Biochemical Methane Potential for Wheat-Based Fuel Ethanol and Beef Feedlot Integration

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By

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ABSTRACT

Assessing the economic and environmental benefits of constructing a biorefinery consisting of an ethanol plant, an anaerobic digester and a beef feedlot depends on understanding the methane potential of substrates available within the system. Fuel ethanol is produced from wheat in Western Canada and literature values for the methane potential of wheat-based ethanol byproducts is scarce.

This study consisted of conducting biochemical methane potential (BMP) assays at thermophilic temperatures on ethanol byproducts typically produced downstream of distillation in wheat-based ethanol facilities. One experiment focused on the methane potential of the byproducts alone and two more experiments focused on the effect of amending the highest potential byproducts with feedlot manure at 1:1 and 2:1 volatile solids ratios of byproduct to manure.

Methane yields for whole stillage, thin stillage and wet cake were 585 ± 32, 547 ± 76 and 495 ± 45 ml/g VS added, respectively. Reliable methane production rate constants for these byproducts could only be determined from the third experiment, but were 0.106, 0.090 and 0.105 day^{-1}, respectively. When feedlot manure was added to the ethanol byproducts, methane yield results were proportionally equal to the ratio of byproduct to manure, except in the case of thin stillage. The combination of thin stillage and feedlot manure yielded 125% and 119% of expected results. Overall, feedlot manure stabilized and increased methane production rate constants for wheat-based ethanol byproducts.

It was concluded from the results that whole stillage should be used in anaerobic digesters when manure is not available near ethanol facilities. When an ethanol plant, feedlot and digester biorefinery is being considered, a mixture of feedlot manure and thin stillage should be digested to achieve proportionally higher methane yields from each substrate.

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ABBREVIATIONS AND SYMBOLS

B – methane yield at time, t

B_∞ – Ultimate methane yield

BMP – Biochemical Methane Potential

CHP – Combined Heat and Power

CH_4 – methane gas

DDGS – Dried Distillers Grain with Solubles

GC – Gas Chromatography

HRT – hydraulic retention time

k – methane production rate constant

OLR – organic loading rate

STP – standard temperature and pressure (0°C and 1.013 bar)

TCOD – Total Chemical Oxygen Demand

TS – Total Solids

t – time

V_R – reactor volume

$V_{substrate}$ – volume of substrate

VFAs – Volatile Fatty Acids

VS/TS – ratio of volatile solids to total solids

VS – Volatile Solids

WDGS – Wet Distillers Grain with Solubles

1. INTODUCTION

Ethanol is a renewable fuel that is used to displace gasoline in combustion engines. It offers reduced greenhouse gas (GHG) emissions compared to gasoline produced from fossil fuels and it provides value-added opportunities in the agriculture sector. Fuel ethanol production also supports rural economies and provides jobs in rural communities. As of September 1, 2010 the Canadian government adopted a renewable fuels standard that requires a 5% blend of ethanol into all sales of gasoline (CRFA, 2010). The adoption of renewable fuels and their mandated use is expected to increase in the coming years alongside concerns about climate change and the environment.

In Canada, fuel ethanol production is dominated by the fermentation and distillation of starchy grains like corn and wheat. The byproducts from ethanol production are processed and dried at high temperatures to create a high protein animal feed called dried distillers grains with solubles (DDGS). For every liter of ethanol produced, between 8 and 15 liters of byproduct effluent requires processing (Saha et al., 2005). Energy consumed to produce DDGS decreases the net energy balance ratio of ethanol production and can negatively impact the carbon footprint of the facility.

As renewable fuel standards increase, the risks of DDGS market saturation and pollution increase as well. Anaerobic digestion of ethanol byproducts could potentially reduce the environmental impact of wastewaters leaving ethanol plants and improve the net energy balance ratio of the process if methane gas is combusted and returns heat and electricity to the process. The methane generating potential of wheat-based ethanol byproducts has not been widely published, so designing anaerobic digesters for ethanol plants is difficult. Information is more publicly available for corn-based ethanol byproducts, but it is not clear if the two types of byproducts have similar methane potentials.

Co-locating an ethanol plant and an anaerobic digester at a beef feedlot could provide even more economic and environmental advantages. Ethanol byproducts can

either be fed to the digester or to the cattle in the feedlot. Anaerobic digestion of manure reduces GHG emissions compared to normal storage practices (Moller et al., 2004) and it can improve methane yields when co-digested with other substrates (Labatut and Scott, 2008). Linking all three components (ethanol plant, beef feedlot and anaerobic digester) creates what is known as a biorefinery where the byproducts of one entity become the inputs for the next and the overall system operates in concert as shown below in Figure 1.1.

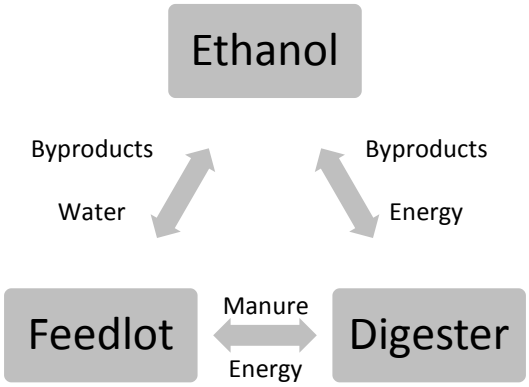

Figure 1.1 Flow of materials through proposed biorefinery

1.1 Problem

Some components of the biorefinery model described above are operating in Western Canada. An Alberta feedlot is generating electricity from the biogas produced by its thermophilic anaerobic digester which runs on feedlot manure and other off-farm substrates. An ethanol facility in Saskatchewan saves energy by feeding wet byproducts to cattle in an adjoined feedlot. A biorefinery containing all three components of ethanol plant, beef feedlot and anaerobic digester has yet to be realized in Western Canada.

In order to analyze the feasibility of a combined feedlot, ethanol plant and anaerobic digester, the methane production potential of ethanol byproducts needs to be understood. Current literature does not provide values for methane production volume or methane production rate constant of wheat-based ethanol byproducts, only for some corn-based byproducts. In addition, the methane potential of co-digesting feedlot manure with wheat-based ethanol byproducts is unknown.

1.2 Objectives

1. Determine the biochemical methane potential of wheat-based ethanol byproducts under thermophilic conditions.
2. Determine the biochemical methane potential of wheat-based ethanol byproducts amended with feedlot manure under thermophilic conditions.

Achieving these objectives included determining the methane yield and methane production rate constant of substrates through biochemical methane potential (BMP) assays. Three rounds of BMP assays were conducted. First, the highest potential byproducts were determined. Then those byproducts were amended with a 1:1 volatile solids ratio of feedlot manure. Finally, a 2:1 volatile solids ratio of ethanol byproduct to feedlot manure was tested to try to maximize methane production through the use of both substrates. Thermophilic temperatures were chosen for the BMP assays because byproducts exit the ethanol distillation process at over 55°C, therefore eliminating the need to heat the substrate before it enters the digester.

1.3 Manuscript Style Thesis

Two distinctly different problems were addressed in this research. In order to logically present the results, two scientific papers were written. One paper focuses directly on the methane potential of wheat-based ethanol byproducts. The other paper focuses on the methane potential of wheat based ethanol products receiving two ratios of feedlot manure.

Both research papers have been incorporated into this manuscript style thesis. A traditional literature review is presented and a more technical review of the available literature is also included in each research paper. The materials and methods used to conduct this research are described in detail in the papers and the results of the experiments are presented in the papers as well. After the papers are presented, a comparison of the results is given, followed by a presentation of the overall conclusions and recommendations determined by this research.

2. LITERATURE REVIEW

2.1 Ethanol – Production

Dry grind ethanol production begins with the cleaning and grinding of grain into a floury consistency. Next, water and α-amylase are added and the mixture is heated to 90°C. This process is called liquefaction and it serves to break long-chain starch polymers into dextrose. The mash is then cooled to 60°C, gluco-amylase is added and the pH is lowered to 4.5. This process serves to convert dextrose into fermentable sugars and it is called saccharification. Finally, ethanol production takes place in batch type reactors by adding yeast species (*Saccharomyces cerevisiae)* which ferment the mash. Fermentation of the available sugars into ethanol is usually achieved within 48 hours and it is the most critical stage of ethanol production because converting the most possible sugars into the most possible ethanol in the shortest amount of time is always the goal. (Wilkie et al., 2000).

After fermentation, continuous distillation is used to separate ethanol from the residual byproducts. A stripper column, heated by steam, boils the mixture of ethanol, fermented grain and water to release ethanol enriched vapours. The vapours pass through a rectifying column and are condensed to 95% pure ethanol. The remaining 5% of water in the mixture is decreased to less than 1% by volume through dehydration in parallel molecular sieve beds. Once the ethanol is 99% pure it is cooled and denatured with gasoline (5% by volume) before being shipped (Wilkie et al., 2000). The gross energy content of ethanol is 23.4 MJ/liter (ORLN, 2011).

Figure 2.1 shows a very general ethanol production process and includes the downstream processing steps necessary to deal with the byproducts of ethanol production.

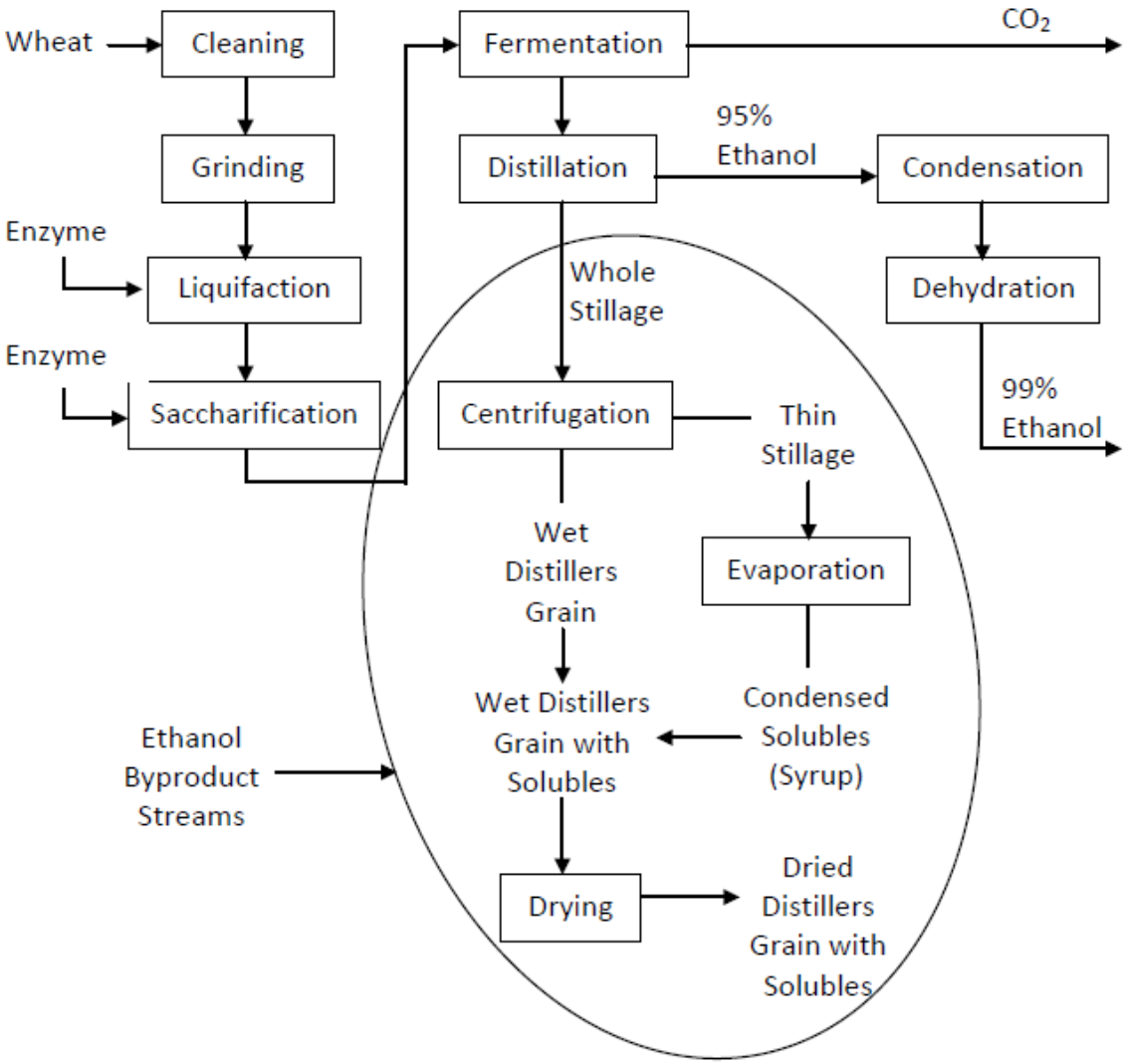

Figure 2.1 Ethanol production process (based on Eskicioglu et al., 2011)

2.2 Ethanol – Byproducts

Byproduct processing begins when whole stillage (leftover from distillation) is collected from the bottom of the distillation column and spun through a centrifuge. The centrifuge produces wet distillers grain (or wet cake) and thin stillage. Some of the thin stillage (approximately 50%) is recycled to liquefaction and the rest is evaporated to concentrate any suspended solids and soluble nutrients into syrup (Agler et al., 2008). Wet distillers grain and syrup are mixed together to form a byproduct stream called wet distiller's grains with solubles (WDGS). Finally, WDGS is dried to produce dried distiller's grains with solubles (DDGS) which is sold into the livestock feed market.

In summary, there are typically six byproduct streams that contribute to downstream processing in an ethanol plant. These streams are whole stillage, thin stillage, wet cake, syrup, WDGS and DDGS. Downstream processing can be a major limitation to ethanol production since drying and evaporation combine to consume approximately 46.8% of the plants total energy needs (Eskicioglu et al., 2011). Bottlenecks in downstream processing can also disrupt system balances and hold up ethanol production on the front end so it should be managed efficiently.

2.3 Ethanol – Biogas Potential

Information about the anaerobic digestion of wheat ethanol byproducts is not widely available, but many studies have been conducted on corn ethanol byproducts. Research has primarily been focused on thin stillage because it requires the most processing energy and it presents the greatest risks to wastewater leaving ethanol plants. In the 1980's, two mesophilic studies on corn thin stillage reported methane yields of 250 – 370 ml CH_4/g TCOD removed (Stover et al., 1984) and 330 ml CH_4/g TCOD removed (Lanting and Gross, 1985). Stover et al. (1984) used suspended growth and fixed film reactors and suggested that this methane production volume could supply 60% of the daily energy consumed by ethanol plants. Lanting and Gross (1985) used up-flow anaerobic sludge blanket (USAB) reactors.

More recently, researchers have studied the thermophilic anaerobic digestion of corn thin stillage (Agler et al., 2008; Schaefer and Sung, 2008).. Thermophilic digestion is sometimes considered a disadvantage compared to mesophilic digestion because it requires higher heating temperatures. In ethanol plants, however, whole stillage exits the distillation column at above 55°C so heating demand is decreased and the metabolic rates achieved by thermophilic digestion provide improved efficiency and economics.

Using completely mixed thermophilic reactors at 30-, 20-, and 15-day hydraulic retention times (HRT) and thin stillage concentrated at 100g/L TCOD and 60g/L VS, Shafer and Sung (2008) observed methane yields ranging between 600 – 700 ml CH_4/g

VS removed. They suggested that natural gas consumption at ethanol plants could be reduced by 43 to 59% with this level of methane production. Another thermophilic study on thin corn stillage used high rate sequencing batch reactors to realize a methane yield of 254 ml CH_4/g TCOD fed on a 10 day HRT (Agler et al., 2008). Anger et al. (2008) also suggested that this amount of methane production would reduce natural gas consumption in conventional dry grind ethanol plants by 51% and translate to an improved net energy balance ratio of ethanol from 1.26 to 1.70.

Information on anaerobic digestion of whole corn stillage is less publically available than thin stillage. In 2011, Eskicioglu et al. published a study of batch and continuous-flow digestion of whole corn stillage from dry-grind ethanol production under thermophilic and mesophilic temperatures. Batch type BMP assays produced methane volumes of 401 ± 17, 406 ± 14, 441 ± 2 and 458 ± 0 ml CH_4/g VS added under mesophilic and 693 ± 17, 560 ± 24, 529 ± 37 and 429 ± 8 ml CH_4/g VS added under thermophilic conditions for whole corn stillage at concentrations of 6348, 12,696, 25,393, and 50,786 mg TCOD/L, respectively (Eskicioglu et al., 2011). Little success was realized during continuous flow experiments with full strength whole corn stillage (254 g TCOD/L) at organic loading rates of 4.25, 6.30 and 9.05 g TCOD/L day. At thermophilic temperatures, the digesters were unstable and at mesophilic temperatures, only a 60 day retention time was stable.

2.4 Manure –Beef Feedlot Production

Canadian beef feedlots range in capacity from a few hundred head to over 40,000 head. The feedlots contributing to this study, Pound-Maker and Highland Feeders Ltd, have capacities of 28, 500 head and 36,000 head, respectively. Calves typically enter feedlots at approximately 350 kg and start on a ration of 30% grain (barley and distillers grain) and 70% forage (barley or corn silage). As calves move towards a finish weight of approximately 550 kg, their diet changes to roughly 80% grain and 20% forage (Pound-Maker, 2005). Cattle gain weight at approximately 1.7 kg per day on high grain diets and are likely to stay in feedlots for a minimum of 120 days

(Canada Beef, 2009). Pound-Maker feedlot is already incorporating ethanol byproducts into the grain portion of the ration its calves receive. Highland Feeders Ltd. sources its grain (a mixture of barley and DDGS) from local producers and grain companies (Highland Feeders, 2011).

The manure generated at beef feedlots can be estimated using standards set by the American Society of Agricultural and Biological Engineers (ASABE). 446 kg beef feeders, on high energy rations, will generate 30 kg/head/day of fresh manure. The manure is estimated at 92% moisture and has a VS/TS ratio of 0.79, therefore beef feeders produce 2.4 kg/head/day TS and 1.9 kg/head/day VS (ASAE, 2005)

2.5 Manure – Biogas Potential

Manure is a widely used feedstock for anaerobic digestion because it decreases the volume of greenhouse gas emissions released during normal manure storage (Moller et al., 2004). Manure is a good substrate for co-digestion with other organic material because it can adjust the carbon-to-nitrogen (C:N) ratio of feedstock (25-30:1 optimal), it can provide buffering capacity (alkalinity) and it can supply essential nutrients that improve methane yields (Labatut and Scott, 2008; Ward et al., 2008). The biogas potential of manure is highly variable and it depends on the type of animal, the animal's feed, climate conditions and the type of bedding used, not to mention the storage conditions of manure before anaerobic digestion occurs (Moller et al., 2004). A typical specific methane yield of beef cattle manure is 328 ml/g VS added (Hashimoto et al., 1981).

2.6 Anaerobic Digestion – Process

Anaerobic digestion is the breakdown of organic matter in an oxygen free environment. Communities of anaerobic microorganisms are fed high organic matter substrates with the goal of producing large volumes of methane rich biogas. Biogas is typically composed of 50-80% methane and 20-40% carbon dioxide, plus trace amounts of other gases such as nitrogen, oxygen, ammonia, and hydrogen sulfide (Naskeo Environment, 2009). Some common inputs for anaerobic digestion include energy

crops, food waste, animal manures, food processing wastes and biosolids. The energy content of biogas depends on the concentration of methane in the final biogas mixture. The gross heating value of methane gas is approximately 39.8 MJ/m^3 at STP (Engineering ToolBox, 2011). Biogas can be upgraded to natural gas quality (>99% methane) and used in the same manner as natural gas, or it can be combusted as is in a combined heat and power (CHP) generator to produce heat and electricity.

Anaerobic digestion requires four sequential reactions to take place in order to convert complex organic materials into biogas. As shown in Figure 2.2, the necessary biochemical processes are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Hecht, 2009). Each process is conducted by a specific set of microorganisms and the products of one stage become the substrates for the next. Healthy and efficient communities of bacteria will only allow products to accumulate temporarily between reactions. The over accumulation of products in one reaction will inhibit the activity of organisms in the next reaction and cause the whole process to fail. Careful design and management of the parameters controlling anaerobic digestion are necessary to achieve efficient conversion of organic matter into biogas.

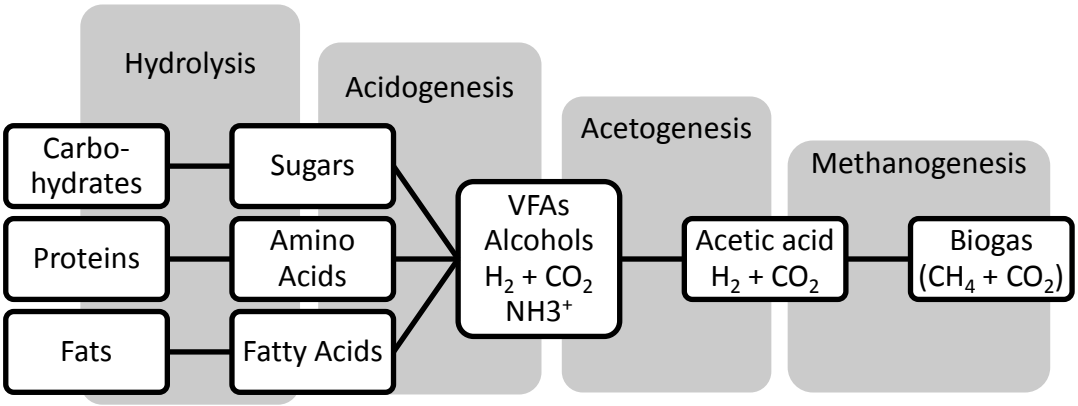

Figure 2.2 Anaerobic digestion process (based on McNeil , 2005)

2.7 Anaerobic Digestion – Environmental Parameters

The environmental parameters controlling anaerobic digestion are temperature, pH, buffering capacity and volatile fatty acid concentration.

