82	<0.0001	1.34	9.22	*97.43*
231	03	231 - 03	48.66	0.35	0.01	<0.0001	0.70	50.94	*100.66*
231	06	231 - 06	46.79	0.35	0.00	<0.0001	1.69	53.12	*101.95*
231	09	231 - 09	48.84	12.25	0.70	<0.0001	1.22	38.03	*101.05*
231	12	231 - 12	40.48	11.76	0.65	<0.0001	0.76	47.19	*100.83*
231	01	231 - 01	51.80	0.35	0.00	<0.0001	1.67	48.18	*102.00*
231	04	231 - 04	55.48	0.66	0.02	0.0123	2.56	43.45	*102.17*
231	07	231 - 07	47.74	15.04	0.94	0.0356	3.14	33.73	*100.62*
231	10	231 - 10	25.53	0.84	0.04	0.0049	2.09	72.05	*100.56*
232	02	232 - 02	45.93	0.35	0.00	<0.0001	1.36	53.28	*100.92*
232	05	232 - 05	50.64	1.73	0.09	0.0053	1.06	48.51	*102.02*
232	08	232 - 08	17.07	19.92	1.12	0.0323	13.32	46.29	*97.74*
232	11	232 - 11	15.24	17.86	2.55	0.0085	14.45	49.53	*99.66*
233	03	233 - 03	48.43	0.36	0.02	<0.0001	1.26	49.34	*99.41*
233	06	233 - 06	40.39	0.36	0.00	0.0042	2.64	58.08	*101.47*
233	09	233 - 09	42.81	13.41	0.78	0.0279	2.67	41.00	*100.69*
233	12	233 - 12	37.47	17.52	1.01	0.0041	0.96	43.34	*100.31*
234	01	234 - 01	51.58	0.36	0.01	<0.0001	1.21	48.63	*101.78*
234	04	234 - 04	51.73	0.87	0.03	0.0085	0.82	48.66	*102.12*
234	07	234 - 07	46.37	17.47	1.00	0.0184	1.93	33.71	*100.49*
234	10	234 - 10	22.15	1.22	0.06	0.0021	0.88	76.21	*100.51*
237	02	237 - 02	43.38	0.39	0.00	<0.0001	1.37	55.14	*100.28*
237	05	237 - 05	41.83	2.60	0.13	0.0002	1.06	55.59	*101.22*

