ANAEROBIC CO-DIGESTION OF DAIRY MANURE WITH CANOLA MEAL

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

ABSTRACT

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There has been an increase of confined animal feeding operations (CAFOs) generating large amounts of manure. When this manure is not handled properly, it generates greenhouse gases (GHGs), odors and water pollution. Anaerobic digestion (AD) is touted as an acceptable approach to address manure management and associated environmental problems. Biogas production from manure alone is limited by low volumes of biogas yield, thus it has a poor economic reputation. Co-digestion of dairy manure with other agricultural wastes has emerged as a promising strategy to enhance the economic viability of AD. Among the agricultural wastes, canola meal (a by-product from extraction of oil from canola seed) was considered as a potential candidate for co-digestion with dairy manure. The purpose of this research was to investigate the suitability and appropriate ratios of canola meal for anaerobic co-digestion with dairy manure.

In this study, various proportions of canola meal: dairy manure (100:0, 10:90, 40:60, 20:80, 0:100) by volume-basis were co-digested in 0.5 L batch bioreactors at a temperature of 35 ± 1^{0}C for 25 d. Two types of canola meal were used in the study; high oil content (HOC) and low oil content (LOC) canola meal with oil contents of 8.0% and 2.5%, respectively. For HOC, the total solids (TS) were high organic loading (HOL, 7.5±2% TS) and low organic loading (LOL, 4.5±2% TS). LOC trials were done at HOL only. In addition, the pretreatment of the canola meal with caustic solution and digestion at high temperature (60 ± 2^{0}C) were evaluated.

Results from this study indicated that at HOL, canola meal is not a viable candidate for anaerobic co-digestion with manure as it lowers biogas production. Manure only digestion performed better than bioreactors augmented with canola meal. The specific methane yield was 352 L/kg VS for manure only and 84 L/kg VS for LOC canola meal only digestion. Nonetheless, at LOL, both 10% and 20% HOC canola meal resulted in increased specific methane of 535 L/kg VS and 445 L/kg VS, respectively. This is 78% and 48% higher than 300 L/kg VS obtained in manure only digestion. Hence, canola meal is beneficial in dairy manure co-digestion at LOL.

At all organic loading levels, canola meal alone digestion had the lowest cumulative biogas production (0.9 L per 0.35 L bioreactor) and specific methane yield (83 L/kg VS). For HOL, the cumulative biogas yield and specific methane yield decreased as the canola meal ratio increased, while at LOL, the decrease was only noted for bioreactors with 40% canola meal. This is suspected to be caused by elevated levels of total volatile fatty acids (VFAs) of more than 4000 mg/L. Two factors are suspected to impact the accumulation of VFAs: the ratio of canola-to-manure in the bioreactor and the organic loading or oil content in the canola meal. In future it will be necessary to look into ways of overcoming the inhibition caused by elevated VFAs.

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CHAPTER 1. INTRODUCTION

Large scale confined livestock operations have emerged over the last few years which generate a significant amount of manure and wastewater. Management of manure is needed to reduce greenhouse gases (GHGs) emissions, odor nuisance and environmental pollution (e.g., water and air pollution). Manure may be viewed as a resource for the production of renewable energy. Environmental concerns associated with liquid manure storage and land disposal can be overcome through anaerobic digestion (AD), where large amounts of manure can be converted to bio-methane, a renewable energy source. The National Research Council estimates the health damage caused by fossil fuels is about $ 120 billion per year (Cohen, 2010). Manure based biogas production provides an opportunity not only to replace the fossil fuels used but also reduce GHG emissions, odor and environmental pollution. Biogas is an energy source that is produced as a result of anaerobic digestion (AD) of organic matter.

Improper handling of dairy manure results in anaerobic decomposition, producing methane (CH_4) and nitrous oxide (N_2O). These gases have higher global warming potential than CO_2, 21 and 310 times (mass ratio) for CH_4 and N_2O, respectively (Lazarus, 2008). One of the most acclaimed methods of reducing this potential in confined animal operations is using manure to produce biogas, which provides an opportunity to capture CH_4, replace fossil fuels directly, and reduce the demand of nitrogen fertilizer (Kaparaju and Rintala, 2011). Even if biogas is combusted it will result in CO_2 which is considered carbon neutral as the carbon in this CO_2 originated from the plants, thus completing the cycle.

Dairy manure remains foremost primary substrate for biogas production, partly due to its abundance (Nielsen and Angelidaki, 2008) and its unique properties, namely high water content making it easy to pump, good buffering capacity and presence of almost all the essential nutrients (Angelidaki and Ellegaard, 2003). Dairy manure based biogas plants are attractive as an energy source and an environmental disposal solution as well as manure borne pathogens reduction (Tafdrup, 1995). In spite of the attractiveness, dairy manure based biogas plants have suffered perpetual problems due to low biogas production (Cuéllar and Webber, 2008). These benefits notwithstanding, North Dakota state is one of the states that do not have an operational dairy manure based biogas plant (USA-EPA, 2010). This is attributed to a number of reasons; one of them is the minimal efficacy of the process to produce enough gas to justify economic viability and sustainability (Lazarus, 2008). In addition, there is a low level of public awareness and interest, poor policy framework and financing (Bilek, 2010; Cohen, 2010).

