Co-Digestion of Hog Manure with Glycerol to Boost Biogas and Methane Production

by

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ABSTRACT

The use of off-farm materials as amendments in anaerobic digestion of manure is an option that is being explored more extensively, due to the benefits of boosting biogas production, and making the process more economical for the farmer. The addition of varying amounts of glycerol, which is a by-product in the biodiesel industry, was used as an amendment to anaerobic digestion of hog manure in lab-scale tests. Four bench-scale anaerobic digesters were operated under mesophilic conditions (35°C) at an SRT of 17.5 days. They were tested to determine how the anaerobic digestion process, and the biogas production, would be affected by the addition of glycerol.

The use of 2% glycerol produced the greatest amount of methane and biogas, however stabilization time was quite high, and the digestion of nutrients in the manure appeared to decrease. The addition of 4% glycerol resulted in an overloading of COD and digester failure. The addition of 1% glycerol resulted in a doubling of the methane and biogas production and the acclimation period was quite short, while the effluent quality remained good. There appeared to be no detrimental effects of using crude, industrial-based glycerol compared to pure, chemical-grade glycerol. Batch tests also confirmed that using smaller additions of glycerol (0.5% and 1%) produced the highest biogas and methane yields, and were recommended as good co-substrates to be used in anaerobic digestion with hog manure.

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ABBREVIATIONS AND SYMBOLS

AD	Anaerobic Digestion
BMP	Biochemical Methane Potential
CH_4	Methane
CHP	Combined Heat and Power
C/N	Carbon to Nitrogen ratio
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
Cu	Copper
FOG	Fats Oils and Grease
GHG	Greenhouse Gas
GTA	Glycerol Trioleate
Ha	Hectare
H_2	Hydrogen Gas
H_2S	Hydrogen Sulphide
HRT	Hydraulic Residence Time
K	Potassium
kW	kilo Watt
kWh	kilo Watt hour
MB	Manitoba
MW	Mega Watt
N	Nitrogen
NaCl	Sodium Chloride
N_2	Nitrogen gas

NH_3	Ammonia
NH_4^+	Ammonium
OFMSW	Organic Fraction of Municipal Solid Waste
P	Phosphorus
PA	Pennsylvania
PO_4	Phosphate
RESOP	Renewable Energy Standard Offer Program
SK	Saskatchewan
SO_2	Sulphur dioxide
SRT	Solids Residence Time
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
USA	United States of America
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSR	Volatile Solids Reduction

CHAPTER 1 INTRODUCTION

1.1 Background

With concerns over the depletion of fossil fuels, and emission of greenhouse gases (GHG), which contribute to global climate change, extensive research is being conducted worldwide to address these environmental problems. Coupled with financial incentives, part of the solution has been to change society's concept of what a "waste product" really is. This has lead to a continuously growing trend towards the recycling and reuse of agricultural, municipal and industrial "waste products". One particular process which follows this trend and has been used for many years, is anaerobic digestion (AD) of animal wastes on a farm-scale. Anaerobic digestion is simply the biological process of converting organic matter into biogas in the absence of oxygen, through the activity of microorganisms. This option addresses these issues by reusing an agricultural "waste product" to produce renewable fuel and reduce GHG emissions, as well as supplying farmers with high quality fertilizer. However, this process has been generally under-utilized in Canada and Manitoba, mainly due to poor economics, which is unfortunate, considering how large the agricultural livestock sector is throughout much of the province and country.

Currently, there are 3 anaerobic digester systems on hog farms in Manitoba, being used for studying the technical and economic feasibility of this technology (Cicek, 2007). They include:

- Topeka Farms, Grunthal, Manitoba
    - 5500 head operation
    - Thermophilic digestion
- Riverbend Colony, Carberry, Manitoba
    - 1500 head operation
    - Mesophilic or Thermophilic digestion
- Cook Feeders, Teulon, Manitoba
    - 6000 head operation
    - Psychrophilic Bio-Terre system

The hog industry in Manitoba is third largest in Canada, behind Quebec and Ontario, providing approximately 21% of the production in Canada (Statistics Canada, 2007). In fact, the number of hogs on farms in Manitoba, at almost 3 million, is more than double the human population in the province, and there continues to be a growing trend towards larger, more centralized farms, with the average number of hogs per farm being about 2300 (Statistics Canada, 2007). On average, each hog produces approximately one ton of manure per year (Agriculture and Agri-Food Canada, 1998), and with the higher density of hogs per farm, there can be greater difficulties with handling, treating, storing and utilizing these large quantities of manure. Some of the associated environmental issues include odor problems, nutrient (P, N) contamination of surface and ground waters, and the emission of ammonia and methane gas into the atmosphere.

In an effort to boost biogas and methane production, which would make the process more economically feasible, co-digestion of agricultural and municipal wastes with different amendments has been explored. These amendments can be a variety of biodegradable waste products that can enhance the anaerobic digestion process, by increasing gas production.

An industrial “waste product” of biodiesel production, which is of particular interest for co-digestion, is glycerol. In Manitoba, biodiesel production is in its infancy, but as with many bio-fuels, interest in full-scale production is increasing, which would lead to a surplus of local glycerol (Biodiesel Advisory Council, 2005).

Addressing the issue of GHG emissions is also very important when trying to develop new energy infrastructure like biogas production, because it is a major public concern. Biogas consists mainly of methane (CH_4) with some carbon dioxide (CO_2), and is emitted when the manure is digested. These gases would normally be released to the atmosphere if they were collected in an open-air lagoon, or at the time of spreading on an agricultural field, which is a common agricultural practice in North America.

Biogas is considered a clean and renewable form of energy, because the carbon comes from plant sources, which are renewable, rather than geological sources which are not. The methane is captured and used; not simply emitted into the atmosphere, which is important because it is a much more potent GHG than CO_2. While CO_2 is perhaps the most well-known GHG, due to its production from the burning of fossil fuels, CH_4 is able to trap heat about 21 times more effectively over a 100 year period (Cakir and Stenstrom, 2005). It has been estimated that anaerobic decomposition of livestock manure contributes about 4% of the global anthropogenic methane emissions, and almost half of that comes directly from pig wastes (USEPA, 2005).

Currently, much research is going into using biogas generated on site to help displace energy inputs, save money and increase energy efficiency, not just on farms but also at industrial and municipal wastewater treatment facilities. While anaerobic digestion is being done on a small scale in Manitoba, there exists a need to enhance the process and make it more economical, especially in a region which uses significant amounts of natural gas in the winter for heating purposes, and has a very low cost of electricity (6¢ / kWh). With the average number of hogs per farm in Manitoba, the electrical capacity per farm would be approximately 35 kW. Currently, this would require significant government subsidies on the cost of biogas-produced energy, or enhancing the anaerobic digestion process by co-digesting with another high-energy feedstock waste such as glycerol, to increase the amount of biogas produced.

1.2 Objectives

The main objective of this study was to evaluate the effect of adding different amounts of glycerol to lab-scale anaerobic digesters with hog manure, under mesophilic conditions. The specific research objectives were:

- To find the optimal glycerol dosage by measuring the effects of biogas and methane production
- To compare the effects of using pure chemical-grade glycerol and crude biodiesel glycerol
- To determine the biochemical methane potential of using raw manure with glycerol in batch tests
- To determine the fate of soluble nitrogen and phosphorus through anaerobic digestion with glycerol

CHAPTER 2 LITERATURE REVIEW

2.1 Anaerobic Digestion Process

Anaerobic digestion, as mentioned earlier, is a naturally occurring process, by which anaerobic microorganisms convert biodegradable organic matter into biogas, in the absence of oxygen. Typically these systems are operated under controlled conditions, however this process is essential to the global carbon cycle and can be seen naturally occurring on the earth's surface, for example in marshes, soils, landfills, and in the intestines of animals, where ever there is the anaerobic decay of organic materials. This anaerobic digestion process occurs with different groups of bacteria in four steps or reactions (figure 2.1): hydrolysis, fermentation (acidogenesis, acetogenesis), and methanogenesis.

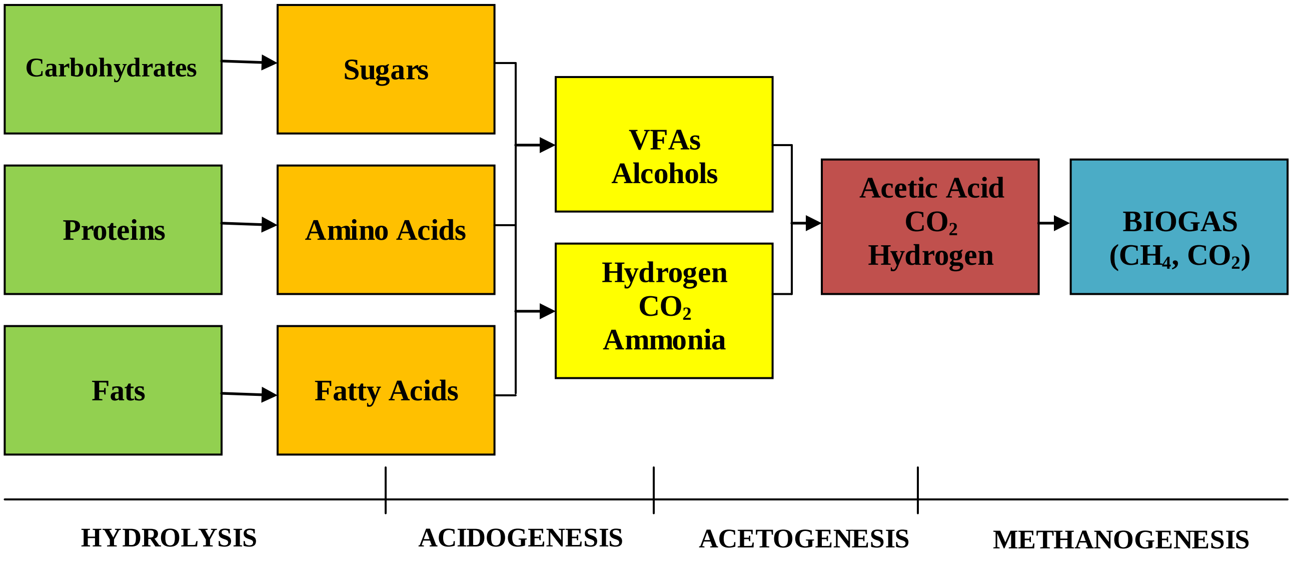


Figure 2.1 Flow diagram of anaerobic digestion process (based on McNeill, 2005).

The various fermentations are carried out by different microorganisms, which naturally produce a number of products. These products accumulate only temporarily because of the synergistic relationship that these fermentative organisms have with each other, where the products from one group of organisms serve as the substrates for the next group of organisms in an anaerobic food chain, leading to the eventual production of methane (Gottschalk, 1986).

In the first step, hydrolysis, particulate matter is converted into soluble compounds that can be further hydrolyzed into monomers used for fermentation (Metcalf and Eddy, 2003). Basically long-chain bio-molecules are broken down by the reactions with water, and with the influence of exoenzymes secreted by bacteria, into short-chain and monomeric soluble products. For example, lipids break down into fatty acids; proteins are converted to amino acids, polysaccharides to monosaccharides, and nucleic acids to purines and pyrimidines (Puchajda, 2006). This step can potentially be rate-limiting if the organic material contains high solids concentration or large amounts of slowly-hydrolyzing cellulose. The reverse is also true, as Palmowsky and Muller (1999) showed that particle size reduction in the feedstock can lead to more rapid digestion.

In the fermentation step, amino acids, sugars and fatty acids are further degraded into intermediary products, such as high carbon VFA's, lactate, succinate, buthanol, and ethanol, by fermentative bacteria called acidogens (Puchajda, 2006). These are then oxidized into the end products of fermentation, which are acetate, hydrogen and carbon dioxide, by acetogenic or syntrophic bacteria (USEPA, 2006). Do to unfavorable thermodynamics, the acetogenic reactions can be rate-limiting, except under conditions where the end products (H_2, formate) are kept at very low concentrations (Gottschalk, 1986). An example of one pathway in glucose fermentation to propionate and acetate is (Boe, 2006):

$$3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O \qquad \text{Eq.(2.1)}$$

Different metabolic pathways will exist depending of several factors such as organic load, pH, or dissolved hydrogen concentrations (Rodriguez, et al., 2006). These different pathways will favor the production of acetate, butyrate, lactate and ethanol. The final stage is methanogenesis, which involves the splitting of acetate into methane and carbon dioxide, with the use of hydrogen as an electron donor and carbon dioxide as the electron acceptor to produce methane. This final step is carried out by a group of Archaea bacteria known as methanogens, which are strict obligate anaerobes (Ferry, 2003). These bacteria are able to grow not only on H_2/CO_2 and acetate, but also on other one-carbon compounds such as formate or methanol, if they are available (Schink, 1997; Stams et al., 2005). There are several reaction pathways related to methanogenesis, depending on the bacteria and process shown below, and favor is given to different reactions under differing conditions (temperature, H_2 partial pressure) (Thauer et al., 1977; Schink, 1997; Batstone et al., 2002):

Hydrogenotrophic methanogenesis	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	Eq.(2.2)
Aceticlastic methanogenesis	$CH_3COOH \rightarrow CH_4 + CO_2$	Eq.(2.3)
Acetate oxidation	$CH_3COOH \rightarrow 4H_2 + CO_2$	Eq.(2.4)
Homoacetogenesis	$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$	Eq.(2.5)

The rate of digestion can also be limited by the growth of these bacteria, which is controlled by temperature, pH, hydraulic and organic loading rate, volatile acid and alkalinity concentrations (Salsali, 2006). While hydrolysis can be rate-limiting in highly particulate substrates, methanogenesis has been shown to be rate-limiting in readily degradable substrates (Vavilin et al., 1997; Bjornsson et al., 2001)

An additional group of microorganisms which are active in the conversion process are known as homoacetogens, and their primary function is to ferment a broad range of components (glucose, H_2, CO_2) during the production of acetic acid (Poulsen, 2003; Boe, 2006). They can however, reverse their action and produce other types of fatty acids other than acetate, if the concentration of acetate, hydrogen or ethanol is too high. The simplified chemistry of the anaerobic digestion process would look like this for the digestion of glucose:

$$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \qquad \text{Eq.(2.6)}$$

This would lead to equal concentrations of CO_2 and CH_4, which is not the case in practice, due to the complexity and variations in substrate. Table 2.1 shows the theoretical methane yields of the various components of manure.

Table 2.1 Theoretical methane yields of various types of organic matter at STP (based on Angelidaki and Ellegaard, 2003).

Substrate	**Composition**	**CH_4 yield (l CH_4/g VS_{rem})**	**CH_4 yield (l CH_4/g CODrem)**	**% CH_4**
Carbohydrate	$C_6H_{10}O_5$	0.415	0.35	50
Protein	$C_5H_7NO_2$	0.496	0.35	50
Lipids	$C_{57}H_{104}O_6$	1.014	0.35	70
Ethanol	C_2H_6O	0.730	0.35	75
Acetate	$C_2H_4O_2$	0.373	0.35	50
Propionate	$C_3H_6O_2$	0.530	0.35	58

The key to having a stable reactor and a healthy bacterial population is making sure that the reactions stay in balance. Under stable conditions, most of the hydrolyzed material will be converted into methanogenic substrates (acetic and formic acid, H_2, and CO_2)

that can be used efficiently for methane production, resulting in high methane content (~ 65%) in the biogas. However, if the process is not in balance and hydrogen is not consumed quickly enough, then alcohols and other fatty acids can build up, creating unfavorable conditions for the methanogens (Poulsen, 2003).

Some of the indicators of system imbalance include changes in: biogas production, biogas composition (CH_4, CO_2, H_2), pH, alkalinity, volatile fatty acids, volatile solids reduction, chemical oxygen demand (COD) reduction, and the carbon to nitrogen (C/N) ratio. The biogas production and composition is closely tied to the level of organic loading. Oftentimes when the digester is overloaded, it will respond with an immediate increase in biogas production, but then a sudden decrease when levels of inhibiting products (acids) are too high and pH drops. Strong imbalances like this can also cause variations in the biogas composition, such as a shift to higher levels of CO_2 and H_2, because they are not consumed fast enough by the H_2-utilizing methanogens (Poulsen, 2003). It is important though, to combine this gas datum with organic loading and composition data, because the changes may simply be due to a change in the feed substrate, which is common. A true indication of imbalance would occur when there is a change in gas production or composition without any change to the feed substrate.

If there is an imbalance in the system, usually it will lead to a buildup of VFAs, which will result in a decrease in pH. This organic acid accumulation will not always result in an immediate drop in pH if the buffering capacity of the material is high, which is often the case with animal manures, so it is not usually the best indicator, but it is relatively easy to measure. The optimum pH for biogas production is between 6.7 and 7.4, with digester performance decreasing when the pH is lower than 6 or higher than 8 (Poulsen, 2003). If the pH falls out of this range, then unionized forms of VFAs and NH_3 become toxic to methanogens, since the un-ionized forms of these compounds can pass through the cell membranes much more easily, destroying the membrane potential (Ros and Zupancic, 2003).

Total alkalinity ($CaCO_3$) is a measure of the buffering capacity, or the ability of the solution to neutralize acids, and it can be a better indicator than pH, because the alkalinity is consumed before a drop in pH is noticed. Alkalinity can also vary with feed composition, so it should be measured in conjunction with VFA concentrations.

As mentioned, the VFA concentrations are an effective indicator of steady state within a digester, because they are a good reflection of bacterial metabolic activity. Both the hydrogen-producing acetogenic, or syntrophic bacteria and the methanogenic bacteria are sensitive and rely on each other for their successful growth. If these bacteria cannot effectively degrade the fatty acids and alcohols, which has been shown to happen under high organic loads, then there will be an accumulation of these intermediate products, leading to a drop in pH (<6), creating a toxic environment for the methanogens (Schink, 2002). Ahring (1995) reported that the relative changes in VFA concentrations are a better indication of imbalance, rather than their absolute value, while Holm-Nielsen et al. (2007) added that changes in VFA concentration acted as an early warning of system instability. Amon et al. (2006), however reported that when VFA concentrations significantly exceed 5 g/l, it caused instability in the AD process and organic overloading was the likely cause. When looking at individual VFA's, it is important to understand that the two-carbon acetate (CH_3COOH) is one of the simplest carboxylic acids, and is the base substrate for the majority of methane production. During acetogenesis, higher carbon VFAs, such as propionate (CH_3CH_2COOH)), butyrate ($CH_3CH_2CH_2COOH$) and valerate ($CH_3CH_2CH_2CH_2COOH$) degrade through various pathways directly to acetate, while iso-valerate and iso-butyrate degrade to lower carbon VFAs, which eventually break down to acetate but require more steps (Boe, 2006). This is important because increases in acetate in an anaerobic digester have been shown to increase metabolic activity and methane production, and that degradation of propionate is the slowest of all VFAs, where a buildup of it can be a good indication of low metabolic activity and slow stabilization (Pind et al., 2003). Hill and Holmberg (1988) reported that increased propionic/acetic acid ratio was observed in problematic digesters. While excessive accumulation of VFAs can inhibit methanogenesis, high hydrogen levels in the digester have also been shown to inhibit propionate and butyrate degrading acetogens (Angelidaki and Ahring, 1993). Therefore a low, stable concentration of VFAs, with the main component being acetate, would be an indication of "healthy" metabolic activity. The reduction in volatile solids and COD from input to output is an indicator of efficiency in the digestion process. If the level of reduction decreases, then it can indicate that there is an accumulation of organic end products which are not being converted to biogas.

Anaerobic digestion can also be inhibited when concentrations of ammonia/ammonium (NH_3/NH_4^+) become too high in a digester. High concentrations of NH_3/NH_4^+ are common in animal manure, especially through the degradation of protein and urea. Acetoclastic methanogens are most sensitive to high concentrations of NH_3/NH_4^+ and are inhibited particularly at high temperatures and pH, where the equilibrium:

$$H^+ + NH_3 \leftrightarrow NH_4^+ \qquad \text{Eq.(2.7)}$$

will tend to shift to the left towards NH_3, but again they can adapt to these conditions over time (Poulsen, 2003; Heinrichs et al., 1990). Lee et al. (2000) found that methanogenic bacteria were inhibited at NH_3/NH_4^+ concentrations over 3 g/l in spite of pH control, and Angelidaki and Ahring (1993) reported ammonia toxicity in hog manure above 4 g/l.

The addition of certain readily degradable organic compounds can create an imbalance and hinder microbial activity. For instance, long-chain organic molecules, like lipids, in high concentrations can result in a fast hydrolysis reaction and an over-production of long-chain fatty acids, because they cannot be consumed fast enough in subsequent reactions. This environment can then inhibit the acetogenic, or syntrophic, and methanogenic bacteria. In a reactor which has adapted to that feed material, however, it can result in a high level of methane production due to the conversion of the high energy content in the lipids.

The C/N ratio is also important for process stability, because it determines the health of the microbial diet. Angelidaki et al. (2003) reported that a C/N ratio of 25 to 32 will increase methane yields, while a lower C/N ratio can cause a risk of excess NH_3-N, which can be inhibitory. A higher C/N ratio can lead to a nitrogen deficiency, which would reduce biomass growth and reduce degradation, as was seen through the digestion of olive mill effluents that typically have a very high COD/N ratio (Angelidaki et al., 2003).