2.7.1 Temperature

The three temperature ranges under which anaerobic digestion can occur are:

Table 2.1 Possible anaerobic digestion temperatures (based on Hecht, 2009)

Temperature	*Range*
Psychrophilic	< 25°C
Mesophilic	25°C to 45°C
Thermophilic	45°C to 70°C

Each temperature range supports a specific type of methanogenic bacteria that are sensitive to temperature fluctuations, as shown in Table 2.1. Temperature directly affects the reaction rates in anaerobic digestion. An increase in temperature will speed up reaction rates and therefore decrease the retention time required to achieve similar levels of biogas production. Figure 2.3 shows how similar levels of biogas or methane can be produced in shorter periods of time under thermophilic conditions (15 – 20 days) compared to mesophilic conditions (30 – 40 days) and psychrophilic conditions (70 – 80 days).

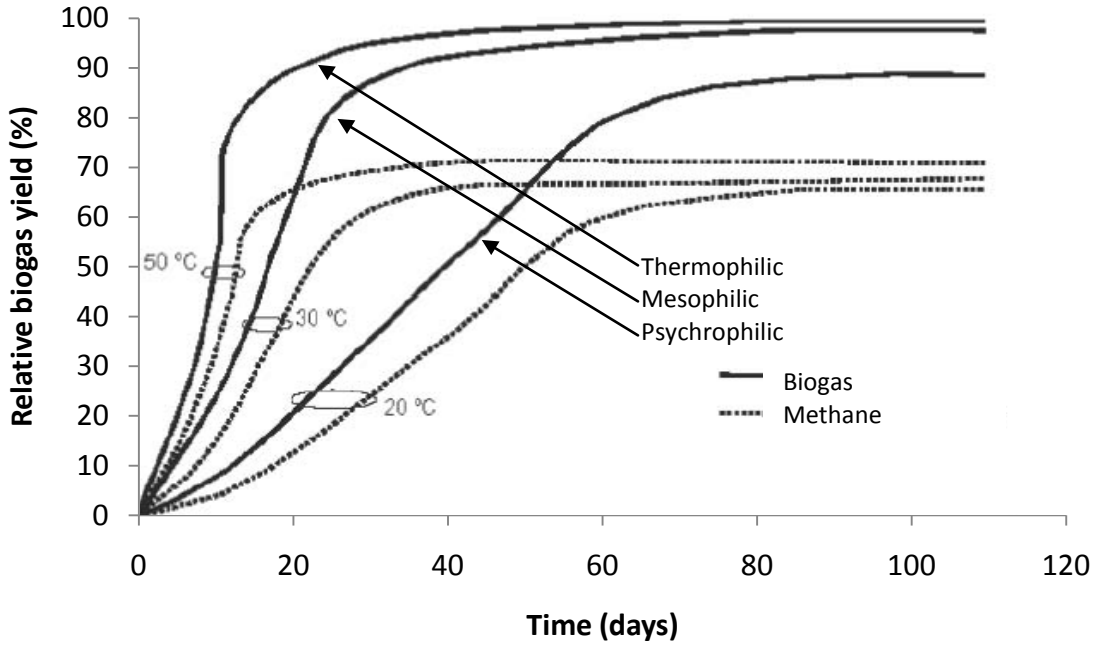

Figure 2.3 Temperature effect on biogas yield (based on Hecht, 2009)

Thermophilic organisms have the fastest growth rate which allows engineers to design smaller systems with shorter hydraulic retention times, but the solubility of some gases (NH_3, H_2, CH_4, H_2S and VFA) also increases with temperature and can have a negative impact on the system if the gas has an inhibitory effect (Hecht, 2009). Thermophilic conditions have shown to improve digestibility and substrate utilization, but the microbes are more sensitive to temperature fluctuations (Hecht, 2009). Additionally, maintaining thermophilic temperatures in a digester requires the highest energy input, also known as parasitic load, which affects the margin between energy input and energy produced.

2.7.2 pH

The pH value of the liquid phase in anaerobic digesters influences the growth rate of methanogenic bacteria as well as the dissociation of compounds that affect the process (ammonia, sulphide, organic acids). pH values between 6.7 and 7.4 are known to optimize methane formation, whereas disruptions in digester performance have been experienced when the range drops below 6 or above 8 (Poulsen, 2003).

The pH value of a substrate and its composition affect the overall pH balance of an anaerobic digester. For example, the degradation of proteins or the presence of ammonia in a substrate will cause an increase in pH, whereas the production and accumulation of VFA will decrease the digester pH. The capability of an anaerobic digester to stabilize pH levels and maintain ultimate biogas production is commonly referred to as the digester's buffering capacity.

2.7.3 Buffering Capacity

The term buffering capacity actually refers to the total alkalinity ($CaCO_3$) in an anaerobic digestion system. It is the ability of the system to neutralize acids and it is an early indicator of digester health. Consumption of buffering capacity precedes drops in pH. Without sufficient buffering capacity to counteract small drops in pH, complete inhibition of methane production is possible. Figure 2.4 is an example of a digester that experienced pH recovery without sufficient buffering capacity recovery.

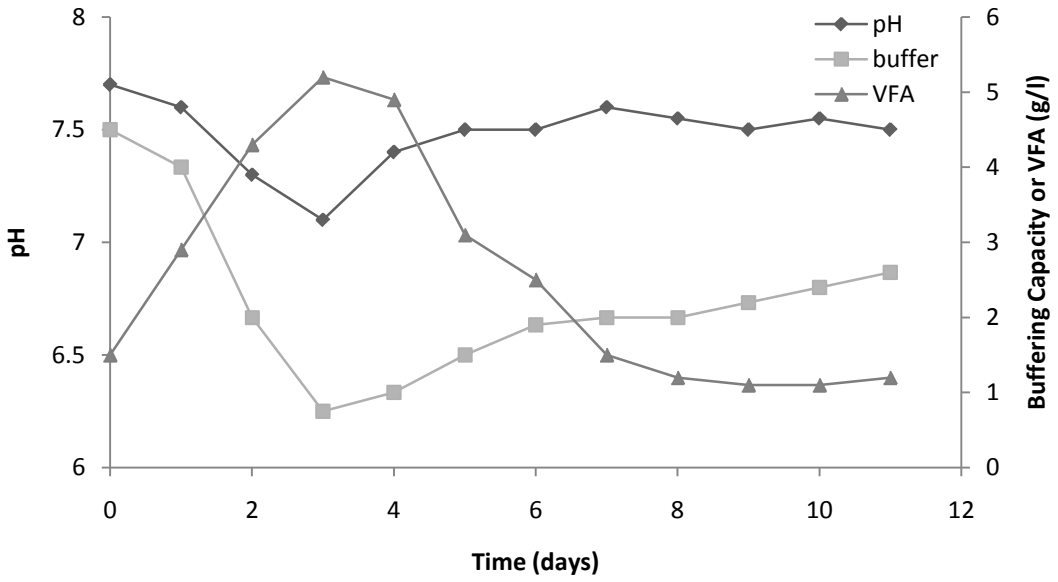

Figure 2.4 Relationship between pH, buffering capacity and VFAs (based on Hecht, 2009)

The buffering capacity of inputs for anaerobic digestion can vary. Cattle manure itself is widely recognized as having good buffering capacity, but its composition can depend on feed, collection methods and climate (Moller et al., 2004)

2.7.4 Volatile Fatty Acids

Figure 2.4 also shows the relationship between volatile fatty acids (VFAs), pH and buffering capacity. VFAs are the intermediate products of acidogenesis and will accumulate if the symbiotic relationship between acetogenic and methanogenic bacteria is sacrificed. The accumulation of VFAs causes a subsequent drop in pH which in turn creates a toxic environment for methanogens (pH<6) (Schink, 2002). The VFA accumulation shown above corresponds with a drop in pH and the consumption of buffering capacity to correct both VFA and pH levels during digester operation.

Monitoring fluctuations of the VFA levels in a specific digester is the most telling sign of process instability (Ahring et al., 1995), whereas comparing VFAs between digesters provides little information due to variations in input material and microbial response. Some VFA accumulations are less concerning than others. For example, acetate feeds methane production directly, so its contribution to the VFA profile is less concerning than say propionate or butyrate which require degradation to acetate

before they are available to methanogens. Increases in acetate have been shown to increase metabolic activity and methane production, whereas increases in propionate have indicated low metabolic activity and slow process stabilization (Pind et al., 2003).

2.8 Anaerobic Digestion – Engineering Parameters

The engineering parameters controlling anaerobic digestion are substrate selection, organic loading rate, hydraulic retention time, and reactor design.

2.8.1 Substrate selection

Biogas production depends heavily on the substrates entering anaerobic digestion systems. A substrate's chemical and physical properties affect the ability of microbes to convert it into methane. Figure 2.5, shows the biogas yield potential of various substrates. Substrates with high caloric values and simple nutrient structures have much higher biogas potentials than watery substrates with tightly bound nutrients. In grasses and vegetables, for example, complex carbohydrate structures like cellulose, hemi-cellulose and lignin bind nutrients and thus degrade very slowly or not at all in anaerobic digesters. Refined fats and carbohydrates, on the other hand, exhibit higher biogas potentials because microbes can easily access and degrade the high energy nutrients.

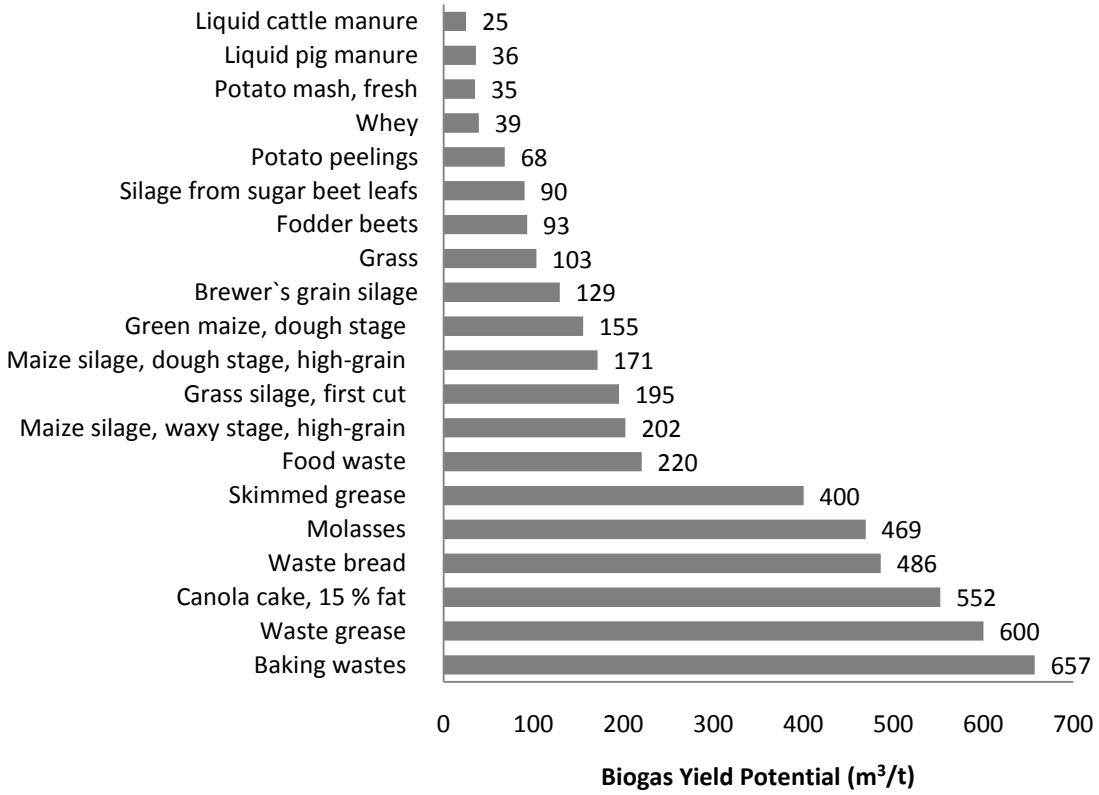

Figure 2.5 Biogas potential of various substrates (data derived from Effenberger, 2010)

Every input for anaerobic digestion has its own biodegradation characteristics. Each will generate different volumes of biogas, require different environmental considerations and respond differently to engineering techniques. The best way to predict a substrate's degradation characteristics and biogas potential is to analyze it using a biochemical methane potential (BMP) test, which is explained in further detail in Section 2.9.

For the most part, higher concentrations of organic matter in a substrate correspond to increased biogas production. The total and volatile solids content of a substrate are important parameters to be determined before BMP analysis for biogas and methane potential. Total solids content (TS) effects the operation of an anaerobic digestion system. Volatile solids (VS) are the organic fraction of a material that could potentially be converted into biogas. Substrates with high volatile solids to total solids

ratio (VS/TS) are expected to produce more biogas per volume of substrate because there is a greater fraction of material available for the microbes to convert into biogas.

2.8.2 Organic loading rate

In order to design anaerobic digesters, engineers need to balance organic loading rate with hydraulic retention time to ensure that the maximum amount of biogas is produced from the substrates entering the system. Organic loading rate, *OLR*, is defined as the amount of organic substrates, *VS*, per active digester volume, V_R, in a given time, *t*.

$$\boldsymbol{OLR = \frac{VS/V_R}{t}} \quad \textbf{(2.1)}$$

Increasing the amount of substrate available for microbes to convert into biogas (*VS*) will increase the rate of biogas production up to a certain point. As long as biochemical processes remain balanced, organic loading may remain steady or even increase. However, if too much hydrolysis is occurring, or if volatile solids entering the system start to inhibit methane production, then biogas production will decline and organic load must be decreased (Hecht, 2009). A demonstration of this relationship is shown in Figure 2.6.

The optimal organic loading rate for a system is affected by the amount of time an input material stays in the digester (hydraulic retention time), the volume of the digester and how effective the microbes in the digester are at converting substrates into biogas.

2.8.3 Hydraulic retention time

Hydraulic retention time, *HRT*, is the mean, theoretical time that any input material spends in the digester. It is defined as the active digester volume, V_R, divided by the volume of substrate, $V_{substrate}$, fed per unit time, *t*.

$$\boldsymbol{HRT = \frac{V_R}{V_{substrate}/t}} \quad \textbf{(2.2)}$$

Engineers determine digester volumes based on a time frame that allows input materials to be converted into biogas before exiting the system. The goal is to keep material in the digester as long as it is producing biogas, but to remove the material

once microbes have used the majority of nutrients from it. Additionally, the growth rate of methanogens in a digester must remain faster than the removal rate of effluent from the digester in order to avoid wash out situations (Shuler and Kargi, 2002).

Figure 2.6 shows how to balance both OLR and HRT for system design and operation. Biogas productivity increases as OLR increases up to a critical level. Biogas yield per kg of volatile solids increases as HRT gets longer and volatile solids are used up. The point at which the OLR and biogas productivity is optimized does not correspond to the maximum biogas yield per kilogram of volatile solids, but a continuous system designed at this HRT and ORL takes advantage of the time period where the rate of biogas yield is the greatest.

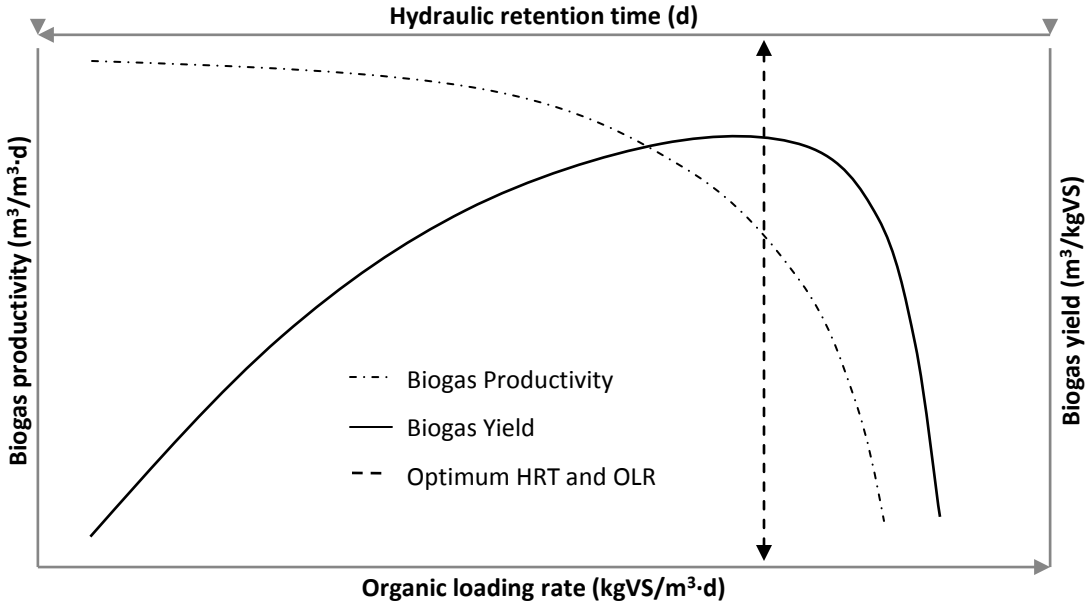

Figure 2.6 Balancing hydraulic retention time with organic loading rate (based on Hecht, 2009)

2.8.4 Reactor design

Many different reactor designs are used to achieve anaerobic digestion. The most common designs are covered lagoon, plug flow and completely stirred tank reactors. Anaerobic digestion has also been conducted in induced blanket reactors, fixed film digesters and batch digesters (US EPA, 2011). Considering all these different reactor designs available to engineers, a standardized test was developed to determine the

biogas potential of substrates being considered for anaerobic digestion. The biochemical methane potential (BMP) test is used to determine a substrate's ultimate biogas potential, which is "the key parameter for assessing design, economic and managing issues for the full scale implementation of anaerobic digestion processes" (Angelidaki, et al., 2009). Engineers use the results of a BMP test to choose reactor types, design gas handling systems and to set operating parameters for anaerobic digesters.

2.9 Anaerobic Digestion – Biochemical Methane Potential

Owen et al. (1979) first proposed the biochemical methane potential (BMP) test as a method for determining the biogas potential of substrates for anaerobic digestion. The basic principles of this method are still employed today as biogas research widens in scope. Variations to the method have been developed and accepted for their contribution to more accurate measuring techniques and ability to generate more consistent and reliable results. Inconsistent reporting of BMP test results in recent years has lead to the development of a few guidelines and standards for the test.

The BMP assay provides information about the maximum amount of methane gas a specific amount of material is capable of producing. It is the most widely used test for comparing and evaluating substrates for anaerobic digestion. BMP assays are relatively inexpensive to perform and can return results more quickly than continuous flow experiments involving anaerobic reactors and many variables (Moody et al., 2009).

The basis of the BMP test is to digest a specific amount of substrate and measure the ultimate methane volume produced. Batch type reactions are conducted using sealed vessels, incubated at a desired digestion temperature. The vessels are inoculated with anaerobic bacteria and a specific ratio is set between the volatile solids of inoculum and substrate. Control vessels are required to account for endogenous metabolism of the inoculum and replicates are required to ensure the results are reliable. Biogas volume is measured using volume displacement or pressure sensing devices. Samples of the produced biogas are analyzed for methane content using gas chromatography.

Finally, the ultimate methane volume and production rate constant is calculated and reported.

A task group of Europe's leading biogas researchers was congregated in 2009 to develop a protocol for reporting the results of BMP tests. The Iowa State University Department of Agricultural and Biosystems Engineering also published BMP methods in 2009 and a German Standard, VDI 4630, was developed in 2006. Angelidaki et al (2009) suggest the following items be reported when communicating BMP test results.

- Date, time of start and end of test
- Substrate, quantity and physical-chemical characteristics
- Inoculum, origin and activity, quantity and physical-chemical characteristics
- Test conditions: temperature, substrate/inoculum ratio, volume of vessel, number of replicates, dilutions
- Methane production profiles with respect to time, including relative average and standard deviations of triplicates
- Results of blank and control methane production (on graphs)
- Specific methane production volume, corrected by subtracting control methane volume, and reported as the volume of methane per gram volatile solid, or per gram COD, or per gram of substrate added.

A typical methane production profile for a triplicate sample with controls is shown in Figure 2.7. Good quality production profiles like this one provide valuable information about a substrate's ultimate methane yield and methane production rate constant.