Biogas is a mixture of principally methane (CH_4) and carbon dioxide (CO_2), of which methane is the energy carrier with an estimated energy value of 37 MJ/m^3 (Khanal, 2009c). A concurrent increase of the volume and methane content in biogas is desirable for maximum economic return and environmental gains (Yiridoe et al., 2009). For the last two to three decades research is ongoing to enhance biogas yield from the AD process. As a result, anaerobic co-digestion has emerged as one of the promising approaches of increasing not only the biogas volume but also the methane yield and, consequently revenues. Anaerobic co-digestion is the simultaneous digestion of more than one substrate. Normally, the substrates have a synergistic effect on each other in terms of microbial activity, and higher conversion of the organic compounds to biogas. The synergistic effect

may be in terms of nutrients, pH, and suppressing toxicity/inhibition. Some of the substrates that have been successfully co-digested with dairy manure include potato wastes (Parawira et al., 2004), energy crops (Lehtomäki et al., 2007), food wastes (El-Mashad and Zhang, 2010; Li et al., 2009a), agro-wastes (Misi and Forster, 2001; Molinuevo-Salces et al., 2010), municipal wastes (Sosnowski et al., 2003; Zupancic et al., 2008) and more recently biofuels by-products (Dhanya, 2009; Kolesárová et al., 2011; Ramachandran et al., 2007).

However, it must be noted that when substrates are not optimized, an antagonistic effect may inevitably lead to inhibition and/or process instability, characterized by lower gas production or even complete failure. Therefore, it is not sufficient to add another substrate to the dairy manure AD without making a conscious effort to determine the type, quantities and form of additions that can bring about the desired results. A number of studies have sought to optimize the addition of co-substrate in terms of organic loading rate (Saev et al., 2010) and accumulation of inhibitors such as volatile fatty acids (Pind et al., 2003) and ammonia (Raposo et al., 2008). The choice of the co-substrates is based on local availability, and suitability in enhancing biogas production.

North Dakota has 7 farms with more than 500 head and 13 others classified as small to medium size dairy farms (200-500 head) spread out in 17 counties (Cohen, 2010). Consequently, North Dakota is ranked 16th nationally among dairy producing states. It is estimated that if manure is collected and used properly, it can produce 4000 MT of biogas, which can produce 14 GWh of electricity (Cohen, 2010). In addition, North Dakota is an agricultural based state that produces large quantities of crops which generate a large quantity of waste and byproducts. Among the notable crops in the state is canola, a small

brownish oilseed, in which the state is the leading producer (about 90%) in the USA (Brown et al., 2008). Canola meal is the by-product of oil extraction from canola seed, and it is currently used as animal feed (USA-EPA, 2010). Canola meal as an animal feed is popular for being rich in proteins and moderate fiber, with a high market selling price of USD 230-250 per short ton (Brown et al., 2008). However, given the expected rapid growth of the canola industry, it is anticipated that the supply will surpass the demand, and it can then be used as a co-substrate with manure. The anaerobic co-digestion of canola meal and manure is an avenue to add more value to both substrates. The overall goal of this study was to determine whether canola meal is a viable co-substrate, and to determine optimal ratio of canola-to-manure to enhance biogas production and methane content when canola meal is co-digested with dairy manure.

1.1 Objectives

The current research attempted to answer two questions as follows:

i. Does canola meal enhance biogas production and methane content when used as a co-substrate with dairy manure?

ii. What are the appropriate canola-to-manure ratio and oil content of canola meal that can be used in dairy manure co-digestion?

In the study, only the technical aspects of canola meal co-digestion with dairy manure will be discussed. These aspects include the suitability of canola meal and the appropriate proportions of canola meal that would positively impact dairy manure AD. Future research might explore the use of other locally available co-substrates in North

Dakota and develop an optimized dairy manure co-substrate strategy for the state based on economic viability and sustainability

1.2 Thesis Organization and Structure

This thesis consists of 6 chapters. Chapter 1 describes the general introduction and overall objectives of this research. Chapter 2 deals with reviews of literature on AD, bioreactors, biogas properties and uses, manure based AD, co-digestion, canola meal as a potential co-substrate and justification for the research. Chapter 3 describes suitability of canola meal for co-digestion with manures. In this chapter, materials and methods are explained. In chapter 4, the organic loading and canola oil content impacts in dairy manure co-digestion is discussed. In chapter 5, a brief discussion on preliminary future research and recommendations is presented. Finally, chapter 6 is the overall general conclusions.