Cycle 2 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
237	08	237 - 08	41.41	23.93	1.40	0.0145	2.53	29.93	*99.22*
237	11	237 - 11	37.17	45.10	2.59	0.0004	2.70	8.07	*95.63*
238	03	238 - 03	39.63	0.36	0.02	<0.0001	3.75	53.54	*97.30*
238	06	238 - 06	34.05	0.37	0.00	<0.0001	2.21	64.28	*100.91*
238	09	238 - 09	37.68	15.80	0.86	0.0083	1.56	43.47	*99.38*
238	12	238 - 12	34.01	25.28	1.43	0.0024	0.83	37.52	*99.08*
239	01	239 - 01	44.01	0.42	0.01	<0.0001	1.42	54.93	*100.81*
239	04	239 - 04	39.19	0.88	0.03	0.0000	1.01	59.63	*100.74*
239	07	239 - 07	38.18	21.21	1.22	0.0103	1.58	37.12	*99.32*
239	10	239 - 10	15.21	1.53	0.08	<0.0001	1.75	81.49	*100.07*
240	02	240 - 02	40.60	0.36	0.00	<0.0001	1.24	57.41	*99.61*
240	05	240 - 05	37.28	2.98	0.15	0.0025	1.20	59.28	*100.90*
240	08	240 - 08	37.37	26.98	1.55	0.0070	2.62	29.73	*98.27*
240	11	240 - 11	36.17	44.51	2.50	0.0001	2.80	9.61	*95.60*
241	03	241 - 03	40.38	0.37	0.03	<0.0001	1.73	54.11	*96.62*
241	06	241 - 06	31.07	0.36	0.00	<0.0001	1.59	67.48	*100.52*
241	09	241 - 09	34.27	17.94	1.00	0.0069	1.13	44.96	*99.30*
241	12	241 - 12	32.89	29.48	1.62	0.0019	0.75	33.71	*98.45*
244	01	244 - 01	15.58	0.35	0.01	<0.0001	12.89	69.89	*98.72*
244	04	244 - 04	31.42	0.87	0.03	<0.0001	1.28	66.89	*100.48*
244	07	244 - 07	30.71	27.47	1.70	0.0039	2.65	36.28	*98.81*
244	0	244 - 10	11.13	1.48	0.08	<0.0001	2.96	83.88	*99.54*
245	02	245 - 02	35.96	0.36	0.00	<0.0001	1.31	61.94	*99.58*
245	05	245 - 05	31.75	3.39	0.19	0.0021	1.07	63.88	*100.28*
245	08	245 - 08	35.63	36.60	2.05	0.0046	0.89	22.05	*97.22*
245	11	245 - 11	38.54	47.25	2.31	<0.0001	1.15	6.02	*95.26*
246	03	246 - 03	37.27	0.38	0.04	<0.0001	0.86	57.58	*96.13*
246	06	246 - 06	26.36	0.37	0.00	<0.0001	1.50	71.94	*100.18*
246	09	246 - 09	29.86	23.67	1.31	0.0092	1.10	43.45	*99.40*
246	12	246 - 12	29.28	31.60	1.95	0.0028	2.52	33.14	*98.48*
247	01	247 - 01	31.69	0.37	0.01	<0.0001	2.75	65.68	*100.50*
247	04	247 - 04	27.14	0.82	0.03	<0.0001	2.85	69.63	*100.46*
247	07	247 - 07	29.90	34.41	1.79	0.0026	1.56	29.73	*97.38*
247	10	247 - 10	11.21	1.64	0.09	<0.0001	1.07	85.54	*99.56*
248	02	248 - 02	32.50	0.36	1.09	<0.0001	2.00	64.76	*100.71*
248	05	248 - 05	27.94	3.31	0.00	0.0089	2.14	67.02	*100.42*
248	08	248 - 08	33.45	41.59	0.00	0.0040	0.75	18.24	*94.04*
248	11	248 - 11	38.11	45.65	0.00	<0.0001	1.09	7.92	*92.77*
251	03	251 - 03	32.17	0.37	4.64	<0.0001	1.34	62.78	*101.29*
251	06	251 - 06	23.35	0.36	0.00	<0.0001	1.27	75.29	*100.28*
251	09	251 - 09	26.14	33.47	0.00	0.0090	0.93	35.47	*96.02*
251	12	251 - 12	30.33	37.60	0.01	<0.0001	0.77	25.87	*94.59*