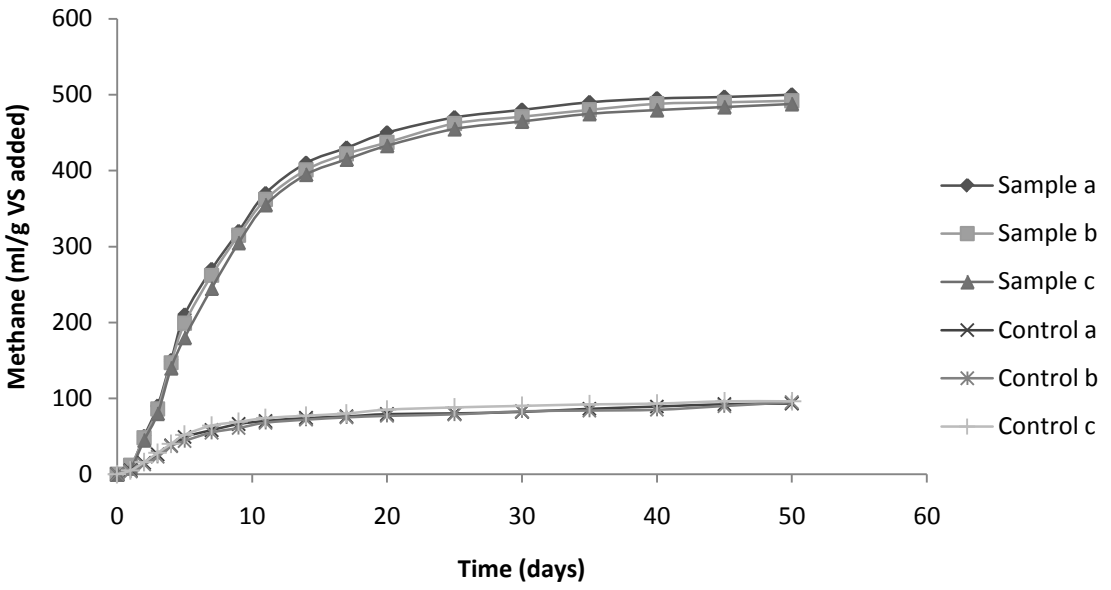

Figure 2.7 Methane production profiles of sample and control (based on Hansen, et al., 2004)

According to Hashimoto et al. (1981) methane production follows a first-order rate of decay. Thus, it is possible to define the methane production rate constant *k* (day^{-1}) using the following equation:

$$\frac{dB}{dt} = -kB \quad \textbf{(2.3)}$$

where, *B* is the methane yield (ml CH_4/g VS), *t* is time and *k* is the methane production rate constant.

When methane yield is determined in a BMP test, *k* can be estimated by taking the reciprocal of the time from the start of the test until *B* equals $0.632B_o$ (Gunaseelan, 2004) or by using a least squares fit of Equation 2.3 to the experimental data. It is also possible to plot experimental data according to the integral of Equation 2.3 and then determine the methane production rate constant, *k*, as the slope of the linear curve obtained (Angelidaki et al., 2009). Equation 2.4 is a linear expression of the integral of Equation 2.3.

$$ln\frac{B_\infty - B}{B_\infty} = -kt \quad \textbf{(2.4)}$$

Regression analysis can then be used to show how closely the experimental data follows first order reaction kinetics. In Figure 2.8 data from Figure 2.7 is graphed according to

Equation 2.4. The slope of the resulting equation is the methane production rate constant, *k*, and the data follows first order reaction kinetics because the coefficient of determination, R^2, is close to 1.0.

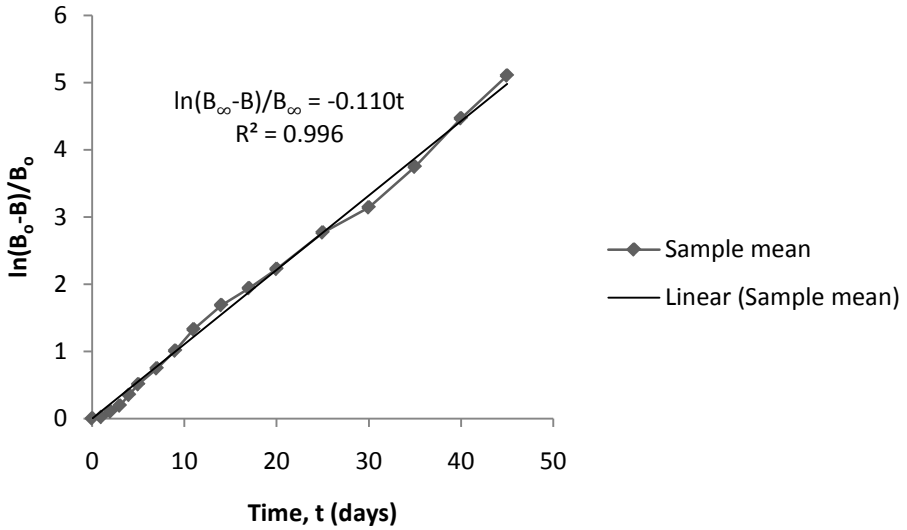

Figure 2.8 Method to determine methane production rate constant, *k* (day^{-1}) (based on Hansen et al., 2004)

3. Biochemical methane potential of wheat-based ethanol byproducts under thermophilic conditions

3.1. Preface

The following scientific paper was written to fulfill the first objective of this research project. The biochemical methane potential (BMP) of six wheat-based ethanol byproduct streams were determined. Pending publication of this paper, methane yields and production rates for whole stillage, thin stillage, wet cake, syrup, wet distillers grains with solubles (WDGS) and dried distillers grains with solubles (DDGS) will be more available for those considering linking an anaerobic digester with a wheat-based ethanol plant in the future.

The paper presented here contains some material that has already been presented in the introduction and literature review sections of this thesis. The materials and methods used to conduct the experiments are outlined in this paper and are followed up with a presentation and discussion of the results. Simple conclusions are drawn at the end of the paper.

The context of this paper as it relates to the overall research project is discussed in Section 5 of the thesis.

3.2. Abstract

Biochemical methane potential (BMP) assays were carried out on byproduct streams typically produced downstream of distillation in conventional wheat-based ethanol plants. In an initial experiment, six byproduct streams were tested in quadruplicate; including whole stillage, thin stillage, wet cake, syrup, WDGS and DDGS. In two subsequent experiments, whole stillage, thin stillage and wet cake were retested in triplicate to ensure reliable results were obtained for the byproducts demonstrating the most energy saving potential. Ultimate methane yield for whole stillage, thin stillage, wet cake, syrup, WDGS and DDGS was 585 ± 46, 549 ± 47, 495 ± 10, 519 ± 24, 518 ± 24 and 516 ± 18 ml CH_4/g VS added, respectively. Methane production rate

constants determined in the first and second experiments did not closely follow first order reaction kinetics. In the final experiment, whole stillage, thin stillage and wet cake had methane production rate constants of 0.106, 0.090 and 0.105 day^{-1}, respectively. Biogas and methane yield, total and volatile solids and pH were recorded for all experiments in the study. The results provide values that can be used for preliminary analysis of the viability of linking anaerobic digestion with wheat based ethanol production.

3.3. Introduction

As of September 1, 2010 the Canadian government adopted a renewable fuels standard that requires a 5% blend of ethanol into all sales of gasoline (CRFA, 2010). In Canada, fuel ethanol production is dominated by the fermentation and distillation of starchy grains like corn and wheat. The byproducts of ethanol production are processed and dried at high temperatures to create a high protein animal feed, DDGS. For every liter of ethanol produced, between 8 and 15 liters of byproduct effluent requires processing (Saha et al., 2005). Energy consumed to produce DDGS decreases the net energy balance ratio of ethanol production and can negatively impact the carbon footprint of ethanol facilities.

As renewable fuel standards increase, the risk of DDGS market saturation increases. Increased fuel ethanol production also increases risks of pollution from CO_2 and organic loading in wastewaters. Anaerobic digestion of ethanol byproducts could potentially reduce the environmental impact of wastewaters leaving ethanol plants and improve the net energy balance ratio of the process if methane gas is combusted and returns heat and electricity to the process. The methane potential of wheat-based ethanol byproducts has not been widely published, so designing anaerobic digesters for wheat-based ethanol plants is difficult. Information is more publicly available for corn-based ethanol byproducts, but it is not clear if the two types of byproducts have similar methane potentials.

3.3.1. Wheat-based ethanol production

Figure 3.1 is a general schematic of the ethanol production process. Downstream processing starts after distillation and can be a major limitation to ethanol production since it consumes approximately 46.8% of the plants total energy needs (Eskicioglu et al., 2011). Bottlenecks in downstream processing can also disrupt system balances and hold up ethanol production on the front end. There are typically six byproduct streams generated during downstream processing. These streams are whole stillage, thin stillage, wet cake, syrup, wet distillers grains with solubes (WDGS) and dried distillers grains with solubes (DDGS).

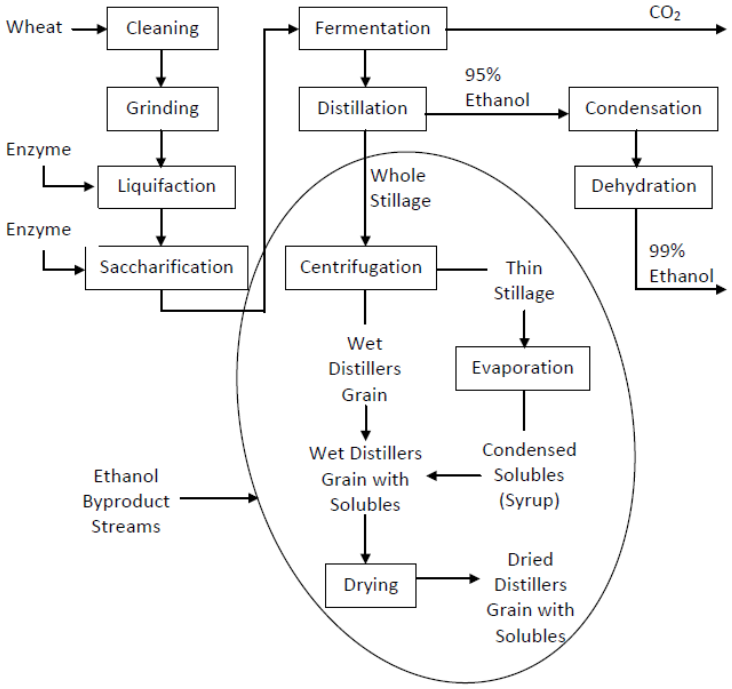

Figure 3.1 Ethanol production process highlighting byproduct streams

3.3.2. Anaerobic digestion of ethanol byproducts

Many studies have been published on anaerobic digestion of corn ethanol byproducts. Research has primarily been focused on thin stillage because it requires the most processing energy and it presents the greatest risks to wastewater leaving ethanol plants. In the 1980's, two mesophilic studies on corn thin stillage reported methane yields of 250 –370 ml CH_4/g TCOD removed (Stover et al., 1984) and 330 ml CH_4/g TCOD

removed (Lanting and Gross, 1985). Stover et al. (1984) used suspended growth and fixed film reactors and suggested that this methane production volume could supply 60% of the daily energy consumed by ethanol plants. Lanting and Gross (1985) used up-flow anaerobic sludge blanket (USAB) reactors.

More recent research has focused on digesting corn thin stillage at thermophilic temperatures. Thermophilic digestion is sometimes considered a disadvantage compared to mesophilic digestion because it requires more energy to reach higher heating temperatures. In ethanol plants, however, whole stillage exits the distillation column at above 55°C so heating demands are decreased and the metabolic rates achieved by thermophilic digestion provide improved efficiency and economics (Agler et al., 2008; Schaefer and Sung, 2008).

Using completely mixed thermophilic reactors at 30-, 20-, and 15-day hydraulic retention times (HRT) and thin stillage concentrated at 100 g/L TCOD and 60 g/L TCOD, Shafer and Sung (2008) observed methane yields ranging between 600 – 700 ml CH_4/g VS removed. They suggested that natural gas consumption at ethanol plants could be reduced by 43 to 59% with this level of methane production. Another thermophilic study on thin corn stillage used high rate sequencing batch reactors to realize a methane yield of 254 ml CH_4/g TCOD fed on a 10 day HRT (Agler et al., 2008). Anger et al. (2008) suggested that this amount of methane production would reduce natural gas consumption in conventional dry grind ethanol plants by 51% and translate to an improved net energy balance ratio of ethanol from 1.26 to 1.70.

In 2011, Eskicioglu et al. published a study of batch and continuous-flow experiments where whole corn stillage was digested under thermophilic and mesophilic conditions. BMP assays produced methane volumes of 401 ± 17, 406 ± 14, 441 ± 2 and 458 ± 0 ml CH_4/g VS added under mesophilic and 693 ± 17, 560 ± 24, 529 ± 37 and 429 ± 8 ml CH_4/g VS added under thermophilic conditions for whole corn stillage at concentrations of 6348, 12,696, 25,393, and 50,786 mg TCOD/L, respectively (Eskicioglu et al., 2011). Little success was realized during continuous flow experiments with full strength whole corn stillage (254 g TCOD/L) at organic loading rates of 4.25, 6.30 and

9.05 g TCOD/L day. At thermophilic temperatures, the digesters were unstable and at mesophilic temperatures, only a 60 day retention time was stable.

3.3.3. Objective

The objective of this study was to determine the biochemical methane potential of wheat-based ethanol byproducts under thermophilic conditions.

3.4. Methods and Materials

3.4.1. Biochemical methane potential (BMP) assay

BMP assays similar to Owen et al. (1979) and Angelidaki et al. (2009) were performed under thermophilic (55 ± 2°C) conditions. Using predetermined total solids (TS) and volatile solids (VS) contents of the various substrates, mixtures of 5% TS and 1:1 VS ratio (substrate:inoculum by mass) were prepared. 300 ml of each mixture was loaded into 1 litre glass assay bottles which were then sealed with a rubber septum and screw cap as shown in Figure 3.2. Samples were taken to measure the actual TS, VS and pH of each prepared mixture. The resulting headspace of the sealed bottles was flushed with pure nitrogen gas for 5 minutes at room temperature. Finally, the assay bottles were loaded into a thermophilic (55 ± 2°C) incubator.

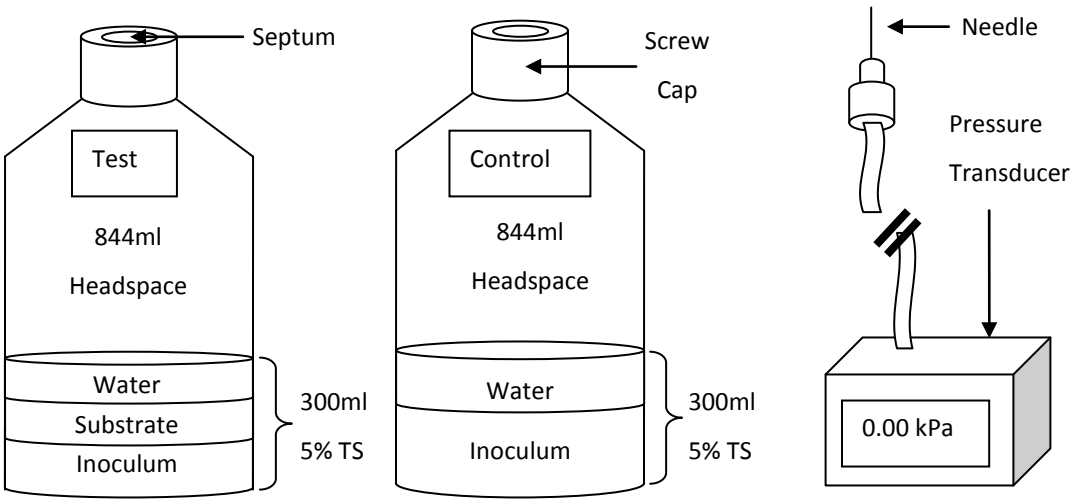

Figure 3.2 Set up for BMP assays

Biogas production was monitored for every bottle throughout the duration of each experiment. Bottles had to be removed from the incubator, but measurements of

biogas volume were done immediately with a needle pressure transducer and calculations were made to convert pressure readings into biogas volumes at standard temperature and pressure. Each time biogas volumes were measured a 20 ml sample of it was taken using a needle syringe. The sample was then transferred to a 5 ml evacuated vial for analysis by gas chromatography (GC). After the biogas samples were taken, the bottles were vented down to 20 mbar, swirled gently and placed back into the incubator.

3.4.2. Analytical methods

Biogas samples were analyzed using gas chromatography (GC). The relative percentages of methane, carbon dioxide, hydrogen, nitrogen and oxygen were determined using a Varion model 450-GC with front and middle TCD detectors. Injector, oven and detector temperatures were 100°C, 50°C and 150°C, respectively. The front column was a Hayesep Q 80/100 CP81069 (1 m x 3.175 mm) using argon make up gas flowing at 20 ml/min. The middle column was a Molsieve 5A 80/100 CP81025 (1 m x 3.175 mm) using helium make up gas flowing at 20 ml/min. The standard gas used for calibrating the GC was composed of H_2(0.5%), CH_4(40%), N_2(1%), O_2(5%), CO_2(bal%).

When the daily biogas production volume dropped below one percent (1%) of the total biogas produced up to that date, the experiment was ended. The bottles were opened and the digestate analyzed for TS, VS and pH.

TS and VS were determined by standard methods (APHA, 1995) with provisions made to avoid losing volatile solids during TS determination. As per Angelidaki et al. (2009) recommendations, TS determination was performed at 70°C, until constant weight (48 hours).

Angelidaki et al. (2009) suggest that ultimate methane yield, B_∞, can be reported as volume of CH_4 per gram VS, or CH_4 per gram COD or CH_4 per gram of sample. In this study, COD was not measured and results are reported as CH_4 per gram VS added to the bottles. Methane production rate constant, k, was determined by the slope of the linear curve obtained when experimental data was plotted according to Equation 3.1

(Angelidaki et al., 2009). The coefficient of determination, R^2, was calculated to show how well the data followed first order rate kinetics.

$$ln\frac{(B_\infty - B)}{B_\infty} = -kt \quad (3.1)$$

3.4.3. Substrate and inoculum characterization

Ethanol byproducts were sampled from a dry-grind wheat-based ethanol plant in Saskatchewan. Samples were collected and then stored at 4°C until needed. TS and VS of each sample were measured to determine quantities needed in each experiment. The same samples of ethanol byproducts were tested in the first and second experiments and fresh samples were obtained for the third experiment.

The same inoculum was used for all three experiments in this study and it was obtained from an anaerobic digester that was operating primarily on feedlot manure. TS and VS of the inoculum were determined before it was frozen at -20°C until needed. The inoculum was thawed and incubated at 55°C for 5, 5 and 7 days, respectively prior to the beginning of each round of experiments.

Table 3.1 lists the TS, VS and VS/TS ratio of substrates used in each experiment.

Table 3.1 Characterization of wheat ethanol byproducts and inoculum (n=3)

Experiment	Substrate	% TS	% VS	% VS/TS
1	Whole Stillage	17.72 ± 0.68	16.26 ± 0.70	91.75 ± 5.31
	Thin Stillage	15.86 ± 0.03	14.16 ± 0.12	89.28 ± 0.76
	Wet Cake	31.89 ± 0.13	30.68 ± 0.11	96.23 ± 0.51
	Syrup	31.21 ± 1.26	27.31 ± 2.11	87.49 ± 7.62
	WDGS	29.42 ± 0.31	26.37 ± 0.13	89.63 ± 1.04
	DDGS	92.74 ± 0.04	80.77 ± 0.62	87.09 ± 0.67
2	Whole Stillage	17.68 ± 0.75	16.26 ± 0.73	91.94 ± 5.66
	Thin Stillage	15.79 ± 0.03	14.07 ± 0.46	89.08 ± 2.90
	Wet Cake	32.45 ± 0.44	31.37 ± 0.42	96.69 ± 1.83
3	Whole Stillage	19.13 ± 1.31	17.72 ± 1.42	92.62 ± 9.77
	Thin Stillage	13.33 ± 0.11	11.77 ± 0.41	88.29 ± 3.14
	Wet Cake	34.07 ± 0.16	33.01 ± 0.17	96.89 ± 0.67
	Inoculum[a]	9.42	6.43	68.25

[a] Limited volumes of inoculum were available, so TS and VS measurements made during another experiment were used and error data was not available.

3.4.4. Experimental set up

Three BMP experiments were performed to determine the ultimate methane yield and methane production rate constant that could be achieved from ethanol byproducts. In the first experiment, quadruplicate samples of the six byproduct streams produced downstream of distillation were tested; including whole stillage, thin stillage, wet cake, syrup, WDGS and DDGS. The three ethanol byproducts exhibiting the most energy saving potential for an ethanol facility were selected from the first experiment and retested in triplicate for the second and third experiments.

3.5. Results and Discussion

Methane yields were corrected to account for endogenous metabolism of the inoculum which was determined by running control assays in each experiment. Methane production profiles are used to show the mean accumulated methane yield, B_o, across replicates in each experiment and error bars represent the standard deviation at each sampling interval. Data points from the methane production profiles were plotted according to Equation 3.1 to determine the methane production rate constant, k, and regression analysis was used to describe the fit of the data to first-order rate kinetics.

3.5.1. Experiment 1

Whole stillage showed the highest methane potential at 645 ± 23 ml CH_4/g VS added. Thin stillage was the second highest at 568 ± 18 ml CH_4/g VS added, but experienced a lag in methane production around day 10, as can be seen in Figure 3.3. Wet cake produced 509 ± 25 which was not significantly different ($p>0.05$) from syrup, WDGS or DDGS which produced 519 ± 24, 518 ± 24, and 516 ± 18 ml CH_4/ g VS added, respectively. The values for methane yield, B_∞, methane production rate constant, k, and pH determined in Experiment 1 are provided in Table 3.2.