CHAPTER 2. LITERATURE REVIEW

2.1 Anaerobic Digestion

Anaerobic digestion (AD) is defined as the biochemical decomposition of organic matter in an environment devoid of oxygen (or its precursors) that is brought about by a consortium of interdependent and symbiotic micro-organism (Chen et al., 1980; Deublein and Steinhausser, 2008; Ghaly, 1996; Khanal, 2009b). This results in the biological gasification of biomass, whose end product is mainly biogas (a mixture of methane, carbon dioxide and traces of hydrogen sulfide and ammonia), and a liquid effluent called digestate (Ghaly, 1996). By definition, AD is bound to occur in the natural environment, exemplified by landfills, marshes, deep ocean gullies, animal guts, rice paddies, and many more (Speece, 1983). AD is historically associated with a number of famed scientists like Robert Boyle, Stephen Hale, and Sir Humphrey Davy, who over the years worked to identify, promote, and popularize its use (Khanal, 2009c). In 1821, Avogadro elucidated methane (CH_4) and in 1884, Louis Pasteur tried to make biogas from the horse dung for lighting Paris streets (Deublein and Steinhausser, 2008). In 1905, Imhoff demonstrated the use of AD to stabilize wastes and produce biogas (Khanal, 2009c). During the World War II, due to the insufficiency of fossil fuel, the use of agricultural wastes to produce biogas was promoted. The impetus seemed to slow down after the war, but the oil crisis of 1970s, had remarkable impact in its renewed interest (Deublein and Steinhausser, 2008). The tempo of its adoption was not sustained until 1990s when a combination of energy provision and environmental concerns came into play, changing the approach, attitude and commitment.

Although, there was overwhelming evidence of AD success over several centuries, recent interest has been partly agitated by the spike in costs and insecurity of fossil fuels (Weiland, 2010) as well as environmental issues, principally emission of greenhouse gases (Clemens et al., 2006). In addition, successful AD provides means to preserve nutrients and reduce the oxygen demand in wastewaters (Cantrell et al., 2008; Yiridoe et al., 2009). By 2007, Europe's energy supply from biogas was about 6 million tons of oil equivalents (mtoe), and was expected to have an annual growth of 20%, and Germany was the leading country with over 4000 agricultural based biogas plants (Wieland, 2010). However, for USA, growth in animal manure based AD processes has been slow until recently when odor control, greenhouse gas emissions (GHGs) and generation of electricity started becoming a priority (Lazarus, 2008). Biogas has been identified as a promising source of renewable energy that can be used in generation of heat and electricity, vehicle fuel and conversion to other chemicals, including methanol that can be integrated with the production of biodiesel (Lantz et al., 2007). Unlike Europe, where provision of energy is a major motivating factor, biogas in USA agricultural sector is viewed as being motivated by reduction of odor, improving air and water quality, and more recently reduction of GHGs (Lazarus, 2008).

The advantages of biogas production from organic wastes notwithstanding, AD in the dairy animal sector is not economically attractive due to low biogas production rates coupled with low methane content. This has been exacerbated by the increasing biogas plant capital and maintenance costs (Lazarus, 2008) as well as the demands for scrupulous attention to the stability of the process (Khanal, 2009a). Under normal conditions, the theoretical methane yield is affected by the type of substrate in use, the carbon content as

shown in Equation 1 (Deublein and Steinhausser, 2008) and the theoretical CH_4 production (Bu) is given as equation 2 (Møller et al., 2004).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 \qquad (1)$$

$$B_u = \frac{(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}) * 22.4}{12n + a - 16b} \quad LCH_4 / kgVS \qquad (2)$$

Based on equation 1 and 2, lipids ($C_{15}H_{31}COOH$), proteins (C_4H_6ON) and carbohydrates ($C_6H_{12}O_6$) will yield biogas with a theoretical methane content of 72%, 63% and 50%, respectively, as in Table 1.

Table 1. Theoretical Biogas Yield Based on Types of Substrate

Substrate	Chemical Composition	COD/VS	CH_4 Yield (STP L/kg VS)	CH_4 Yield (STP L/kg COD)
Carbohydrates	$(C_6H_{12}O_6)_n$	1.19	415	350
Proteins	C_4H_6ON	1.49	496	350
Lipids	$C_{15}H_{31}COOH$	2.90	1014	350

Assumption: 100% organic matter converted to CH_4 & CO_2, and N_2 to NH_3; STP= Standard Temperature and Pressure. **Source:** Holm-Nielsen, 2009.