Other factors to consider include nutrient and substrate concentration, retention time, and solids content, which all have an effect on system performance. Typically manure will already contain all of the macro and micronutrients necessary for methane production without any additional supplements. The solids content and retention time give information as to how readily available the food source is to the bacteria, and how much time they have to digest it.

Anaerobic digestion can have several advantages over other wastewater treatment methods. These include: energy production, increased volatile solids reduction (VSR), reduced biomass production, lower nutrient requirements, smaller volume requirements, higher allowable loadings, and energy savings due to the avoidance of aeration. These advantages become more obvious in wastewaters with high biodegradable COD concentrations and elevated temperatures (Metcalf and Eddy, 2003).

There are some design considerations to keep in mind however, which can affect the economics of the system. They include: longer start-up times (months), longer solids retention times (SRT's), the potential for odors, and corrosive materials. With proper wastewater characterization and process design, these problems can be managed effectively, and provide a desirable environment for the bacteria. Other design considerations include: toxicity, mixing, volatile solids (VS) loading, temperature, hydraulic retention time (HRT) and SRT. In complete-mix anaerobic systems without sludge recycle, HRT equals SRT, which is typicallly in the range of 15-30 days to provide adequate stabilization (Metcalf and Eddy, 2003).

Anaerobic digesters can have different designs and be operated under different conditions. Typically covered lagoons or complete-mix digesters are the most efficient designs for hog farms, while plug-flow digesters are used mainly for cattle or dairy operations that have higher solids contents. Complete-mix digesters in designed tanks have much more control and predictable end products, while covered lagoons will also undergo biological anaerobic digestion, but rates of gas production will tend to be lower and less predictable.

The characteristics of the manure waste will help determine the most appropriate handling procedures, as well as digester options. Figure 2.2 illustrates the different options available depending on the solids content.

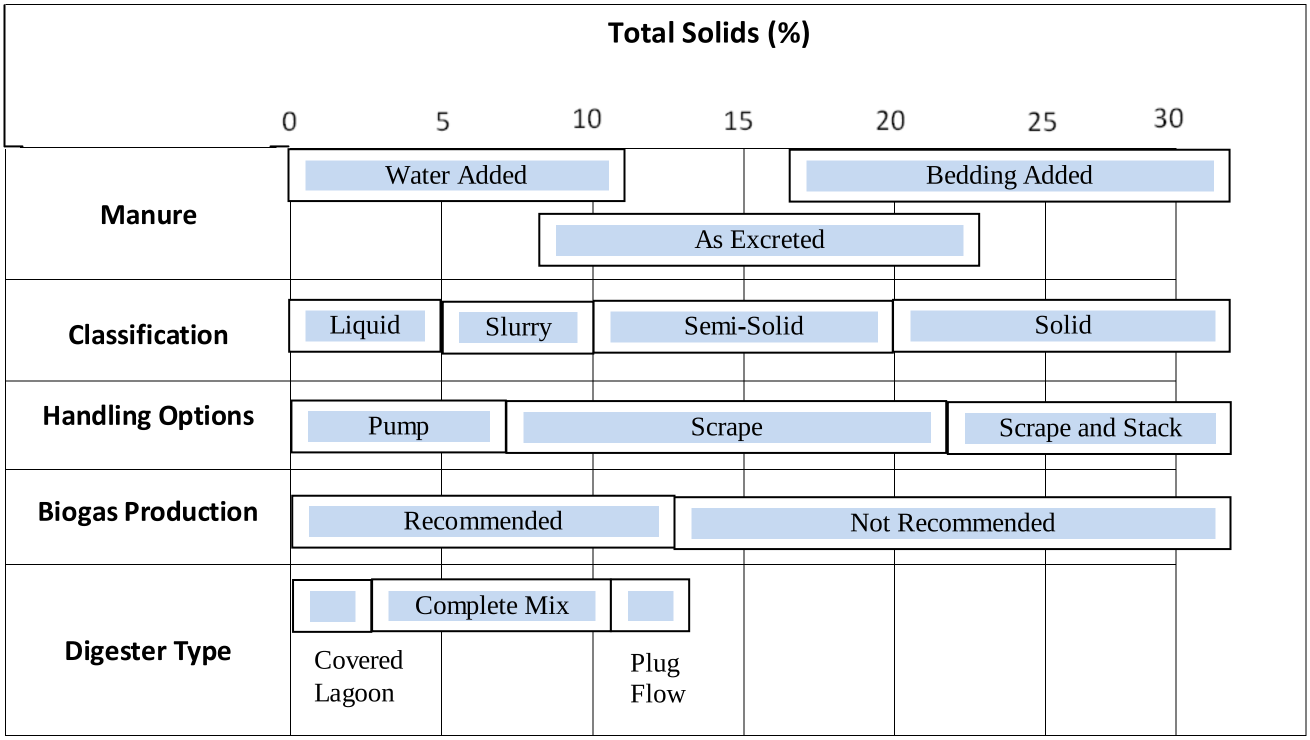


Figure 2.2 Summary of the basic considerations for manure handling and processing based on fluid properties (based on US EPA Agstar, 2002).

These digesters can be operated under mesophilic (30-40°C), thermophilic (50-60°C), or psychrophilic (5-25°C) temperatures and with varying retention times; each with their own advantages and disadvantages. Typically thermophilic and mesophilic temperatures are preferred because they allow for higher amounts of organic loading, increased digester capacity, and shorter retention times.

The biogas produced can be used as a fuel for heating boilers or for electrical generation, or both in a co-generation unit. Very often these systems are self sustainable, meaning they can provide enough energy to heat the digesters themselves, and possibly even produce a net supply of energy which can be used in other parts of the farm or sold to the electrical grid. Typically higher-strength wastewaters will produce a greater amount of methane, but again this depends on the incoming COD concentration and digestion temperature. At mesophilic temperatures (~35°C), COD concentrations greater than 1500-2000 mg/l are needed to produce enough methane to heat the wastewater without an external source (Metcalf and Eddy, 2003). The total amount of biogas produced can be estimated based on the percentage of VS reduction, and typically is in the range of 0.75-1.12 m^3/kg of volatile solids destroyed (Metcalf and Eddy, 2003). Approximately 1 m^3 of biogas (65% CH_4), can produce 1.7 kWh, with a net zero emission of CO_2 (Oleszkiewicz, 2006).

As mentioned, anaerobic digestion produces biogas, which typically contains about 65-70% methane (CH_4) and 25-30% carbon dioxide (CO_2) by volume, with trace amounts of nitrogen (N_2), hydrogen (H_2) and hydrogen sulfide (H_2S) gases (Metcalf and Eddy, 2003). The quantity and quality of the biogas is very important for the economy of generating facilities which depend on the purity of the biogas to sell for electricity or heat generation.

The production of hydrogen sulfide is a problem, because it can be highly odorous and corrosive to metals. This results when high concentrations of sulfates and sulfites are present in wastewater. These compounds can serve as electron acceptors for sulfate-reducing bacteria, producing hydrogen sulfide (H_2S), which can be toxic to methanogenic bacteria at high concentrations, and can compete with methanogenic bacteria for COD, reducing the amount of methane gas produced (Metcalf and Eddy, 2003).

2.2 Co-digestion of Wastes

The use of co-substrates in anaerobic digestion is a growing development, because the many associated benefits have made this technology an attractive option for energy production. Co-digestion of manure with waste supplements can enhance the anaerobic digestion process, because these co-substrates can supply nutrients which may be deficient, and have an overall positive synergistic effect in the digestion medium, leading to stable digestion and enhanced gas yields (Yadvika et al., 2004). Often times the co-substrates used are other offsite waste materials which have little other use and require disposal. One goal for using organic waste products is to reduce the amount of waste going to other treatment systems (i.e. Landfills, incinerators), and also to recycle the nutrients in those wastes back into the agricultural sector. If these organic industrial wastes are more easily degradable than manure, then they will have the potential for higher gas yields and VFA production, making it important to control the hydraulic and organic loading. Some examples include the use of other manures, food waste, organic municipal solid waste, corn silage, slaughterhouse waste, and fats-oils-grease (FOG) wastes. Co-digestion with manure is important because manure is an excellent substrate that has high water content, provides the necessary nutrients for AD, and acts as a buffer to aid in digestion and help overcome some of the operational problems (Angelidaki et al., 1997). The methane yields of some organic wastes are compared in table 2.2, showing that the organic oil wastes which have the highest energy content (Joules), also have the highest methane yields.

Table 2.2 Comparison of methane yields of different organic wastes (based on Angelidaki et al., 2003)

Type of Organic Waste	**Composition of Waste**	**% Organic Content**	**Methane Yield (m^3/ton)**
Livestock manure	Cellulose, carbohydrates, lipids, proteins	-	10-20
Pure Glycerol	Sugar alcohol	-	470
Floatation sludge (dewatered)	65-70% proteins, 30-35% lipids	13-18	40-60
Fish oil sludge	30-50% lipids and other organics	80-85	450-600
Organic household waste	Carbohydrates, lipids, proteins	20-30	150-240
Whey	75-80% lactose, 20-25% proteins	7-10	40-55
Soya oil/margarine	90% vegetable oil	90	800-1000
Sewage sludge	Carbohydrates, lipids, proteins	3-4	17-22

The advantages of increasing biogas and methane production can lead to economic benefits, which are important to offset the capital and operational costs of the anaerobic digestion units and power generation equipment. Other than the increased methane production, tipping fees charged to the polluter for waste disposal can also increase revenues. Some challenges do exist however, such as the transportation costs associated with hauling extra waste products to the digester site, and the possibility of pre-treatment or post-treatment required, for the disinfection of certain wastes. Permits may also be required for accepting some off-farm materials which may pose a health risk when they are spread on agricultural fields.

The benefits are being realized not just on a farm-scale, but also on a municipal level. At the wastewater treatment facility in Millbrae, California, the use of grease trap waste in municipal waste digestion saved the plant roughly $80,000 annually through energy savings, by increasing their biogas production by 150%, plus additional revenue was received from tipping fees (Figure 2.3) (Chung et. al., 2007).

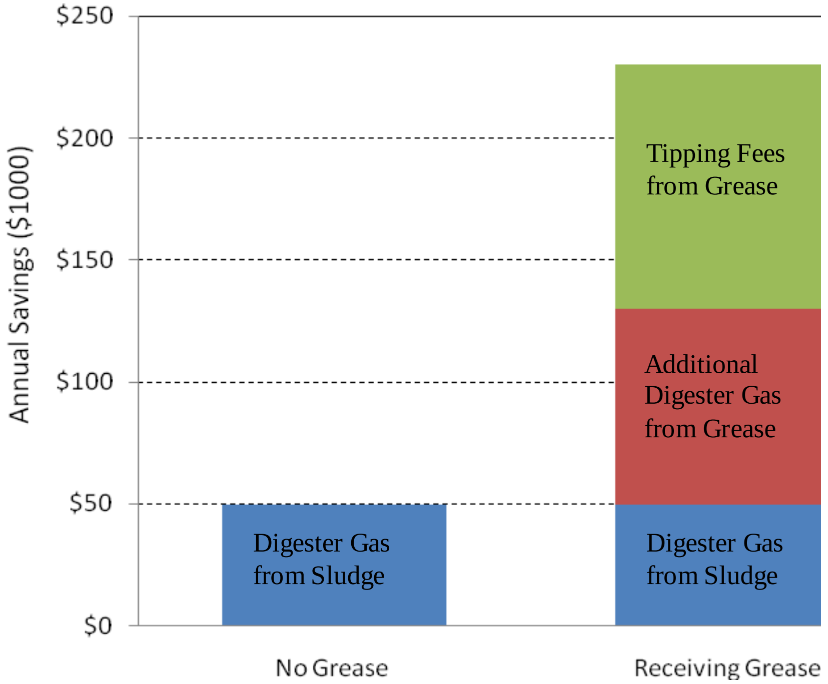


Figure 2.3 Annual savings from the addition of grease trap waste at the Millbrae WWTP in California (based on Chung et al., 2007)

The use of grease waste materials in co-digestion is being done by various other organizations in an effort to offset the rising costs of energy and fuel, as well as to reduce the problems produced by grease such as odour production and sewer blockages. The largest contributor of FOG wastes are restaurants, food preparation facilities, and industrial processing plants, which often release these wastes directly into the sewers. This FOG, when properly digested, has proven to produce methane at higher production rates than other organic matter, as it is able to enhance the degradation of other organic feed stocks in the digester. At the city of Riverside wastewater treatment plant in California, 20-30% additions of FOG to the digester sludge have resulted an almost doubling of the biogas production, which has lead to significant energy savings (Bailey, 2007).

There are also many examples of co-digestion of animal manure with many different waste products that have been experimented with in the last several years and most showed positive results. Kaparaju and Rintala (2005) showed that pig manure could be co-digested with up to 20% of potato waste, and increase methane yields ($m^3\ kg^{-1} VS_{add}$) up to 150% in a semi-continuous, mesophilic digester. In another batch study co-digesting hog manure with poultry manure, results showed approximately 3 times more biogas and methane production than digestion with hog manure alone, and also higher levels of biogas and methane yield, demonstrating the complementary nature of these two wastes (Magbanua et al., 2001). Callaghan et al. (1999) ran several batch co-digestion experiments with cattle manure slurries, and found that methane production was higher with fruit and vegetable waste, poultry manure, fish offal and dissolved air floatation sludge than with the digestion of cattle slurry alone. Specific methane yields ($m^3\ CH_4$/kg VS removed) were also higher in co-digestions with fish offal and brewery sludge. In a batch experiment co-digesting 5% oil bleaching earth (which has high lipids content) with hog manure, results showed almost 3 times the methane production, and a methane yield (ml CH_4/g VS_{add}) 2.4 times that of digestion with manure alone (Campos et al., 1999).

A similar waste product to grease that could be used in anaerobic digestion applications is glycerine, or glycerol. Glycerol is a readily biodegradable sugar alcohol, which is a byproduct of biodiesel production, and is of particular interest in the province of Manitoba, due to the anticipated increase in the biodiesel industry and the associated waste products (Biodiesel Advisory Council, 2005). Glycerol has many other commercial applications, such as in the manufacture of detergents, cosmetics, and in food processing, however its market value is greatly affected by its availability and purity.

There is limited research on the use of glycerol in anaerobic digesters. In one research study involving the mixture of cattle manure with 2% glycerol trioleate (GTA), significantly better specific methane yields (224 ml CH_4/g VS day to 382 ml CH_4/g VS day) and a higher removal of organic matter (37% to 51%) were observed, compared with a digester operated solely with manure. The reactor with mixed waste also exhibited a microbial community with higher densities, and higher numbers of methanogenic microorganisms (Mladenovska et.al, 2003). The work by Amon et al. (2006) using batch digesters showed that with the addition of glycerine to hog manure in varying amounts, a consistent increase in methane production could be observed along with a co-fermentation effect. The co-fermentation effects were the highest with glycerine additions of 3-6% to hog manure that had a total solids content of approximately 4%. They also suggested that the addition of glycerine should not exceed 6% by volume to ensure stable operation. The work of Holm-Nielsen et al. (2007) on co-digestion of manure with glycerol in semi-continuous lab digesters, showed that loading of 3% (vol) glycerol was fairly easy to manage and gave increasing biogas yields, however, when the glycerol concentration exceeded 5-7 g/l in the digester, methane was significantly reduced from the organic overloading.

This research study focused on the addition of varying amounts of glycerol to hog manure in a continuous-feed digester system, in an attempt to boost biogas and methane yields. A variety of glycerol-to-manure feeding ratios were investigated in order to determine suitable feeding regimes and evaluate thresholds for carbon overloading.

Both chemical-grade or “pure” glycerol, and crude (unrefined) glycerol (from a biodiesel production process) were tested to asses the impact of impurities in the crude glycerol on digestion performance. Ma et al. (2008) tested both pure and crude glycerol in a co-digestion experiment with potato wastewater, and reported that the biogas and methane production increased by about 1.5 times from the control. When comparing the pure and crude glycerol, the results were very similar between them, but that it would be more profitable to co-digest with crude glycerol, because it is a cheaper waste product.

2.3 Characteristics of Farm Wastes and Practices

Manitoba’s pig production is a major contributor to the provincial agricultural sector. The challenge for producers is to identify and manage environmental problems related to their operations, by using sustainable practices and technologies.

It is important to get an understanding of the waste products produced on the farm and the common farming practices, to be able to make an application like anaerobic digestion work in Manitoba and similar areas.

The typical approach by hog producers in much of North America has been to collect manure with the wash water from the barn and pump it into an uncovered storage tank or earthen lagoon, where it is held until it is time to spread it on the agricultural field. This approach is the cheapest method of waste disposal, however there are intangible costs associated that are often not considered, such as the potential for surface and ground water pollution, odour emissions, and GHG emissions.

Most large-scale hog operations use a confined barn system, so they can control the environmental conditions within. They specifically do not use bedding so that the manure can be handled as liquid slurry, which gets washed by a hydraulic flushing system into a floor pit covered by a slatted floor (Hatfield et al., 1998). The slurry is then pumped to a storage tank or lagoon, which can typically store manure for 120-180 days, or longer, before spreading to nearby fields. Spreading usually occurs in the spring and fall, so the storage needs to be large enough to handle the loading during the other seasons. This storage phase is where several problems can arise as mentioned, like odours, leaks, overflow, and over-application of effluent, but it is also where anaerobic digestion can occur. When open lagoon systems are used, anaerobic digestion still occurs below the surface, but the VFAs and ammonia that are produced are allowed to volatilize through the surface and are emitted into the atmosphere, creating strong odours, and causing the loss of nitrogen (Cheng and Liu, 2002).

Depending on the method of collection, hog manure slurry will contain 90-99% water, 1.9% phosphorus (P), 7.2% nitrogen (N), and 3.2% potassium (K), along with some copper (Cu) and anti-microbial drug residues, which are added to enhance growth and improve health (Hatfield et al., 1998). The presence of anti-microbial products would obviously be detrimental for the use of anaerobic digestion, however when added to feed at recommended levels, inhibition is not likely (Nielsen, 2006). Studies have shown that nutrients found in manure (N,P,K) are not destroyed, but simply transformed through the AD process. Unlike carbon, which leaves the digester when it is transformed into biogas, total amounts of N,P, and K are the same in the influent and effluent of a digester. Typically organic nitrogen and phosphorus which are bound in particulate form, are released in soluble forms of NH_3 and PO_4. These soluble forms are much easier for plants to uptake and use, making the digestion process advantageous for producing a valuable fertilizer product. This can make the release of nutrients easier for farmers to predict, therefore over-fertilizing fields is reduced and less chemical fertilizer is needed. While N and P are in soluble forms, it makes them more susceptible to runoff, leaching, or even volatilization in the case of NH_3 (Aldrich, 2005). Some of the common characteristics of hog manure found in literature are shown in table 2.3.

Table 2.3 General characteristics of liquid Hog Manure (Moller, et al., 2002; Hatfield, 1998; Oleszkiewicz, 2000; Cheng et al., 2007)

Parameter	**Typical Values**
COD	14,000 – 45,000 mg/l
TS	10,000 – 70,000 mg/l
% TS	3% - 7%
% VS/TS	60%
TKN	1,300 – 4,200 mg/l
TP	400 – 1,300 mg/l
PO4-P	200 - 800 mg/l
NH3-N	800 – 3,600 mg/l
pH	7 – 7.5

As can be seen in table 2.3, the physical and chemical characteristics of hog manure can vary greatly, depending on various factors, such as age of the animal, the animal feed composition, and the manure collection methods.

Some of the technologies that have been explored for hog manure processing in Manitoba include: solid-liquid separation, composting, and anaerobic digestion. While there is no clear best solution, these technologies are being considered with the following objectives in mind (Cicek, 2007):

- Nutrient management (P,N)
- Renewable energy production
- Odour control and reduction
- Greenhouse gas reduction

Pigs are generally fed imported feed in a barn and the manure is collected and then spread on fields. This practice requires careful nutrient management, to ensure that the levels of nutrients applied do not exceed the amount taken up by the crops. This can pose a problem if highly concentrated hog-farming operations generate quantities of manure that are too large to spread on the surrounding land, and require longer distance transport. If spreading of manure is done incorrectly or in the wrong season, it can lead to contamination of ground or surface water with soluble N and P, which is a large problem in Manitoba. The Red River in Manitoba, currently suffers from enhanced nutrient loads, most of which comes from human density and the agricultural sector (Jones and Armstrong, 2001).

Modern farm digesters in Europe will typically have highly controlled conditions (temperature, pH, organic loading) and are very efficient, however their cost is also quite high, which is one of the reasons many small operations in North America use the cheaper covered lagoon systems. Table 2.4 gives a comparison of the AD technology available on a farm scale.

Table 2.4 Overview of AD technology and how suitable it is for hog manure processing (based on US EPA, 2002).

Technology Option	**Description**	**Level of Control**	**Relative Cost**	**Suitability of Hog**
Covered lagoon	Earthen pit with plastic cover	Low	Low	High
Complete mix	Reactor with agitator and environmental controls	High	High	High
Plug flow	Concrete heated pit with plastic cover	Med	Med	Med-low

2.4 Glycerol Production

In the production of biodiesel, triglycerides (organic fatty acids) are mixed with alcohol in the presence of a catalyst (sodium hydroxide) to produce biodiesel and glycerin. This process is referred to as transesterification (Figure 2.4).