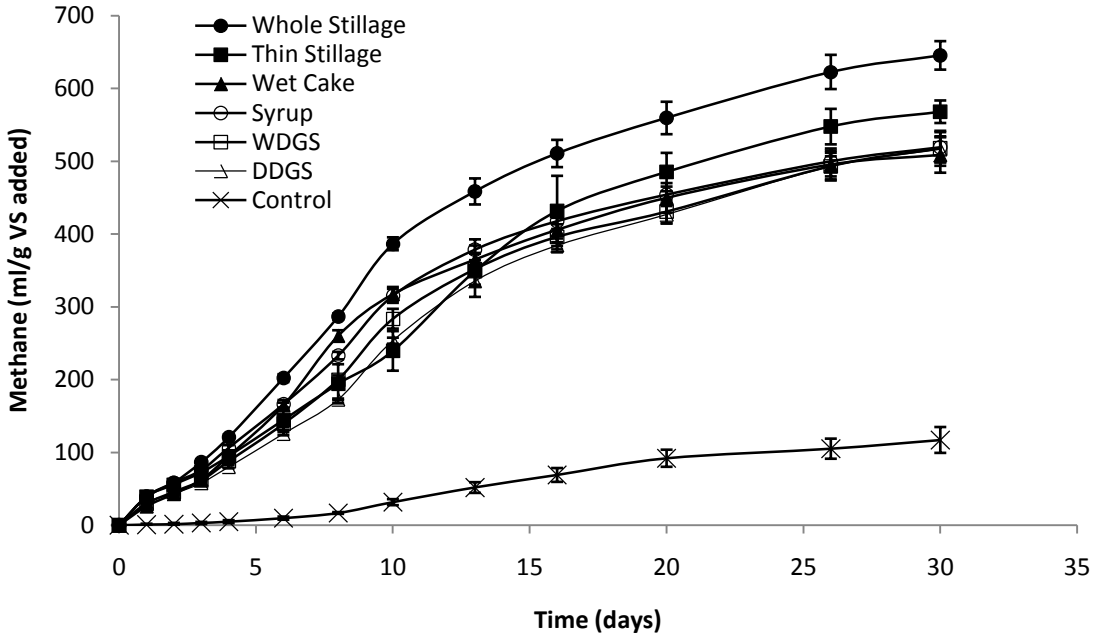

Figure 3.3 Experiment 1 methane production profiles

Table 3.2 Experiment 1 methane yield, methane production rate constant and pH

	Methane yield	Methane production rate constant		pH	
Substrate	B_∞ (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	645 ± 23[a]	0.107	0.943	7.71	7.81
Thin Stillage	568 ± 18[a]	0.099	0.890	7.15	7.84
Wet Cake	509 ± 25	0.115	0.934	7.65	7.77
Syrup	519± 24	0.108	0.955	7.12	7.89
WDGS	518 ± 24	0.097	0.936	7.07	7.79
DDGS	516 ± 18	0.094	0.912	7.24	7.83
Control	117 ± 4	NA	NA	8.10	7.86

[a] Significantly different ($p<0.05$)

Methane production rate constants, k, were 0.107, 0.099, 0.115, 0.108, 0.097, and 0.094 day^{-1} for whole stillage, thin stillage, wet cake, syrup, WDGS and DDGS, respectively. Unfortunately, methane production rate constants did not closely follow first order reaction kinetics. The poor fit of Experiment 1 data to first order reaction kinetics can be attributed to a lag in biogas production at the start of the experiment. For example, the lag phase for thin stillage, seen in Figure 3.3, is reflected by the R^2 value for thin stillage being the lowest in the group. No significant increases in methane

production were realized until after day 5, indicating that the inoculum may have required a longer incubation period.

Thin stillage, syrup and WDGS mixtures had lower pH values than the other byproducts because they contained higher levels of soluble organics. By the end of Experiment 1 all byproduct mixtures self adjusted to pH values between 7.77 and 7.89.

From this experiment it was decided that whole stillage, thin stillage and wet cake would be selected for repetitive BMP analysis. Whole stillage and thin stillage exhibited the highest methane yields and the methane yield of wet cake was not significantly different from syrup, WDGS or DDGS. Digesting wet cake could also avoid downstream heating requirements in an ethanol facility and it showed a higher methane production rate constant than the other byproducts.

3.5.2. Experiment 2

Whole stillage showed the highest methane yield again in the second experiment at 578 ± 14 ml CH_4/g VS added as shown in Figure 3.4. Thin stillage and wet cake produced similar volumes to each other at 483 ± 59 and 493 ± 32 ml CH_4/g VS added, respectively. Whole stillage methane production was significantly less in Experiment 2 compared to Experiment 1 and Figure 3.4 shows that thin stillage was highly variable and slow to start. Wet cake methane yield reached similar volumes in the first two experiments. The values for methane yield, B_∞, production rate, k, and pH determined in Experiment 2 are provided in Table 3.3.

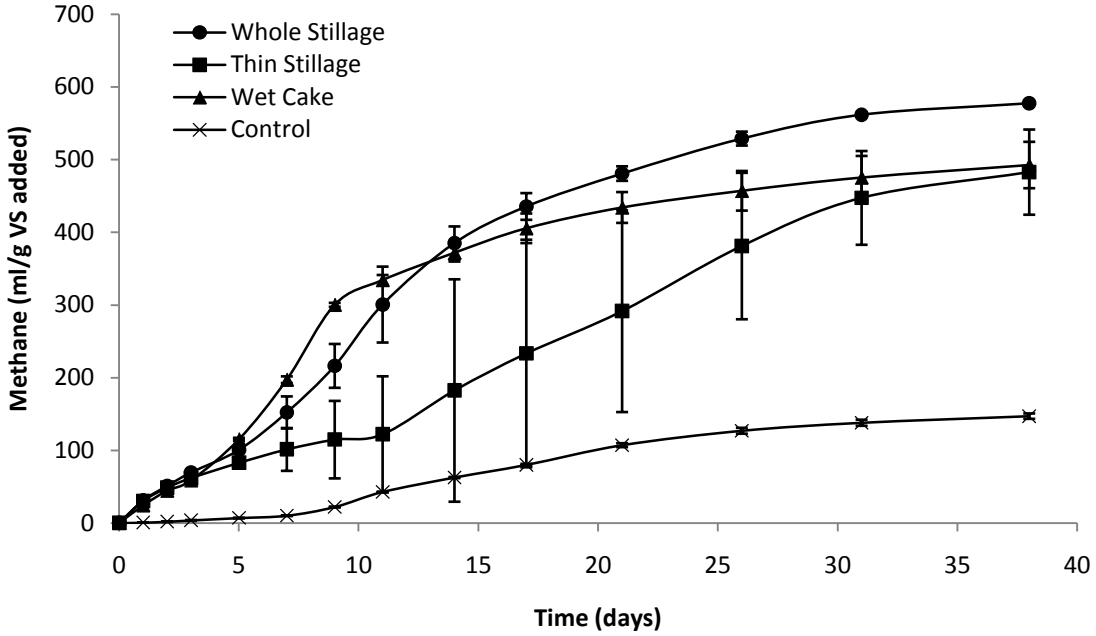

Figure 3.4 Experiment 2 methane production profiles

Table 3.3 Experiment 2 methane yield, methane production rate constant and pH

	Methane yield	Methane production rate constant		pH	
Substrate	B_∞ (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	578 ± 14[a]	0.094	0.927	7.76	7.81
Thin Stillage	483 ± 59	0.058	0.821	7.37	7.89
Wet Cake	493 ± 32[a]	0.102	0.987	7.75	7.66
Control	147 ± 4	NA		8.01	7.87

[a] Significantly different ($p<0.05$)

Methane production rate constants, k, were 0.094, 0.058 and 0.102 day^{-1} for whole stillage, thin stillage and wet cake, respectively and wet cake most closely followed first order reaction kinetics. The variability between bottles for thin stillage can be seen in Figure 3.4 and also by its low R^2 value in Table 3.3. Figure 3.4 shows early gains in wet cake methane production which are reflected by its high methane production rate constant and R^2 value. pH values were similar between the first two experiments and again self adjusted to between 7.66 and 8.89. Thin stillage pH started

a little higher and wet cake pH moved down instead of up, but overall neither change contributed to a variation of the results.

The variability observed in this experiment and the differences from values obtained in Experiment 1 were contributed to storage of ethanol byproducts for 40 days while the first experiment was being conducted. It is possible that the chemical and physical structure of the byproducts changed during this time. A lag phase at the start of Experiment 2 was again attributed to poor inoculum activation. Higher methane production from the control assay in Experiment 2 also suggests that microbial activity at the beginning of that experiment may have been compromised compared to the first and third experiments. Fresh byproduct samples were collected for Experiment 3 and the inoculum incubation period was extended to 7 days.

3.5.3. Experiment 3

In the final experiment, thin stillage out performed whole stillage and wet cake with a methane yield of 592 ± 37 ml CH_4/g VS added. Whole stillage and wet cake produced 533 ± 18 and 485 ±19 ml CH_4/g VS added, respectively. The methane production curves shown in Figure 3.5 represent robust methane production for all byproducts. There were no lag phases and the majority of methane was produced in the first 15 days of the experiment. Table 3.4 provides the methane yield, B_∞, methane production rate constant, k, and pH determined in Experiment 3.

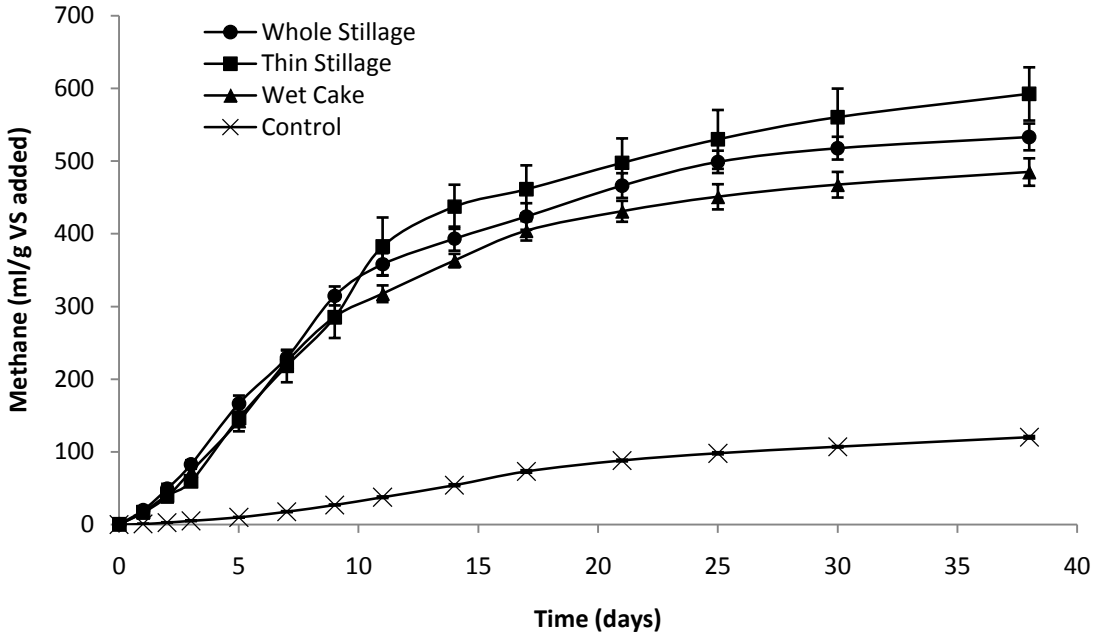

Figure 3.5 Experiment 3 methane production profiles

Table 3.4 Experiment 3 methane yield, methane production rate constant and pH

	Methane yield	Methane production rate constant		pH	
Substrate	B_∞ (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	533 ± 18	0.106	0.977	7.41	7.70
Thin Stillage	592 ± 37	0.090	0.983	7.31	7.78
Wet Cake	485 ± 19[a]	0.105	0.990	7.58	7.57
Control	120 ± 2	NA		8.03	7.84

[a] Significantly different ($p<0.05$)

Methane production rate constants most closely followed first order reaction kinetics in this experiment, as demonstrated by high R^2 values in Table 3.4. Whole stillage and wet cake had higher methane production rate constants than thin stillage, indicating that they achieved peak methane yields in a shorter period of time. The pH values in the third experiment follow closely with the values seen in the previous two experiments. Whole stillage started out a lower pH, but all the mixtures again self adjusted to between 7.57 and 7.84.

Variability among thin stillage replicates improved substantially in Experiment 3, but was still the highest of all byproducts. Wet cake produced similar amounts of

methane as that observed in the first two experiments. Thin stillage results were similar to Experiment 1 and whole stillage methane production was once again significantly lower in the third experiment than in the other experiments. Decreased variation between assay bottles containing the same substrate and improved fit of the data to first order reaction kinetics were attributed to the use of fresh substrates and the extension of the inoculum incubation period.

3.5.4. Summary and significance

Table 3.5 is a summary of the methane yields observed for each byproduct across all three experiments. The column second from the right is the calculated mean and standard deviation of whole stillage, thin stillage and wet cake for the entire study. Notice that the whole stillage values fall within the range of values obtained for by Eskicioglu et al. (2011) for corn stillage.

Table 3.5 Summary of methane yields (ml CH_4/g VS added)

Substrate	Experiment 1	Experiment 2	Experiment 3	Entire Study	Corn
Whole Stillage	645 ± 23[a]	578 ± 14[a]	533 ± 18	585 ± 46	429-693
Thin Stillage	568 ± 18[a]	483 ± 59	592 ± 37	547 ± 47	
Wet Cake	509 ± 25	493 ± 32[a]	485 ± 19[a]	495 ± 10[a]	
Syrup	519± 24				
WDGS	518 ± 24				
DDGS	516 ± 18				

[a] Significantly different ($p<0.05$)

Overall, whole stillage showed the highest average methane yield across all the experiments at 585 ± 46 ml CH_4/g VS added. Thin stillage showed the next highest methane yield, but with widest range of variability at 547 ± 47 ml CH_4/g VS added. Wet cake produced similar volumes of methane gas across all three experiments and averaged 495 ± 10 ml CH_4/g VS added. Similar results were obtained for thin stillage when fresh substrates were used in Experiments 1 and 3. One possible explanation for lower methane production from whole stillage in Experiment 3 is that the fresh

substrate had slightly higher TS content than the previous material and thus, less total substrate was used in the assays.

Consider that for every litre of ethanol produced, ten litres of whole stillage are generated with 18% TS and 92%. VS/TS. For every liter of ethanol produced approximately 1656 grams of volatile solids are generated. If full scale anaerobic digestion of whole stillage achieves 75% of the biochemical methane potential determined in this study, every litre of ethanol produced could also produce 725 litres of methane gas. That is just over 28 MJ of energy that could be harnessed from every liter of ethanol produced. Ethanol itself contains 23.4 MJ/L of energy (ORLN, 2011). 28 MJ is also equivalent to 8 kWh of heat or burning 1 kg of coal.

3.6. Conclusion

Whole stillage, thin stillage and wet cake methane yields were 585 ± 46, 549 ± 47 and 495 ± 10 ml CH_4/g VS added, respectively, across three experiments adding up to ten replicates each. Syrup, WDGS and DDGS specific methane yields were 519 ± 24, 518 ± 24 and 516 ± 18 ml CH_4/g VS added, respectively, from one experiment with three replicates each. Methane production rate constants did not follow first order reaction kinetics in the first two experiments, but in the third experiment, rates were 0.106, 0.090 and 0.105 day^{-1} for whole stillage, thin stillage and wet cake, respectively and followed first order reaction kinetics.

The significance of this study is two-fold. The biochemical methane potential of wheat-based ethanol byproducts is now known, so now engineers designing anaerobic digestion projects have statistics to support their estimates. Additionally, estimates show that for every litre of wheat-based ethanol produced another 28 MJ of energy in the form of methane gas could possibly be harnessed by anaerobic digestion of whole stillage.

4. Biochemical methane potential of wheat-based ethanol byproducts with feedlot manure under thermophilic conditions

4.1. Preface

The following scientific paper was written to fulfill the second objective of this research project. The effect of adding feedlot manure to the most digestible ethanol byproducts was determined using biochemical methane potential (BMP) assays. Pending publication of this paper, methane yields and production rates for wheat-based whole stillage, thin stillage and wet cake with 1:1 and 2:1 ratios of wheat-based ethanol byproduct to feedlot manure will be more accessible information for those considering linking an anaerobic digester with a wheat-based ethanol plant and a feedlot in the future.

The paper presented here contains some material that has already been presented in the Introduction and Literature Review sections of this thesis. The materials and methods used to conduct the experiments are outlined in the paper and are followed up with a presentation and discussion of the results. Simple conclusions are drawn at the end of the paper.

The context of this paper as it relates to the overall research project is discussed in Section 5 of the thesis.

4.2. Abstract

Biochemical methane potential (BMP) assays were conducted on byproducts from dry-grind wheat-based ethanol plants receiving two ratios of feedlot manure. Whole stillage, thin stillage and wet cake were tested alone and with 1:1 and 2:1 ratios (VS basis) of byproduct to feedlot manure. The resulting methane yield for ethanol byproducts with 1:1 VS ratio of manure were 389 ± 15, 446 ± 12 and 344 ± 12 ml CH_4/g VS added for whole stillage, thin stillage and wet cake, respectively. When ethanol byproducts were amended with feedlot manure at 2:1 VS ratio (byproduct:manure), the

methane yields were 399 ± 18, 523 ± 13 and 367 ± 12 ml CH_4/g VS added for whole stillage, thin stillage and wet cake, respectively. Methane yield and production rate, total and volatile solids and pH were recorded for all experiments. With the exception of thin stillage, methane yields of ethanol byproducts reached expected values for manure amended versions based on the ratio of byproduct to manure. However, manure amended thin stillage produced 125% and 119 % of the expected methane yield for the 1:1 and 2:1 ratio experiments, respectively.

4.3. Introduction

As of September 1, 2010 the Canadian government adopted a renewable fuels standard that requires a 5% blend of ethanol into all sales of gasoline (CRFA, 2010). In Canada, fuel ethanol production is dominated by the fermentation and distillation of starchy grains like corn and wheat. The byproducts of ethanol production are processed and dried at high temperatures to create a high protein animal feed, dried distillers grains with solubles (DDGS). For every liter of ethanol produced, between 8 and 15 liters of byproduct effluent requires processing (Saha et al., 2005). Energy consumed to produce DDGS decreases the net energy balance ratio of ethanol production and can negatively impact the carbon footprint of ethanol facilities.

As renewable fuel standards increase, the risk of DDGS market saturation increases. Increased fuel ethanol production also increases risks of pollution from CO_2 and organic loading in wastewaters. Anaerobic digestion of ethanol byproducts could potentially reduce the environmental impact of wastewaters leaving ethanol plants and improve the net energy balance ratio of the process if methane gas is combusted and returns heat and electricity to the process. The methane potential of wheat-based ethanol byproducts has not been widely published, so designing anaerobic digesters for wheat-based ethanol plants is difficult. Information is more publicly available for corn-based ethanol byproducts, but it is not clear if the two types of byproducts have similar methane potentials.

Co-locating an ethanol plant and an anaerobic digester at a beef feedlot could provide even more economic and environmental advantages. Co-digestion of feedlot manure with ethanol byproducts is possible and ethanol byproducts can either be fed to the digester or to the cattle in the feedlot. Linking all three components (ethanol plant, beef feedlot and anaerobic digester) creates what is known as a biorefinery where the byproducts of one entity become the inputs for the next and the overall system operates in concert as shown below in Figure 4.1.

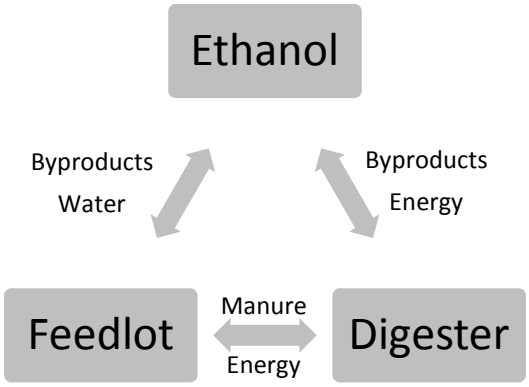

Figure 4.1 Flow of materials through proposed biorefinery

4.3.1. Wheat-based ethanol production

Figure 4.2 is a general schematic of the ethanol production process. Downstream processing starts after distillation and can be a major limitation to ethanol production since it consumes approximately 46.8% of the plants total energy needs (Eskicioglu et al., 2011). Bottlenecks in downstream processing can also disrupt system balances and hold up ethanol production on the front end. There are typically six byproduct streams generated during downstream processing. These streams are whole stillage, thin stillage, wet cake, syrup, wet distillers grains with solubles (WDGS) and dried distillers grains with solubes (DDGS).