The organic matter content may be determined based on either volatile solids (VS) or chemical oxygen demand (COD). The methane yield based on COD is 350 L/kg $COD_{removed}$ (Holm-Nielsen et al., 2009). The relative ease of degradation increases in the order: cellulose, hemicelluloses, proteins, lipids and carbohydrates, implying that substrates with relatively high lipids degrade faster than substrate whose composition is more of cellulose and hemicelluloses. Carbohydrates are divided into two broad categories: easily

degradable (VS_e) and slowly degradable (VS_s), which can be determined by equations below (Møller et al., 2004).

$$VS_e = VS\text{-}VS_{protein}\text{-}VS_{lipid}\text{-}VS_{VFA}\text{-}\ VS_s \quad (3)$$

$$VS_s = VS_{crude\ fiber}\text{-}VS_{lignin} \quad (4)$$

Even though lipids are shown in Table 1 to have high methane yield, more often they suffer from accumulation of long chain fatty acids (LCFAs) causing process inhibition (Hashimoto, 1983). The widely accepted mechanism of inhibition of lipids and LCFAs is by absorption on the surface of the microbial population, preventing any further hydrolysis (Fernández et al., 2005). In addition, LCFAs may inhibit even their own hydrolysis (Neves et al., 2009b). By recognizing the differences in substrate composition, various organic material that may be possible candidates for biomethanization have been reported with difference in biogas yield as in Table 2.

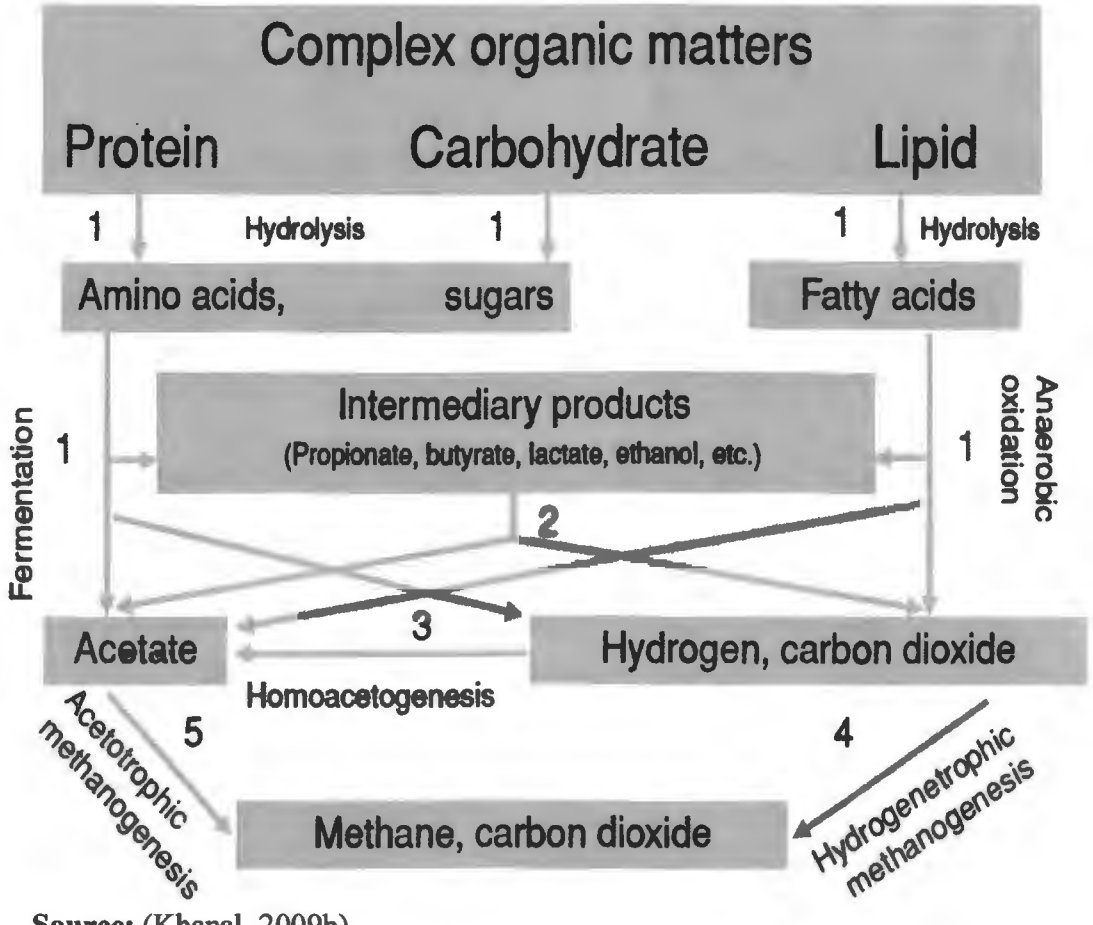
Table 2. Feedstock Characterization & Biogas Yield

Feedstock	DM, %	ODM, %	C/N ratio	BY, L/kg ODM	BYw, L/kg wet
Cow manure	7-15	65-85	3-10	200-400	25
Pig manure	3-13	65-85	6-20	350-550	27
Chicken manure	10-20	70-80	3-10	350-550	51
Vegetable waste	10-20	65-85	NR	400-700	75
Corn silage	15-40	75-95	NR	500-900	200
Grass Silage	30-50	80-90	NR	500-700	220
Fat slurry	8-50	70-90	NR	600-1300	310