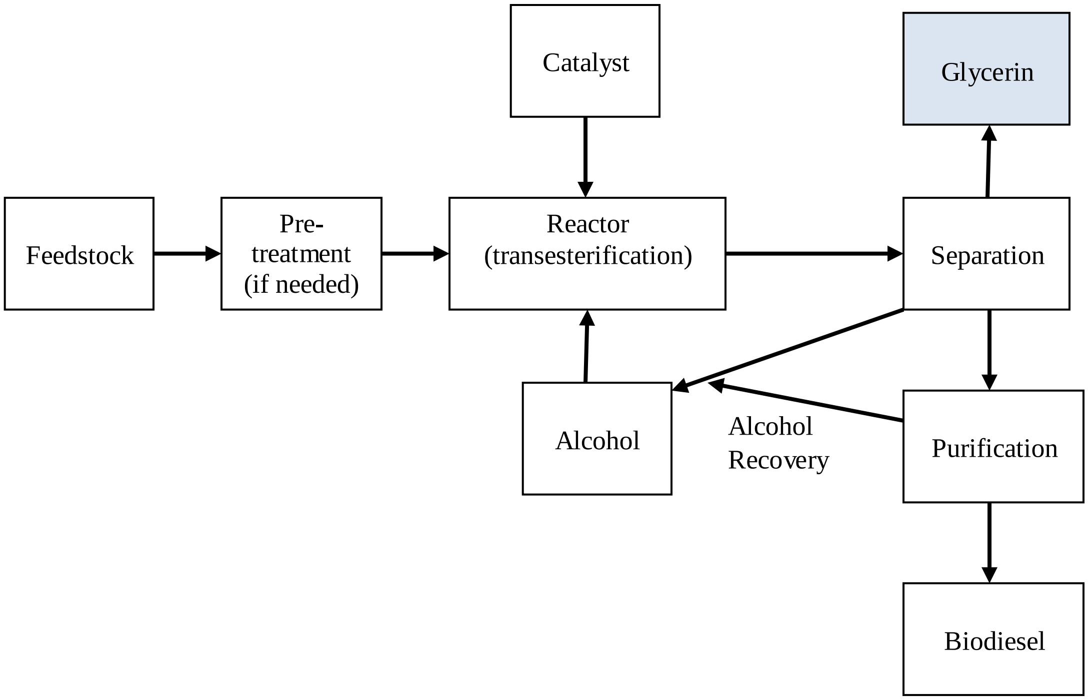


Figure 2.4 Schematic of biodiesel production process (based on Schafer et al., 2007)

Through the transesterification reaction, an impure biodiesel is produced in which approximately 10-20% of the end product is glycerol. Within this crude glycerol, there is approximately 8% methanol and other contaminants, such as water, catalyst residues, salts and soaps (Kemp, 2006; Mittelbach and Remschmidt, 2004).

Glycerol $C_3H_5(OH)_3$ is a low-toxicity sugar alcohol, which is used in many applications in the food and beverage industry, and the pharmaceutical industry, such as in the production of toothpastes, soaps and skin care products. It is also a high calorific feedstock, making its use in anaerobic digestion a potential benefit, especially if it becomes a large waste by-product of the biodiesel industry in Manitoba.

The demand for glycerol would largely be tied to the demand for biodiesel, therefore if biodiesel supply increases, then the price of glycerol would decrease. There are 2 potential sources of feedstock for large scale production of biodiesel in Manitoba, they are: animal fats (tallow) and canola oil (Biodiesel Advisory Council, 2005). The demand for biodiesel is growing worldwide because it is a cleaner fuel then conventional diesel, which reduces air pollutants and GHG emissions. Germany is leading the way in biodiesel production, and the United States is also strongly promoting its use. It is anticipated that production will increase in Canada due to regulatory mandates from the government on adding biodiesel to regular diesel in different blends at the pumps. This would help generate a local glycerol market, and if the level of glycerol refinement needed was low, where “crude” glycerol could be used, for example in anaerobic digestion, it could be beneficial for both sides.

The co-digestion of glycerol is advantageous to some other wastes, because it is readily digestible, has high levels of COD, and can be stored for long periods before being used in biogas production (Ma et al., 2008). Some characteristics of pure (chemical grade) and crude glycerol are shown in table 2.5, as measured by Ma et al., 2008.

Table 2.5 Some basic chemical characteristics of pure and crude glycerol (based on Ma et al., 2008)

Parameter	**Theoretical**	**Pure**	**Crude**
COD (g/l)	1,540	1,200	1,120
Density (kg/l)	1.26	1.21	1.22
VFA (g/l)	0	0.145	0.337

According to this data, the “pure” or chemical grade glycerol, was not entirely pure because there were low levels of VFA’s present, and the crude glycerol had a lower level of COD than the pure glycerol, indicating that it may have undergone methanol recovery in the biodiesel production process.

Glycerol can be fermented by various microorganisms to different organic acids, solvents, and 1,3 propanediol (Lengeler et al., 1998). Figure 2.5 shows the most common metabolic pathway in the fermentation of glycerol by a mixed culture.

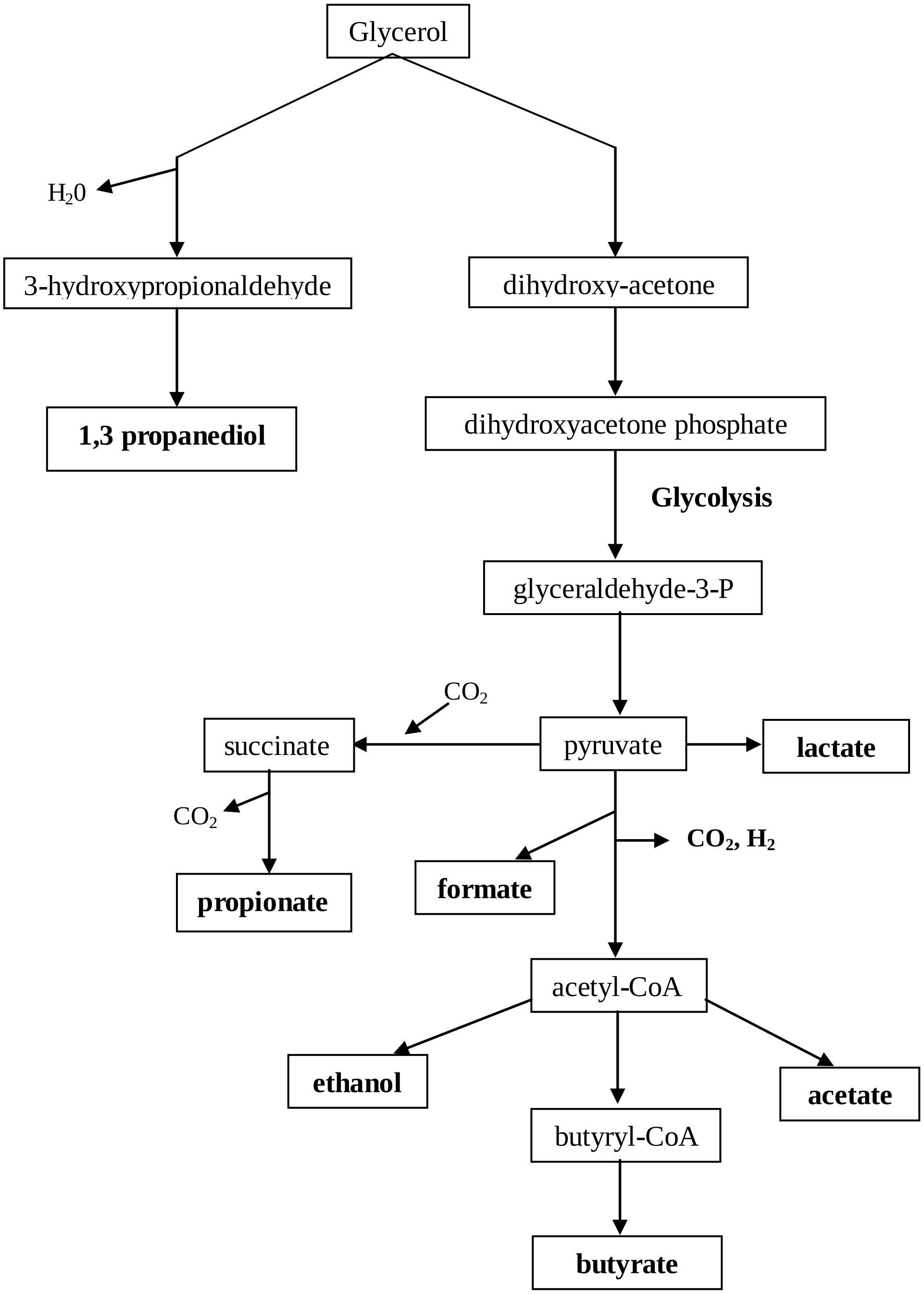


Figure 2.5 Metabolic pathway of glycerol fermentation including the multiple pathways of glycolysis (based on Temudo et al., 2008)

If glycerol is used as a co-substrate in AD, it is important to establish a stabilized mixed culture of acetogens and methanogens, which can efficiently convert the glycerol to methane (Drake, 1994). First the acetogens convert the glycerol to acetic acid, as shown in the equation below:

$$7C_3H_5(OH)_3 + 2CO_2 \rightarrow 7CH_3COOH + 2H_20 \qquad \text{Eq.(2.8)}$$

This is followed by the conversion of acetic acid to methane by acetoclastic methanogens:

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Eq.(2.9)}$$

Acetic acid can also be formed from CO_2 and H_2 in a different pathway, while different methanogenic bacteria can also utilize other carbon sources (CO_2, formate) to produce methane (Gottschalk, 1986).

2.5 Biogas Development

The production and utilization of biogas in farm digesters has been occurring for over a hundred years, but the first real scientific attention came during the energy crisis in the 1970's, and again more recently as energy prices have continued to rise (Angelidaki et al., 2003). The desire for stable, alternative energy solutions has lead to many options, and biogas development is one of them, for both energy production as well as waste treatment. Both Asia and Europe were the first to utilize small farm-scale digesters and it is estimated that there are over 6 million of these digesters, with the majority being found in Asia. These small-scale digesters are designed for single farms or several neighboring farms, and are usually low-cost, low-control systems where the biogas is used as cooking fuel. Currently, Europe is leading the way in anaerobic digestion technology, specifically in Germany and Denmark, where governments have invested significantly in this renewable energy option. There are over 600 farm-based digesters in Europe, with almost a half of them installed within the last 15 years, mainly due to changes in environmental policy (Lusk, 1998). Modern digesters in Europe have high levels of control and are either smaller farm-scale biogas plants, or larger, centralized facilities, that service several different farms. They tend to be highly efficient, and use off-farm materials in co-digestion to increase biogas production.

The worldwide capacity of electricity generation from AD is estimated to be close to 6300 mega watts (MW), which as mentioned, is found mostly in Asia. Of the estimated 307 MW capacity from AD in Europe, Germany currently has the largest development at 150 MW, followed by Denmark (40 MW), Italy (30 MW), and Austria and Sweden (20 MW each), with Denmark having the most co-digestion plants (Zhang, 2008). Since 2001, the total production of crude biogas from European Union (EU) countries has been increasing, and is equivalent to 2.7 million tons of oil per year (Demirbas and Balat, 2006).

Of the more than 1500 biogas plants in Europe, only 5% are being run at psychrophilic conditions, and 8% are thermophilic, while the rest (87%) are mesophilic digesters (Poulsen, 2003). Typical biogas plants have a gas storage tank that they can use for production of heat and electricity when needed, and many advanced plants now use a combined heat and power (CHP) plant design, or a cogeneration unit to fully maximize the energy and heat potential. The heat can be used on-site for heating the digester, or sold to nearby consumers and electricity is sold to the electrical grid. The CHP units tend to be quite efficient, utilizing 90% of the energy produced (Poulsen, 2003). First, between 25 and 40% of the biogas is converted to electricity in a gas motor connected to a power generator, and then as the engine is cooled, the remaining energy is used to generate hot water that can be used on site or distributed (Hilborn et al., 2007). The available waste heat could offset the cost of propane at $0.75/gal at a value of approximately 2800 Btu for every kWh generated (Lusk, 1998). The application of fuel cell technology in biogas plants is also an interesting one that is currently being explored, because of the possibility of zero emissions.

The main components and design of a farm-scale and centralized biogas plant are shown in figure 2.6.

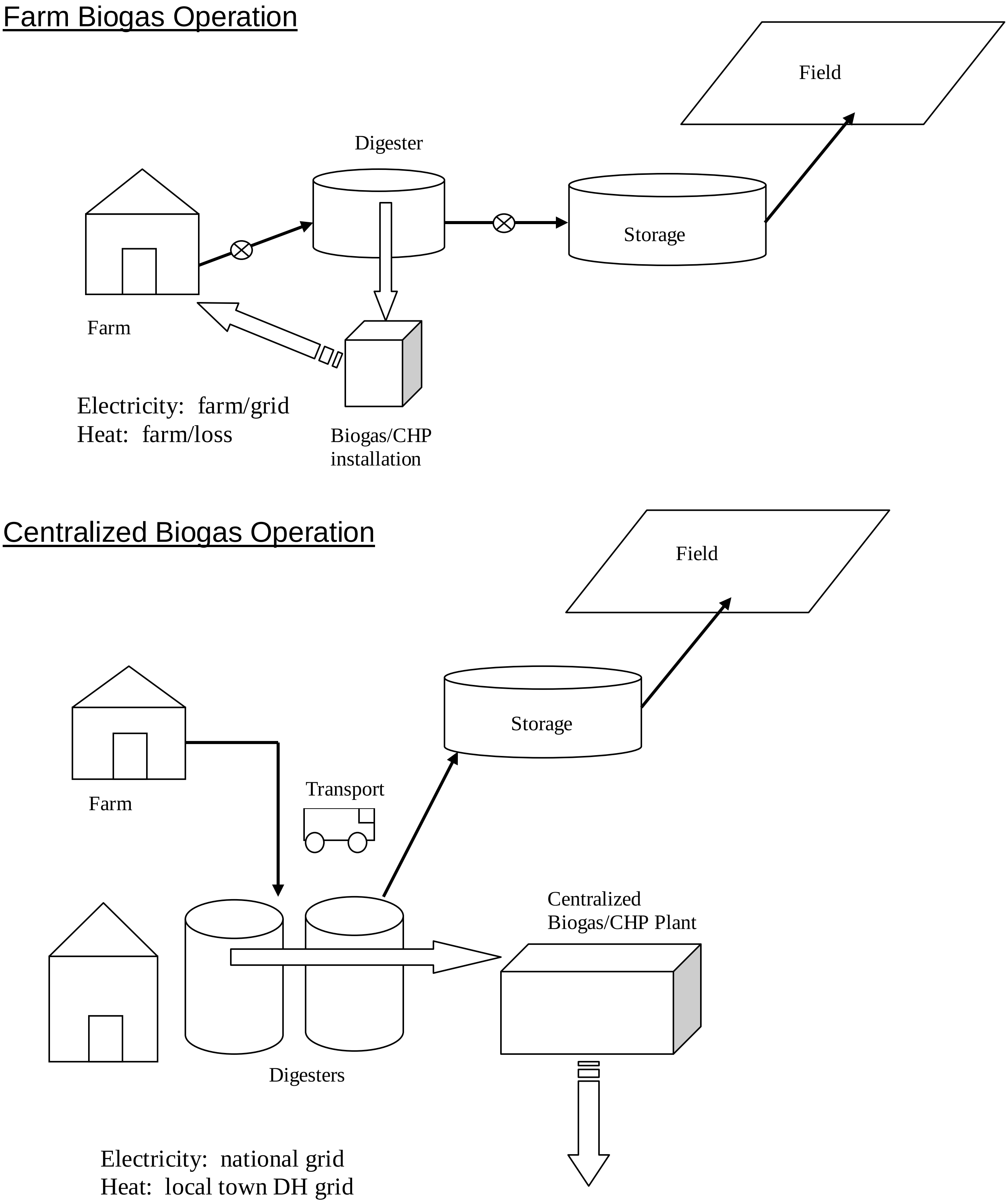


Figure 2.6 Conceptualization of a typical farm-scale and centralized manure biogas plant in Europe (based on Angelidaki et al., 2003)

There are several different operations utilized at biogas plants for efficient and stable operation, such as (Angelidaki et al., 2003):

- Transport/pumping
- Stirring/mixing
- Macerating/grinding
- Heat exchanging
- Biogas treatment and cleaning
- Biogas compression, storage and transportation
- Filtration/separation

As mentioned earlier, biogas is a mixture of CH_4 and CO_2, with trace amounts of H_2S, NH_3, and N_2. It is a “wet” gas that also contains water vapour which must be removed by condensation, before the gas can be used. If there are no restrictions on SO_2 emissions, then the biogas can be used in a boiler directly after the removal of water vapour (Poulsen, 2003). However, H_2S may need to be removed, due to emission standards, or if the gas is being used in an internal combustion motor, where the H_2S can cause corrosion of the metal parts. Another option being tested is using biogas to displace natural gas in pipelines, or for vehicle fuels. This option requires more intensive gas cleaning with the removal of H_2S and CO_2, to bring the level of methane up to commercial standards, which in Manitoba is 96% (D. Miller, Manitoba Hydro, 2008). Feeding upgraded biogas into the natural gas grid is already being done in Sweden, Switzerland and The Netherlands (Weiland, 2003). Methane from biogas is similar to “natural” gas, except that natural gas also contains ethane, propane and butane, giving it a higher calorific value (Lusk, 1998). Compressed biomethane from a biogas plant in Linkoping, Sweden is being used in vehicles to fuel all of the city’s transit buses as well as other vehicles, greatly reducing the CO_2 emissions (IEA Bioenergy Task 37, 2008). Biogas has a lower calorific value than natural gas (table 2.6), therefore cleaning needs to be done to improve its quality.

Table 2.6 Calorific value of biogas and natural gas at standard temperature and pressure (based on Angelidaki et al., 2003)

Gas Composition	**Biogas (65% CH_4)**	**Biogas (55% CH_4)**	**Natural Gas (96% CH_4)**
Upper calorific value (KWh/m^3) at STP	7.1	6.0	12.0
Lower calorific value (KWh/m^3) at STP	6.5	5.5	10.8

The heating value of methane is about 2.5×10^4 KJ m^{-3}, which is equivalent to about 1 kg of raw coal (Zeng et al., 2007). When compared to natural gas, biogas has a heating value between 500 and 700 Btu, while natural gas has a heating value of 1000 Btu (Goodfellow, 2007).

Many of the new, large biogas plants in Denmark, once stabilized can operate at quite high efficiencies, where only 10-15% of the energy produced needs to be recycled back into the digestion process (Angelidaki et al., 2003).

The co-digestion of manure with industrial and municipal organic wastes has been widespread in Denmark, with 22 centralized co-digestion plants in operation since the late 1980's (Angelidaki and Ellegaard, 2003). These are large-scale manure treatment plants that service many local farmers, digesting slurry manures with supplementary organic wastes. There are also a large number of smaller farm-scale biogas plants and together, their growth will help the nation to reach its long-term renewable energy goals.

The largest biogas plant in Sweden, in Kristianstad, is a co-digestion plant which treats 15% organic fraction of municipal solid waste (OFMSW), 18% industrial organic waste, and 67% manure. The biogas yield is about 40 m^3/ton, and the production of biogas is enough to cover the heating requirements of 600-800 households (Angelidaki et al., 2003). A flow diagram of the inputs and outputs from an integrated centralized co-digestion plant are shown in figure 2.7.