Solid State Digestion Gas Analysis

Cycle 3 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
253	01	253 - 01	14.75	0.34	-0.01	<0.0001	1.28	84.01	*100.38*
253	04	253 - 04	28.32	0.01	0.07	<0.0001	4.29	67.51	*100.20*
253	07	253 - 07	30.76	2.72	0.08	<0.0001	2.38	64.36	*100.31*
253	10	253 - 10	25.96	0.35	0.30	<0.0001	3.00	70.47	*100.08*
255	03	255 - 03	57.67	0.01	0.31	0.0075	1.54	42.54	*102.09*
255	06	255 - 06	45.26	0.01	2.99	<0.0001	1.26	51.34	*100.86*
254	02	254 - 02	41.12	0.36	0.03	0.0075	1.45	58.41	*101.38*
254	05	254 - 05	40.01	0..01	1.43	0.0024	0.67	58.72	*100.83*
254	08	254 - 08	37.40	3.87	0.01	0.0203	1.49	58.05	*100.83*
254	11	254 - 11	31.54	4.94	0.03	0.0160	2.71	61.14	*100.38*
255	09	255 - 09	39.51	3.55	0.02	0.1276	1.88	55.86	*100.95*
255	12	255 - 12	35.45	6.08	0.11	0.0253	1.39	57.66	*100.70*
258	01	258 - 01	48.92	0.01	0.00	0.0027	1.45	51.19	*101.57*
258	04	258 - 04	49.92	0.40	2.70	0.0193	4.25	44.31	*101.59*
258	07	258 - 07	42.94	14.58	0.01	0.0383	2.72	38.82	*99.11*
258	10	258 - 10	34.65	2.58	0.05	0.0127	1.95	61.47	*100.72*
259	02	259 - 02	63.36	0.01	2.26	0.0052	1.56	34.85	*102.04*
259	05	259 - 05	52.22	1.67	0.07	0.0072	0.96	47.12	*102.05*
259	08	259 - 08	49.72	12.80	0.01	0.1038	2.06	35.44	*100.13*
259	11	259 - 11	15.05	9.67	0.00	0.0112	14.55	58.61	*97.89*
260	03	260 - 03	64.01	0.01	3.03	0.0042	1.24	33.77	*102.07*
260	06	260 - 06	46.03	0.34	4.27	0.0039	2.58	48.09	*101.32*
260	09	260 - 09	46.66	11.08	0.01	0.0933	2.37	40.08	*100.30*
260	12	260 - 12	40.62	32.34	0.01	0.0047	0.67	22.57	*96.22*
261	01	261 - 01	53.69	0.38	0.00	0.0001	1.56	46.41	*102.04*
261	04	261 - 04	46.42	0.48	0.01	0.0246	5.28	49.25	*101.46*
261	07	261 - 07	45.27	20.74	1.13	0.0786	1.90	30.95	*100.07*
261	10	261 - 10	29.04	4.16	0.22	0.0068	1.84	65.43	*100.70*
262	02	262 - 02	61.68	0.35	0.00	0.0020	1.53	37.30	*100.87*
262	05	262 - 05	50.24	2.77	0.14	0.0051	1.07	47.79	*102.01*
262	08	262 - 08	53.14	18.96	1.04	0.0828	0.79	26.43	*100.44*
262	11	262 - 11	36.42	36.10	2.20	0.0063	3.34	18.87	*96.93*
265	03	265 - 03	50.02	0.36	0.01	0.0011	3.12	44.73	*98.23*
265	06	265 - 06	42.05	0.35	0.00	0.0012	2.32	55.02	*99.75*
265	09	265 - 09	47.98	17.65	0.94	0.0357	0.97	33.12	*100.70*
265	12	265 - 12	36.84	49.67	2.76	0.0065	1.03	5.34	*95.65*
266	01	266 - 01	45.78	0.53	0.01	<0.0001	1.71	53.55	*101.59*
266	04	266 - 04	20.71	0.49	0.02	0.0146	12.40	65.46	*99.11*
266	07	266 - 07	42.74	27.37	1.52	0.0382	1.50	26.19	*99.35*
266	10	266 - 10	21.90	5.95	0.37	0.0042	2.24	69.62	*100.07*
267	02	267 - 02	48.50	0.36	0.00	0.0008	2.57	48.83	*100.26*
267	05	267 - 05	44.09	4.24	0.22	0.0062	0.92	52.00	*101.47*
267	08	267 - 08	49.32	25.14	1.28	0.0301	0.96	22.86	*99.58*
267	11	267 - 11	35.77	47.54	2.64	0.0031	1.27	8.58	*95.80*
268	03	268 - 03	48.53	0.38	0.02	0.0003	0.89	47.75	*97.56*
268	06	268 - 06	39.15	0.37	0.00	0.0007	1.47	60.38	*101.37*
268	09	268 - 09	44.54	19.99	1.10	0.0192	0.93	33.43	*100.00*
268	12	268 - 12	36.46	52.19	2.71	0.0066	0.55	2.96	*94.87*