DM=Dry matter, ODM=Organic dry matter, BY=Biogas yield, BYw=Biogas Yield), C/N=Carbon Nitrogen ratio, NR= Not reported.
Source: Chen et al., 1980; Møller et al., 2004

2.2 Microbiology of Anaerobic Digestion

Anaerobic digestion (AD) is a four steps biochemical transformation of complex organic matter into carbon dioxide, methane and intermediates. This process involves five different groups of bacteria as shown in Figure 1 (Khanal, 2009b). The bacteria obtain energy by catabolizing degradable matter to end-products CO_2 and CH_4 (Deublein and Steinhausser, 2008). These steps are hydrolysis, fermentation (acidogenesis), acetogenesis and methanogenesis brought about by hydrolytic enzymes, fermentative bacteria, syntropic bacteria, acetoclastic methanogens, and hydrogen-oxidizing methanogens, respectively (Henson, 2007). Some of these processes have been described in the following sections.



Source: (Khanal, 2009b)

Figure 1. Biochemical Conversion Stages

Hydrolysis:- This is the initial cleaving of complex macromolecules by different types of exo-enzymes secreted by the bacterial population. The rate of hydrolysis is controlled mainly by: pH, substrate composition, particles size (Henson, 2007).

Fermentation (Acidogenesis):- At the onset, conversion of sugar monomers to pyruvate ($C_3H_4O_3$), then to a variety of short chain organic compounds, primarily acetate, propionate, butyrate, alcohols, CO_2, and hydrogen, hence referred as acid-forming or acidogenesis (Henson, 2007). In slight variation of pH from 4.5 to 6.5, prevents the conversion of hydrogen into methane by the hydrogenotrophic methanogens (Khanal, 2009a). From bioenergy point of view, biohydrogen from this route is attractive especially when temperatures are elevated to thermophilic range but suffers from low yield in the range of 3.76 Mole H_2 per Mole of sucrose fermented (Khanal, 2009a).

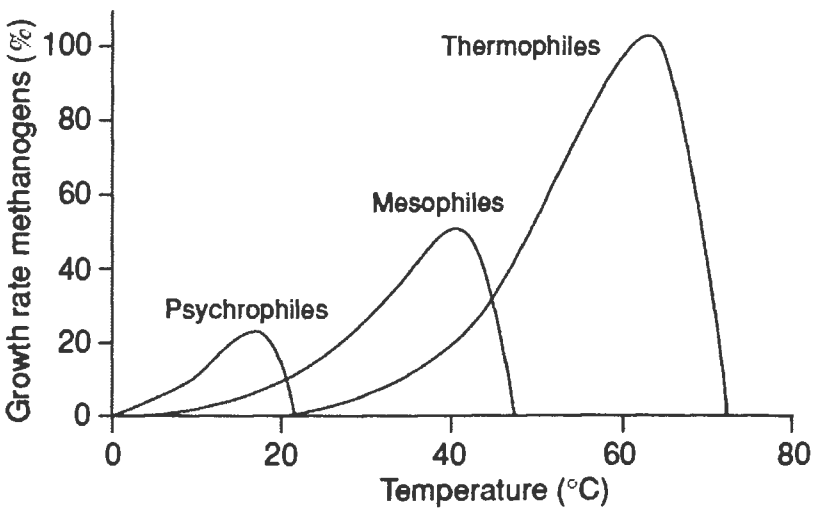
Acetogenesis:- Short chain simple organic acids are oxidized to acetate, hydrogen and CO_2 by the syntrophic bacteria. Any interruption at this stage causes accumulation of hydrogen that in turn inhibits the process. In two-stage AD configuration, this inhibition is minimized by collecting biogas rich in H_2 and CO_2 (Blonskaja et al., 2003).

Methanogenesis:- This is the final phase, where methane is produced from two different microbial groups, of which one third methane is from hydrogen and the other two thirds are from acetic acid as per the reactions shown below (Henson, 2007).

$$4H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O \qquad (5)$$

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad (6)$$

The second equation (6) plays a major role in the removal of acetate that contributes to the acidity. In addition, the methanogens remove hydrogen, allowing sustained growth of the syntrophic bacteria that makes acetate from propionate and butyrate. The activities of methanogens is heavily influenced by the temperature, the highest growth is at about 60-65^{0}C (Khanal, 2009a) as represented in Figure 2.