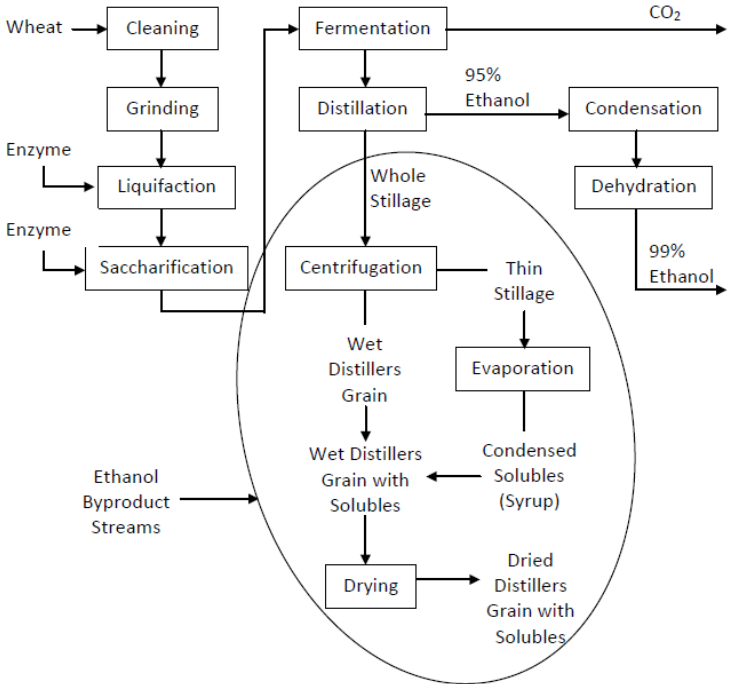

Figure 4.2 Ethanol production process highlighting byproduct streams

4.3.2. Anaerobic digestion of ethanol byproducts

Many studies have been published on anaerobic digestion of corn ethanol byproducts. Research has primarily been focused on thin stillage because it requires the most processing energy and it presents the greatest risks to wastewater leaving ethanol plants. In the 1980's, two mesophilic studies on corn thin stillage reported methane yields of 250 –370 ml CH_4/g TCOD removed (Stover et al., 1984) and 330 ml CH_4/g TCOD removed (Lanting and Gross, 1985). Stover et al. (1984) used suspended growth and fixed film reactors and suggested that this methane production volume could supply 60% of the daily energy consumed by ethanol plants. Lanting and Gross (1985) used up-flow anaerobic sludge blanket (USAB) reactors.

More recent research has focused on digesting corn thin stillage at thermophilic temperatures. Thermophilic digestion is sometimes considered a disadvantage compared to mesophilic digestion because it requires more energy to reach higher heating temperatures. In ethanol plants, however, whole stillage exits the distillation column at above 55°C so heating demands are decreased and the metabolic rates

achieved by thermophilic digestion provide improved efficiency and economics (Agler et al., 2008; Schaefer and Sung, 2008).

Using completely mixed thermophilic reactors at 30-, 20-, and 15-day hydraulic retention times (HRT) and thin stillage concentrated at 100 g/L TCOD and 60 g/L TCOD, Shafer and Sung (2008) observed methane yields ranging between 600 – 700 ml CH_4/g VS removed. They suggested that natural gas consumption at ethanol plants could be reduced by 43 to 59% with this level of methane production. Another thermophilic study on thin corn stillage used high rate sequencing batch reactors to realize a methane yield of 254 ml CH_4/g TCOD fed on a 10 day HRT (Agler et al., 2008). Anger et al. (2008) suggested that this amount of methane production would reduce natural gas consumption in conventional dry grind ethanol plants by 51% and translate to an improved net energy balance ratio of ethanol from 1.26 to 1.70.

In 2011, Eskicioglu et al. published a study of batch and continuous-flow experiments where whole corn stillage was digested under thermophilic and mesophilic conditions. BMP assays produced methane volumes of 401 ± 17, 406 ± 14, 441 ± 2 and 458 ± 0 ml CH_4/g VS added under mesophilic and 693 ± 17, 560 ± 24, 529 ± 37 and 429 ± 8 ml CH_4/g VS added under thermophilic conditions for whole corn stillage at concentrations of 6348, 12,696, 25,393, and 50,786 mg TCOD/L, respectively (Eskicioglu et al., 2011). Little success was realized during continuous flow experiments with full strength whole corn stillage (254 g TCOD/L) at organic loading rates of 4.25, 6.30 and 9.05 g TCOD/L day. At thermophilic temperatures, the digesters were unstable and at mesophilic temperatures, only a 60 day retention time was stable.

4.3.3. Anaerobic digestion of manure

Manure is a widely used feedstock for anaerobic digestion because it decreases the volume of greenhouse gas emissions released during normal manure storage (Moller et al., 2004). Manure itself is a good substrate for co-digestion with other organic material because it can adjust the carbon-to-nitrogen (C:N) ratio of feedstock (25-30:1 optimal), it can provide buffering capacity (alkalinity) and it can supply essential nutrients that improve methane yields (Labatut and Scott, 2008; Ward et al., 2008). The

biogas potential of manure is highly variable and it depends on the type of animal, the animal's feed, climate conditions and the type of bedding used, not to mention the storage conditions of manure before anaerobic digestion occurs (Moller et al., 2004). A typical specific methane yield of beef cattle manure is 328 ml/g VS added (Hashimoto et al., 1981).

4.3.4. Objective

The objective of this study was to determine effect of feedlot manure on the biochemical methane potential of wheat ethanol byproducts under thermophilic conditions.

4.4. Methods and Materials

4.4.1. Biochemical methane potential (BMP) assay

BMP assays similar to Owen et al. (1979) and Angelidake et al., (2009) were performed under thermophilic (55 ± 2°C) conditions in this study. Using predetermined total solids (TS) and volatile solids (VS) content of the various substrates, mixtures of 5% TS and 1:1 VS ratio (substrate:inoculum by mass) were prepared. When feedlot manure amendments were added, the substrate:inoculum VS ratio remained 1:1 and the substrate itself was composed of either 1:1 or 2:1 VS ratios of ethanol byproduct to manure. 300 ml of each mixture was loaded into 1 litre glass assay bottles which were then sealed with a rubber septum and screw cap as shown in Figure 4.3. Samples were taken to measure the actual TS, VS and pH of each prepared mixture. The resulting headspace of the sealed bottles was flushed with pure nitrogen gas for 5 minutes at room temperature. Finally, the assay bottles were loaded into a thermophilic (55 ± 2°C) incubator.

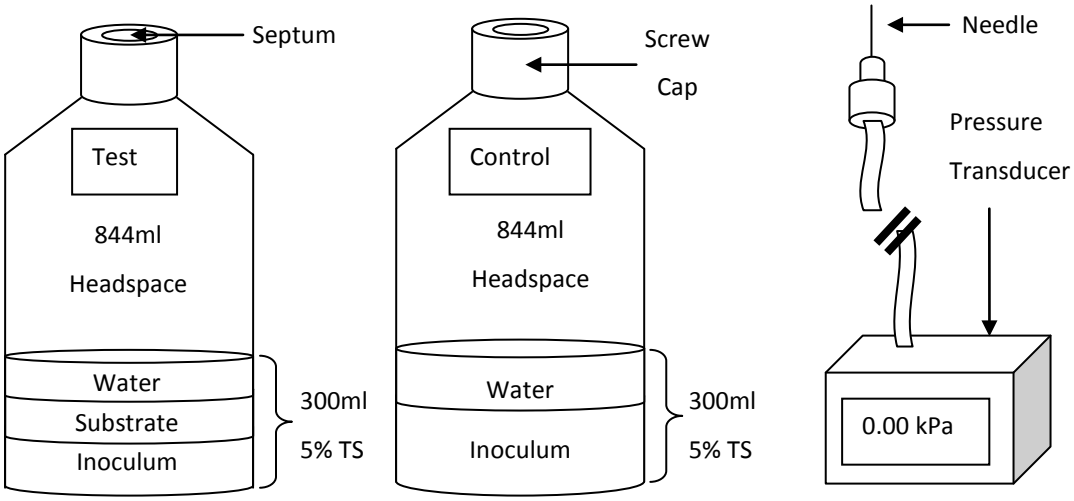

Figure 4.3 Set up for BMP assays

Biogas production was monitored for every bottle throughout the duration of each experiment. Bottles had to be removed from the incubator, but measurements of biogas volume where done immediately with a needle pressure transducer and calculations were made to convert pressure readings into biogas volumes at standard temperature and pressure. Each time biogas volumes were measured a 20 ml sample of it was taken using a needle syringe. The sample was then transferred to a 5 ml evacuated vial for analysis by gas chromatography (GC). After the biogas samples were taken, the bottles were vented down to 20 mbar, swirled gently and placed back into the incubator.

4.4.2. Analytical methods

Biogas samples were analyzed using gas chromatography (GC). The relative percentages of methane, carbon dioxide, hydrogen, nitrogen and oxygen were determined using a Varion model 450-GC with front and middle TCD detectors. Injector, oven and detector temperatures were 100°C, 50°C and 150°C, respectively. The front column was a Hayesep Q 80/100 CP81069 (1 m x 3.175 mm) using argon make up gas flowing at 20 ml/min. The middle column was a Molsieve 5A 80/100 CP81025 (1 m x 3.175 mm) using helium make up gas flowing at 20 ml/min. The standard gas used for calibrating the GC was composed of H_2(0.5%), CH_4(40%), N_2(1%), O_2(5%), CO_2(bal%).

When the daily biogas production volume dropped below one percent (1%) of the total biogas produced up to that date, the experiment was ended. The bottles were opened and the digestate analyzed for TS and VS. The pH of each bottle was also measured.

TS and VS were determined by standard methods (APHA, 1995) with provisions made to avoid losing volatile solids during TS determination. As per Angelidaki et al. (2009) recommendations, TS determination was performed at 70°C, until constant weight (48 hours).

Angelidaki et al. (2009) suggest that specific methane production can be reported as volume of CH_4 per gram VS, or CH_4 per gram COD or CH_4 per gram of sample. In this study, COD was not measured and results are reported as CH_4 per gram VS added to the bottles. Methane production rate constant, *k*, was determined by the slope of the linear curve obtained when experimental data was plotted according to Equation 4.1 (Angelidaki et al., 2009). The coefficient of determination, R^2, was calculated to show how well the data followed first order rate kinetics.

$$ln\frac{(B_\infty - B)}{B_\infty} = -kt \quad \textbf{(4.1)}$$

4.4.3. Substrate inoculum characterization

Ethanol byproducts were sampled from a dry-grind wheat-based ethanol plant in Saskatchewan. Samples were collected and then stored at 4°C until needed. TS and VS of each sample were measured to determine quantities needed in each experiment. Ethanol byproducts tested in the 1:1 experiment had been stored at 4°C for more than 30 days, while the byproducts for the 2:1 experiment were stored for less than one week.

Manure samples were collected from an Alberta beef feedlot for the 1:1 experiment and from a Saskatchewan beef feedlot for the 2:1 experiment. The Alberta feedlot manure sample was collected in the late spring, transported in less than two hours and stored at 4°C until used. The Saskatchewan sample was collected in the early

fall and was in transport for approximately twelve hours before being stored at 4°C until needed.

The same inoculum was used for both experiments in the study. The inoculum was obtained from an anaerobic digester while it was operating primarily on feedlot manure. The TS and VS of the inoculum were determined before it was frozen at -20°C until needed. The inoculum was thawed and incubated 5 and 7 days prior to the beginning of each round of experiments.

Table 4.1 lists the TS, VS and VS/TS ratio for ethanol byproducts, manures and inoculum used in each experiment.

Table 4.1 Characterization of wheat ethanol byproducts, manure and inoculum (n=3)

Experiment	Substrate	% TS	% VS	% VS/TS
1:1	Whole Stillage	17.68 ± 0.75	16.26 ± 0.73	91.94 ± 5.66
	Thin Stillage	15.79 ± 0.03	14.07 ± 0.46	89.08 ± 2.90
	Wet Cake	32.45 ± 0.44	31.37 ± 0.42	96.69 ± 1.83
	Manure	32.59 ± 4.46	23.63 ± 3.48	72.51 ± 14.57
2:1	Whole Stillage	19.13 ± 1.31	17.72 ± 1.42	92.62 ± 9.77
	Thin Stillage	13.33 ± 0.11	11.77 ± 0.41	88.29 ± 3.14
	Wet Cake	34.07 ± 0.16	33.01 ± 0.17	96.89 ± 0.67
	Manure	38.66 ± 3.16	17.53 ± 0.86	45.34 ± 4.33
	Inoculum[a]	9.42	6.43	68.25

[a] Limited volumes of inoculum were available, so TS and VS measurements made during another experiment were used and error data was not available.

4.4.4. Experimental set up

Two BMP experiments were performed to determine the ultimate methane yield and methane production rate constant that could be achieved from ethanol byproducts receiving two different ratios of feedlot manure. Based on the results from a previous experiment by Annand et al. (2011), whole stillage, thin stillage and wet cake received manure amendments of 1:1 and 2:1 VS ratios (byproduct:manure). Methane production of manure amended ethanol byproducts was compared to the results achieved by Annand et al. (2011) for un-amended byproducts.

4.5. Results and Discussion

Methane yields were corrected to account for endogenous metabolism of the inoculum which was determined by running control assays in each experiment. Methane production profiles are used to show the mean accumulated methane yield, B_o, across replicates in each experiment and error bars represent the standard deviation at each sampling interval. Data points from the methane production profiles were plotted according to Equation 4.1 to determine the methane production rate constant, *k*, and regression analysis was used to describe the fit of the data to first-order rate kinetics.

4.5.1. 1:1 Experiment

Two graphs are presented to show how a 1:1 VS ratio manure amendment affected the methane potential of ethanol byproducts. Figure 4.4 shows methane production profiles for whole stillage, thin stillage and wet cake without manure amendment. Methane yields for these byproducts were 578 ± 14, 473 ± 59 and 493 ± 32 ml CH_4/g VS added, respectively. Figure 4.5 shows methane production profiles for whole stillage, thin stillage and wet cake with manure amendments as well as one for manure itself. Methane yields for these byproducts with 1:1 VS ratio manure and for manure alone were 389 ± 15, 446 ± 12, 344 ± 12 and 230 ± 16 ml CH_4/g VS added, respectively.

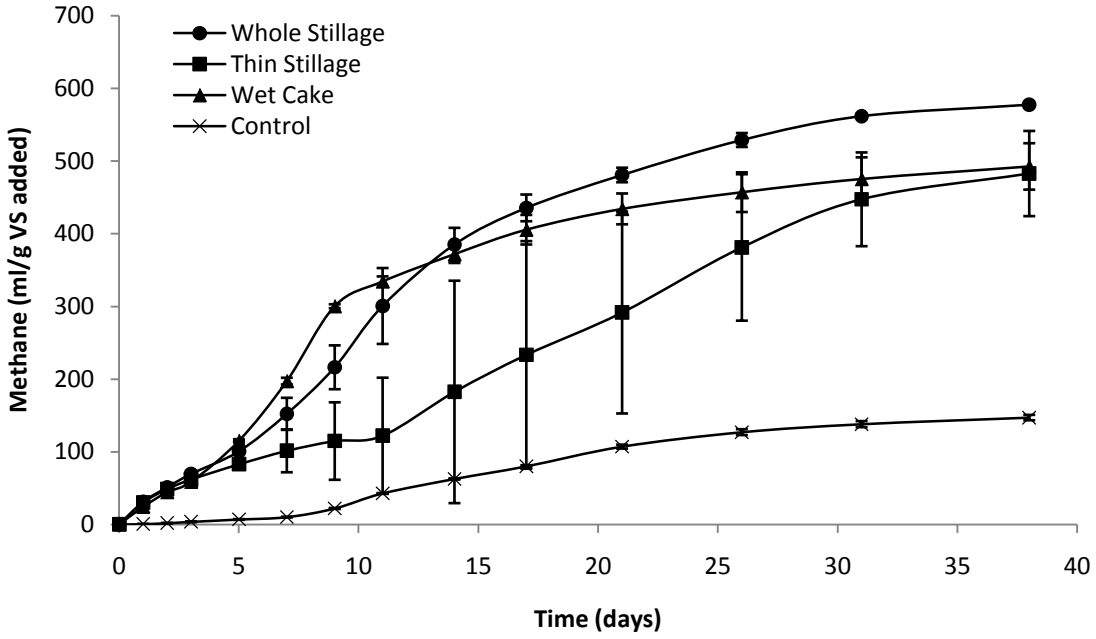

Figure 4.4: Un-amended methane production profiles

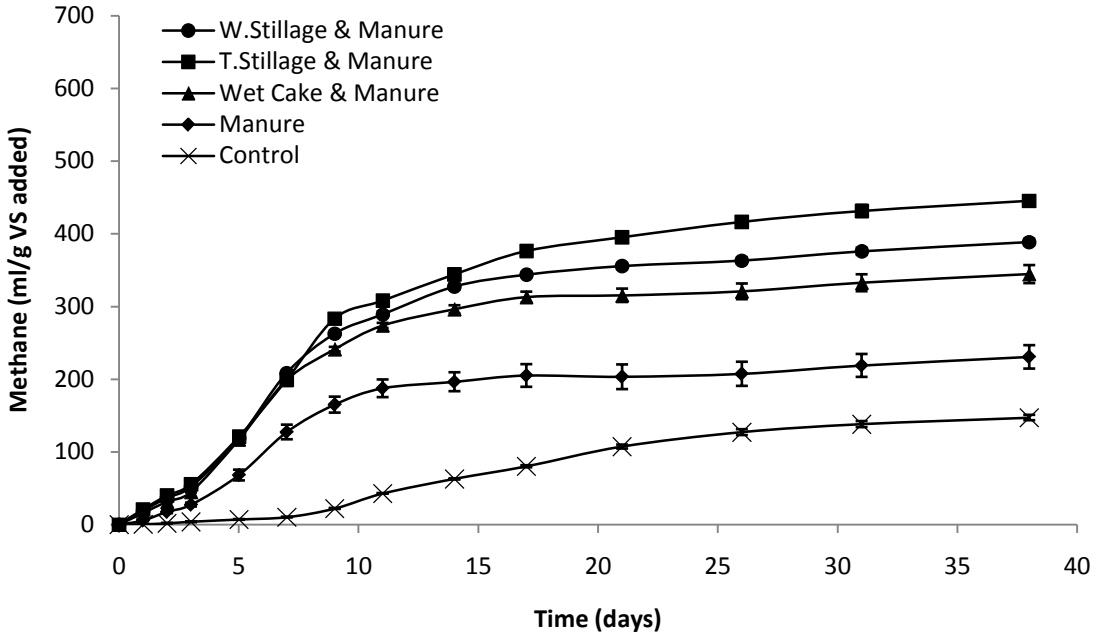

Figure 4.5 Manure amended methane production profiles (1:1 VS ratio)

The effect of manure amendment on ethanol byproduct methane potential can be seen by comparing Figures 4.4 and 4.5. As expected, methane yields are less in Figure 5 because the biogas potential of manure is less than that of ethanol byproducts.

Manure amendment had a very obvious stabilizing effect on ethanol byproducts, especially thin stillage. The variability observed between bottles in Figure 4.4 was virtually eliminated in Figure 4.5 and thin stillage became the top methane yielding byproduct instead of whole stillage when manure was added.

Table 4.2 provides the values for methane yield, B_∞, methane production rate constant, k, and pH measurements determined in this 1:1 VS ratio experiment.

Table 4.2 1:1 Experiment methane yield, methane production rate constant and pH

	Methane yield	Methane production rate constant		pH	
Substrate	B_∞ (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	578 ± 14[a]	0.094	0.927	7.76	7.81
Thin Stillage	483 ± 59	0.058	0.821	7.37	7.89
Wet Cake	493 ± 32[a]	0.102	0.987	7.75	7.66
Whole Stillage & Manure	389 ± 15	0.113	0.980	7.97	7.59
Thin Stillage & Manure	446 ± 12	0.110	0.989	7.67	7.64
Wet Cake & Manure	344 ± 12	0.115	0.958	7.97	7.55
Manure	230 ± 16	0.104	0.915	8.19	7.45
Control	147 ± 4	NA		8.01	7.87

[a] Significantly different from manure amended counterpart ($p<0.05$)

Figure 4.5 also shows much faster methane production rate constants than Figure 4.4; a fact that is reiterated by the methane production rate constant, k, values in Table 4.2. Whole stillage and thin stillage methane production did not fit first order kinetics very well (low R^2 values), but the manure amended versions of these byproducts did. Wet cake followed first order kinetics well, but the manure amended wet cake achieved an even faster methane production rate constant, 0.102 day^{-1} compared to 0.115 day^{-1}, respectively. The pH values of manure amended byproducts also started higher than their un-amended counterparts, which may have led to faster methane production rate constants caused by improved buffering capacity and micronutrient availability.

4.5.2. 2:1 Experiment

Two graphs are presented to show how a 2:1 VS ratio of ethanol byproduct to manure affected the methane potential of ethanol byproducts. Figure 4.6 shows methane production profiles for whole stillage, thin stillage and wet cake without manure amendment. Methane yields for these byproducts were 533 ± 18, 592 ± 37 and 485 ± 19 ml CH_4/g VS added, respectively. Figure 4.7 shows methane production profiles for whole stillage, thin stillage and wet cake with manure amendments as well as one for manure itself. Methane yields for these byproducts receiving manure amendment and for manure alone were 399 ± 18, 523± 13, 367 ± 12 and 136 ± 12 ml CH_4/g VS added, respectively.