Cycle 3 - Sample JulDay	rep	ID	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	O_2 (%)	N_2 (%)	*Total (%)*
269	01	269 - 01	37.20	0.40	0.01	<0.0001	3.30	59.90	*100.82*
269	04	269 - 04	37.86	0.67	0.02	0.0090	2.78	59.63	*100.97*
269	07	269 - 07	35.26	26.73	1.71	0.0235	3.74	31.36	*98.82*
269	10	269 - 10	20.55	7.69	0.45	0.0023	1.46	69.84	*100.01*
272	02	272 - 02	42.23	0.38	0.00	0.0002	1.17	56.48	*100.27*
272	05	272 - 05	37.68	4.74	0.27	0.0071	0.71	57.58	*100.99*
272	08	272 - 08	42.27	19.33	1.69	0.0152	3.30	34.05	*100.65*
272	11	272 - 11	33.70	52.80	2.53	0.0030	1.42	11.28	*101.73*
274	03	274 - 03	38.49	0.37	0.02	0.0000	0.85	57.94	*97.67*
274	06	274 - 06	31.33	0.36	0.00	0.0006	1.26	68.33	*101.28*
274	09	274 - 09	38.76	27.05	1.54	0.0105	0.80	31.17	*99.34*
274	12	274 - 12	34.80	53.76	2.69	0.0048	0.71	4.68	*96.64*
274	01	274 - 01	32.25	0.38	0.02	<0.0001	2.68	65.53	*100.86*
274	04	274 - 04	32.31	0.73	0.03	0.0068	2.16	65.81	*101.05*
274	07	274 - 07	35.46	40.25	2.01	0.0136	1.61	24.06	*103.41*
274	10	274 - 10	19.31	10.83	0.63	0.0012	1.26	68.20	*100.23*
275	02	275 - 02	37.73	0.37	0.01	0.0001	1.40	61.11	*100.61*
275	05	275 - 05	34.51	4.86	0.27	0.0074	0.86	60.87	*101.37*
275	08	275 - 08	16.09	14.16	1.88	0.0116	13.50	54.28	*99.92*
275	11	275 - 11	32.88	47.93	2.40	0.0026	1.62	15.22	*100.05*
276	01	276 - 01	30.38	0.36	0.02	<0.0001	2.65	67.25	*100.66*
276	03	276 - 03	32.61	0.36	0.00	<0.0001	2.48	62.94	*98.39*
276	06	276 - 06	28.97	0.38	0.00	0.0005	1.35	70.35	*101.05*
276	09	276 - 09	36.86	33.07	1.65	0.0090	0.74	29.17	*101.50*
276	12	276 - 12	34.59	57.19	2.86	0.0052	0.57	4.68	*99.90*
279	01	279 - 01	29.41	0.37	0.01	<0.0001	1.38	69.51	*100.68*
279	04	279 - 04	29.31	0.78	0.03	0.0063	1.01	69.96	*101.09*
279	07	279 - 07	33.08	48.24	2.41	0.0095	1.13	20.17	*105.04*
279	10	279 - 10	19.58	14.97	0.93	0.0017	1.08	63.51	*100.08*
280	02	280 - 02	29.85	0.39	0.01	0.0000	2.79	67.66	*100.69*
280	05	280 - 05	27.62	4.65	0.28	0.0083	2.24	65.86	*100.65*
280	08	280 - 08	35.30	47.74	2.39	0.0094	1.22	17.15	*103.81*
280	11	280 - 11	31.77	44.32	2.22	0.0026	1.76	23.19	*103.25*
281	03	281 - 03	29.47	0.38	0.01	<0.0001	1.38	67.17	*98.41*
281	06	281 - 06	24.78	0.37	0.00	0.0001	1.35	74.68	*101.18*
281	09	281 - 09	31.12	48.80	2.44	0.0072	1.14	21.36	*104.87*
281	12	281 - 12	33.27	58.82	2.94	0.0038	0.55	6.00	*101.59*
282	01	282 - 01	25.71	0.38	0.10	<0.0001	1.96	72.44	*100.59*
282	04	282 - 04	26.55	0.75	0.03	0.0051	1.11	72.85	*101.29*
282	07	282 - 07	31.24	49.71	2.49	0.0099	1.01	19.09	*103.54*
282	10	282 - 10	19.97	17.42	1.06	0.0025	1.10	60.52	*100.07*

Solid State Digestion Biogas Volume Calculations

Volume Calculations @ operating T&P

T1 = 38.81 °C

T2 = 36.58 °C

T3 = 29.02 °C

Using average Box Temperature over the duration of the cycle

P= 2 "H20 = 0.0049 atm guage pressure

Absolute pressure = 1.0049 atm 101821.5 Pa

Volume Calculations @ Standard Temperature and Pressure (STP)