Source: (Khanal, 2009a)

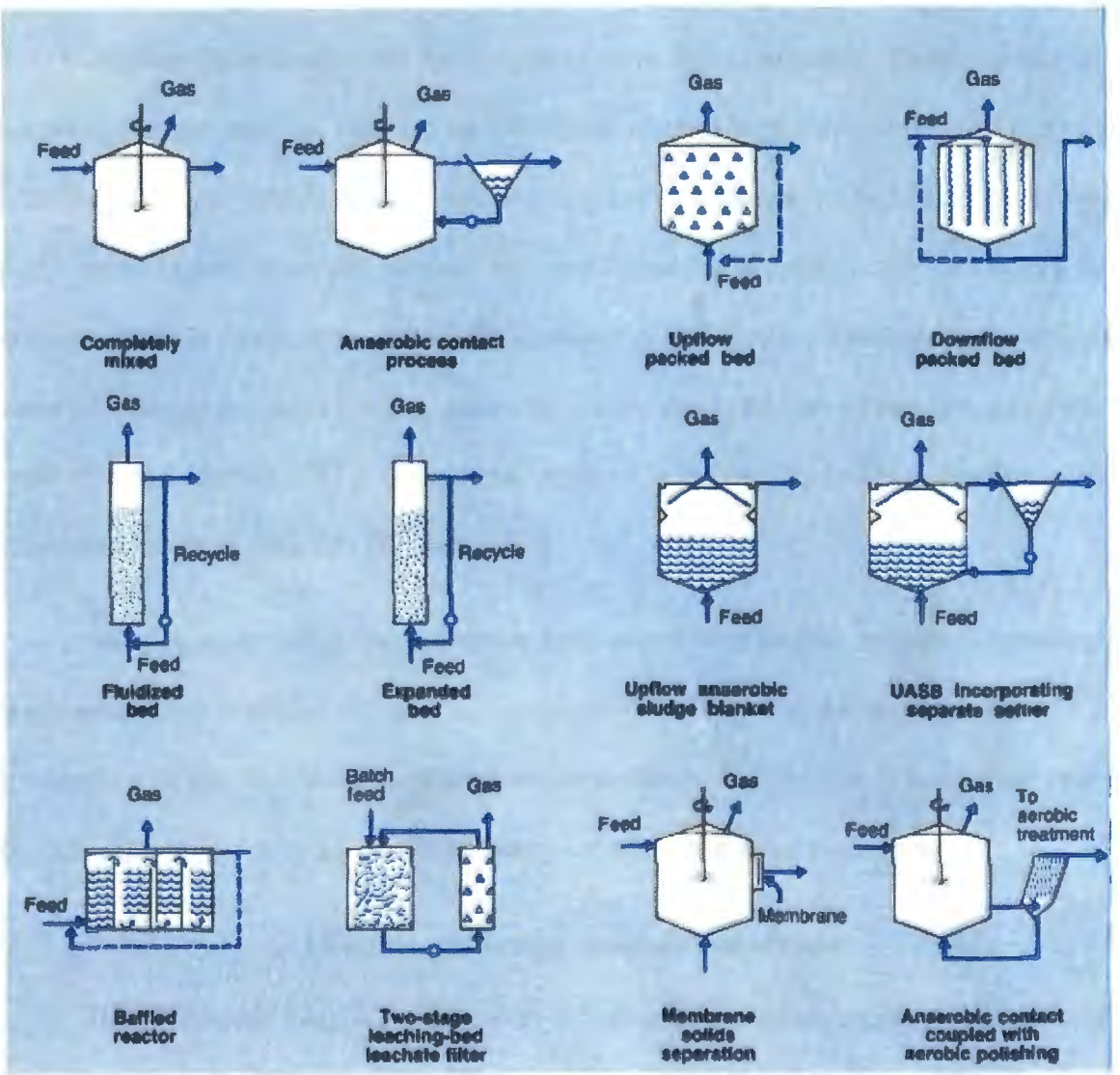
Figure 2. Relative Growth of Methanogens at Different Temperatures

Volatile fatty acids (VFAs), despite being known to cause inhibition, they are the most important intermediate products formed in AD processes (Liu et al., 2007). Accumulation of non-dissociated VFAs (acetate, propionate, isobutyrate, n-butyrate, isovalerate and n-valerate) in the bioreactor results in reduction of pH, making the process unstable and eventually leading into reduction of biogas production. The amount of biogas produced is closely tied to VFAs level in the digestate. Accumulation of VFAs leads to decrease in pH, resulting in an overall impact of poor quality gas characterized by high CO_2 content (Boe et al., 2010). In addition VFAs could result in reduced adenosine tri-phosphate (ATP) production and more VFAs are produced as the hydrogen ions are

diverted from the acetate metabolic pathway (Stafford, 1982). Accumulation of VFA can be used to signal an impending failure in the AD process, since it is a main factor in the process preceding methanogenesis (Speece, 1996).

2.3 Bioreactor Classification

Various configurations of biogas reactors are as described by (Speece, 1983) and represented in Figure 3.