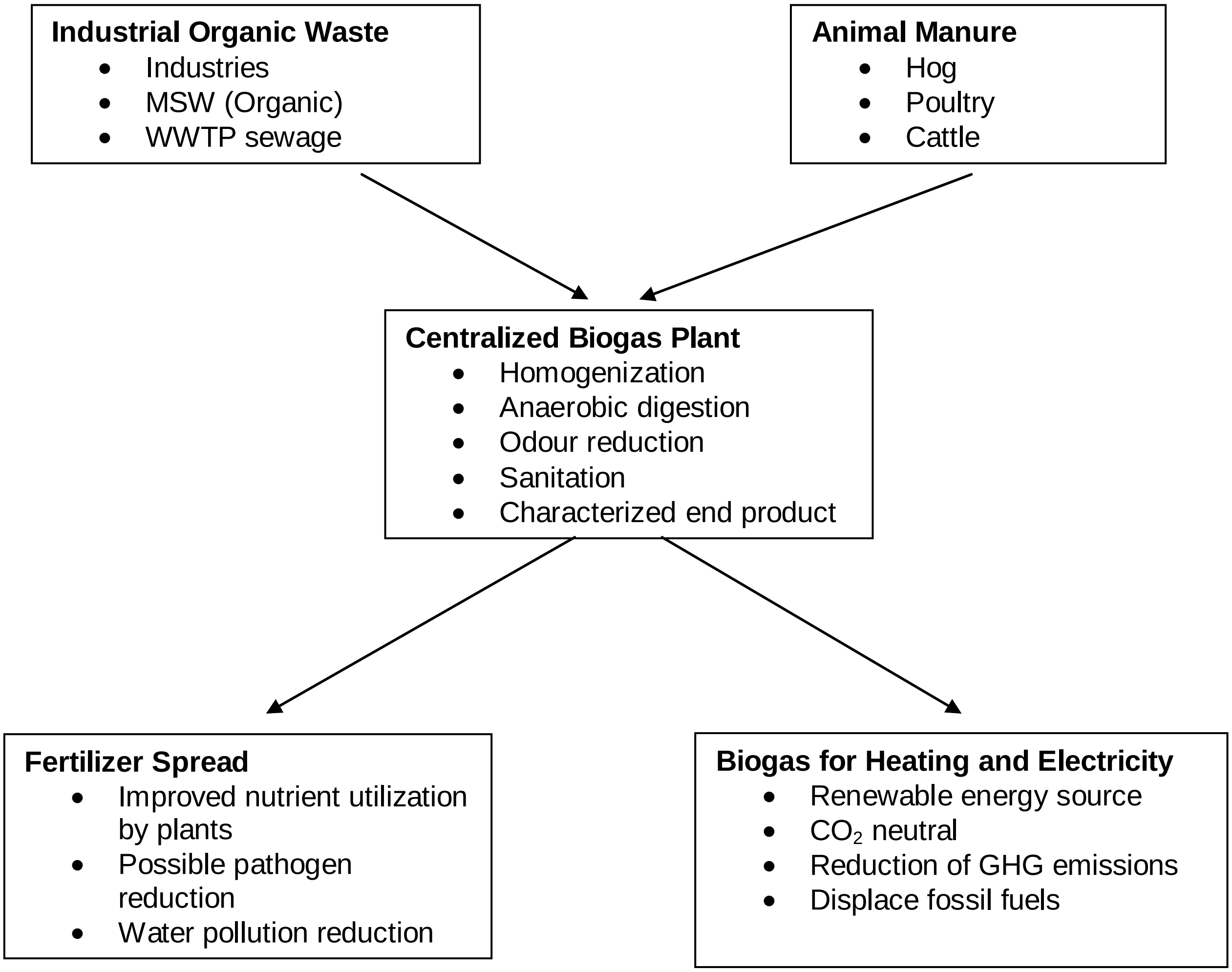


Figure 2.7 Flow diagram of the integrated agricultural/industrial co-digestion process

Many of the agricultural digesters, especially in Germany, are being run on energy crops, such as corn silage, to focus on energy generation, in response to government incentives for biogas production. Weiland (2003) has reported that most biogas plants (~95%) in Germany are farm-scale, with only a few being large centralized facilities. Of these biogas plants, more than 90% are being operated with co-substrates which include a variety of energy crops, digesting with manure. Corn silage is the main energy crop used in Germany for co-digestion, with an energy yield of 16,000 kWh of electricity (plus equal heat) per hectare (ha) (Hilborn et al., 2007).

Unlike Europe, experience with anaerobic digestion in Canada is quite limited. Farm-based anaerobic digestion in North America only began out of necessity for odour control due to urban encroachment (Lusk, 1998). Currently, Canada has approximately 16 farm-scale anaerobic digesters operating or being built (DeBruyn, J., P.Eng., OMAFRA, 2007).

The government of Ontario is actively pursuing AD development in the agricultural sector, with hopes of utilizing biogas for electricity production. In 2006, the Ontario government implemented a Renewable Energy Standard Offer Program (RESOP), which guaranteed farmers a higher rate for biogas-produced electricity, along with a financial assistance program designed to reduce the capital costs of digester construction (Hilborn et al., 2007). There are currently three agricultural biogas systems operating in Ontario, with several more in the design and construction phases.

As mentioned earlier, Manitoba has three anaerobic digesters in operation, which are being used mainly to study their technical and economic feasibility (Cicek, 2007). In order for AD to be successful in Manitoba, it would need to be economically competitive with other forms of energy, as well as be technically reliable. Based on an estimation of

15 W per 100kg hog and a hog population in Manitoba of approximately 3 million, there is potential for 45 MW of electrical power generation from AD (Cicek, 2007). This would only account for about 0.8% of the total production capacity of 5461MW from Manitoba Hydro, but it would still be a considerable amount of green energy production in the province. Currently, the Manitoba government is not as active as the Ontario government in pursuing AD for electricity generation, due to the well-established and cost effective hydroelectricity industry in the province. In a Manitoba context, it may be more efficient to use the biogas produced for heating purposes or the displacement of natural gas instead of electricity generation, due to the very low electricity costs in the province and the large amount of subsidies that would be required. The most efficient use of biogas for heating would be to connect it to a district heating system without having long transmission lines, which is more common in Europe. This can pose a problem however, since farm digesters are usually located some distance from the nearest town which could utilize it. Whatever the end-use for the biogas, the feasibility of using AD in Manitoba will depend on regulations that are put in place by the government to promote renewable energy production. Another obstacle is the cold climate, which can severely reduce the positive energy balance from gas generation. Therefore, more research in temperature control systems and improved insulation needs to be done, as well as taking a closer look at other cold climate digesters, like the hog manure complete-mix digester in Saskatoon, SK., which is operating under similar conditions. The research pilot-scale digester in Glenlea, MB, which is being housed in a passive solar building, should also provide interesting data for enhancing the energy balance in cold climates when it is operating.

2.6 Economics of Anaerobic Digestion

As mentioned earlier, the income for a biogas plant comes from a few sources: the sale of energy, the sale of fertilizer, tipping fees for receiving off-farm waste, and possibly government subsidies. If cost of energy production is too high, then the other option is simply flaring the biogas, to eliminate odours and GHG emissions, however this is also not an economically viable option.

The costs associated with biogas production consist of capital costs (installation) and operational costs (maintenance). Capital costs are mostly influenced by the size of the digester, the heat recovery equipment, and the energy production equipment required, while the operational costs are related to the amount of waste material being treated. These are both dependant on the cost of construction materials and labour, both of which vary depending on the global location. More technologically advanced plants will also cost more than simply designed ones which have less process control. A typical modern anaerobic digester can cost anywhere from $40-$80 per 100 kg hog (USEPA Agstar, 2002). Another estimate from Mueller (2007), was a cost of approximately 8.1 cents per kW for producing biogas electricity in the USA.

The economics surrounding anaerobic digestion have been the biggest stumbling block towards its widespread use in Canada and North America. This has generally been the case due to abundant low-cost energy production from hydro, especially in Manitoba, where consumers only pay approximately 6 ¢/KWh. For cold climate AD to become economically viable in Canada, premium rates (minimum 20 ¢/KWh) would need to be paid to the operator/farmer for biogas-generated electricity, along with subsidies for capital investment (Cicek, 2007). In Ontario, the RESOP guaranties operators/farmers a minimum energy price of 11 ¢/KWh for biogas-generated electricity, along with capital investment funding of up to $400,000 per biogas system (Hilborn and DeBruyn, 2007). In 2004, the German government adopted a feed-in tariff to encourage renewable energy production from biomass, which forced German utilities to pay a premium price of between 11 and 15 cents per kilowatt-hour for biogas produced electricity. There were also additional bonuses for projects which used animal manure, CHP and other advanced technologies, plus there were also financial incentives up to $20,190 offered for the development of small biogas projects (<70KW) (Anders, 2007). It is these kinds of incentives and progressive thinking by governing bodies which has spurred the biogas industry on in Germany and many other parts of Europe, while the lack of them is what has kept North America far behind in this respect.

There seems to be a desire however, to pursue biogas production in North America as governments are investing more into developing renewable fuel technologies, due to the unpredictable nature of fossil fuels and their environmental consequences. There are currently 18 hog manure-based AD systems in the United States, and table 2.7 compares the estimated costs of complete-mix digesters to lagoon systems, while table 2.8 shows the actual costs of installed systems in the USA.

Table 2.7 Estimated Costs of Manure Treatment Options for 2500 Sows (Iowa) (based on Moser et al., 2007).

Treatment Option	**Estimated Treatment Cost**	**Estimated Storage Cost**	**Estimated Total Cost**	**Estimated Return as Propane ($/yr)***	**Cost per hog**
Single cell lagoon (lined)	$372,115	Included	$372,115	0	$55.73
2 cell lagoon, 1 covered	$353,578	$106,491	$460,069	$29,110	$68.90
Complete mix digester	$348,204	$106,491	$454,695	$35,171	$68.09

* electricity cost at $0.05/kWh

Table 2.8 Costs of installed and operating AD systems in the USA (based on US EPA AgSTAR, 2007)

Location and Year Built	**Size**	**AD System**	**Capital Cost**	**Biogas End-use**	**CH_4 Reduction (Mt CH_4/yr)***
Pennsylvania, 2004	4,400 grower/ finishing hogs	Mesophilic, mixed, covered lagoon	$256,620	Heat and Electricity (47 kW)	55
Colorado, 1999	5,000 sow, 1,200 grower	Complete-mix digester, mesophilic	$368,000	Electricity (63 kW)	157
Illinois, 1998	8,300 finishing hogs	Mesophilic, mixed, covered lagoon	$140,000	Hot water and flare (36 kW)	285
North Carolina, 1997	4,000 sows farrow to wean	Covered lagoon	$290,000	Electricity and hot water (108 kW)	140

*(Mt CH_4/yr = Metric tons of methane per year)

Many of the older digester systems that were installed on hog farms in the 80's, were used mostly for controlling odour, however some have been very successful ventures financially as well. Rocky Knolls Farms digester in Lancaster PA., cost approximately $325,000 to install, and produces up to $100,000 of useable energy every year, while Valley Pork in PA., cost $250,000 to install and produced about $65,000 in energy (Moser et al., 2007). The reason that these farms succeeded, was because they had good professional design, operation and management, which is where many previous farm digestion operations failed. Farm digesters are expensive when compared to other manure storage options, and they do require some expert knowledge, however if they are designed and operated properly, they can prove to be valuable investments under a proper economic structure.

2.7 Summary

Anaerobic digestion is a multi-step, biological process which converts insoluble organic matter into CH_4 and CO_2. This process however, requires the particular control of certain environmental parameters (pH, temperature, loading, SRT) to operate in an efficient manner. If any one of these parameters is out of the “optimal” range it can be the result of, or result in, a system imbalance, which is important because successful digestion depends on the symbiotic relationship of the bacterial species. The benefits of AD, are not simply the production of a renewable fuel, but also the reduction of GHG emissions and odours, which are a problem for many open lagoon treatment systems found on farms in Canada. Co-digestion of different waste products is an interesting option, since it can enhance the AD process and increase biogas production. The result is an alternative waste disposal method and increased energy yields, which lead to greater profits. This idea is being used now on many farm-scale digesters, especially in Europe, where the excess biogas produced is used for electricity and heat generation.

The process of enhancing energy production from co-digestion of manure with other waste products may be the first step towards the feasibility of AD in Canada, along with government incentive programs, which have be shown to drive this technology in other parts of the world.

CHAPTER 3 MATERIALS AND METHODS

3.1 Semi-Continuous Experiment

Mesophilic, complete-mix anaerobic digesters were chosen for determining the effects glycerol addition has on the digestion process, and biogas production. Four bench-scale, complete-mix anaerobic digesters were operated at 35±1°C in a constant-temperature environmental chamber, at the University of Manitoba. The working volume of each digester was 3.5 litres with an additional 0.5 litres of headspace. The biogas produced was collected in a separate bottle, which contained an acid-salt solution to prevent the gases from dissolving into the liquid. This bottle was connected to a similar bottle in series, for equalization of the liquid to determine the volume of gas produced. The configuration of the digesters is presented in figure 3.1.

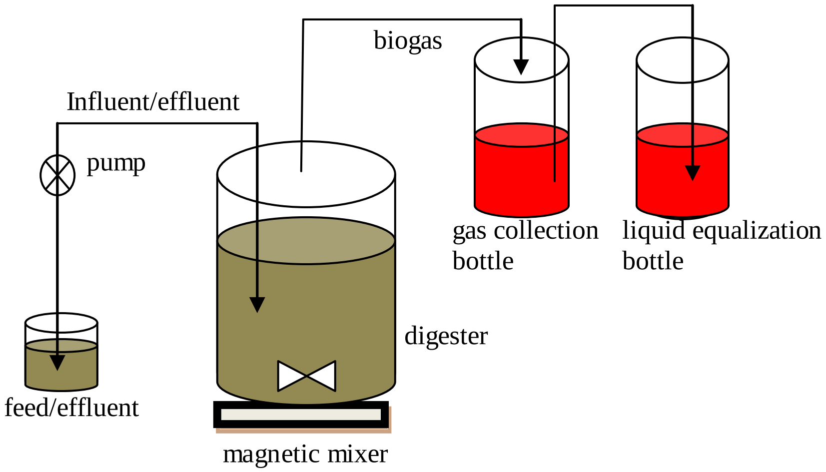


Figure 3.1 Configuration of the lab-scale anaerobic digestion laboratory set-up

The feed and effluent of the digester were manually pumped in and out of the same port with a peristaltic pump, with the effluent being pumped out first. The tubing used for feeding and effluent extraction was cleaned periodically, due to the buildup of residuals. The digesters were constantly mixed with a magnetic mixer. All ports were sealed and the reactor was not opened during the experiment, to ensure anaerobic conditions were maintained, and to prevent biogas from escaping. The gas transfer tubing was also coated in a sealant to prevent biogas from permeating through. Photographs of the digester set-up can be found in Appendix B.

Dilute hog manure and glycerol were fed to the reactors at a rate of 200 ml/day, resulting in a hydraulic retention time of 17.5 days. The raw hog manure was obtained from Cook Feeders farm, a 6000 head finishing hog operation, located in Teulon, Manitoba. This manure was delivered to the University of Manitoba, whenever needed, where it was screened through a 1.2 mm sieve to remove any large particles which might cause clogging in the tubing and restrict mixing. The manure was then sealed and stored at 4°C, until needed, to reduce the amount of bacterial activity. The manure was also diluted with varying amounts of water to equalize the organic loading before feeding. This dilution occurred each day as the feed was prepared, and then the feed was placed in the environmental chamber with the digesters, to allow the temperature to equalize at 35°C and prevent any temperature shock loading effect.

These digesters were fed semi-continuously (once a day) with the control reactor being fed only hog manure, while the other three being fed mixtures of manure with 1% (1.6 ml), 2% (3.2 ml) and 4% (6.3 ml) glycerol by weight, which was equivalent to 1.7, 2.3, and 4.3 times the COD load respectively. The digester fed 4% glycerol was used in a second run at a later time, after re-seeding, for a total of 5 runs. The amount of glycerol addition was chosen based on the additional COD load and the calculation of volume equivalent can be found in Appendix A. For the first 4 runs, the glycerol used was laboratory grade (99% pure). For the 5th run, crude glycerol, obtained from a biodiesel production unit in Manitoba, (containing some residual salts and methanol) was added at the 1% (by weight) level to compare with the “pure” glycerol. The reactors were operated for 57-88 days (runs of varying length). The digesters were originally seeded with digested anaerobic sludge from the mesophilic anaerobic digesters at the North End Water Pollution Control Center in Winnipeg, Manitoba, however the re-seeding which occurred later on, used digested effluent from the control reactor in the experiment.

3.1.1 Sample Collection and Analysis

The biogas produced was sampled from the digester headspace for quality analysis, and collected in gas collection bottles for volume determination using liquid displacement. The biogas production rates were recorded daily and gas samples were taken from the reactor headspace regularly (at least once per week).

The digester effluent was wasted every day, and samples were taken typically twice per week from this effluent. The sampling location was the center of the digester, and the sample size was 200 ml. The digester effluent was characterized for alkalinity, pH, COD, total solids (TS), and volatile solids (VS) on a regular basis according to standard methods (APHA 2005). These parameters were measured immediately after the samples were collected. COD, TS, and VS analysis was always done in duplicate. Alkalinity was measured according to standard methods, titration method 2320B, where the standard acid (0.02N H_2SO_4) was recorded and used to calculate alkalinity concentration according to equation 3.1 shown below:

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{ml\ sample} \qquad \text{Eq.(3.1)}$$

where :
A = mL of standard acid used and
N = normality of standard acid

Volatile solids reduction was calculated using the mass balance approach below:

$$VSR = \frac{\text{loss in volatile solids}}{\text{sum of volatile solids inputs}} \qquad \text{Eq.(3.2)}$$

Gas composition analysis (CH_4 and CO_2), as well as volatile fatty acid (VFA) analysis, was done using gas chromatography (Varian CP8410, GC) with a flame ionization detector. Gas composition analysis was done once per week, and VFA samples were taken twice per week, but frozen immediately and analyzed once every two weeks.

Total COD was measured twice per week with a modification of the standard methods (APHA, 2005), using HACH method 8000 digestion vials.

Samples were also analyzed for NH_3-N, total nitrogen (TN), PO_4, and total phosphorus (TP). These samples were taken and analyzed every second day for a two-week period, once the digesters were well-established and had stable gas production. Total and soluble phosphorus analysis was done in duplicate, according to standard methods (APHA, 2005) using a Lachat QuickChem 8500 flow injection analyzer. Total nitrogen and NH_3-N analysis was done in duplicate, with a modification of standard methods (APHA, 2005), using HACH method 10031 for NH_3-N, and HACH method 10072 for TN. A one-time 50 ml sample of crude glycerol was taken and sent to Manitoba Hydro Laboratories in Selkirk, Manitoba for analysis of salts and methanol.

The theoretical methane potential of glycerol was calculated using the Buswell formula shown below (Ma et al., 2008):

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_20 \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3$$

$$C_3H_5(OH)_3 \rightarrow 1.75CH_4 + 1.25CO_2 + 0.5H_2O \qquad \text{Eq.(3.3)}$$

Statistical analysis on data (standard deviation and two-tailed T test) was done using Microsoft Excel 2007.

3.2 Batch Experiment

The purpose of this batch experiment was to determine the biogas and methane potential by using different ratios of pure and crude glycerol to raw hog manure.

Two separate batch experiments were run using smaller 160 ml serum bottles, under mesophilic conditions (35°C±1), in a temperature-controlled environmental chamber. Both experiments used raw, unscreened and undiluted hog manure (~5% TS) obtained from the same source as in the semi-continuous experiment (Cook Feeders Farm). The hog manure was delivered to the university lab in chilled coolers and stored at 4°C until used. The inoculum used as a seed, was the effluent from the digested, semi-continuous control reactor, which was fed only dilute hog manure. All of the serum bottles were filled to 60 ml with seed and hog manure, plus glycerol, except for one reactor which used no hog manure. This left approximately 100 ml of headspace in the bottles which was flushed with nitrogen gas to minimize oxygen contamination. The bottles were sealed with rubber stoppers and clamped with metal caps for the duration of the experiment. Both experiments were run for 16 days. The bottles were shaken by hand twice a day to encourage mixing. Each condition was run in duplicate to determine experimental error.

3.2.1 Batch Experiment 1

The first batch experiment was run with six different combinations of seed with raw hog manure, and pure glycerol in varying concentrations (See table 3.1). The control reactor contained 90% seed (54 ml) with 10% hog manure (6 ml). Reactors 1-4 contained a similar mixture of seed and hog manure, but with slightly less seed, to allow for the addition of 0.5%, 1%, 2%, and 4% of glycerol (by weight). Reactor 5 contained 100% seed (60 ml), with 1% glycerol, and was run to determine the effect of glycerol, without hog manure, on gas production.

Table 3.1 Set-up for both batch experiments with volumes of feed added

Reactor	**Inoculum (ml)**	**Hog Manure (ml)**	**Glycerol (% by weight)**	**Glycerol (ml)**	**Total Volume (ml)**
Control	54	6	0	0	60
R1	53.76	6	0.5%	0.24	60
R2	53.5	6	1%	0.5	60
R3	53	6	2%	1	60
R4	52.1	6	4%	1.9	60
R5	59.5	0	1%	0.5	60

3.2.2 Batch Experiment 2

The second batch experiment that was run was virtually identical to the first batch experiment, but crude glycerol was used instead of pure glycerol. Experiment 2 was done at a later time, so fresh manure and seed were used in this experiment, but all of the other environmental conditions and feeding ratios were kept the same as in the first experiment (See table 3.1).

3.2.3 Sample Collection and Analysis

Samples were collected and analyzed for TS, VS, COD, pH, CH_4, CO_2, and total biogas. Liquid samples were analyzed at the beginning and end of the experiments, while gas samples were taken every few days throughout experiments. Volume of biogas produced was measured using syringe displacement, while CH_4 and CO_2 samples were taken from the headspace and were analyzed in the biogas by gas chromatography (Varian CP8410, GC). Measurements of pH, TS, and VS were done according to Standard Methods (APHA, 2005), while COD was measured with a modification of the standard methods (APHA 2005), using HACH method 8000 digestion vials.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Semi-Continuous Experiment

During the start-up period, before adding glycerol to any of the digesters, gas leaks were repaired, and stable, similar gas production was observed in all 4 digesters. This start-up period lasted approximately 60 days (figure 4.1).