T = 0 °C 273.15 K

P = 1 atm 101325 Pa

Volume @ STP $V_2=V_1(P_1/P_2)(T_2/T_1)$ Where P2 & T2 are STP

Molecular Mass

M_{O2} 31.9988 g/mol

M_{CO2} 44.00995 g/mol

M_{CH4} 16.04303 g/mol

M_{N2} 28.0134 g/mol

Gas Constant (R) = 8.314 $(m^3*Pa)/(mol*K)$

Ideal Gas Law = PV = nRT

1 m3 = 1000 L

1 Metric tonr 1000000 g

Cycle 1 Volume % = Mol%

Container	Mass Difference (g)	CO2_PC	CH4_PC	H2_PC	H2S_PC	O2_PC	N2_PC	% Sum	Ave Molecular Weight	n (mol)	Volume(V_1) (m^3)	Volume @ STP (m^3)	Scaled to 1MT, V (m^3)@ STP on a Wet Basis	Scaled to 1MT, V (m^3)@ STP on a Dry Basis	Scaled to 1MT, V (m^3)@ STP on VS Basis	L/gVS @ STP
1	35.62	64.55%	0.01%	3.86%	0.01%	2.08%	38.23%	1.09	39.78	0.895	0.023	0.020	23.49	77.32	97.60	0.10
2	38.14	73.88%	0.01%	2.22%	0.02%	1.49%	31.41%	1.09	41.79	0.913	0.023	0.020	23.79	85.64	193.38	0.19
3	37.44	61.77%	0.01%	2.62%	0.03%	2.03%	40.98%	1.07	39.32	0.952	0.024	0.021	25.08	91.59	217.91	0.22
4	46.2	66.22%	1.57%	4.16%	0.02%	2.26%	35.36%	1.10	40.02	1.154	0.029	0.026	30.24	127.58	233.56	0.23
5	42.32	76.57%	3.95%	0.87%	0.02%	1.46%	27.48%	1.10	42.50	0.996	0.025	0.022	25.89	91.99	216.18	0.22
6	47.37	59.08%	0.03%	2.12%	0.02%	2.80%	44.28%	1.08	39.31	1.205	0.031	0.027	31.53	107.25	222.05	0.22
7	37.76	60.33%	23.61%	0.13%	0.03%	1.40%	25.42%	1.11	37.91	0.996	0.025	0.022	25.37	123.39	211.82	0.21
8	38.72	57.74%	21.41%	0.12%	0.05%	1.67%	29.81%	1.11	37.73	1.026	0.026	0.023	25.94	122.02	235.32	0.24
9	37.43	62.15%	20.96%	0.12%	0.04%	2.25%	25.56%	1.11	38.60	0.970	0.025	0.022	24.74	118.12	192.63	0.19
10	20.77	35.45%	1.48%	2.77%	0.04%	3.58%	61.37%	1.05	34.18	0.608	0.015	0.014	15.99	69.07	92.09	0.09
11	27.64	35.43%	15.47%	0.29%	0.01%	2.25%	48.92%	1.02	32.50	0.850	0.022	0.019	22.31	100.01	125.85	0.13
12	39.17	28.35%	3.01%	1.09%	0.00%	2.30%	69.34%	1.04	33.12	1.183	0.030	0.027	31.19	126.52	164.73	0.16

Cycle 1 Mass%

Container	Mass Difference (g)	CO2_PC	CH4_PC	O2_PC	N2_PC	% Sum
1	35.62	71.41%	0.00%	1.67%	26.92%	1.00
2	38.14	77.80%	0.00%	1.14%	21.05%	1.00
3	37.44	69.14%	0.01%	1.65%	29.20%	1.00
4	46.2	72.82%	0.63%	1.80%	24.75%	1.00
5	42.32	79.29%	1.49%	1.10%	18.11%	1.00
6	47.37	66.15%	0.01%	2.28%	31.56%	1.00
7	37.76	70.04%	9.99%	1.18%	18.79%	1.00
8	38.72	67.35%	9.10%	1.42%	22.13%	1.00
9	37.43	70.87%	8.71%	1.87%	18.55%	1.00
10	20.77	45.65%	0.69%	3.36%	50.30%	1.00
11	27.64	47.98%	7.64%	2.22%	42.17%	1.00
12	39.17	37.67%	1.46%	2.22%	58.65%	1.00

Individual Volume Calculations

CO2_PC	CH4_PC	O2_PC	N2_PC	Volume(V_1) (m^3)	Volume @ STP (m^3)	Scaled to 1MT, V (m^3)@ STP on a Wet Basis	Scaled to 1MT, V (m^3)@ STP on a Dry Basis	Scaled to 1MT, V (m^3)@ STP on VS Basis	L/gVS @ STP
0.0147	0.0000	0.0005	0.0087	0.024	0.021	24.63	81.09	102.35	0.10
0.0172	0.0000	0.0003	0.0073	0.025	0.022	25.40	91.46	206.51	0.21
0.0150	0.0000	0.0005	0.0099	0.025	0.022	26.28	95.98	228.36	0.23
0.0195	0.0005	0.0007	0.0104	0.031	0.027	31.88	134.47	246.19	0.25
0.0194	0.0010	0.0004	0.0070	0.028	0.024	28.34	100.70	236.63	0.24
0.0181	0.0000	0.0009	0.0136	0.033	0.029	33.48	113.89	235.81	0.24
0.0153	0.0060	0.0004	0.0065	0.028	0.025	28.10	136.66	234.60	0.23
0.0151	0.0056	0.0004	0.0078	0.029	0.025	28.70	134.99	260.35	0.26
0.0154	0.0052	0.0006	0.0063	0.027	0.024	27.45	131.02	213.67	0.21
0.0055	0.0002	0.0006	0.0095	0.016	0.014	16.29	70.37	93.82	0.09
0.0077	0.0034	0.0005	0.0106	0.022	0.019	22.78	102.08	128.46	0.13
0.0085	0.0009	0.0007	0.0209	0.031	0.027	32.13	130.31	169.67	0.17