Source: Speece, 1983

Figure 3. Various Biogas Reactor Configurations

Bioreactors are commonly classified based on the mode of operation either as batch or continuous. Continuous bioreactors are further classified as one stage and two-stage while batch bioreactors are either pure batch or fed-batch. In terms of wetness of substrate, AD may be classified as wet or dry, depending on the total solids (Liu et al., 2007). The wet AD processes are differentiated as liquid form (TS<5%) and slurry form (5%>TS<15%) while dry AD processes are when TS >20%. Dairy manure AD is more often operated as slurry.

Another classification will be in terms of either low or high rate, where low rate are unmixed systems operated with 1-2 kg COD/m^3/d whereas high rate operate with 5-30 kg COD/m^3/d (Khanal, 2009b). In this classification, low rate bioreactors will include lagoons, septic tanks, imhoff tanks and standard low rate biogas while the high rate bioreactors are systems such as continuous stirred tank reactor (CSTR), plug flow reactors, upflow anaerobic sludge blanket (UASB), anaerobic filters, fluidized bed bioreactors and many more (Khanal, 2009c). CSTR is the most common in treating agricultural residues, with total solids of about 10% TS (Weiland, 2010).

In some other works, the bioreactors have only been classified in terms of operating temperatures, pychrophilic (15-25^{0}C), mesophilic (33-40^{0}C) and thermophilic (55-65^{0}C). Irrespective of the classification of bioreactors as shown in Figure 3, a number of other factors affect biogas production. These factors are presented in the next section.

2.4 Factors Affecting Biogas Production

In AD process, biogas production is influenced by process design factors such as reactor type, configuration, mixing (Kaparaju et al., 2008), number of stages (Blonskaja et

al., 2003), and shape of bioreactor (Ward et al., 2008). In addition, environmental factors such as temperature, pH, and presence or absence of inhibitors play a crucial role (Khanal, 2009a). Other factors will include the substrate type (Yadvika et al., 2004), biogas potential (Labatut et al., 2011), inoculum to substrate ratio (Raposo et al., 2008), and the carbon to nitrogen (C/N) ratio (El-Mashad and Zhang, 2010). Virtually all these factors must be optimized, including the cost of implementation for a successful co-digester (Bekkering et al., 2010) and operational issues such as hydraulic retention times (HRT) (Cuetos et al., 2008), level of accumulation of VFAs (Parawira et al., 2004), and the need for either pre-treatment or use of additives (Ward et al., 2008). From Table 1, co-substrate rich in lipids, proteins, and carbohydrates are the prime feedstock candidates. Of particular importance is the C/N ratio, the buffer capacity and levels of free ammonia or presence of inhibition (Alvarez and Lidén, 2008; Chen et al., 2008). It is yet to be fully established on how ammonia inhibition is affected by the temperature of operation other than its influence on fatty acids (Chen et al., 2008). Traditionally, imbalances in the digesters have been monitored directly by changes in pH (Alastriste-Mondragon et al., 2006), but measuring alkalinity is more appropriate, and is easy to rectify the imbalances by addition of carbonate salts in the earlier stages (Ward et al., 2008). In general, a successful AD will be characterized by low presence of VFAs, less than 50 mM (Ahring, 1995), neutral pH, and a partial alkalinity (PA) of more than 1.2 g $CaCO_3$/L (Mshandete et al., 2005; Parawira et al., 2004). Whereas most of the co-digestion is done in the wet fermentation, an attempt to move to dry fermentation to reduce the reactor volume is likely to be desirable in the future. In addition to dry systems, two-stage continuous configurations operating at thermophilic temperatures results in higher conversion rates (Sosnowski et al., 2003).

2.5 Biogas Properties

Some biogas properties are given in Table 3, showing that it is flammable when the methane content is more than 45% (Deublein and Steinhausser, 2008). Impurities in biogas (ammonia, hydrogen sulfide and water vapor) have an overall impact on the quality, with carbon dioxide (CO_2) known to reduce the energy content, increase storage volumes, and cause corrosion. This is worsened by the presence of water vapor in biogas. Hydrogen sulfide (H_2S) is known to be poisonous, damages catalyst, causes corrosion and even affects burning (Chen et al., 1980). For most gas utilization units, the biogas needs to be dried and desulfurized (Weiland, 2010). Both carbon dioxide and ammonia have been shown to destroy alkali fuel cells (Deublein and Steinhausser, 2008).