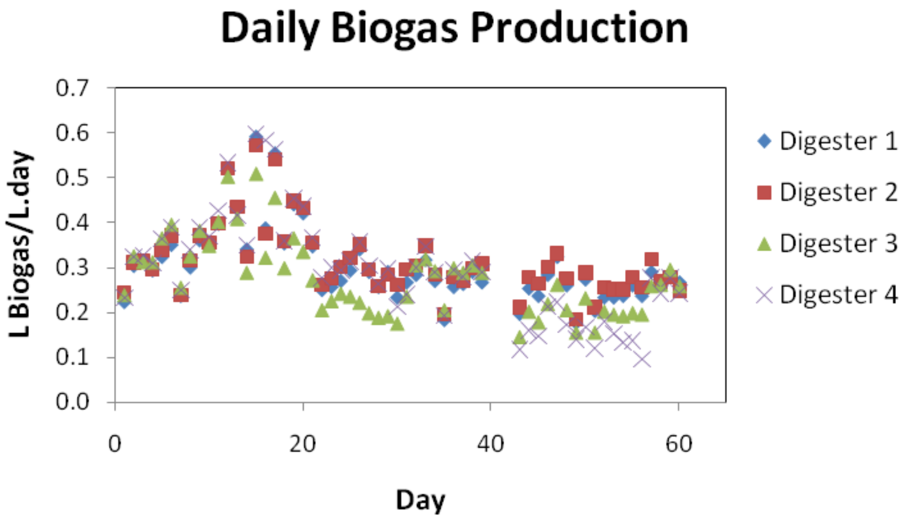


Figure 4.1 Daily biogas production in all four digesters during the start-up period, before glycerol addition

Once glycerol was added to the three treatment digesters at varying levels, stable gas production was observed in the 1% crude glycerol digester after 8 days of glycerol feeding. The 1% pure glycerol digester was operating steadily after approximately 20 days of feeding, while initially the 2% and 4% glycerol digesters never achieved steady gas production. The values shown below for the 2% glycerol digester were determined during the second attempted run, which did not stabilize until approximately 45 days after the second glycerol feeding. The results shown below were average values from the digesters for the experimental period, once they were determined to be operating at a steady state, as indicated by a constant methane yield and low VFA-to-alkalinity ratio described by Schaefer and Sung (2008). The feed, effluent, and gas characteristics of all 5 digester runs from the semi-continuous experiment are summarized in table 4.1.

Table 4.1 Characteristics of the feed, effluent and gas for all 5 anaerobic digester runs

Treatment	*COD loading (g/l day)*	*pH*	*Alkalinity (mg/l $CaCO_3$)*	*% Volatile Solids Reduction*	*% COD removal*	*% CH_4*	*CH_4 Yield (m^3 CH_4/kg COD_{rem})*
Control (manure only)	1.05 (±0.20)	7.6 (±0.02)	6515 (±554)	43 (±11)	61 (±7)	71 (±2)	0.4 (±0.14)
1% pure glycerol + manure	1.8 (±0.23)	7.4 (±0.03)	6408 (±434)	42 (±12)	70 (±3)	63 (±2)	0.37 (±0.06)
1% crude glycerol + manure	1.96 (±0.25)	7.5 (±0.03)	6758 (±488)	48 (±9)	74 (±3)	67 (±1)	0.36 (±0.05)
2% pure glycerol + manure	2.46 (±0.26)	7.3 (±0.07)	5610 (±194)	67 (±3)	72 (±2)	66 (±4)	0.41 (±0.06)
4% pure glycerol + manure	4.11 (±0.38)	NA	NA	NA	NA	NA	NA

NA – not available, due to digester instability

4.1.1 Shock Loading Performance.

During the first run, the two digesters which were fed 2% and 4% glycerol amendment showed almost an immediate doubling of biogas and methane production after just 3 days of feeding glycerol, however both failed and were not able to reach a steady state (Figure 4.2).

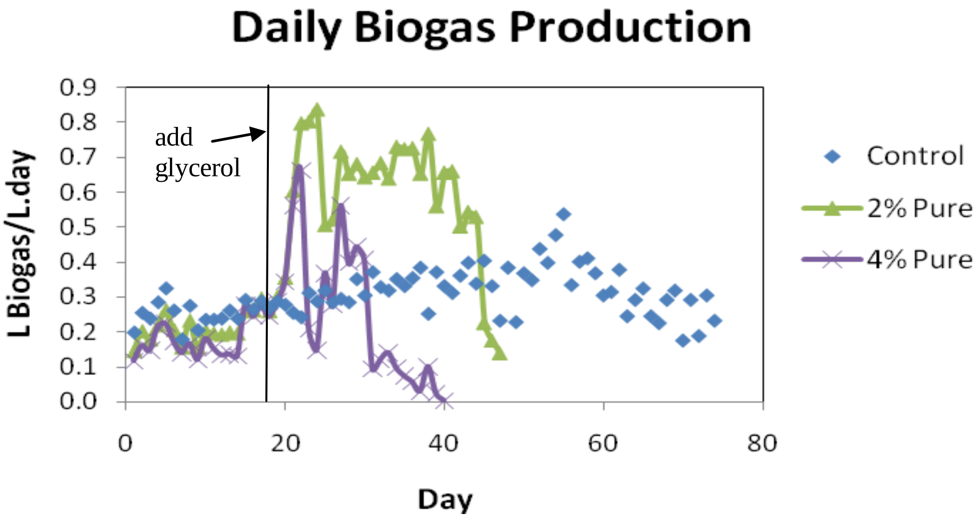


Figure 4.2 Biogas production after a shock loading with 2% and 4% glycerol amendment

The quick response in gas production was likely due to the breakdown of the readily biodegradable soluble COD in the glycerol, however due to the sustained high COD loading, a build-up of intermediate VFAs occurred, which lead to a substantial pH drop and resulted in an unstable system (Figure 4.3).

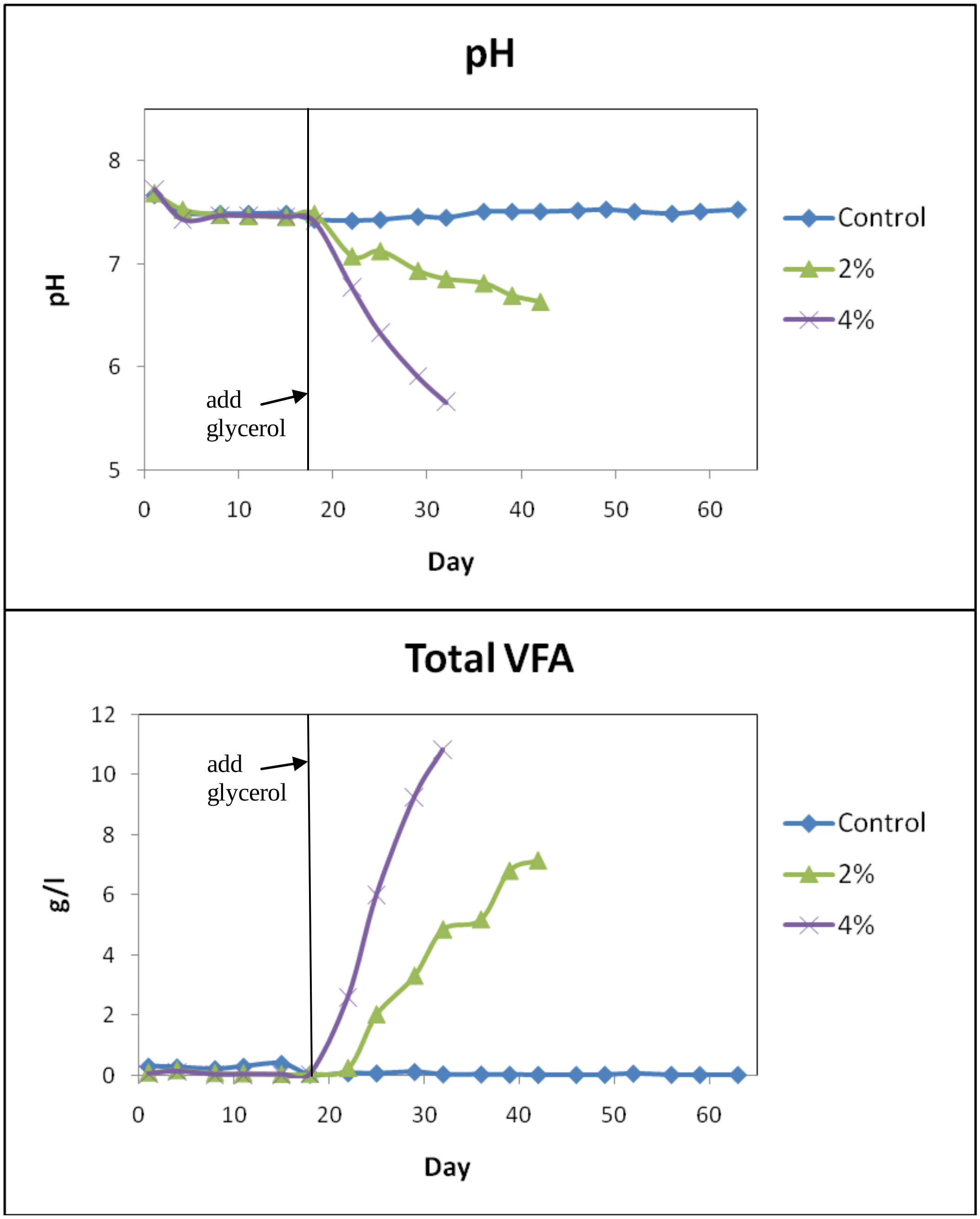


Figure 4.3 The effect of high COD shock loading (2% and 4% glycerol) on pH and VFA

The 4% glycerol digester exhibited a sharp decline in gas production after feeding glycerol for approximately 5 days, and essentially stopped producing biogas after 12 days. This corresponded to a steady drop in pH down to a low of 5.66, and a build- up of high levels of VFAs to levels over 10000 mg/l; an environment which inhibited methanogenic activity. The feeding of glycerol was stopped, however the bacteria in this digester were not able to recover, and re-seeding was required to re-establish it. The 2% glycerol digester showed a slower shock effect, but it also failed to stabilize. It began building up high levels of VFAs to a maximum of over 7000 mg/l after approximately 25 days, which corresponded to the sharp reduction in biogas and methane production, at which time glycerol feeding was stopped and the reactor was allowed to recover by feeding only hog manure. During this run there was no shock loading effect observed with the 1% pure glycerol digester and gas production remained relatively stable throughout operation (Figure 4.4).

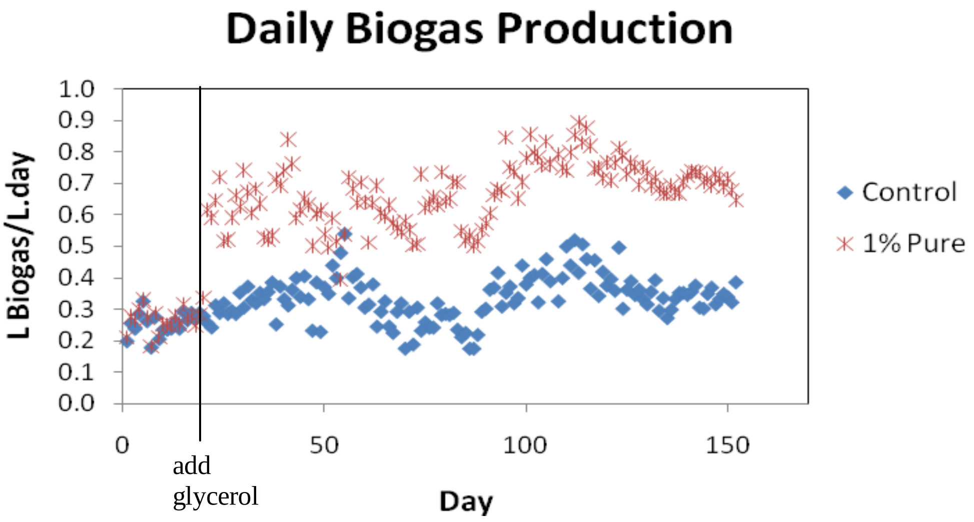


Figure 4.4 Biogas production with the addition of 1% pure glycerol

In addition to stable gas production throughout, VFA production was relatively low and stable in the 1% pure glycerol digester, which was similar to the values in the control reactor on average (Figure 4.5).

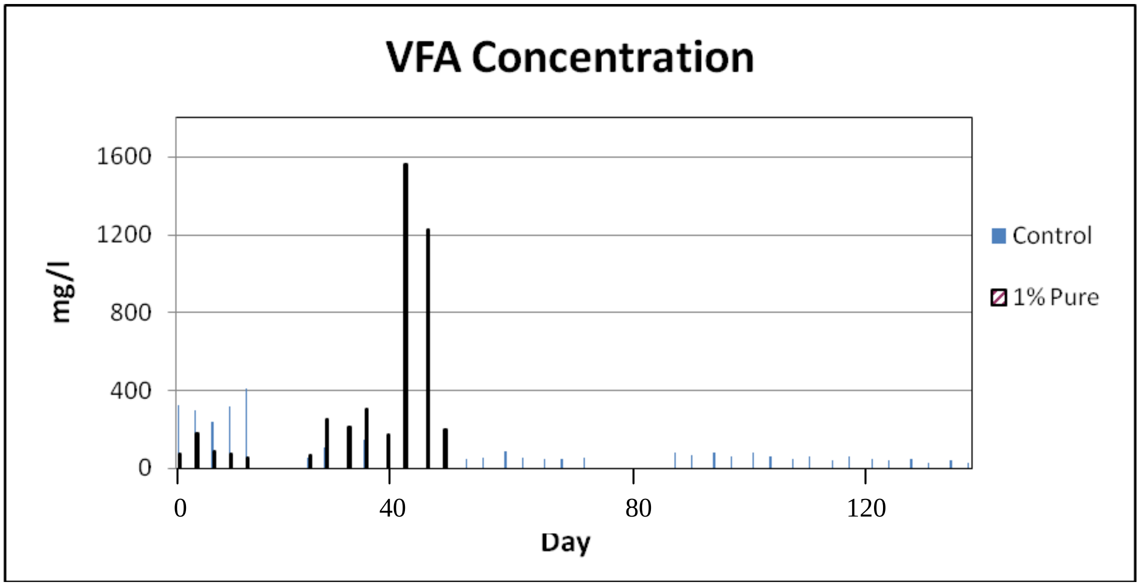


Figure 4.5 VFA concentration in the 1% pure glycerol digester compared to the control digester

This demonstrates that the bacteria were able to handle the addition of almost double the COD load without suffering from a build-up of intermediate acids, which the acetogenic and methanogenic bacteria could not utilize.

4.1.2 Digester Recovery

The digester with the 2% glycerol amendment was allowed to recover after the shock loading by feeding it only hog manure for a period of 45 days, until it had stabilized to control conditions, and then it was fed 2% glycerol again to test the bacterial tolerance to the sustained high COD loading. During the second feeding stage with 2% glycerol, the bacteria in the digester were more resilient, and after 88 days, this digester was producing approximately 2.5 times the methane than the control digester, and almost 3 times the biogas. This could also be an indication of the glycerol-utilizing bacteria out-competing the slower growing heterotrophs. Once again VFA's built up in the digester initially, but only to a maximum of approximately 4000 mg/l after 25 days, before they began to decrease as they were being consumed and converted to biogas. Gas production in this digester showed a steady increase throughout the run, until it stabilized, indicating that bacteria continued to convert the excess VFA's into biogas and methane as seen in figure 4.6, while the methane content in the biogas also increased over time from a low of 49% to a high of 75%. The VFA and gas composition data is shown in figure 4.12.

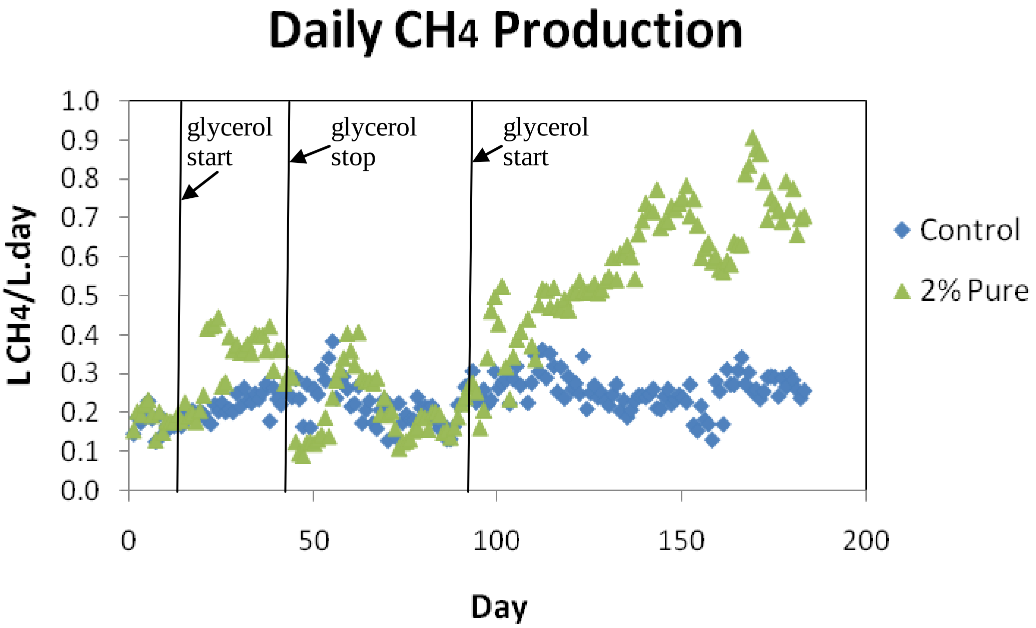


Figure 4.6 Methane production in the 2% glycerol digester before and after bacterial recovery

4.1.3 Pure vs Crude Glycerol

Two digesters were operated side by side and compared; one with a feed of hog manure plus 1% pure glycerol and the other with a feed of hog manure plus 1% crude glycerol, to determine whether impurities in the crude glycerol, such as methanol and salts, have any detrimental effect on the AD process as it relates to biogas and methane production. The feed COD for the 1% crude glycerol digester was slightly higher than the 1% pure glycerol digester, indicating that there was some additional carbon component with a higher COD than glycerol, such as methanol, in the crude glycerol. Analysis confirmed that methanol was present in the crude glycerol sample, as well as salts (See table 4.2).

Table 4.2 Results of composition analysis of crude glycerol

Component	**Analysis Method**	**Quantity**
Sodium (Na)	ICP	10000 mg/l
Calcium (Ca)	ICP	6.95 mg/l
Magnesium (Mg)	ICP	4.03 mg/l
Potassium (K)	ICP	7.52 mg/l
Methanol	GC/FID	>1%

Unfortunately exact methanol content could not be determined, however literature confirms that methanol content in crude glycerol is approximately 8% in conventional biodiesel production systems which incorporate methanol recycling and reuse (Kemp, 2006; Mittelbach and Remschmidt, 2004).

During the comparison experiment, which lasted 57 days, both digesters behaved similarly, with the 1% crude glycerol digester showing slightly higher methane content, COD removal, and volatile solids removal, and the 1% pure glycerol digester showing a slightly higher biogas yield. Overall, biogas and methane production were very similar, with both producing approximately double the amount of biogas compared to the control reactor that contained manure alone at steady state operation (figure 4.7 and figure 4.8).