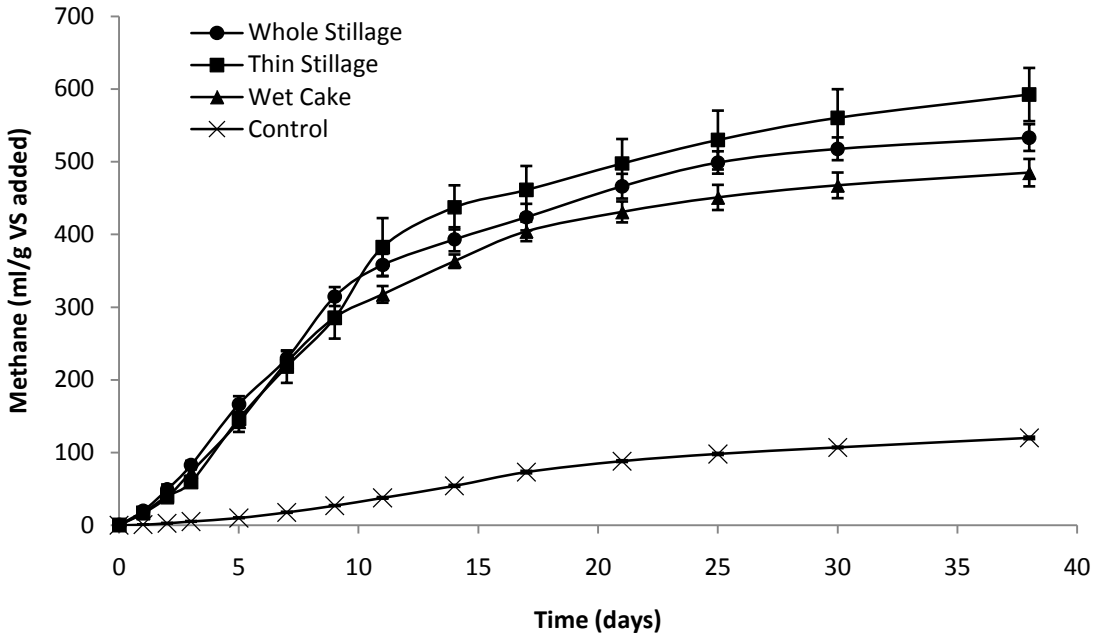

Figure 4.6 Un-amended methane production profiles

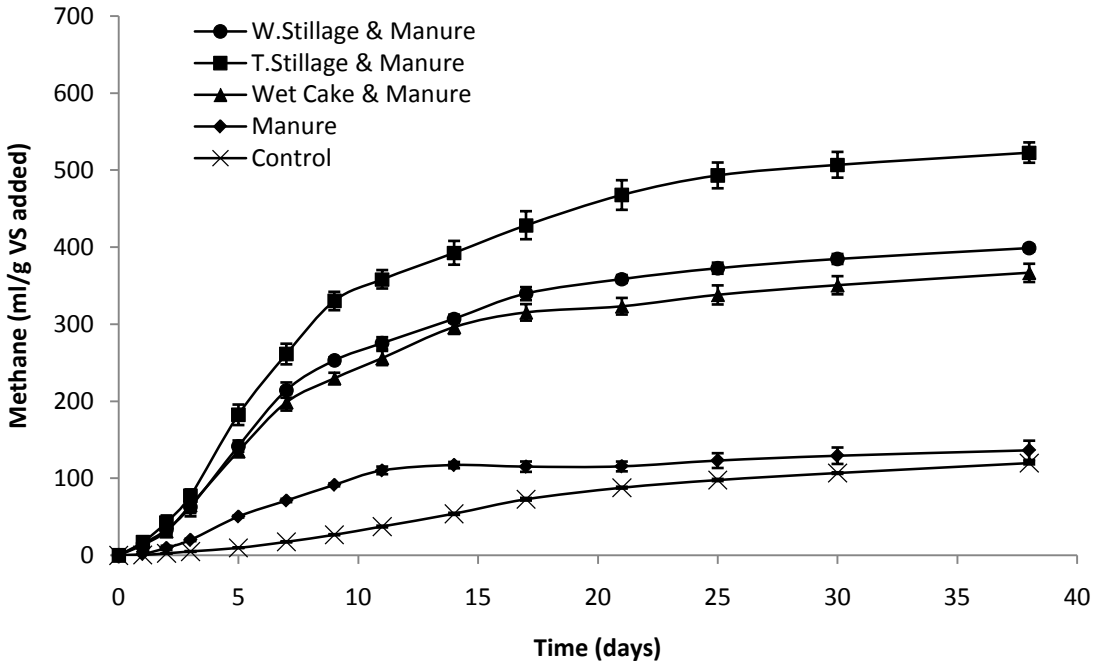

Figure 4.7 Manure amended methane production profiles (2:1 VS ratio)

The effect of manure amendment on ethanol byproduct methane potential can be seen by comparing Figures 4.6 and 4.7. It was again expected that methane yields would be less in Figure 4.7 because the biogas potential of manure is less than that of ethanol byproducts. In contrast to the 1:1 Experiment discussed above, methane production of un-amended ethanol byproducts was relatively stable in this experiment and thin stillage yielded the highest methane volumes in both the un-amended and manure amended trails.

Table 4.3 provides the values for methane yield, B_∞, production rate, k, and pH determined in the 2:1 Experiment.

Table 4.3 2:1 Experiment methane yield, methane production rate constant and pH

	Methane yield	Methane production rate constant		pH	
Substrate	B_∞ (ml/g VS added)	k (day^{-1})	R^2	Initial	Final
Whole Stillage	533 ± 18a	0.106	0.977	7.41	7.70
Thin Stillage	592 ± 37[a]	0.090	0.983	7.31	7.78
Wet Cake	485 ± 19[a]	0.105	0.990	7.58	7.57
Whole Stillage & Manure	399 ± 18	0.109	0.995	7.68	7.59
Thin Stillage & Manure	523 ± 13	0.110	0.988	7.71	7.66

Wet Cake & Manure	367 ± 12	0.105	0.990	7.81	7.44
Manure	136 ± 12	0.102	0.933	8.18	7.42
Control	120 ± 2	NA		8.03	7.84

[a] Significantly different from manure amended counterpart ($p<0.05$)

Faster methane production rate constants can be seen by the earlier rise in methane production profiles in Figure 4.7, compared to Figure 4.6. Table 4.3 also shows that methane production rate constants, *k*, were faster for manure amended ethanol byproducts and that all the trials in this experiment followed first order reaction kinetics as demonstrated by high R^2 values, except for manure. Manure amendment caused thin stillage methane production rate constant to increase by 0.02 day^{-1}. The pH values of manure amended byproducts also started higher than their un-amended counterparts, which may have led to faster methane production rate constants caused by improved buffering capacity and micronutrient availability.

4.5.3. Summary and significance

Table 4.4 outlines the actual and expected methane yields of manure amended ethanol byproducts for both experiments. For the 1:1 experiment the expected methane yields are half of the ethanol byproduct yield plus half of the manure yield. For the 2:1 experiment the expected methane yields are two thirds of the ethanol byproduct yield plus one third of the manure yield. The manure amended byproduct yields are also expressed as a percent of the un-amended trials. This shows that offsetting ethanol byproduct use for manure still results in similar methane yields, especially for thin stillage.

Table 4.4 Effect of manure amendment on methane yield

		Methane yield, B_∞ (ml/g VS added)			
Experiment	Substrate	actual	expected	% of expected	% of un-amended
1:1	Whole Stillage & Manure	389 ± 15	404 ± 21	96%	67%
	Thin Stillage & Manure	446 ± 12	357 ± 35	**125%**	**92%**
	Wet Cake & Manure	344 ± 12	362 ± 24	95%	70%
2:1	Whole Stillage & Manure	399 ± 18	401 ± 23	100%	75%
	Thin Stillage & Manure	523 ± 13	440 ± 32	**119%**	**88%**

Wet Cake & Manure	367 ± 12	369 ± 22	100%	76%

Amending thin stillage with feedlot manure produced 125% and 119% of the expected methane in the 1:1 and 2:1 experiments, respectively. The other ethanol byproducts responded as expected to manure amendments and produced methane at the ratios of their respective inputs. Higher fractions of manure in the 1:1 experiment produced less methane gas for whole stillage and wet cake, but the opposite occurred for thin stillage. Manure amended thin stillage produced 92% of the methane produced by thin stillage alone in the 1:1 experiment versus 88% in the 2:1 experiment. Theoretically, more of the higher producing ethanol byproduct would have been available for conversion to methane in the 2:1 experiment.

Possible explanations for this situation are that in the 1:1 experiment un-amended thin stillage may not have reached its full methane potential in the 38 day duration of the experiment. Thin stillage methane production was also slow to start and highly variable in the 1:1 experiment, so manure may have supplied the necessary nutrients and microbial stability that allowed the amended version to perform so well.

4.6. Conclusion

In a 1:1 VS ratio experiment whole stillage, thin stillage and wet cake responded to the addition of feedlot manure to achieve methane yields of 389 ± 15, 446 ± 12 and 344 ± 12 (ml CH_4/g VS added), respectively. In a 2:1 VS ratio experiment (byproduct:manure) whole stillage, thin stillage and wet cake responded to feedlot manure addition with methane yields of 399 ± 18, 523 ± 13 and 367 ± 12 (ml CH_4/g VS added), respectively. Feedlot manure stabilized the anaerobic digestion process and improved the methane production rate constants for all ethanol byproducts in this study.

A synergistic co-digestion relationship was exposed between feedlot manure and thin stillage. Equal parts of feedlot manure and thin stillage produced 92% of the methane produced by thin stillage alone. Similarly, 2 parts thin stillage were offset by 1 part feedlot manure and produced 88% of the methane produced by thin stillage alone.

Adding feedlot manure to BMP assays of ethanol byproducts resulted in expected methane volumes from whole stillage and wet cake, but unexpectedly high volumes of methane from thin stillage.

5. DISCUSSION

Two scientific papers have been presented to describe how three rounds of experimentation were used to fulfill two research objectives. The first paper described the results of three rounds of BMP assays on wheat-based ethanol byproducts. The second paper described the results of adding feedlot manure to ethanol byproducts at different ratios. The most significant findings of all three experiments were highlighted in the papers, but a discussion of the experiments as a whole is still necessary. An example of how data was collected and calculated is provided in Appendices A-D.

5.1. Methane yield

The following graph represents the methane yield data obtained across all three experiments. Syrup, WDGS and DDGS are not shown here because they were not exposed to repetitive testing, nor did their values contribute to the overall significance of the research. The results are presented in Figure 5.1, from left to right, in the order that the experiments were conducted.

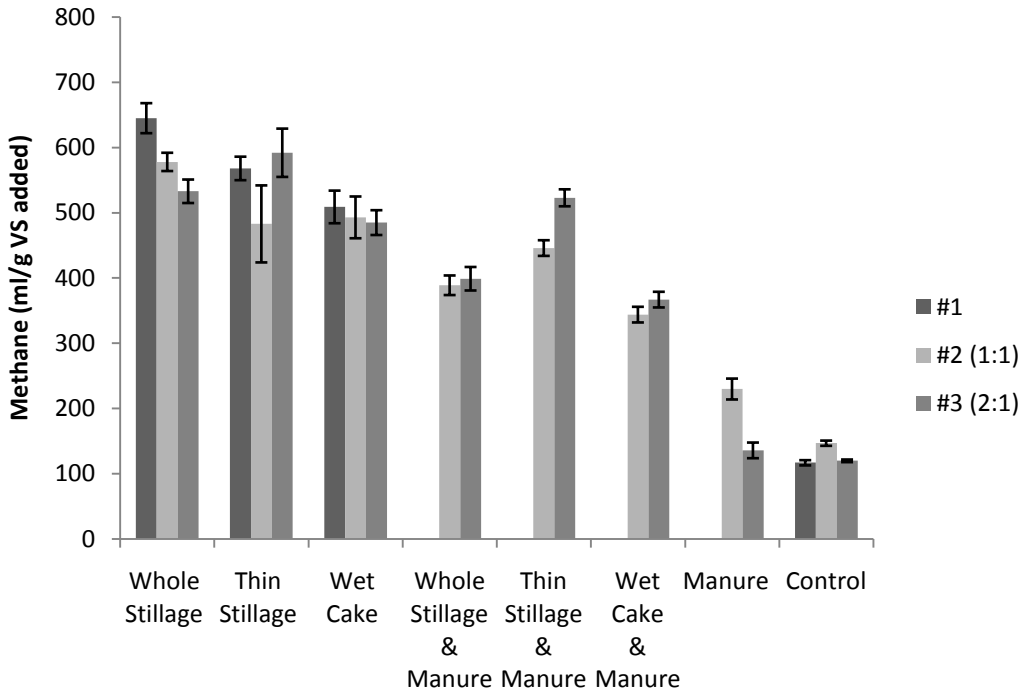

Figure 5.1 Methane yield results from all three experiments

As discussed in the first paper, the average methane yield across all experiments was the highest for whole stillage at 585 ± 46 ml/g VS added. Even though the error was small within each experiment, Figure 5 shows the difference in results across all experiments. The average methane yield for thin stillage was lower at 547 ± 47 ml/g VS added. Wet cake values were tighter in Figure 5.1 and the average methane yield was 495 ± 10 ml/g VS added. If converted into m^3/tonne of fresh substrate, these methane yields are similar to that of brewer's grain silage shown in Figure 2.5.

Figure 5.1 also shows the effects of feedlot manure on methane yields. The relationship between un-amended and manure amended samples can be seen, but it is difficult to describe the results beyond what happened within each experiment. Ideally, substantially increased methane production would have been seen in Experiment 3 (2:1 VS ratio) over Experiment 2 (1:1 VS ratio) because more of the higher yielding ethanol byproduct was present. Unfortunately, the use of new substrates for Experiment 3 made it difficult to compare results between the two experiments.

Perhaps the biggest reason for not seeing increased methane yields for manure amended assays in the third experiment can be attributed to the use of a lower yielding feedlot manure. The manure sample collected for the third experiment had far less volatile solids availability than the sample used in the second experiment. It only had approximately 46% VS/TS compared to 72% VS/TS for the sample in the second experiment. It is possible that with a higher yielding manure substrate, results from the 2:1 ratio experiment would have surpassed those of the 1:1 ratio experiment by a greater margin than that seen in Figure 5.3.

Analyzing expected methane yields versus those actually obtained in each experiment sheds light on the impact of manure amendments. Expected methane yields in Figures 5.2 and 5.3 were calculated based on the ratio of the contributing substrates. With the exception of thin stillage, actual methane yields were on par with expectations in Experiments 2 and 3.

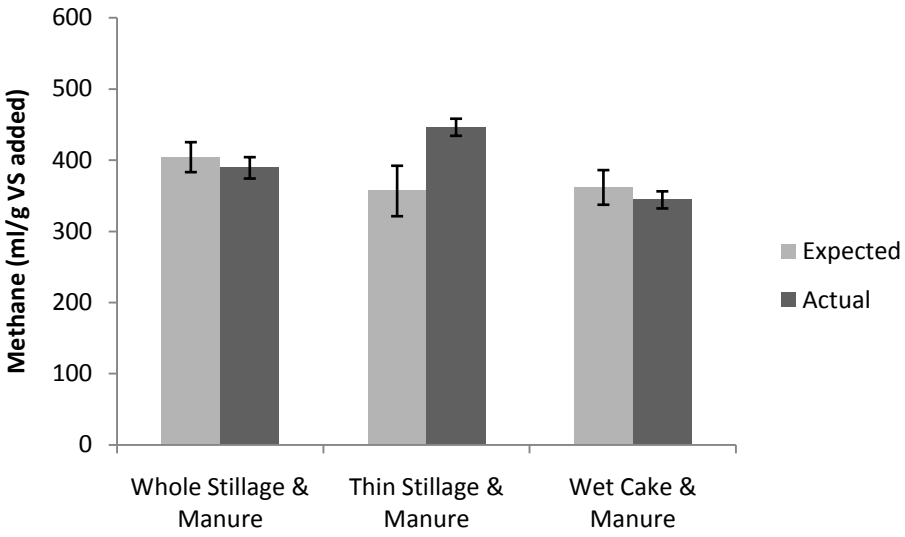

Figure 5.2 Expected and actual methane yields for Experiment 2

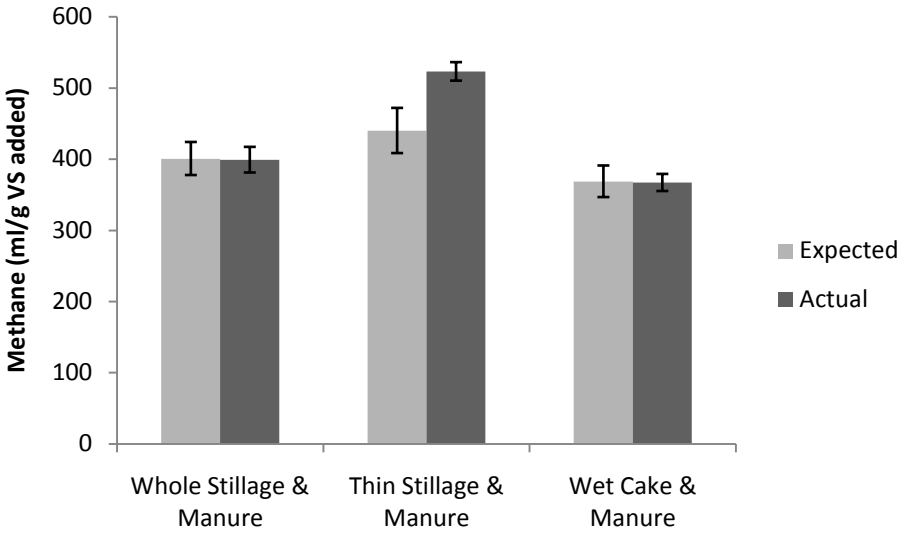

Figure 5.3 Expected and actual methane yields for Experiment 3

For thin stillage, samples amended with feedlot manure exceeded expected methane yields by a significant margin ($p<0.05$). Figures 5.2 and 5.3 highlight the spread between expected and actual methane yields in both experiments, respectively. It is unclear why this synergistic relationship exists between thin stillage and feedlot manure. Attempting to explain the relationship in Experiment 2 by suggesting that the methane yield for thin stillage alone was inhibited and therefore decreased

expectations does not hold up when trying to explain the results of Experiment 3 using the same logic. In Experiment 3, the increased methane yield over Experiment 2 was expected because of a higher methane yield for thin stillage alone, but that expectation was again surpassed by thin stillage amended with feedlot manure.

Without more analysis of the substrates used and digestate produced in these experiments, it is impossible to draw conclusions as to why thin stillage amended with feedlot manure outperformed expectations for both the 1:1 and 2:1 VS ratio experiments. It can be concluded that a synergistic relationship occurs between the two substrates and that feedlot manure should be added to the anaerobic digestion of thin stillage to take advantage of this synergy.

5.2. Methane production rate constant

The methane production rate constant was calculated for two reasons. First, to describe the speed at which each substrate was capable of producing methane gas, *k*. Second, to describe how well the BMP assays followed first order reaction kinetics. The following table provides the methane production rate constant, *k*, and corresponding coefficient of determination, R^2, for substrates across all three experiments.

Table 5.1 Methane production rate constants, k, and R^2 values for all experiments

	Experiment 1		Experiment 2		Experiment 3	
Substrate	k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2
Whole Stillage	0.107	0.943	0.094	0.927	0.106	0.977
Thin Stillage	0.099	0.890	0.058	0.821	0.090	0.983
Wet Cake	0.115	0.934	0.102	0.987	0.105	0.990
Whole Stillage & Manure	NA	NA	0.113	0.980	0.109	0.995
Thin Stillage & Manure	NA	NA	0.106	0.988	0.110	0.988
Wet Cake & Manure	NA	NA	0.115	0.958	0.105	0.990
Manure	NA	NA	0.104	0.915	0.102	0.933

Methane production rate constants for ethanol byproducts without manure generally did not follow first order reaction kinetics for the first and second experiments, as demonstrated by low R^2 values. It was therefore difficult to compare the results across all experiments. Instead, confidence is placed on the results from the third experiment because higher R^2 values show that the data closely followed first

order reaction kinetics. First order reaction kinetics is expected when proper BMP assays are performed. A longer inoculum incubation period and the use of fresh substrates in Experiment 3 contributed to improved reaction kinetics.

The addition of feedlot manure to the BMP assays resulted in faster reaction speeds and improved fit to first order kinetics. Ethanol byproducts amended with manure in Experiments 2 and 3 both exhibited faster *k* values and higher R^2 values, shown in Table 5.1. This strengthens the argument for including feedlot manure in anaerobic digestion of ethanol byproducts.

The most notable improvement in reaction kinetics was observed when manure was added to thin stillage in Experiment 2. Figures 5.4 and 5.5 show what actually happened in the bottles containing thin stillage in this experiment. The extreme variability between bottles in Figure 5.4 is virtually eliminated in Figure 5.5. A slightly lower methane yield is achieved by the manure amended bottles in Figure 5.5, but the data follows a tight, first order reaction curve that is expected from BMP assays and closely resembles the one described by Figure 2.6 in Section 2.9 of this thesis.