Solid State Digestion Biogas Volume Calculations

Volume Calculations @ operating T&P

T1 =	38.81	°C		
T2 =	36.58	°C		
T3 =	29.02	°C		
Using average Box Temperature over the duration of the cycle				
P=	2	"H20 =	0.0049 atm	guage pressure
Absolute pressure =	1.0049	atm	101821.5 Pa	

Volume Calculations @ Standard Temperature and Pressure (STP)

T =	0 °C	273.15 K	
P =	1 atm	101325 Pa	
Volume @ STP	$V_2=V_1(P_1/P_2)(T_2/T_1)$	Where P2 & T2 are STP	

Molecular Mass

M_{O2}	31.9988	g/mol
M_{CO2}	44.00995	g/mol
M_{CH4}	16.04303	g/mol
M_{N2}	28.0134	g/mol

Gas Constant (R) = 8.314 $(m^3*Pa)/(mol*K)$

Ideal Gas Law = PV = nRT

1	m3 =	1000	L
1	Metric tonr	1000000	g

Cycle 2 Volume % = Mol%

Container	Mass Difference (g)	CO2_PC	CH4_PC	H2_PC	H2S_PC	O2_PC	N2_PC	% Sum	Ave Molecular Weight	n (mol)	Volume(V1) (m3)	Volume @ STP (m3)	Scaled to 1MT, V (m3)@ STP on a Wet Basis	Scaled to 1MT, V (m3)@ STP on a Dry Basis
1	17.60	36.32%	0.31%	0.01%	0.01%	3.66%	60.74%	1.01	34.22	0.514	0.013	0.012	14.05	56.08
2	15.90	39.56%	0.26%	0.16%	0.01%	1.54%	58.90%	1.00	34.44	0.462	0.012	0.010	12.42	66.17
3	17.40	40.19%	0.31%	0.68%	0.01%	1.61%	56.03%	0.99	33.95	0.513	0.013	0.011	14.08	70.95
4	19.20	46.76%	0.66%	0.22%	0.01%	1.89%	52.61%	1.02	36.03	0.533	0.013	0.012	14.71	74.27
5	19.60	43.65%	2.24%	0.09%	0.00%	1.14%	53.97%	1.01	35.05	0.559	0.014	0.013	15.23	76.14
6	16.90	35.85%	0.31%	0.00%	0.00%	1.73%	62.86%	1.01	33.99	0.497	0.013	0.011	13.75	70.59
7	24.40	41.55%	18.77%	1.19%	0.04%	2.10%	37.01%	1.01	32.34	0.755	0.019	0.017	20.03	101.15
8	22.20	38.50%	24.64%	1.05%	0.01%	3.10%	31.03%	0.98	30.58	0.726	0.018	0.016	19.08	90.35
9	22.70	38.47%	17.42%	0.71%	0.01%	1.34%	41.67%	1.00	31.83	0.713	0.018	0.016	19.01	122.85
10	14.70	23.11%	1.04%	0.33%	0.02%	1.17%	74.50%	1.00	31.58	0.465	0.012	0.010	12.53	63.68
11	30.10	37.16%	38.03%	1.91%	0.00%	3.50%	15.88%	0.96	28.02	1.074	0.027	0.024	29.13	76.37
12	16.50	35.53%	22.37%	0.98%	0.00%	1.04%	39.02%	0.99	30.49	0.541	0.014	0.012	14.94	85.10

Scaled to 1MT, V (m3)@ STP on VS Basis	L/gVS @ STP
135.88	0.14
184.89	0.18
181.93	0.18
218.43	0.22
185.71	0.19
157.22	0.16
138.56	0.14
129.43	0.13
187.88	0.19
82.95	0.08
96.33	0.10
120.22	0.12