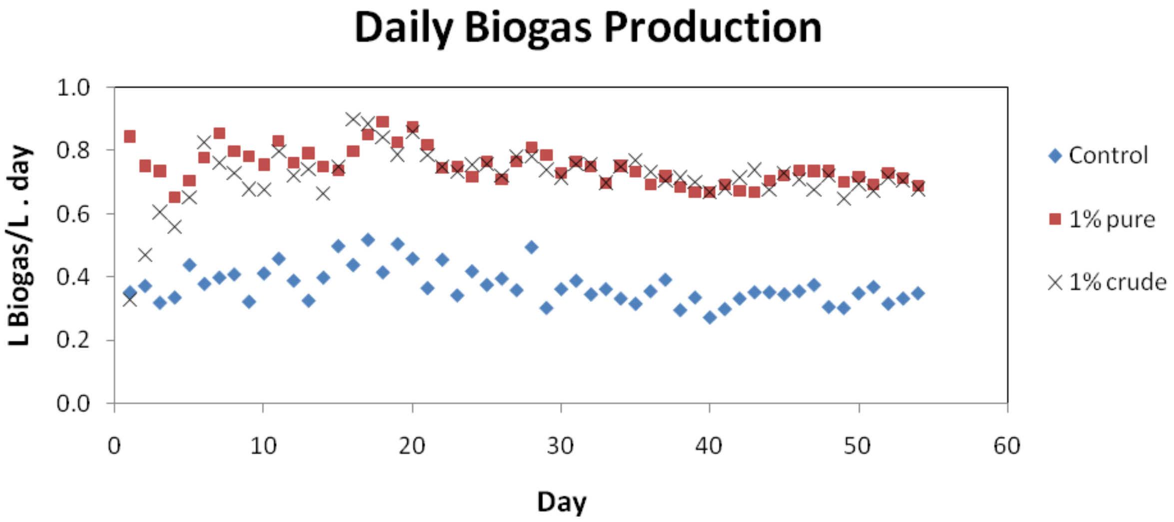


Figure 4.7 Comparison of biogas production in digesters with 1% pure and 1% crude glycerol amendment

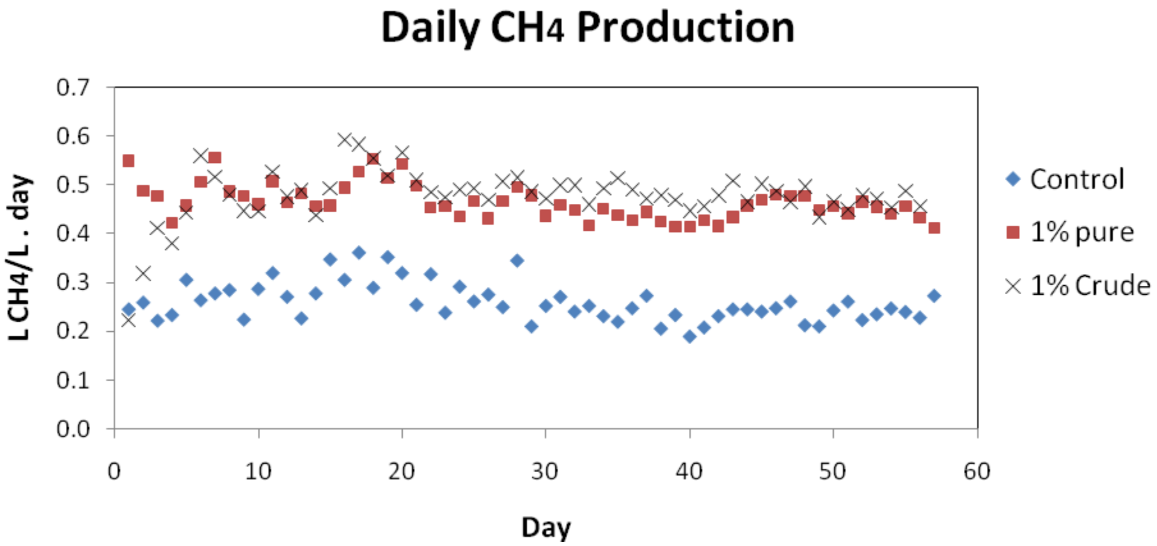


Figure 4.8 Comparison of methane production in digesters with 1% pure and 1% crude glycerol amendment

The 1% pure glycerol digester had been running for 76 days before this experiment began, which explains why the level of biogas and methane production is at a higher level initially than the digester with the 1% crude glycerol addition. The 1% crude glycerol digester however, stabilized quite quickly and reached similar gas production as the 1% pure glycerol digester within approximately 7 days. Overall, the crude glycerol digester behaved virtually the same, or slightly better than, the pure glycerol digester and did not exhibit any detrimental effects due to contaminants such as methanol or salts which were present. Calculations of salt accumulation in the digester fed with crude glycerol are found in appendix C. These calculations show an eventual steady state concentration of salt (Na) in the digester, of 80 mg/l after approximately 90 days. This sodium concentration would not be expected to cause toxicity to methanogenic bacteria (Bashir and Matin, 2004).

4.1.4 Biogas and Methane Production/Yield.

As seen in figures 4.7 and 4.8, the biogas production followed a similar trend to the methane production in both of the 1% glycerol digesters, producing approximately double the methane and biogas of the control digester. In the 2% glycerol digester, the daily increase in methane production (0.391 L) was lower than the increase in biogas production (0.683 L) when compared to the control, due to the lower methane content in the biogas. The theoretical methane potential from glycerol alone, based on the Buswell formula and the ideal gas law, is 0.47 litres of methane per gram of pure glycerol, and 0.44 litres of methane per gram of crude glycerol (Ma et al. 2008). In the 1% and 2% pure glycerol digesters, the actual methane yield of glycerol was 0.37 and 0.35 l/g glycerol respectively, and 0.4 l/g glycerol for the 1% crude glycerol digester (Table 4.3). The higher methane yield in the crude glycerol may have been the result of the methanol in the crude glycerol, which is another source of easily degradable COD for the bacteria to utilize.

Table 4.3 Comparison of methane yield for glycerol in the pure and crude glycerol digesters

Parameters	*1% pure glycerol*	*1% crude glycerol*	*2% pure glycerol*
Methane production (l CH_4/g glycerol)	0.37	0.4	0.35
Theoretical methane production (l CH_4/g glycerol)	0.47	0.44	0.47
% Theoretical production	79	91	74

The actual methane yield of glycerol in the digesters was based on the measured methane produced from the manure plus glycerol digesters, minus the methane produced from the control digester with manure alone. The actual methane yield should always be lower than the theoretical value, because part of the substrate feed will always be used for cell growth, and some substrate will leave the digester without being degraded (Poulsen 2003).

The biogas yield was quite similar among the 4 digesters, ranging between 0.53 and 0.62 m^3 Biogas/kg COD_{rem}, with the 2% glycerol digester showing the highest biogas yield and the 1% crude glycerol digester having the lowest biogas yield (figure 4.9).

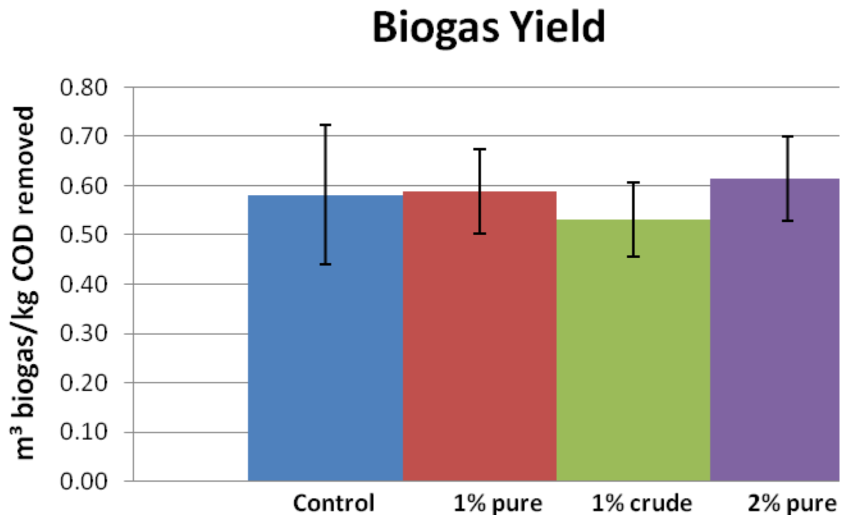


Figure 4.9 Average biogas yield of digesters with 1% and 2% glycerol amendment

There was no significant difference in the biogas or methane yield ($p>0.05$) between all three treatments and the control digester. The lower biogas yield in the 1% crude glycerol digester is the result of having a higher COD load, but the same biogas production as the 1% pure glycerol digester. The methane yield was also similar among all 4 digesters, ranging between 0.4 and 0.36 m^3 CH_4/kg COD_{rem} (figure 4.10), with both of the 1% glycerol digesters showing slightly lower CH_4 yields than the control, and the 2% glycerol digester having a slightly higher CH_4 yield. The theoretical methane yield of manure is 0.35 m^3/kg COD_{rem}, but the exact yield depends on various environmental conditions, such as feedstock, temperature and microbial populations (Lusk, 1998).

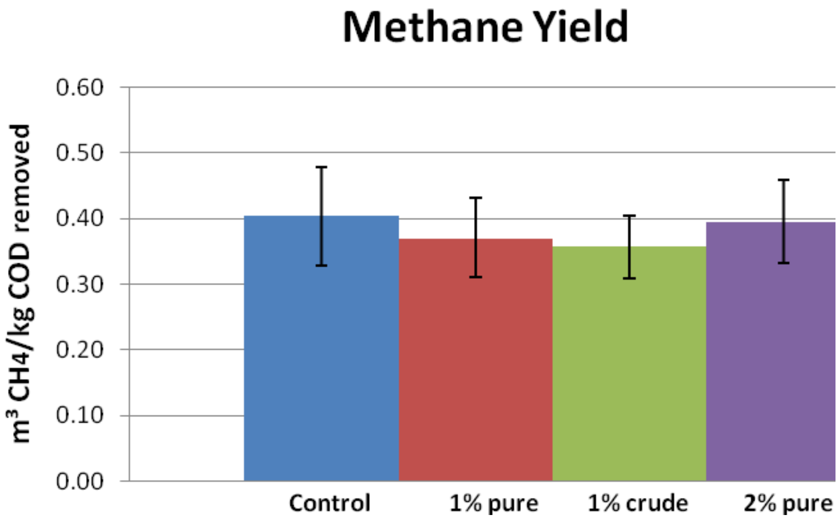


Figure 4.10 Average methane yield of digesters with 1% and 2% glycerol amendment

The addition of glycerol, despite increasing the amount of overall biogas and methane produced, actually resulted in a reduction of methane composition in the biogas, while increasing the composition of carbon dioxide. The methane content in the biogas of the control digester was the highest at 71% CH_4, but remained relatively high in all 3 glycerol treatments, and ranged from 63% CH_4 in the 1% pure glycerol digester to 67% CH_4 in the 1% crude glycerol digester. The 2% glycerol reactor had a surprisingly high CH_4 content of 66%. The majority of the remaining gas produced in the glycerol digesters was carbon dioxide (figure 4.11).

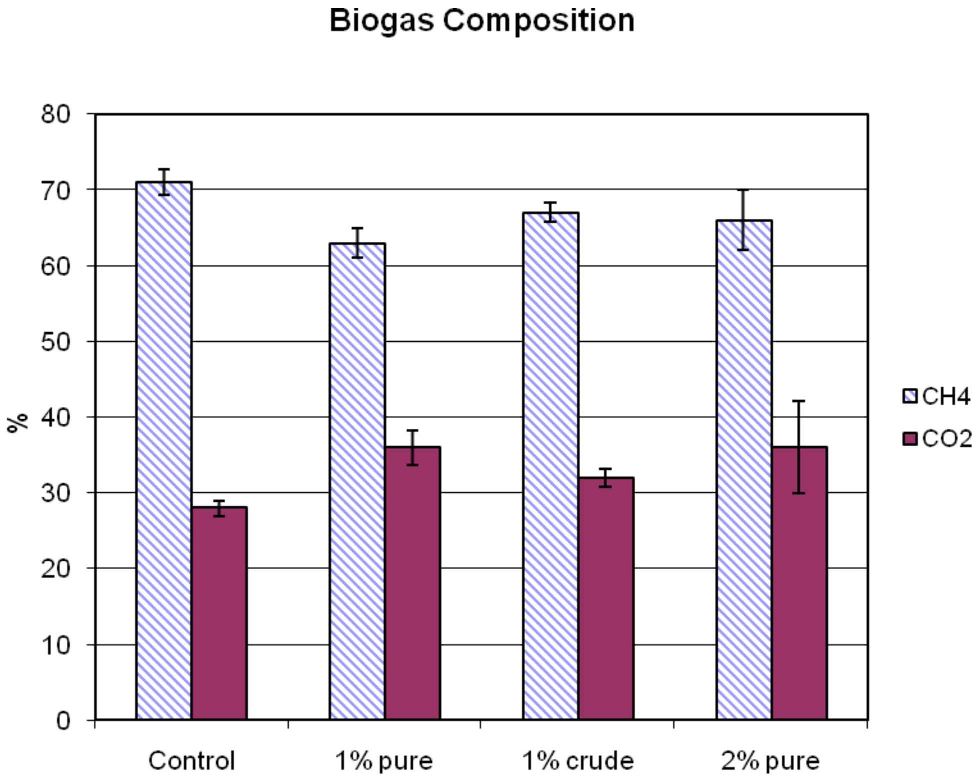


Figure 4.11 Average composition of biogas in all 4 digesters

Throughout the stabilization period, a decrease in methane concentration corresponded to an increase in overall VFA production and more specifically the production of propionic acid, while a decrease in propionic acid led to a higher methane content in the biogas, as can be seen in figure 4.12.

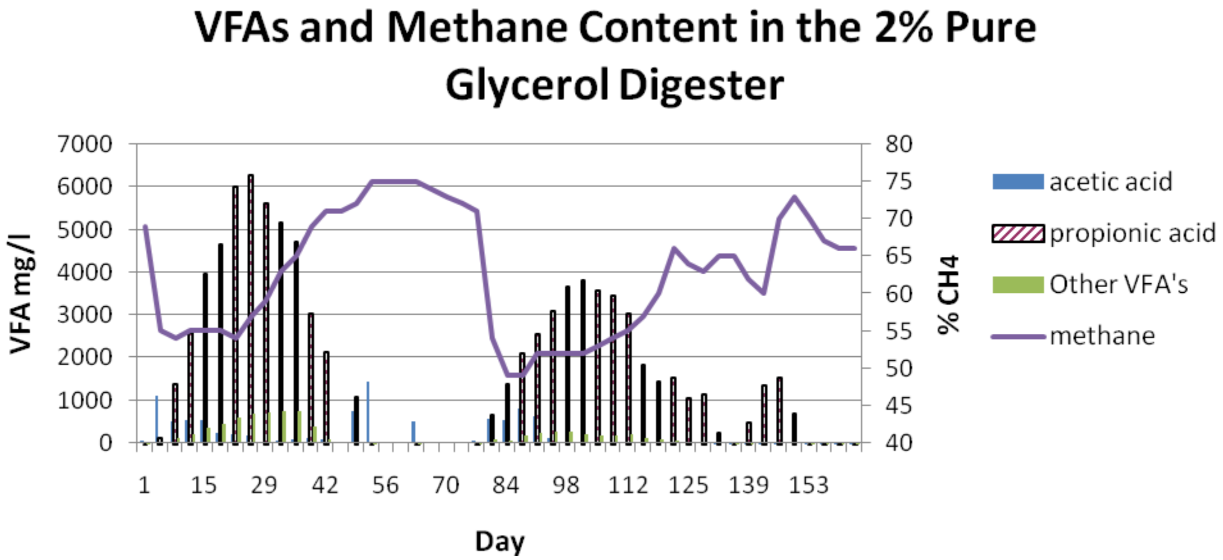
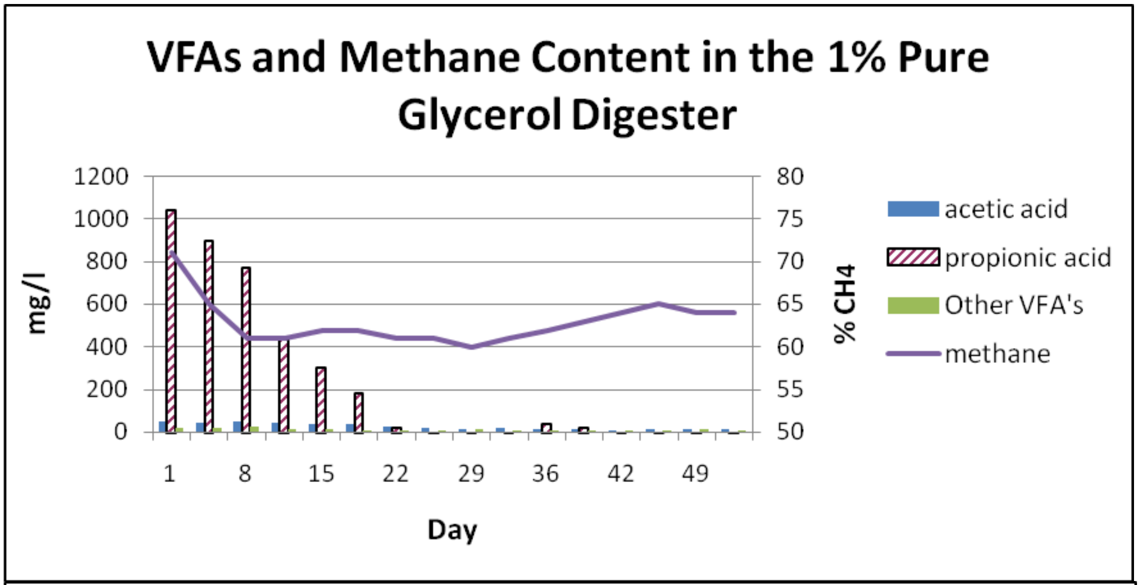


Figure 4.12 The effect that change in propionic acid concentration has on methane content in the 2% glycerol digester

This relationship was seen most dramatically in the 2% glycerol digester, whereas levels of VFAs in the 1% glycerol digesters remained relatively low throughout the experiment (Figure 4.13). As mentioned earlier, the degradation of propionic acid has been reported to be the slowest of all VFAs, making it a good indicator of the microbial activity of propionate degraders. Therefore, a build-up of propionic acid, as was seen in the 2% glycerol digester, suggests that initially, the acidogenic bacteria were actively breaking down glycerol to propionic acid at a faster rate than the acetogenic bacteria were able to convert it to acetic acid. Further microbial analysis would need to be done to fully understand the microbial growth rates and activity of the various acetogenic and methanogenic species.



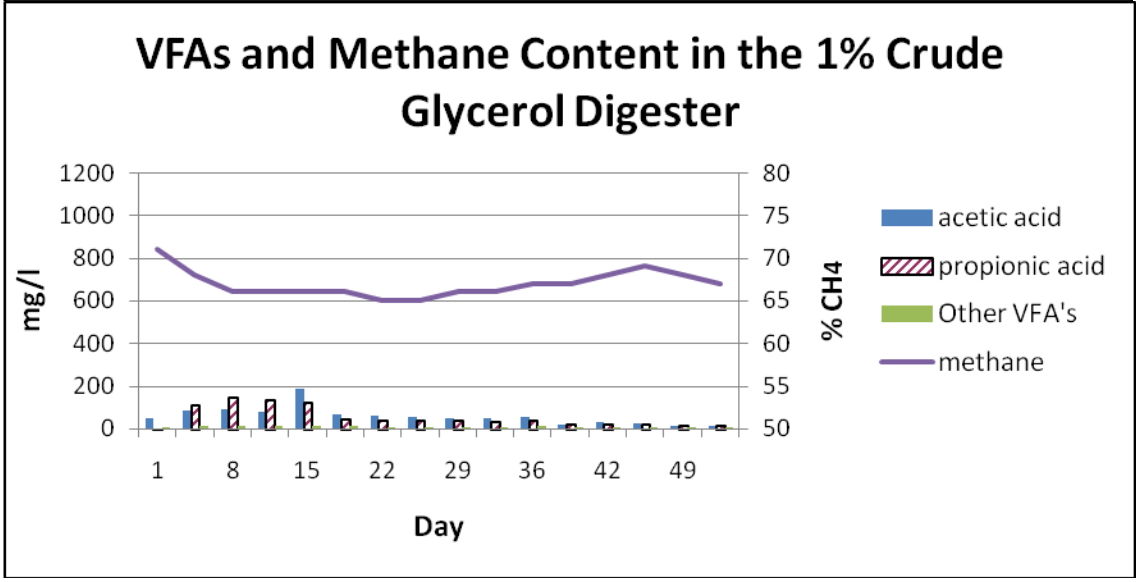


Figure 4.13 Comparison of methane composition and VFA concentration in 1% glycerol digesters

The increase in CO_2 concentration may have simply been the result of a decrease in the production of methane, due to inhibition from the increased organic load. A longer SRT may be required to achieve higher quality biogas (increased methane) with higher levels of glycerol addition, because it would allow all of the bacteria cultures more time to fully consume the glycerol and manure substrate. A study by Qatibi et al. (1991) revealed the anaerobic degradation of glycerol by a mixed culture, lead to the formation of 1,3 propanediol prior to propionate and acetate formation. In that study, the propionate accumulated to higher levels than the acetate and also degraded slower.

4.1.5 Nutrient Analysis

Analysis was done on nitrogen and phosphorus in the digesters to determine their fates and how they change through digestion with and without glycerol addition. Typically through the anaerobic digestion process, organic nitrogen and phosphorus are hydrolyzed through microbial activity, to more soluble forms (NH_3 and PO_4). These soluble forms of N and P are much easier for plants to uptake, making the effluent a valuable fertilizer. Results of nutrient analysis done on effluent of the four digesters (control, 1% pure, 1% crude, and 2% pure) is summarized in table 4.4.

Table 4.4 Nutrient analysis of digester effluent measuring N and P

Digester Treatment	**TN (mg/l)**	**NH_3-N (mg/l)**	**% NH_3-N/TN**	**TP (mg/l)**	**PO_4 (mg/l)**	**% PO_4/TP**
Control	1686 ±79	1240 ±62	74	378 ±15	219 ±25	58
1% Pure glycerol + manure	1686 ±79	1179 ±50	70	378 ±15	229 ±16	61
1% Crude glycerol + manure	1686 ±79	1238 ±55	73	378 ±15	232 ±38	61
2% Pure glycerol + manure	1686 ±79	1093 ±42	65	378 ±15	194 ±11	51

The TN and TP values in table 3 were averaged together from all of the digesters, as it was assumed that TN and TP are the same before and after digestion, and that glycerol does not contain any N or P. From this analysis, it appears that both NH_3-N and PO_4

production was quite similar among the control and 1% glycerol digesters, but was significantly lower ($p<0.05$) in the 2% glycerol digester. This suggests that there was a "normal" level of hydrolysis of the manure occuring in the digesters with 1% glycerol addition. It also suggests that there was a reduction in the level of hydrolysis of manure in the digester with 2% glycerol addition. A reduction in hydrolysis could indicate that the bacteria were overloaded with glycerol and could not degrade the manure as effectively, because their metabolism was shifted towards utilizing the more easily degradable glycerol rather than the manure. A more thorough bacterial analysis would need to be conducted in order to determine whether there was a shift to larger populations of glycerol utilizing bacteria.