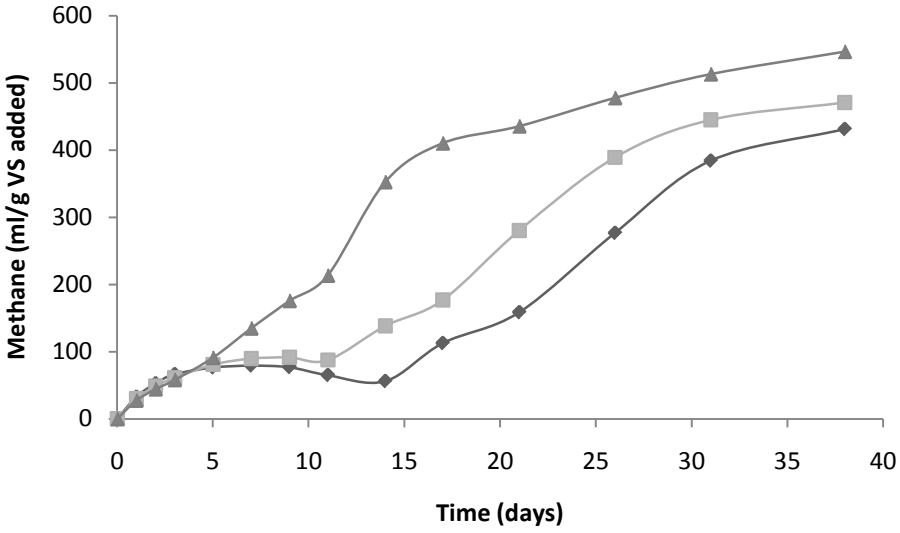

Figure 5.4 Un-amended thin stillage methane production profiles

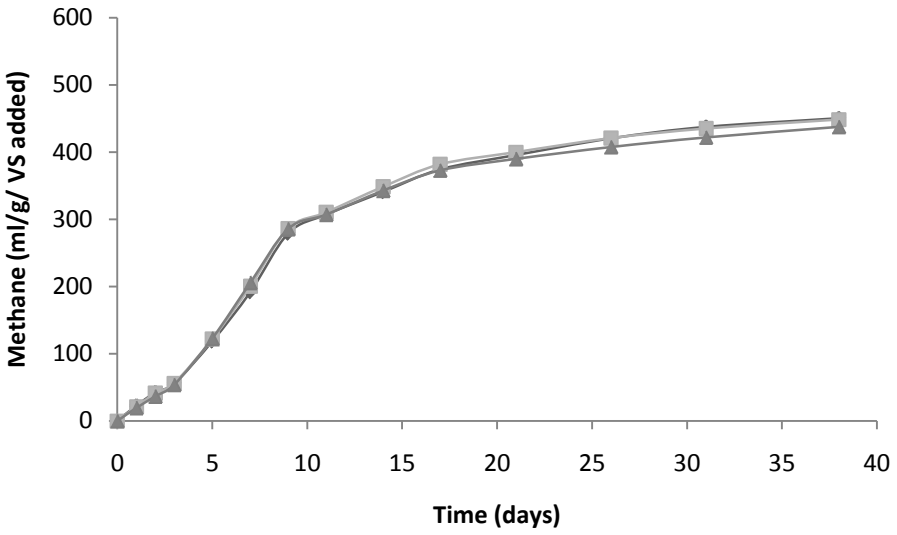

Figure 5.5 Manure amended thin stillage methane production profiles

These results show why manure should be added to thin stillage for anaerobic digestion. This synergy also strengthens the argument for including an ethanol plant, beef feedlot and anaerobic digester as three components of a biorefinery. Selecting substrates with high methane production rate constants and predictable profiles like the one shown in Figure 5.5 gives engineers more confidence in designing anaerobic digesters with shorter hydraulic retention times.

5.3. Selecting Substrates

A mass balance of the first three ethanol byproducts produced downstream of distillation was performed to determine if there was an advantage to using one byproduct stream over the others. Following the suggestion from Saha et al. (2005) that between 8 and 15 liters of byproduct are produced for each liter of ethanol, it was estimated that 10 liters of whole stillage was produced per liter of ethanol. Byproduct processing begins with the centrifugation of whole stillage into thin stillage and wet cake. Total solids contents from Table 1 in each paper were used to determine that 10 liters of whole stillage would be separated to 8.24 liters of thin stillage and 1.76 liters of wet cake.

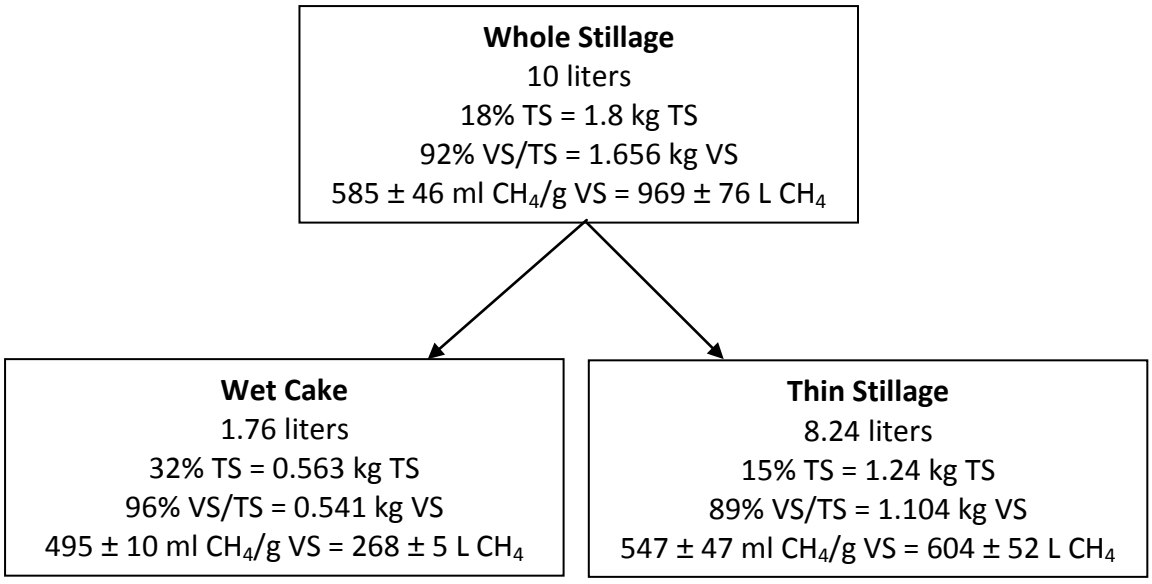

Figure 5.6 Mass balanced methane potentials for ethanol byproducts

The volume of each byproduct was multiplied by its total solids content and percent volatile solids to determine the grams of each byproduct that would be available for conversion to methane gas. Using the average methane values determined in this research, it was possible to find the volume of methane gas that could be produced from the byproducts streams on a mass balance basis, rather than just on a per gram basis. Figure 5.6 depicts the calculations involved and the resulting mass balanced methane potentials. As expected, the sum of thin stillage and wet cake methane potentials reach the potential of whole stillage when maximum error values are considered.

In terms of choosing a substrate, the mass balance calculations still show that whole stillage yields the highest methane volume per liter of ethanol produced at 969 L CH_4/L ethanol. When feedlot manure is not available to create synergies with thin stillage, whole stillage should be the substrate of choice for anaerobic digestion. Digesting some of it would reduce the load entering the centrifuge and the downstream processes while producing the most potential methane gas with the least variability. Further analysis of the cost benefits for digesting each byproduct stream were not conducted as part of this research.

5.4. Volatile Solids Conversion to Biogas

A mass balance between the mass of volatile solids added and the mass of biogas produced was conducted to check the results. Conversion ratios can be found in Appendix E. The average mass of biogas produced by each bottle was divided by the average mass of VS added to each bottle. Mass of biogas was calculated by adding together the mass of each component gas; determined from their measured volumes and densities (0.72 kg/m^3 and 1.97 kg/m^3 for CH_4 and CO_2, respectively) at STP.

Similar conversion ratios (g biogas/g VS added) were observed for like byproducts across all three experiments. According to VDI 4630 (2006), a realistic conversion ratio is approximately 85% for carbohydrates and 50% to 70% for plant fats and proteins. Average conversion ratios for whole stillage, thin stillage and wet cake were 79% ± 6%, 80% ± 6%, and 70% ± 4%, respectively. For the same ethanol byproducts amended with manure, average conversion rates were 59% ± 5%, 69% ± 5%, 55% ± 3%, respectively. The average conversion ratio for manure was 39% ± 3% and 34% ± 3% for the controls. Individual conversion ratios are provided in Appendix E.

These conversion ratios coincide with literature values based on the relative composition of each substrate mixture. It was therefore assumed that the biogas produced in each bottle was a direct result of the volatile solids added to the bottle.

6. CONCLUSIONS

In order to assess the feasibility of constructing a biorefinery consisting of an ethanol plant, beef feedlot and anaerobic digester it was necessary to determine the biochemical methane potential of the substrates available at the proposed biorefinery.

Initially, biochemical methane potential (BMP) tests were conducted on all of the byproducts typically produced during downstream processing in an ethanol plant. From that experiment, it was determined that whole stillage, thin stillage and wet cake were the most suitable byproducts for anaerobic digestion because the benefits of converting them into methane gas outweighed the costs of using them to produce the other ethanol byproducts of syrup, WDGS and DDGS which all require further heat and processing costs to be produced.

In two additional rounds of BMP testing, whole stillage, thin stillage and wet cake were tested again but also with 1:1 and 2:2 VS ratios of byproduct to feedlot manure. The goal of the second and third round of testing to was to ensure repeatability of results and to determine the effect of different ratios of feedlot manure on the methane potential of ethanol byproducts; something for which there was no previously available published data.

BMP assays provide two results. First, they provide the ultimate methane yield that a substrate is capable of producing, which in the case of these experiments was expressed in terms of the volume of methane gas per gram of volatile solids added (ml CH_4/g VS added). Second, they allow for the calculation of a methane production rate constant to be determined for specific substrates. Both of these results are important pieces of information for engineers trying to select substrates and design anaerobic digesters to maximize methane generation. Average results for ultimate methane yield across all three experiments showed whole stillage achieving the highest volume at 585 ± 46 ml/g VS added. Thin stillage was second at 547 ± 47 ml/g VS added and wet cake was third at 495 ± 10 ml/g VS added.

When manure was added to these wheat-based ethanol byproducts it became difficult to compare results across the two experiments because different substrates were used. Calculating the expected methane yields for each experiment based on the ratio of ethanol byproducts and manure inputs was the best way of analyzing the results for manure treatments. Actual methane yields attained in each experiment fit closely with expected results, except for thin stillage and manure. Surprisingly both experiments showed actual methane yields for manure amended thin stillage to exceed the expected results. In fact, thin stillage receiving 1:1 and 2:1 ratios of feedlot manure outperformed expectations by 125% and 119%, respectively and achieved 92% and 88% of the methane yield realized by thin stillage alone for each experiment.

Adding feedlot manure to ethanol byproducts both stabilized and increased methane production rate constants. Manure amended byproducts closely followed first order reaction kinetics and achieved faster methane production rate constants than their un-amended versions in both experiments.

The synergistic relationship between thin stillage and feedlot manure should be taken advantage of when designing an anaerobic digester to fit into the biorefinery model of ethanol plant, beef feedlot and anaerobic digester. The results shown here suggest that a high value substrate like thin stillage can be offset by a low value substrate like manure to produce higher than expected methane yields and exceed expectations based on input ratios.

On the other hand, if designing an anaerobic digester for integration with an ethanol plant alone, it appears that using whole stillage is a more reliable option that produces higher levels of methane gas.

7. RECOMMENDATIONS

Confidence in the results obtained in this research could be improved by conducting additional rounds of BMP assays. More assays similar to those conducted in the second and third experiments would provide greater depth to a database of the methane potential of wheat-based ethanol byproducts. Varying the ratio of feedlot manure used in the assays and ensuring that fresh substrates were always collected would be of great value. More assays that follow first order reaction kinetics will strengthen the database and allow for more accurate predictions of methane production from wheat-based ethanol byproducts.

Obviously BMP assays are not the only step necessary before a full scale anaerobic digester can be designed and constructed at an ethanol plant. Achieving that step will also require using the results of the BMP assays to design and build a laboratory sized anaerobic digester with continuous flow capabilities. Running a lab scale anaerobic digester on ethanol byproducts and feedlot manure will be necessary to determine the balance of organic loading rate (OLR) and hydraulic retention time (HRT) that should provide optimal methane production in a full scale design.

Better analysis of the composition of the substrates and inoculum used in these experiments would also benefit future research on this topic. Understanding the nutrients present in each substrate would allow for better reasoning as to why some perform differently than others. Knowing how substrate composition effects methane production could lead to adjusting substrate composition in favour of more methane production. Also, the concentration of nutrients in the digestate from these experiments should be determined to shed lights on the digestion process as well as provide fertilizer values for the plant available nutrients remaining in the digestate.

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APPENDIX A – Equations

Biogas volume adjusted to STP

Equation A.1 was used to convert pressure readings into biogas volumes at standard temperature and pressure.

$$V_o = \frac{P * V_H * To}{P_o * T} \quad \textbf{(A.1)}$$

Where

V_o volume of dry gas at standard temperature and pressure, in ml

P pressure of gas phase at time of reading, in mbar

V_H volume of head space in bottle; 844 ml

T_o standard temperature; 273 K

P_o standard pressure; 1013 mbar

T temperature of gas phase at time of reading, in K

Normalized biogas composition

Equation A.2 was used to normalize the measured biogas composition so that GC values obtained would only reflect the contribution of biogas.

$$C_{corr} = C_{CH_4(CO_2)} \left(\frac{100}{C_{CH_4} + C_{CO_2}}\right) \quad \textbf{(A.2)}$$

Where

C_{corr} corrected concentration of biogas component in dry gas, in % by volume

$C_{CH_4(CO_2)}$ measured concentration of methane (or carbon dioxide) in the gas, in % by volume

C_{CH_4} measured methane concentration in the gas, in % by volume

C_{CO_2} measured carbon dioxide concentration in the gas, in % by volume

Inoculum biogas volume

Equation A.3 was used to calculate the volume of biogas attributed to endogenous metabolism of the inoculum.

$$V_{inoc} = \frac{V_{cont} * m_{inoc}}{m_{cont}} \quad \textbf{(A.3)}$$

Where

V_{inoc} volume of gas produced by inoculum, in ml

V_{cont} volume of gas produced in control bottle, in ml

m_{inoc} mass of inoculum used in treatment bottle, in g VS added

m_{cont} mass of inoculum used in control bottle, in g VS added

Corrected biogas yield

Equation A.4 was used to correct the biogas volume by subtracting the inoculum biogas volume and dividing by the mass of volatile solids added.

$$V_{substrate} = \frac{V_{total} - V_{inoc}}{m_{substrate}} \quad \textbf{(A.4)}$$

Where

$V_{substrate}$ volume of gas produced by substrate, in ml/g VS added

V_{total} volume of gas produced in test bottle, in ml

V_{inoc} volume of gas produced by inoculum, in ml

$m_{substrate}$ mass of substrate used in test bottle, in g VS added

Wet gas samples

According to VDI 4360, gas measurements do not need to be corrected for moisture if CH_4 and CO_2 are measured simultaneously. However, the standard gas used to calibrate the GC was dry and the experimental gas samples were not. The percent gas compositions may, therefore, have been underestimated in these experiments.

APPENDIX B – Sample GC data

Table B.1 Gas chromatography for whole stillage biogas samples in Experiment 3

				Gas composition					
Day	Sample	ID	INJ	%H2	%O2	%N2	%CH4	%CO2	%Total
0				0	0	0	0	0	0
1	WSTILL	1	1	0.527	0.270	40.729	5.245	27.114	73.885
1	WSTILL	1	2	0.530	0.297	40.679	5.215	27.026	73.747
1	WSTILL	2	1	0.486	0.267	39.936	5.881	27.770	74.340
1	WSTILL	2	2	0.482	0.287	39.892	5.882	27.673	74.216
1	WSTILL	3	1	0.508	0.304	39.447	6.300	27.825	74.384
1	WSTILL	3	2	na	na	na	na	na	na
2	WSTILL	1	1	0.488	4.912	0.952	39.866	51.305	97.523
2	WSTILL	1	2	0.486	4.922	1.003	39.817	51.225	97.453
2	WSTILL	2	1	0.035	0.253	26.049	14.153	41.120	81.610
2	WSTILL	2	2	0.035	0.281	26.060	14.117	41.006	81.499
2	WSTILL	3	1	0.029	0.244	25.094	15.969	40.874	82.210
2	WSTILL	3	2	0.029	0.268	25.084	15.908	40.761	82.050
3	WSTILL	1	1	0.034	0.267	18.020	25.296	43.984	87.601
3	WSTILL	1	2	0.034	0.293	18.046	25.202	43.818	87.393
3	WSTILL	2	1	0.030	0.250	16.545	29.539	42.096	88.460
3	WSTILL	2	2	0.028	0.283	16.577	29.410	41.930	88.228
3	WSTILL	3	1	0.026	0.245	15.732	31.855	41.120	88.978
3	WSTILL	3	2	0.026	0.269	15.766	31.731	41.023	88.815
5	WSTILL	1	1	0.010	0.245	8.826	48.141	35.456	92.678
5	WSTILL	1	2	0.011	0.264	8.855	48.044	35.325	92.499
5	WSTILL	2	1	0.011	0.240	8.366	50.004	34.249	92.870
5	WSTILL	2	2	0.012	0.250	8.389	49.878	34.114	92.643
5	WSTILL	3	1	0.013	0.234	8.366	50.032	34.223	92.868
5	WSTILL	3	2	0.011	0.259	8.383	49.868	34.089	92.610
7	WSTILL	1	1	0.017	0.231	5.352	55.567	32.835	94.002
7	WSTILL	1	2	0.017	0.250	5.390	55.430	32.745	93.832
7	WSTILL	2	1	0.017	0.227	5.057	55.926	32.803	94.030
7	WSTILL	2	2	0.016	0.242	5.090	55.779	32.693	93.820
7	WSTILL	3	1	0.015	0.225	5.068	55.630	33.000	93.938
7	WSTILL	3	2	0.015	0.249	5.113	55.503	32.877	93.757
9	WSTILL	1	1	0.009	0.234	2.986	64.164	27.709	95.102
9	WSTILL	1	2	0.009	0.257	3.019	64.016	27.597	94.898
9	WSTILL	2	1	0.009	0.240	2.995	64.533	27.307	95.084
9	WSTILL	2	2	0.009	0.260	3.036	64.361	27.205	94.871
9	WSTILL	3	1	0.010	0.246	3.038	64.094	27.542	94.930
9	WSTILL	3	2	0.010	0.265	3.084	63.936	27.472	94.767
11	WSTILL	1	1	0.012	0.270	2.269	66.984	26.170	95.705
11	WSTILL	1	2	0.011	0.287	2.312	66.756	26.093	95.459
11	WSTILL	2	1	0.013	0.256	2.334	66.365	26.712	95.680
11	WSTILL	2	2	0.013	0.278	2.372	66.188	26.646	95.497
11	WSTILL	3	1	0.012	0.261	2.325	66.114	27.004	95.716
11	WSTILL	3	2	0.012	0.279	2.362	65.929	26.922	95.504
14	WSTILL	1	1	0.011	0.254	1.725	67.359	26.933	96.282
14	WSTILL	1	2	0.011	0.279	1.775	67.121	26.831	96.017
14	WSTILL	2	1	0.011	0.251	1.810	67.059	26.995	96.126
14	WSTILL	2	2	0.011	0.281	1.868	66.856	26.889	95.905

14	WSTILL	3	1	0.010	0.255	1.808	67.003	26.990	96.066
14	WSTILL	3	2	0.010	0.285	1.869	66.733	26.871	95.768
17	WSTILL	1	1	0.010	0.241	1.366	68.801	26.352	96.770
17	WSTILL	1	2	0.010	0.258	1.420	68.592	26.273	96.553
17	WSTILL	2	1	0.013	0.243	1.455	67.691	27.263	96.665
17	WSTILL	2	2	0.013	0.268	1.524	67.451	27.177	96.433
17	WSTILL	3	1	0.014	0.250	1.507	66.919	28.002	96.692
17	WSTILL	3	2	0.014	0.278	1.577	66.704	27.900	96.473
21	WSTILL	1	1	0.012	0.246	1.114	69.009	26.272	96.653
21	WSTILL	1	2	0.011	0.264	1.167	68.830	26.202	96.474
21	WSTILL	2	1	0.010	0.228	1.124	69.333	26.049	96.744
21	WSTILL	2	2	0.010	0.252	1.169	69.157	25.978	96.566
21	WSTILL	3	1	0.009	0.230	1.262	68.995	26.509	97.005
21	WSTILL	3	2	0.009	0.257	1.329	68.758	26.418	96.771
25	WSTILL	1	1	0.013	0.274	1.131	65.763	29.865	97.046
25	WSTILL	1	2	0.013	0.293	1.191	65.560	29.786	96.843
25	WSTILL	2	1	0.013	0.258	1.122	65.778	29.813	96.984
25	WSTILL	2	2	0.012	0.277	1.176	65.557	29.705	96.727
25	WSTILL	3	1	0.014	0.251	1.134	66.856	28.629	96.884
25	WSTILL	3	2	0.014	0.277	1.187	66.626	28.526	96.630
30	WSTILL	1	1	0.011	8.039	21.667	38.243	19.052	87.012
30	WSTILL	1	2	0.010	9.730	26.062	32.813	16.496	85.111
30	WSTILL	2	1	0.010	8.323	22.447	37.289	18.433	86.502
30	WSTILL	2	2	0.012	9.989	26.761	31.850	15.885	84.497
30	WSTILL	3	1	0.007	11.194	29.871	28.055	13.515	82.642
30	WSTILL	3	2	0.011	12.456	33.106	23.819	11.656	81.048
38	WSTILL	1	1	0.012	0.274	1.012	62.990	33.314	97.602
38	WSTILL	1	2	0.012	0.299	1.082	62.831	33.213	97.437
38	WSTILL	2	1	0.012	0.282	1.070	63.758	32.394	97.516
38	WSTILL	2	2	0.012	0.313	1.147	63.580	32.297	97.349
38	WSTILL	3	1	0.014	0.277	1.048	63.439	32.919	97.697
38	WSTILL	3	2	0.014	0.305	1.122	63.213	32.803	97.457