Cycle 2 Mass%

Container	Mass Difference (g)	CO2_PC	CH4_PC	O2_PC	N2_PC	% Sum
1	17.6	46.71%	0.15%	3.42%	49.72%	1.00
2	15.9	50.55%	0.12%	1.43%	47.90%	1.00
3	17.4	52.10%	0.15%	1.52%	46.23%	1.00
4	19.2	57.12%	0.29%	1.68%	40.91%	1.00
5	19.6	54.80%	1.03%	1.04%	43.13%	1.00
6	16.9	46.42%	0.15%	1.62%	51.81%	1.00
7	24.4	56.55%	9.31%	2.08%	32.06%	1.00
8	22.2	55.41%	12.93%	3.24%	28.42%	1.00
9	22.7	53.19%	8.78%	1.35%	36.68%	1.00
10	14.7	32.20%	0.53%	1.18%	66.08%	1.00
11	30.1	58.36%	21.77%	4.00%	15.87%	1.00
12	16.5	51.29%	11.77%	1.09%	35.85%	1.00

Individual Volume Calculations

CO2_PC	CH4_PC	O2_PC	N2_PC
0.0047	0.0000	0.0005	0.0079
0.0046	0.0000	0.0002	0.0069
0.0052	0.0000	0.0002	0.0073
0.0063	0.0001	0.0003	0.0071
0.0062	0.0003	0.0002	0.0076
0.0045	0.0000	0.0002	0.0079
0.0079	0.0036	0.0004	0.0071
0.0071	0.0045	0.0006	0.0057
0.0069	0.0031	0.0002	0.0075
0.0027	0.0001	0.0001	0.0088
0.0101	0.0103	0.0010	0.0043
0.0049	0.0031	0.0001	0.0053

Volume(V_1) (m^3)	Volume @ STP (m^3)	Scaled to 1MT, V (m^3)@ STP on a Wet Basis	Scaled to 1MT, V (m^3)@ STP on a Dry Basis
0.013	0.012	14.19	56.66
0.012	0.010	12.46	66.34
0.013	0.011	13.82	69.64
0.014	0.012	14.99	75.69
0.014	0.013	15.38	76.90
0.013	0.011	13.86	71.11
0.019	0.017	19.92	100.57
0.018	0.016	18.56	87.89
0.018	0.016	18.80	121.50
0.012	0.010	12.51	63.57
0.026	0.023	27.55	72.22
0.013	0.012	14.63	83.36

Scaled to 1MT, V (m^3)@ STP on VS Basis	L/gVS @ STP
137.28	0.14
185.37	0.19
178.56	0.18
222.62	0.22
187.57	0.19
158.39	0.16
137.77	0.14
125.89	0.13
185.82	0.19
82.80	0.08
91.10	0.09
117.77	0.12

Solid State Digestion Biogas Volume Calculations

Volume Calculations @ operating T&P

T1 =	38.81	°C		
T2 =	36.58	°C		
T3 =	29.02	°C		
Using average Box Temperature over the duration of the cycle				
P=	2	"H20 =	0.0049 atm	guage pressure
Absolute pressure =	1.0049	atm	101821.5 Pa	

Volume Calculations @ Standard Temperature and Pressure (STP)

T =	0 °C	273.15 K	
P =	1 atm	101325 Pa	
Volume @ STP	$V_2=V_1(P_1/P_2)(T_2/T_1)$	Where P2 & T2 are STP	

Molecular Mass

M_{O2}	31.9988	g/mol
M_{CO2}	44.00995	g/mol
M_{CH4}	16.04303	g/mol
M_{N2}	28.0134	g/mol