4.1.6 Summary

This semi-continous experiment with manure and glycerol showed some very interesting trends. First, this experiment showed that in a low-solids digestion slurry of hog manure, a shock loading of 2.5 times and over the regular COD load from 2% and 4% glycerol addition, led to a system failure through the build-up of intermediate VFAs, and subsequently a drop in pH to toxic levels. The digestion slurry however, was able to handle a doubling of the COD load from adding 1% glycerol, and essentially produce twice the amount of biogas and methane, and produce a similar quality effluent as the digestion of hog manure alone. It was also demonstrated that the bacteria which were shock loaded and failed earlier with the 2% glycerol addition, could recover and eventually develop a tolerance to higher glycerol loading over time. This would imply that a step-wise approach could be taken to slowly increase the amount of glycerol in the feed to higher levels, without causing a system imbalance and failure. Unfortunately there was insufficient time in this study to undergo a step-wise feeding regime.

When comparing the crude and pure glycerol, it was clear that there were no detrimental effects experienced from the contaminants (methanol and salts) in the crude glycerol, and that biogas and methane production was virtually the same as that with the pure glycerol. It would be worthwhile however, to see if there are any long-term effects from the possible build-up of salts in the digester, which was not observed in this study because it would require a period of close to 90 days of operation and have a steady state concentration of 80 mg/l (Appendix C). After 52 days of operation, the concentration of sodium in the digester was 76 mg/l.

The biogas and methane yields of the glycerol digesters were fairly similar to that of the control digester, indicating that the conversion of soluble carbon to biogas had a similar level of efficiency. There was however, a distinct trend seen in the 2% glycerol digester, where, due to the higher COD loading, propionic acid built-up and resulted in a decrease in the proportion of CH_4 and subsiquently an increase in the proportion of CO_2, in the biogas produced. This was also evident in the nutrient analysis, where the 2% glycerol digester showed significantly less hydrolytic NH_3-N and PO_4 production. It appeared that this was the result of an overloading of glycerol, which may have caused the bacteria to partially neglect the manure feed for the glycerol feed.

4.2 Batch Experiments

The batch tests, or biochemical methane potential (BMP) tests, were run to determine which level of glycerol loading would be optimal and at which level overloading is observed. This was done with raw manure (unscreened and undiluted) to simulate a higher solids slurry, which is more commonly found on hog farms in Manitoba. Results of both batch tests are summarized in table 4.5.

Table 4.5 Results of batch tests with pure and crude glycerol

Reactor	**Glycerol Addition (% by weight)**	**COD Load (g)**	**pH (after test)**	**% TS**	**% COD removal**	**Biogas Yield (m^3/kg COD$_{add-rem}$)**		**Methane Yield (m^3/kg COD$_{add-rem}$)**	
Experiment 1 (pure glycerol)						**COD add**	**COD rem**	**COD add**	**COD rem**
Control	0	0.73	7.95	1.21	59	0.33	0.56	0.13	0.22
R1	0.5	1.06	7.65	1.14	41	0.25	0.61	0.09	0.21
R2	1	1.29	6.82	1.12	3	0.17	0.51	0.03	0.13
R3	2	2.00	5.89	1.14	1	0.11	0.36	0.01	0.12
R4	4	3.32	5.66	1.19	3	0.05	0.38	0.003	0.1
R5*	1	0.81	6.75	1.03	55	0.37	0.68	0.11	0.2
Experiment 2 (crude glycerol)									
Control	0	1.28	7.6	2.43	33	0.23	0.7	0.12	0.33
R1	0.5	1.71	7.7	2.57	46	0.28	0.6	0.15	0.34
R2	1	2.09	7.69	2.68	57	0.3	0.52	0.17	0.3
R3	2	2.72	6.7	3.17	12	0.11	0.91	0.03	0.29
R4	4	4.22	5.7	3.67	6	0.08	0.49	0.02	0.12
R5*	1	1.5	7.56	1.88	54	0.29	0.55	0.15	0.29

* reactors contained only seed and glycerol, no manure was added.

4.2.1 Batch Experiment 1

In the first batch test using pure glycerol as an amendment at various levels, the control reactor showed a steady production of biogas throughout the 16 days, but it did not have the highest production or yield, that was R5, which contained only seed and glycerol (Figure 4.14) (Table 4.5).

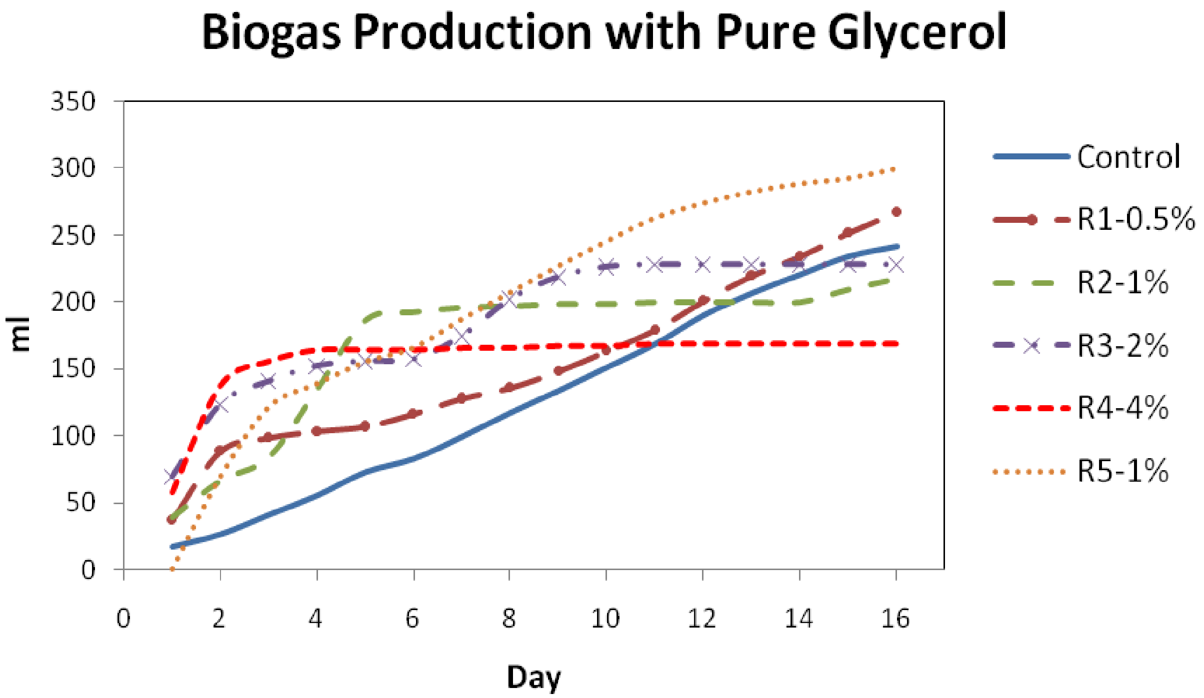


Figure 4.14 Biogas production in batch test with varying amounts of pure glycerol with manure

Biogas production in R5 was 24% higher than the control reactor. All of the reactors showed higher biogas production initially (after 2 days), than the control, indicating that the bacteria were able to quickly utilize the glycerol. R4, which had the highest glycerol concentration (4%), produced the largest amount of biogas initially, but essentially stopped producing any gas after 3 days, indicating that after 3 days, conditions in the reactor had become toxic to the methanogenic bacteria, which was indicated by the drop in pH down to 5.66 by the end of the 16 days. A similar trend was seen with R3, which contained less glycerol (2%), but gas production did not stop until day 10, while R2 (1%) appeared to have stopped producing gas after 5 days, but started producing gas again after 14 days, indicating the bacteria were able to recover from toxic conditions and once again utilize the by products which had built up earlier. R1 showed an initially higher biogas production as the bacteria utilized the glycerol, and then gas production rate slowed down to a very similar one as the control, indicating that the bacteria could switch to feeding on the manure once the glycerol was used up.

Methane production showed a similar trend to biogas production, where the control, R1 and R5 all had the highest levels of production (Figure 4.15).

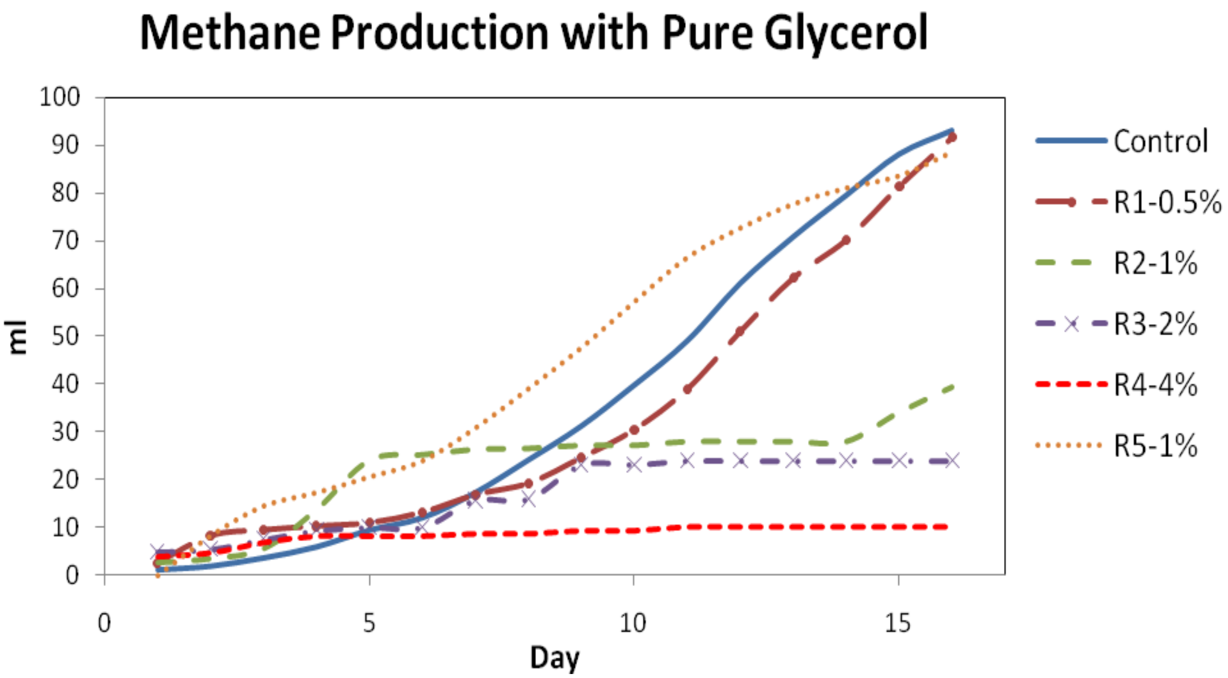


Figure 4.15 Methane production in batch test with varying amounts of pure glycerol

The concentration of CH_4 in the biogas was highest in the control reactor, which is a similar trend to what was seen in the semi-continous reactors, where the addition of glycerol caused a shift to higher levels of CO_2 and lower levels of CH_4 in the biogas. While biogas production was initially high, R2, R3, and R4 all showed progressively lower concentrations of methane, and higher concentrations of CO_2, as the amount of glycerol added increased. This may have been the result of a buildup of intermediate by-products (higher C-chain VFAs) which could not be further degraded and consumed, due to inactivity of acetogenic and methanogenic bacteria. This buildup of VFAs was confirmed by the decrease in the pH of all three of these reactors.

4.2.2 Batch Experiment 2

In the second batch test with crude glycerol, the results showed that R2, which contained 1% glycerol, had the most steady gas production rate, and produced the most biogas by the end of the 16 days (Figure 4.16).

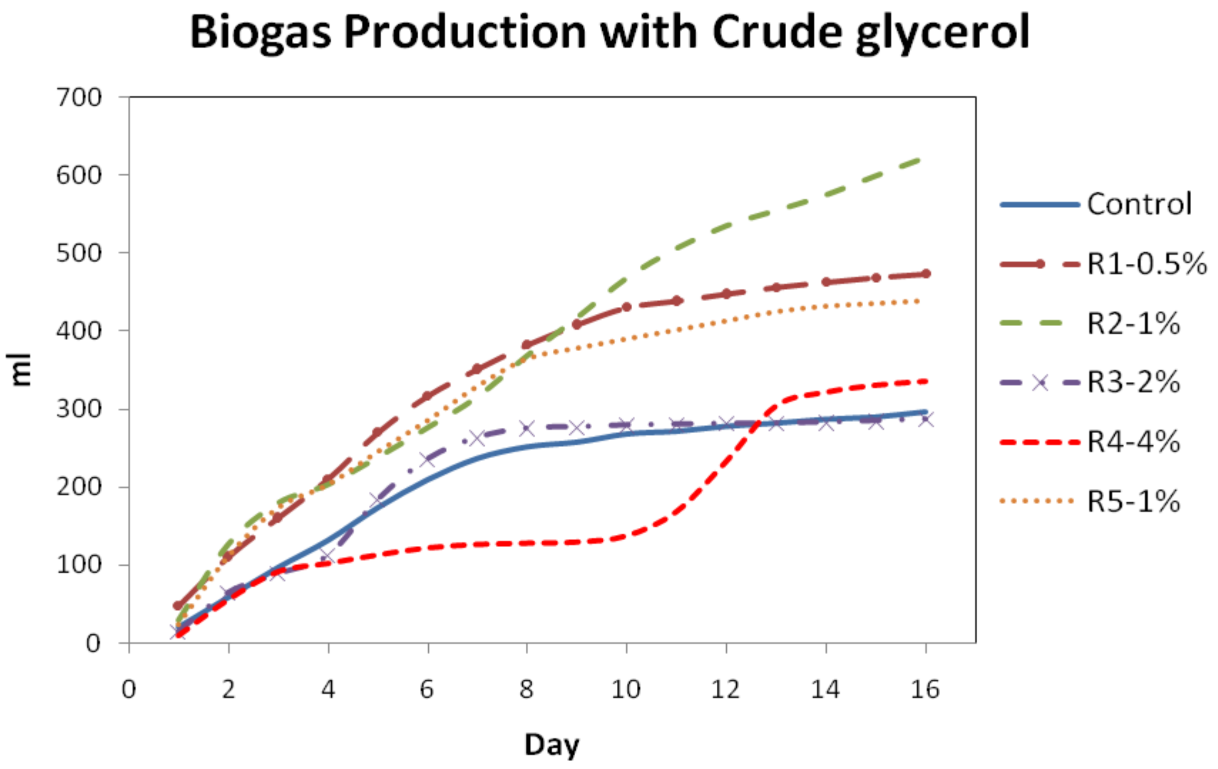


Figure 4.16 Biogas production in batch test with varying amounts of crude glycerol

The biogas production in R2 was more than double that of the control reactor and the biogas yield was also higher. R1 and R5 had similar biogas production rates up until day 8-9, and then gas production slowed down. The total gas production and biogas yield were both higher in R1 and R5 than in the control. Biogas production rates in R3 and the control reactor were very similar and both slowed down after 8 days. R4, which had the highest glycerol additon (4%), showed gas production until day 3, when it stopped, but then started again at day 10, until day 13. This could be an indication of bacterial recovery in a toxic environment, which was indicated by the drop in pH down to 5.7 by the end of the 16 days.

Methane production showed very similar trends to the biogas production, with R2 (1%) having steady CH_4 production throughout, and the most CH_4 production by the end (Figure 4.17).

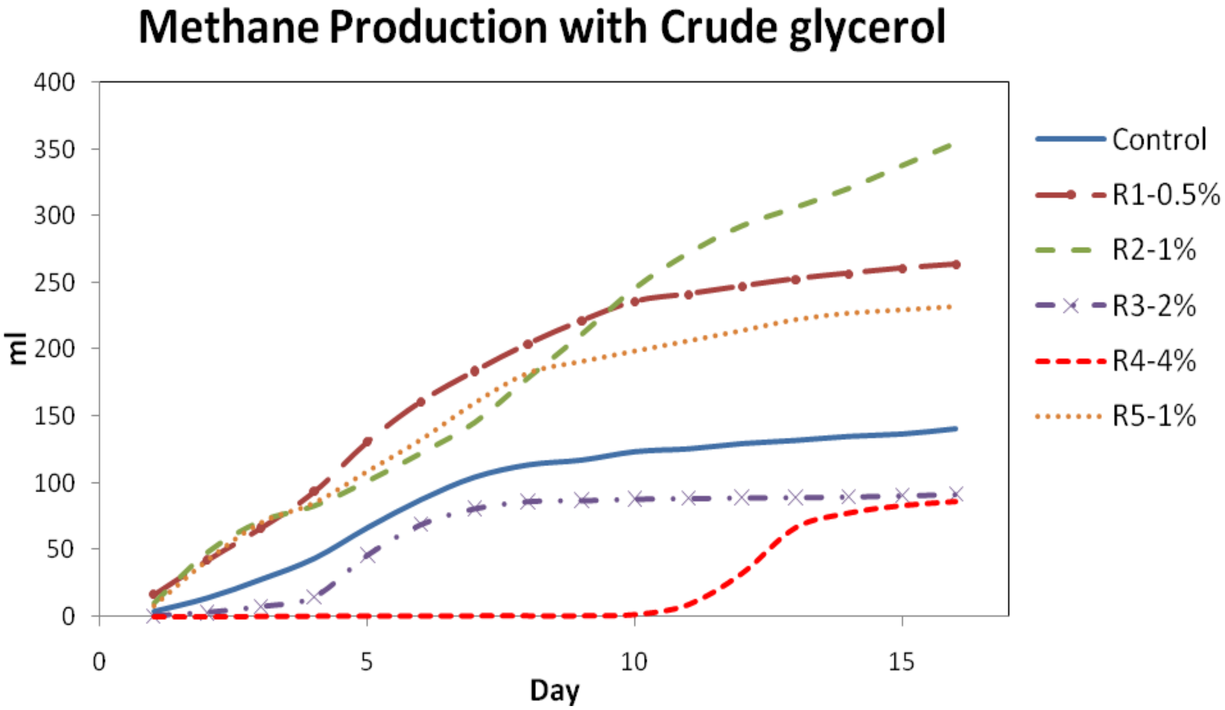


Figure 4.17 Methane production in batch test with varying amounts of crude glycerol

In fact, by the end of the experiment R2 had produced approximately 2.5 times as much CH_4 as the control reactor, while R1 and R5 had produced almost double the amount of CH_4. All three of these reactors also had higher % CH_4 content in the biogas than the control reactor, while R3 and R4 both had a lower % CH_4 content and a higher % CO_2 in the biogas. In R2, R1, and R5, the pH was approximately 7.6 by the end of the experiment, which was the same as that of the control reactor, indicating that there was no buildup of acidic by-products (VFAs), resulting in high biogas and methane production, and yields. The behavior of R1 and R2 would suggest that anaerobic digestion was enhanced with the addition of 1% or less glycerol, because the rate of methane production was higher than the control with manure alone, and the methane content of the biogas was also higher throughout the experiment. This is also supported by the fact that the COD removal increased with additions of 0.5% and 1%, and there was no noticible build up of acidic by-products by the end.

4.2.3 Summary

Even though batch test's 1 and 2 were operated under similar conditions, and fed similar substrates, the results were quite different. Most notably was the much quicker and higher gas production seen in batch test 2. This may have been attributed to the seed, which was taken from the same source in both tests, but it may have required a longer acclimation period in batch test 1. This would explain why the biogas and methane production rate in the control reactor of batch test 1 were higher at the end of the experiment, than the control reactor in batch test 2, which slowed down significantly after day 8. It may also be the result of a higher solids concentration in the raw manure in the second batch test, in which case the bacteria would feed on the easily digestible portion of the manure feed first, but then slow down because there is a higher proportion of less digestible material. The methane production and yields were also different among the different treatments between batch tests. In the first batch test the control reactor had a similar methane production and yield to the low-glycerol treatments, whereas in the second batch test, the methane production and yield was much higher in the low-glycerol treatments than in the control reactor. Therefore it would suggest that using a smaller amount of glycerol (1% or less) would have a positive effect on the anaerobic digestion process and result in the greatest methane and biogas yields. It would also suggest that using a higher dosage of glycerol (2% or more) in a shock loading would cause a buildup of intermediate acids, resulting in a drop of pH and a toxic environment for acetogenic and methanogenic bacteria. A step loading approach may also be a better solution, to allow the bacteria to aclimate for some time with lower dosages of glycerol before adding 2% or more.

CHAPTER 5 SUMMARY AND CONCLUSIONS

Four lab-scale, anaerobic digesters were operated in a semi-continuous mode under mesophilic conditions, in order to test how different amendments of both pure and crude glycerol would affect the digestion of hog manure. A similar experiment was also run in smaller batch tests to determine the BMP. Glycerol was of interest because it is an easily biodegradable carbon source, and is a by-product of the biodiesel industry. Measurements of gas production and composition were of particular interest because of the potential for energy production. Chemical constituents such as COD and VFAs, along with nutrient analysis were performed to determine how the anaerobic digestion process was affected by these feed products. Based on the results of the study, the following conclusions can be made:

1. Shock loading the anaerobic digesters with 2% glycerol (by weight) or more, caused a build-up of intermediate VFAs, which created a toxic environment for the microbes and an unstable system, which stopped producing biogas.
2. The shock-loaded digester was able to recover over time and stabilize with a loading of 2% glycerol after having built up a tolerance for the high COD loading.
3. The addition of 1% glycerol maintained a stable AD process while producing approximately double the amount of biogas and methane, and had similar effluent quality to that of the control digester with hog manure alone.
4. The co-digestion of crude glycerol, which had salts and methanol contaminants, showed no detrimental affects to the AD process and had very similar gas and methane production to that of the digester fed with an equal amount of pure glycerol.