APPENDIX C – Sample biogas volume data

Table C.1 Biogas volume calculations for whole stillage in Experiment 3

			Pressure		Volume	Measured		Normalized		NET CH_4	NET CO_2
Day	Sample	ID	PSI	mbar	ml (STP)	$\%CH_4$	$\%CO_2$	$\%CH_4$	$\%CO_2$	(ml)	(ml)
1	WSTILL	1	11.6	800	501	5.23	27.07	16.19	83.81	81	420
1	WSTILL	2	11.9	820	515	5.88	27.72	17.50	82.50	90	425
1	WSTILL	3	12.6	869	549	6.30	27.83	18.46	81.54	101	448
2	WSTILL	1	8.7	600	416	39.84	51.27	43.73	56.27	182	234
2	WSTILL	2	9.0	621	430	14.14	41.06	25.61	74.39	110	320
2	WSTILL	3	9.6	662	459	15.94	40.82	28.08	71.92	129	330
3	WSTILL	1	7.3	503	349	25.25	43.90	36.51	63.49	127	222
3	WSTILL	2	8.5	586	406	29.47	42.01	41.23	58.77	168	239
3	WSTILL	3	9.1	627	435	31.79	41.07	43.63	56.37	190	245
5	WSTILL	1	15.3	1055	731	48.09	35.39	57.61	42.39	421	310
5	WSTILL	3	14.3	986	684	49.95	34.16	59.39	40.61	406	278
7	WSTILL	1	10.2	703	488	55.50	32.79	62.86	37.14	307	181
7	WSTILL	2	10.8	745	516	55.85	32.75	63.04	36.96	325	191
7	WSTILL	3	10.7	738	512	55.57	32.94	62.78	37.22	321	190
9	WSTILL	1	13.8	951	660	64.09	27.65	69.86	30.14	461	199
9	WSTILL	2	12.1	834	578	64.45	27.26	70.28	29.72	407	172
9	WSTILL	3	12.0	827	574	64.02	27.51	69.94	30.06	401	172
11	WSTILL	1	7.2	496	344	66.87	26.13	71.90	28.10	247	97
11	WSTILL	2	6.8	469	325	66.28	26.68	71.30	28.70	232	93
11	WSTILL	3	7.4	510	354	66.02	26.96	71.00	29.00	251	103
14	WSTILL	1	7.2	496	344	67.24	26.88	71.44	28.56	246	98
14	WSTILL	2	6.7	462	320	66.96	26.94	71.31	28.69	228	92
14	WSTILL	3	6.5	448	311	66.87	26.93	71.29	28.71	222	89
17	WSTILL	1	7.0	483	335	68.70	26.31	72.31	27.69	242	93
17	WSTILL	2	6.6	455	316	67.57	27.22	71.28	28.72	225	91
17	WSTILL	3	5.9	407	282	66.81	27.95	70.50	29.50	199	83
21	WSTILL	1	7.3	503	349	68.92	26.24	72.43	27.57	253	96
21	WSTILL	2	7.6	524	363	69.25	26.01	72.69	27.31	264	99
21	WSTILL	3	7.5	517	359	68.88	26.46	72.24	27.76	259	100
25	WSTILL	1	5.2	359	249	65.66	29.83	68.76	31.24	171	78

25	WSTILL	2	5.7	393	272	65.67	29.76	68.81	31.19	188	85
25	WSTILL	3	6.4	441	306	66.74	28.58	70.02	29.98	214	92
30	WSTILL	1	3.8	262	182	35.53	17.77	66.65	33.35	121	61
30	WSTILL	2	3.8	262	182	34.57	17.16	66.83	33.17	121	60
30	WSTILL	3	4.1	283	196	25.94	12.59	67.33	32.67	132	64
38	WSTILL	1	4.4	303	210	62.91	33.26	65.41	34.59	138	73
38	WSTILL	2	3.6	248	172	63.67	32.35	66.31	33.69	114	58
38	WSTILL	3	4.2	290	201	63.33	32.86	65.84	34.16	132	69

Note: Calculated from data in Table B1 and Equations A1 and A2.

Table C.2 Un-corrected biogas and methane volumes for whole stillage in Experiment 3

UNCORRECTED DAILY BIOGAS (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	501	416	349	731	488	660	344	344	335	349	249	182	210
WSTILL 2	0	515	430	406	617	516	578	325	320	316	363	272	182	172
WSTILL 3	0	549	459	435	684	512	574	354	311	282	359	306	196	201
Average	0	522	435	397	677	505	604	341	325	311	357	276	186	**194**
SD	0	25	22	44	58	15	48	15	17	27	7	29	8	**20**
UNCORRECTED ACCUMULATED BIOGAS (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	501	917	1266	1997	2485	3145	3489	3833	4168	4517	4765	4947	5157
WSTILL 2	0	515	946	1352	1969	2485	3063	3388	3709	4024	4388	4660	4842	5014
WSTILL 3	0	549	1008	1443	2126	2638	3212	3565	3876	4158	4517	4823	5019	5219
Average	0	522	957	1354	2031	2536	3140	3481	3806	4117	4474	4749	4936	**5130**
SD	0	25	46	88	84	88	74	89	87	80	75	82	89	**105**
UNCORRECTED DAILY METHANE (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	81	182	127	421	307	461	247	246	242	253	171	121	138
WSTILL 3	0	101	129	190	406	321	401	251	222	199	259	214	132	132
Average	0	91	140	162	398	318	423	243	232	222	259	191	125	**128**
SD	0	10	37	32	29	10	33	10	13	22	6	22	6	**12**
UNCORRECTED ACCUMULATED METHANE (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	81	263	390	812	1118	1579	1827	2073	2314	2567	2738	2859	2997
WSTILL 2	0	90	200	368	734	1059	1466	1698	1926	2151	2415	2603	2724	2838
WSTILL 3	0	101	230	420	826	1147	1548	1800	2021	2220	2479	2693	2825	2957
Average	0	91	231	393	791	1108	1531	1775	2007	2229	2487	2678	2803	**2931**
SD	0	10	31	26	50	45	59	68	74	82	76	69	70	**83**

Table C.3 Inoculum biogas and methane volumes for whole stillage in Experiment 3

INOCULUM DAILY BIOGAS (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
Average	0	85	58	46	54	68	72	78	112	124	99	66	62	89
SD	0	7	8	3	8	5	4	4	2	8	12	2	6	6
INOCULUM ACCUMULATED BIOGAS (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
Average	0	85	143	189	244	312	384	462	574	698	796	862	924	1013
SD	0	7	3	5	5	3	4	4	6	8	9	10	6	10
INOCULUM DAILY METHANE (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
Average	0	3	9	11	22	34	41	48	74	85	68	44	40	59
SD	0	0	2	1	3	3	2	3	1	5	9	1	4	4
INOCULUM ACCUMULATED METHANE (ml)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
Average	0	3	12	23	44	79	120	168	242	327	395	439	479	538
SD	0	0	1	2	2	3	3	5	6	8	5	6	5	8

Note: Calculated using Equation A3 with 4.49 g VS inoculum added to match whole stillage VS added

Table C.4 Corrected biogas and methane yields for whole stillage in Experiment 3

CORRECTED DAILY BIOGAS (ml/g VS added)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	93	80	67	151	94	131	59	52	47	56	41	27	27
WSTILL 2	0	96	83	80	125	100	113	55	46	43	59	46	27	19
WSTILL 3	0	103	89	87	140	99	112	61	44	35	58	53	30	25
Average	0	97	84	78	139	97	118	59	47	42	58	47	28	**24**
SD	0	5	5	10	13	3	11	3	4	6	2	6	2	**4**
CORRECTED ACCUMULATED BIOGAS (ml/g VS added)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	93	172	240	391	484	615	674	726	773	829	869	896	923
WSTILL 2	0	96	179	259	384	484	597	652	698	741	800	846	873	891
WSTILL 3	0	103	193	279	419	518	630	691	736	771	829	882	912	937
Average	0	97	181	259	398	496	614	673	720	762	819	866	894	**917**
SD	0	5	10	20	19	20	17	20	19	18	17	18	20	**23**
CORRECTED DAILY METHANE (ml/g VS added)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	17	39	26	89	61	93	45	38	35	41	28	18	18
WSTILL 2	0	19	23	35	77	65	81	41	34	31	44	32	18	12
WSTILL 3	0	22	27	40	86	64	80	45	33	25	43	38	20	16
Average	0	20	29	34	84	63	85	44	35	30	43	33	19	**15**
SD	0	2	8	7	6	2	7	2	3	5	1	5	1	**3**
CORRECTED ACCUMULATED METHANE (ml/g VS added)														
Day	0	1	2	3	5	7	9	11	14	17	21	25	30	38
WSTILL 1	0	17	56	82	171	232	325	370	408	443	484	512	530	548
WSTILL 2	0	19	42	77	154	218	300	341	375	406	450	482	500	512
WSTILL 3	0	22	49	89	174	238	318	364	396	422	464	502	523	539
Average	0	20	49	82	166	229	314	358	393	424	466	499	518	**533**
SD	0	2	7	6	11	10	13	15	17	18	17	15	16	**18**

Note: Calculated from data in Tables C2 and C3 using Equation A4

APPENDIX D – Sample biogas and methane graphs

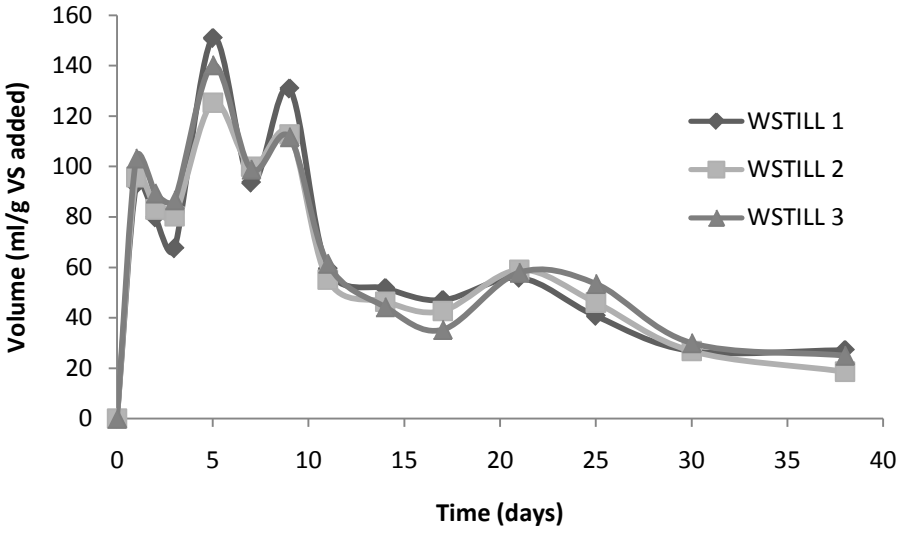

Figure C.1 Whole stillage daily biogas yield

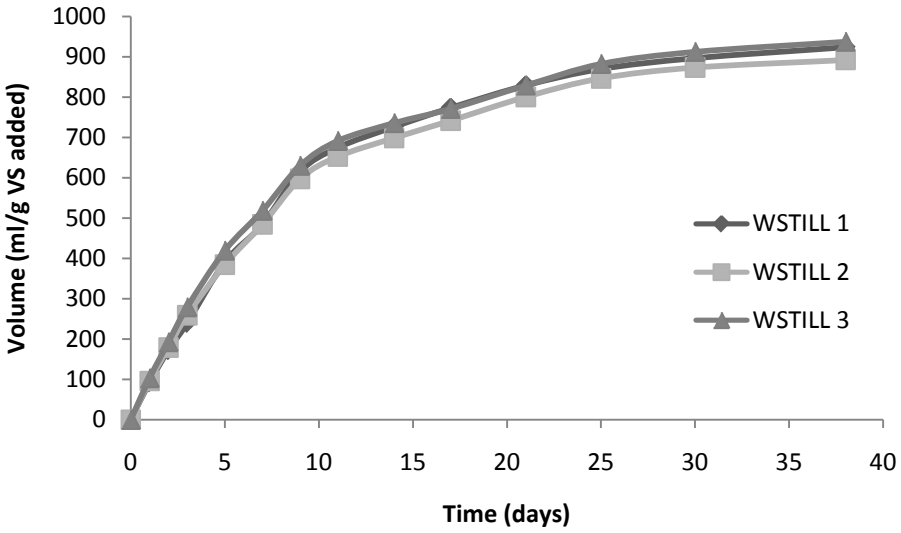

Figure C.2 Whole stillage accumulated biogas yield

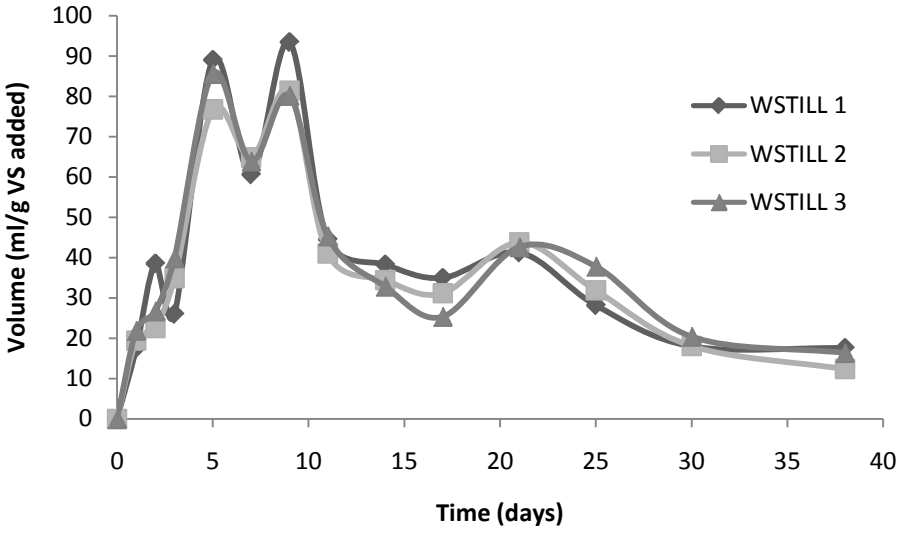

Figure C.3 Whole stillage daily methane yield

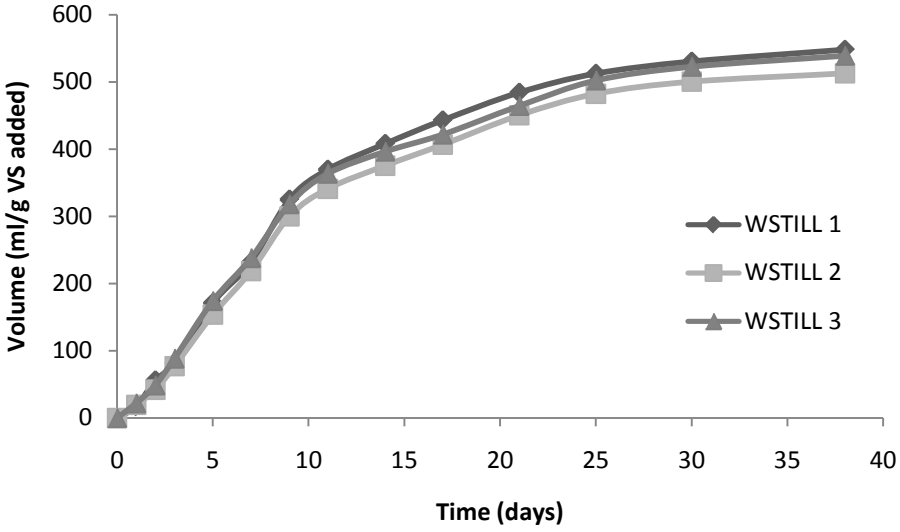

Figure C.4 Whole stillage accumulated methane yield

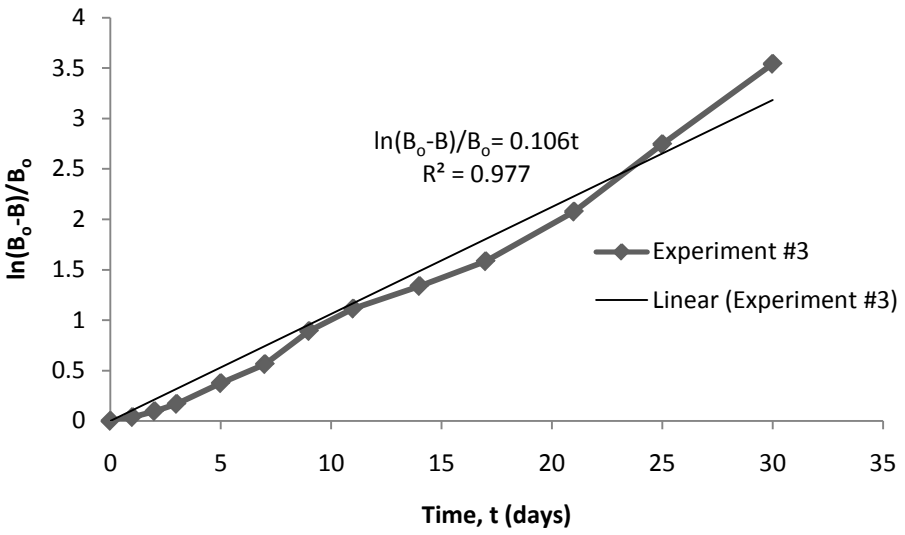

Figure C.5 Methane production rate constant, k, as slope of linear curve obtained.

APPENDIX E – Volatile solids conversion

Table E.1 Volatile solids conversion to biogas

	VS Added		VS Destroyed		Biogas Volume		CH_4	CO_2	Biogas Mass[a]		% Converted (g/g)	
#1	g	sd	g	sd	ml	sd	%	%	g	sd	(Biogas/VS Added)	
Whole stillage	8.34	0.48	3.55	0.50	5625	153	57%	43%	7.11	0.11	85%	5%
Thin stillage	8.77	0.48	4.10	0.60	5538	114	54%	46%	7.16	0.08	82%	5%
Wet cake	10.25	0.13	5.01	0.18	5713	236	56%	44%	7.25	0.16	71%	2%
Syrup	8.03	0.28	3.65	0.30	4842	122	53%	47%	6.34	0.11	79%	3%
WDGS	9.49	0.20	4.60	0.31	5498	181	55%	45%	7.07	0.14	74%	2%
DDGS	9.49	0.16	4.58	0.19	5529	137	54%	46%	7.13	0.10	75%	2%
Control	8.16	0.08	2.34	0.12	2024	37	47%	53%	2.79	0.03	34%[c]	1%
#2 (1:1 ratio)	VS Added		Destroyed		Biogas Volume		CH_4	CO_2	Biogas Mass[a]		% Converted	
Whole stillage	8.64	0.37	3.87	0.38	5592	54	57%	43%	7.03	0.02	81%	4%
Thin stillage	8.60	0.01	4.19	0.15	5272	261	53%	47%	6.86	0.27	80%	3%
Wet cake	9.62	0.22	4.44	0.23	5503	291	57%	43%	6.90	0.20	72%	3%
Whole stillage & Manure	9.19	0.56	3.68	0.58	4431	31	57%	43%	5.58	0.02	61%	4%
Thin stillage & Manure	8.42	0.38	2.44	0.44	4475	52	57%	43%	5.63	0.03	67%	3%
Wet cake & Manure	9.31	0.17	3.12	0.23	4150	107	56%	44%	5.25	0.08	56%	1%
Manure	9.03	0.56	2.34	0.62	3239	148	54%	46%	4.18	0.10	46%[c]	3%
Control	7.45	0.22	1.14	0.23	2200	74	52%	48%	2.92	0.04	39%[c]	1%
#3 (2:1 ratio)	VS Added		Destroyed		Biogas Volume		CH_4	CO_2	Biogas Mass[a]		% Converted	
Whole stillage	8.98	0.12	4.21	0.14	5130	105	57%	43%	6.44	0.09	72%[c]	1%
Thin stillage	8.88	0.18	4.27	0.22	5609	225	56%	44%	7.10	0.19	80%	3%
Wet cake	9.84	0.30	4.53	0.30	5227	154	57%	43%	6.58	0.11	67%[d]	2%
Whole stillage & Manure	8.46	0.44	3.29[b]	0.49	3878	25	57%	43%	4.90	0.02	58%	3%
Thin stillage & Manure	7.33	0.45	2.81[b]	0.84	4128	67	57%	43%	5.19	0.06	71%	4%
Wet cake & Manure	8.79	0.35	3.11[b]	0.37	3777	96	57%	43%	4.77	0.07	54%	2%
Manure	7.81	0.12	2.32[b]	0.25	1891	98	53%	47%	2.48	0.07	32%[c]	1%
Control	7.91	0.71	1.37	0.71	1783	17	53%	47%	2.33	0.02	29%[c]	3%

[a] Biogas mass calculated using CH_4 and CO_2 gas densities of 0.72 kg/m^3 and 1.97 kg/m^3, respectively, at STP (0°C and 101.3 kPa)

[b] Error in VS measurement caused by loss of VS during TS measurement

[c] Significantly different from counterpart in other experiments ($p<0.05$)

[d] Significantly different from counterpart in other experiments ($p<0.10$)