Gas Constant (R) = 8.314 $(m^3*Pa)/(mol*K)$

Ideal Gas Law = PV = nRT

1	m3 =	1000	L
1	Metric tonr	1000000	g

Cycle 3 Volume % = Mol%

Container	Mass Difference (g)	CO2_PC	CH4_PC	H2_PC	H2S_PC	O2_PC	N2_PC	% Sum	Ave Molecular Weight	n (mol)	Volume(V1) (m3)	Volume @ STP (m3)	Scaled to 1MT, V (m3)@ STP on a Wet Basis	Scaled to 1MT, V (m3)@ STP on a Dry Basis
1	18.50	35.96%	0.35%	0.02%	0.00%	1.92%	62.82%	1.01	34.09	0.543	0.013	0.012	15.14	55.46
2	20.90	46.35%	0.32%	0.33%	0.00%	1.78%	52.09%	1.01	35.61	0.587	0.014	0.013	16.09	85.85
3	21.00	45.83%	0.27%	0.49%	0.00%	1.64%	50.98%	0.99	35.02	0.600	0.015	0.013	16.82	69.48
4	19.40	33.92%	0.54%	0.36%	0.01%	4.16%	61.85%	1.01	33.67	0.576	0.014	0.013	16.27	94.79
5	20.70	40.91%	3.28%	0.38%	0.01%	1.06%	55.71%	1.01	34.48	0.600	0.015	0.013	16.66	82.62
6	19.20	36.80%	0.31%	1.04%	0.00%	1.66%	61.17%	1.01	33.91	0.566	0.014	0.013	15.96	85.97
7	23.10	37.09%	28.79%	1.42%	0.03%	2.00%	31.88%	1.01	30.51	0.757	0.019	0.017	20.70	122.25
8	25.80	40.46%	20.29%	1.19%	0.04%	3.33%	35.47%	1.01	32.06	0.805	0.020	0.018	21.67	109.46
9	22.90	40.78%	23.03%	1.10%	0.04%	1.26%	34.88%	1.01	31.82	0.720	0.018	0.016	19.69	94.90
10	15.50	23.87%	7.99%	0.50%	0.00%	1.74%	66.13%	1.00	30.87	0.502	0.012	0.011	13.74	48.48
11	28.70	31.02%	34.76%	1.72%	0.01%	3.81%	28.13%	0.99	28.33	1.013	0.025	0.023	28.49	136.49
12	29.10	36.00%	44.29%	2.00%	0.01%	0.78%	14.84%	0.98	27.36	1.064	0.026	0.024	29.91	117.05

Scaled to 1MT, V (m3)@ STP on VS Basis	L/gVS @ STP
124.57	0.12
226.56	0.23
147.34	0.15
254.75	0.25
181.40	0.18
205.02	0.21
192.43	0.19
180.61	0.18
142.35	0.14
64.05	0.06
176.93	0.18
154.46	0.15

Cycle 3 Mass%

Container	Mass Difference (g)	CO2_PC	CH4_PC	O2_PC	N2_PC	% Sum
1	18.5	46.42%	0.16%	1.80%	51.62%	1.00
2	20.9	57.28%	0.14%	1.60%	40.98%	1.00
3	21	57.60%	0.12%	1.50%	40.78%	1.00
4	19.4	44.33%	0.26%	3.95%	51.46%	1.00
5	20.7	52.22%	1.52%	0.98%	45.27%	1.00
6	19.2	47.76%	0.15%	1.56%	50.53%	1.00
7	23.1	53.50%	15.14%	2.10%	29.27%	1.00
8	25.8	55.53%	10.15%	3.32%	30.99%	1.00
9	22.9	56.41%	11.61%	1.27%	30.71%	1.00
10	15.5	34.03%	4.15%	1.80%	60.01%	1.00
11	28.7	48.19%	19.69%	4.30%	27.82%	1.00
12	29.1	57.92%	25.97%	0.91%	15.20%	1.00

Individual Volume Calculations

Container	CO2_PC	CH4_PC	O2_PC	N2_PC
1	0.0048	0.0000	0.0003	0.0084
2	0.0067	0.0000	0.0003	0.0075
3	0.0068	0.0000	0.0002	0.0075
4	0.0048	0.0001	0.0006	0.0088
5	0.0061	0.0005	0.0002	0.0083
6	0.0051	0.0000	0.0002	0.0085
7	0.0069	0.0054	0.0004	0.0060
8	0.0080	0.0040	0.0007	0.0070
9	0.0072	0.0041	0.0002	0.0062
10	0.0030	0.0010	0.0002	0.0082
11	0.0078	0.0087	0.0010	0.0070
12	0.0094	0.0116	0.0002	0.0039

Container	Volume(V_1) (m^3)	Volume @ STP (m^3)	Scaled to 1MT, V (m^3)@ STP on a Wet Basis	Scaled to 1MT, V (m^3)@ STP on a Dry Basis
1	0.014	0.012	15.29	56.04
2	0.015	0.013	16.17	86.32
3	0.015	0.013	16.61	68.59
4	0.014	0.013	16.35	95.23
5	0.015	0.014	16.82	83.41
6	0.014	0.013	15.95	85.92
7	0.019	0.017	20.65	121.95
8	0.020	0.018	21.58	108.97
9	0.018	0.016	19.68	94.85
10	0.012	0.011	13.71	48.35
11	0.024	0.022	27.84	133.38
12	0.025	0.023	28.68	112.27

Scaled to 1MT, V (m^3)@ STP on VS Basis	L/gVS @ STP
125.87	0.13
227.78	0.23
145.45	0.15
255.94	0.26
183.14	0.18
204.89	0.20
191.96	0.19
179.80	0.18
142.28	0.14
63.87	0.06
172.90	0.17
148.14	0.15