5. The build-up of propionic acid in the 2% glycerol digester was evidence of an unstable system, and resulted in a reduction in the CH_4 content of the biogas down to 49% and an increase in the CO_2 content up to 49%.
6. Nutrient analysis showed that the 2% glycerol digester produced significantly less soluble nitrogen and phosphorus, suggesting a lower level of manure degradation with a high glycerol loading.
7. Batch tests with higher solids slurry gave best results with glycerol additions of 0.5% and 1%, producing 2 to 2.5 times the biogas and methane as the control reactors, with both pure and crude glycerol. Results from batch tests with crude glycerol suggest an enhanced anaerobic digestion with the 0.5% and 1% additions.

It was concluded that the use of glycerol as an amendment to anaerobic digestion of hog manure can enhance digestion at doses of 1% or less, by increasing both biogas and methane production. It was also shown that bacteria only require a short acclimation period to utilize the glycerol, and could exert a boost in biogas production almost immediately. It is not entirely clear what effect a higher dosage of glycerol can have on the AD process in the long-term, but the performance of the 2% glycerol digester suggests that incremental glycerol additions may provide a stronger and healthier microbial population that can adapt quickly. The results of co-digestion using crude glycerol from the biodiesel industry, indicate that it should be able to be used as a co-substrate in a digester, without any additional refining.

CHAPTER 6 ENGINEERING SIGNIFICANCE

The benefits of boosting biogas and methane production in anaerobic digestion through co-digestion are obvious. By having minimal input costs from using a waste byproduct of the local biodiesel industry such as glycerol, farmers could be greatly assisted in offsetting high infrastructure and capital costs, especially if tipping fees were associated with receiving the waste. There are however, many intangible advantages of anaerobic digestion which are often not considered, because of their unknown economic benefits. These include: the reduction of odours and pathogens, the production of a more concentrated fertilizer product in the digestate, the reduction of soil and water contamination, the reduction of greenhouse gas emissions, and increasing production of green energy in the province. In the case of using glycerol, it also benefits the local biodiesel industry, because the glycerol can be taken and used without further refining. While it is difficult to determine the nutrient value of hog manure, Sutton (1992) estimated the potential value of fertilizer to be between $2.50 to $3.50, per market hog sold. Estimates in Manitoba suggest that the value for nitrogen and phosphorus from hog manure is in the range of $30 to $40 million a year, and is often given away to crop producers (Cicek, 2007).

Below are some considerations for the cost of building a farm-scale anaerobic digester in Manitoba. Based on the average size of a hog farm in Manitoba, which is 2300 hogs, and a conservative estimate of 70$ per hog, based on the USEPA estimates, the cost for a complete-mix digester would be:

2300 x $70 = **$161,000**.

The cost of a cogeneration unit would also need to be included, and this can vary considerably depending on the technology used and the location (heat balance) of the digester, however a general estimate would be approximately $1000/kW (Lusk, 1998). Based on the estimate of 15 W per 100 kg hog, or 35 kW per farm, a cogeneration unit would cost:

35kW x $1000/kW = **$35,000**

Therefore the total cost would be:

$161,000 + $35,000 = **$196,000**

The return on investment from selling electricity, at a price for electricity at 6 ¢/kWh, would be:

35kW x 8000hr/yr x $0.06 = **$16,800/yr**

This is assuming that the hog barn always has 2300 hogs, and that the digester is operating for 8000 hours a year. With this return on investment, it would take 11.7 years to pay off the capital cost. Undoubtedly, this would be a long-term investment for any farmer, and with an unpredictable hog market, it is likely a large risk that, not surprisingly, many farmers would not want to take. Of course, if the economics were to improve, from the price of natural fertilizer, tipping fees, enhanced biogas production, or government subsidies on green energy produced, then AD on farms in Manitoba may not be that unreasonable. If the Manitoba government, however simply continues to invest in Hydro power, then electricity generation from farm digesters may not be feasible, and other uses for the biogas should be explored, such as for heating homes, or vehicle fuels.

CHAPTER 7 RECOMMENDATIONS FOR FUTURE WORK

Based on this study, recommendations for future research would be as follows:

1. Due to some lab-scale constraints in this study, screened and diluted hog manure was used (1% TS). For future study, it is recommended to use raw hog manure (unscreened and undiluted), which has a higher solids concentration (~5% TS) in semi-continuous digesters, to better simulate a real-world digester, and test what effect glycerol or other co-substrates have on the AD process.
2. Adding glycerol in incrementally increasing amounts is recommended, to determine the maximum glycerol loading after bacterial acclimation.
3. Operating the digesters under a longer SRT is recommended, to determine the optimal SRT for a particular load of manure with glycerol, which demonstrates the most efficient VSR.
4. Operating the 2% glycerol digester in a long-term experiment is recommended, to determine digester performance after a lengthy acclimation and stabilization period.
5. Performing a full microbial analysis during an experiment with co-digestion of manure and glycerol is recommended, to determine the different microbial species growth rates at different times in the digestion process.
6. A study of estrogenic activity through co-digestion is also recommended, to determine whether the microbial activity would cause it to increase or decrease.
7. This research indicated that co-digestion of hog manure with glycerol has good potential for enhancing the AD process. A pilot-scale application of this study should be the next step.

REFERENCES

Agriculture and Agri-food Canada Research Branch. 1998. Research Strategy for Hog Manure Management in Canada. http://dsp-psd.pwgsc.gc.ca/Collection/A42-77-1998E.pdf. Accessed on May 11, 2007.

Ahring, B.K., Sandberg, M., and I. Angelidaki. 1995. Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Applied Microbiology and Biotechnology* 43: 559-565.

Aldrich, B.A. 2005. Cornell University, Dept. of Biological and Environmental Engineering.

Amon, Th., Amon, B., Kryvoruchko, V., Bodiroza, V., Potsch, E. and W. Zollitsch. 2006. Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. *International Congress Series* 1293: 217-220.

Anders, S. 2007. Biogas production and use on California's dairy farms, a survey of regulatory challenges. Energy Policy Initiaties Center, University of San Diego. http://www.sandiego.edu/epic/publications/documents/BiogasRegulatoryPaper_FINAL_001.pdf (2008/08/29).

Angelidaki, I., and B.K. Ahring. 1993. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Applied Microbiology and Biotechnology* 38: 560-564.

Angelidaki, I. and B.K. Ahring. 1997. Anaerobic digestion in Denmark. Past, present and future. In: III Curs d'Enginyeria Ambiental. Lleida, 336-342.

Angelidaki, I., Ellegaard, L., and B.K. Ahring. 2003. Applications of the anaerobic digestion process. *Advances in Biochemical Engineering/Biotechnology* 82: 1-33.

Angelidaki, I. and L. Ellegaard. 2003. Co-digestion of manure and organic wastes in centralized biogas plants, status and future trends. *Applied Biochemistry and Biotechnology* 109: 95-105.

APHA, (American Public Health Association) 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Wastewater Association and Water Environment Federation, Washington, DC.

Baily, R. S. 2007. Anaerobic digestion of restaurant grease wastewater to improve methane gas production and electrical power generation potential. In WEFTEC 07, 6793-6805. San Diego, California.

Bashir, B.H., and A. Matin. 2004. Sodium toxicity control by the use of magnesium in an anaerobic digester. *Journal of Applied Sciences and Environmental Management* 8(1): 17-21.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhna, S.V., Pavlostathis, S.G., Rozzi, A., Sanders W.T.M., Siegrist, H., and V.A. Vavilin. 2002. Anaerobic digestion model No. 1 (ADM1). IWA Publishing, London, UK.

Biodiesel Advisory Council. 2005. *Biodiesel Made in Manitoba*. Report for the Government of Manitoba.

Bjornsson, L., Murto, M., Jantsch, T.G., and B. Mattiason. 2001. Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. *Water Research* 35 (12): 2833-2840.

Boe, K. 2006. Online monitoring and control of the biogas process. PHD Thesis. Institute of Environment and Resources, Technical University of Denmark.

Cakir, F.Y., and M.K. Stenstrom. 2005. Greenhouse gas production: A comparison between aerobic and anaerobic wastewater treatment technology. *Water Research* 39: 4197-4203.

Callaghan, F.J., Wase, D.A.J., Thayanithy, K., and C.F. Forster. 1999. Co-digestion of waste organic solids – batch studies. *Bioresource Technology* 67(2): 117-122.

Campos, E., Palatsi, J. and X. Flotats. 1999. Co-digestion of pig slurry and organic wastes from food industry. In Proceedings of the II International Symposium on Anaerobic Digestion of Solid Wastes, 192-195. Barcelona, June.

Cicek, N. 2007. Assessment of potential hog manure processing strategies in Manitoba. In *A Report on Current Knowledge of Key Environmental Issues Related to Hog Production in Manitoba*. Clean Environment Commission, 247-271. Winnipeg, Manitoba.

Cheng, J., and B. Liu. 2002. Swine wastewater treatment in anaerobic digesters with floating medium. *American Society of Agricultural Engineers* 45(3): 799-805.

Cheng, J., Roos, K.F., and L.M. Saele. 2007. Evaluation system for swine waste treatment and energy recovery. USEPA Agstar. http://www.epa.gov/agstar/resources/lag_perf.html.

Chung, G., York, R., and S. Rank. 2007. Making cogeneration pencil out: anaerobic co-digestion of grease trap waste. In *WEFTEC*, 4506-4512. San Diego, California.

Demirbas, M.F., and M. Balat, 2006. Recent advances on the production and utilization trends of biofuels: a global perspective. *Energy Covers Manag* 47: 2371-2381.

Drake, H.L. 1994. Acetogenesis, Acetogenic bacteria, and the acetyl-CoA "wood/ljungdahl" pathway: past and current perspectives. In *Acetogenesis*, p.3-60. New York, NY: Chapman & Hall.

Ferry, J.G. 2003. One-carbon metabolism in methanogenic anaerobes. In Biochemistry and Physiology of Anaerobic Bacteria. eds. Ljungdahl, L.G., Adams, M.W., Barton, L.L., Ferry, J.G., and M.K. Johnson, 143-156. New York, N.Y.: Springer-Verlag.

Goodfellow Agricola Consultants Inc. 2007. Anaerobic digestion for Bioelectricity production. In The Elorin Bioenergy Feasibility Study.

Gottschalk, G. 1986. Bacterial fermentations. In *Bacterial Metabolism*, p.208-282. New York, NY: Springer-Verlag

Hatfield, J.L., Brumm, M.C., and S.W. Melvin. 1998. Swine manure management. United States Department of Agriculture, Agriculture Research Service. http://www.ars.usda.gov/is/np/agbyproducts/agbychap4.pdf

Heinrichs, D.M., Poggi-Varaldo, H.M., and J.A. Oleszkiewicz. 1990. Effects of ammonia on anaerobic digestion of simple organic substrates. *Journal of Environmental Engineering* 116: 698-710.

Hilborn, D., DeBruyn, J., and S. Clarke. 2007. Anaerobic Digestion and the renewable energy standard offer program. Factsheet 07-051, October. Ontario: Ministry of Agriculture, Food and Rural Affairs.

Hilborn, D. and J. DeBruyn. 2007. Considerations and opportunities for building a farm-based anaerobic digester system in Ontratio. Infosheet, August. Ontario: Ministry of Agriculture, Food and Rural Affairs.

Hill, D.T. and R.D. Holmberg. 1988. Long chain volatile fatty acid relationships in anaerobic digestion of swine waste. *Biological Wastes* 23: 195-214.

Holm-Nielsen, J.B., Lomborg, C.J., Oleskowicz-Popiel, P., and K.H. Esbensen. 2007. On-line near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. *Biotechnology and Bioengineering* 99(2): 302-313.

IEA Bioenergy Task 37, 2008. Energy from biogas and landfill gas. http://www.iea-biogas.net/documente/casestudies/linkoping_final.pdf. 2008/10/07.

Jones, G. and N. Armstrong. 2001. Long-term trends in total nitrogen and total phosphorus concentrations in Manitoba streams. Manitoba Conservation Report No. 2001-07.

Kaparaju, P., and J. Rintala. 2005. Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. *Resources, Conservation and Recycling* 43: 175-188.

Kemp, William H. 2006. *Biodiesel Basics and Beyond.* Tamworth, ON: Aztext Press.

Lee, S.M., Jung, J.Y., and Y.C. Chung. 2000. Measurement of ammonia inhibition of microbial activity in biological wastewater treatment process using dehydrogenase assay. *Biotechnology Letters*. 22: 991-994.

Lengeler, J.W., Drews, G., and H.G. Schlegel. 1998. *Biology of prokaryotes*. Oxford: Blackwell Science. p.955.

Lusk, P. 1998. Methane recovery from animal manures: the current opportunities casebook. National Renewable Energy Laboratory.

Magbanua Jr., B.S., Adams, T.T., and P. Johnston. 2001. Anaerobic co-digestion of hog and poultry waste. *Bioresource Technology* 76: 165-168.

Ma, J., Wambeke, M. V., Carballa, M., and W. Verstraete. 2008. Improvement of the anaerobic digestion of potato processing wastewater in a UASB reactor by co-digestion with glycerol. *Biotechnology Letters* 30(5): 861-867.

McNeil Technologies, Inc., 2005. Colorado Agriculture IOF Technology Assessments: Anaerobic Digestion.

Metcalf and Eddy Inc. 2003. Wastewater Engineering, Treatment and Reuse, Chpt. 10, 14. Tata McGraw-Hill Publishing, New Dehli. pp. 984-1018, 1505-1533.

Mittelbach, M., Remschmidt, C. 2004. *Biodiesel, The Comprehensive Handbook*. Graz, Austria: Martin Mittelbach.

Mladenovska, Z., Dabrowski, S., and B. K. Ahring. 2003. Anaerobic digestion of manure and mixture of manure with lipids: biogas reactor performance and microbial community analysis. *Water Science and Technology* 48(6): 271-278.

Moller, H.B., Sommer, S.G., and B.K. Ahring. 2002. Separation efficiency and particle size distribution in relation to manure type and storage conditions. *Bioresource Technology* 85: 189-196.

Moser, M.A., Mattocks, R.P., Gettier, S., and K.F. Roos. 2007. Keeping the neighbors happy – reducing odor while making biogas. US EPA Agstar. http://www.epa.gov/agstar/resources/proj_sums.html (04/09/08)

Mueller, S. 2007. Manure's allure: variation of the financial, environmental and economic benefits from combined heat and power systems integrated with anaerobic digesters at hog farms across geographic and economic regions. *Renewable Energy* 32: 248-256.

Nielsen, H.B. 2006. Control parameters for understanding and preventing process imbalances in biogas plants: Emphasis on VFA dynamics. PHD Dissertation, BioCentrum-DTU, Technical University of Denmark.

Oleszkiewicz, J., 2006. Theory of Wastewater Treatment. The University of Manitoba, Lecture Notes.

Oleszkiewicz, J.A. 2000. Changes in hog manure in a flow-through anaerobic storage tank. *Environmental Technology* 21: 597-600.

Palmowski, L., and J. Muller. 1999. Influence of the size reduction of organic waste on their anaerobic digestion. In: Mata-Alvarez, J.,Tilche, A., Cecchi, F. (Eds.), Proceedings of the Second International Symposium on Anaerobic Digestion of Solid Wastes, Barcelona, vol. 1. June, 137-144.

Pind, P.F., Angelidaki, I., and B.K. Ahring. 2003. Dynamics of the anaerobic process: Effects of volatile fatty acids. *Biotechnology and Bioengineering* 82(7): 791-801.

Poulsen, Tjalfe G. 2003. Anaerobic digestion. In *Solid Waste Management*. Aalborg University, Denmark.

Puchajda, B. M., 2006. Increased energy recovery and enhanced pathogen removal through anaerobic digestion of thickened wastewater sludge. Unpublished Ph.D. thesis. Winnipeg, Manitoba: Department of Civil Engineering, University of Manitoba.

Qatibi, A-I., Bories, A., and J.-L. Garcia. 1991. Sulfate reduction and anaerobic glycerol degradation by a mixed microbial culture. *Current Microbiology* 22: 47-52.

Rodriguez, J., Kleerebezem, R., Lema, J.M., and M.C.M. Van Loosdrecht. 2006. Modelling product formation in anaerobic mixed culture fermentations. *Biotechnology and Bioengineering* 93(3): 592-606.

Ros, M., and G.D. Zupancic. 2003. Thermophilic anaerobic digestion of waste activated sludge. *Acta Chim. Slov*. 50: 359-374.

Schaefer, S. H., and Sung, S. 2008. Retooling the ethanol industry: thermophilic anaerobic digestion of thin stillage for methane production and pollution prevention. *Water Environment Research* 80(2): 101-108.

Schafer, P., Trueblood, D., Fonda, K., and C. Lekven. 2007. Grease processing for renewable energy, profit, sustainability, and environmental enhancement. In WEFTEC 07, pp. 4497-4505. San Diego, California.

Schink, B. 1997. Energetics of sytrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews* 61(2): 262-280.

Schink, B. 2002. Synergistic interactions in the microbial world. *Antoine Van Leeuwenhoek* 81: 257-261.

Stams, A.J.M., Plugge, C.M., De Bok, A.F.M., Van Houten, B.H.G.W., Lens, P., Dijkmen, H., and J. Weijma. 2005. Metabolic interactions in methanogenic and sulfate-reducing bioreactors. *Water Science and Technology* 52(1): 13-20.

Statistics Canada – Manitoba Agriculture, food and rural Initiatives. 2007. http://www.gov.mb.ca/agriculture/statistics/pdf/23-010hog-statistics-jan07.pdf. Accessed on May 11, 2007.

Temudo, M.F., Poldermans, R., Kleerebezem, R., and M.C.M. Van Loosdrecht. 2008. Glycerol fermentation by open mixed cultures: A chemostat study. *Biotechnology and Bioengineering* 100(6): 1088-1098.

Thauer, R.K., Jungermann, K. and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews* 41(1): 100-180.

USEPA Agstar, Office of Air and Radiation. Winter 2002. Managing Manure with Biogas Recovery Systems, Improved Performance at Competitive Costs. EPA-430-F-02-004

USEPA, United States Environmental Protection Agency. 2006. Biosolids Technology Factsheet, Multi-Stage Anaerobic Digestion. Office of Water EPA 832-F-06-031.

USEPA, United States Environmental Protection Agency. 2007. Methane. http://www.epa.gov/methane/sources.html (2008/08/15).

Vavilin, V.A., Lokshina, L.Y., Rytov, S.V., Kotsyurbenko, O.R., Nozhevnikova, A.N., and S.N. Parshina. 1997. Modelling methanogenesis during anaerobic conversion of complex organic matter at low temperatures. *Water Science and Technology* 36: 531-538.

Weiland, P. 2003. Production and energetic use of biogas from energy crops and wastes in Germany. *Applied Biochemistry and Biotechnology* 109: 263-274.

Yadvika, S., Sreekrishnan, T.R., Kohli, S. and V. Rana. 2004. Enhancement of biogas production from solid substrates using different techniques – a review. *Bioresource Technology* 95: 1-10.

Zhang, Z., 2008. Anaerobic digestion. The California Energy Commission. http://www.energy.ca.gov/pier/renewable/biomass/anaerobic_digestion/index.html (2008/08/29).

Zeng, X., Ma, Y., and L. Ma. 2007. Utilization of straw in biomass energy in China. *Renewable and Sustainable Energy Reviews* 11: 976-987.

APPENDICES

APPENDIX A

CALCULATIONS OF THE APPLIED VOLUMES OF GLYCEROL INTO EACH OF THE DIGESTERS

The volume of glycerol added to each of the digesters was based on the weight of the manure feed and the weight of the glycerol.

Assuming: manure feed has a density of 1g/ml
glycerol has a density of 1.262 g/ml

For 1% glycerol: 200 ml = 200 g
200 g x 0.01 = 2 g
2 g /1.262 g/ml = 1.6 ml of glycerol added

For 2% glycerol: 200 g x 0.02 = 4 g
4 g/1.262 g/ml = 3.2 ml of glycerol added

For 4% glycerol: 200 g x 0.04 = 8 g
8 g/1.262 g/ml = 6.3 ml of glycerol added

APPENDIX B

PHOTOGRAPHS OF THE SEMI-CONTINUOUS EXPERIMENT

APPENDIX C

CALCULATIONS OF SALT (NaCl) ACCUMULATION IN THE DIGESTERS

NaCl added to the reactor each day

10,000 mg/l NaCl x 0.0016 L = **16 mg NaCl**

Concentration of NaCl in reactor after day 1

16 mg/3.5 L = **4.57 mg/l**

NaCl removed from reactor after day 1

4.57 mg/l x 0.2 L = **0.91 mg**

NaCl remaining in reactor after day 1

16 mg – 0.91 mg = **15.09 mg**

Repeat calculation until steady state is achieved in the digester, at the time when 16 mg of NaCl is added to the digester and 16 mg of NaCl are removed. This steady state is achieved on approximately day 90, and the concentration of NaCl in the digester would be 80 mg/l.