Table 3. Biogas Properties

Property	Value
Composition	Methane (55-70%), CO_2 (30-45%) and others:H_2S, ammonia
Molar mass	16.043 kg/Kmol
Energy content	20.5-26.1 MJ/m^3
Density	1.2 kg/m^3
Explosion Limits	6-12% biogas in air
Smell	Bad eggs, unless de-sulfurized to become odorless

Source: Chen et al., 1980; Deublein and Steinhausser, 2008

By using various combinations of substrates, especially material rich in fats and lipids, and longer retention times, the CO_2 content in biogas can be influenced (Lastella et al., 2002). The resultant CO_2 content can be reduced by the $Ca(OH)_2$ precipitation, consequently increasing the actual methane content to a value of 82% (Ghaly, 1996). Dependent on the application and favorable economics, biogas may be cleaned and

upgraded to natural gas quality (Raven and Gregersen, 2007). Biogas is fairly comparable to both natural and landfill gas, which are constituted principally of methane gas and used for low cost energy. Thus, it is mainly useable in all applications that currently run on natural gas as shown in the next section.

2.6 Uses of Biogas

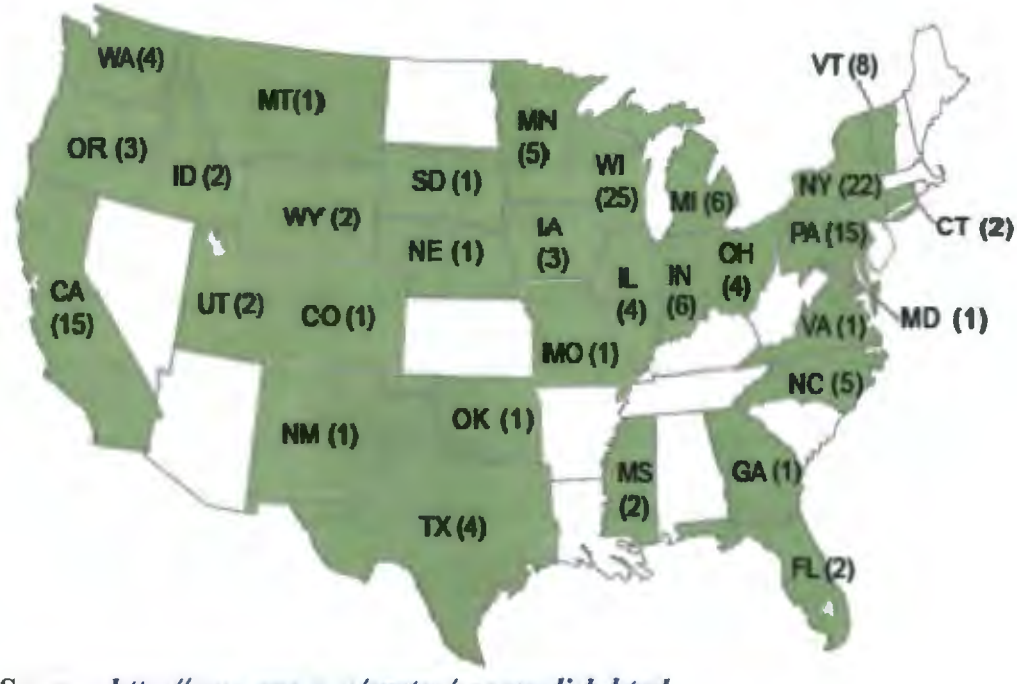
There are about four well known and highly regarded ways of harnessing the energy in biogas, namely: production of electricity, heat, combined heat and electricity, and upgrade to methane that can be injected in the natural gas supply (Bekkering et al., 2010). Other secondary uses of biogas include use in fuel cells, production of methanol and use as vehicle fuel (Holm-Nielsen et al., 2009; Poeschl et al., 2010). Most of these applications will require biogas purification to remove H_2S (known to cause corrosion), and adsorption of CO_2 to enhance energy content (Lastella et al., 2002).

2.7 Animal Manure Based AD

Customarily animal manure, mainly from cattle, swine and to a lesser extent poultry has been the main AD substrates (Lehtomäki et al., 2007; Yadvika et al., 2004). Dairy manure is the main primary substrate in co-digestion, partly due to its abundance (Nielsen and Angelidaki, 2008) and its unique properties. These properties are high water content making it easier to pump, good buffering capacity and presence of almost all the essential nutrients (Angelidaki and Ellegaard, 2003). Improper handling of manure leads to emission of methane and nitrous oxide that have been identified as GHGs, that resulted in 272.3 million metric tons (MMT) of CO_2 equivalent in 2005 in USA alone, with an increasing trend as the activities in the sector grow (Cuéllar and Webber, 2008). Animal manure AD is beleaguered with innumerable problems, such as the deficiency in biogas production and

methane yields (El-Mashad and Zhang, 2010). Despite the low yields, untreated or poorly managed manure becomes an environmental bane that causes odor nuisance, water pollution, pathogen emission, impacts wastewater strength and nutrient leaching (Cantrell et al., 2008; Nielsen and Angelidaki, 2008; Yiridoe et al., 2009).

Irrespective of the high initial capital and maintenance costs associated with farm based AD; a properly optimized biogas digester can provide several benefits, some of them not necessarily monetary (Yiridoe et al., 2009). However, most of the existing dairy manure based ADs in USA that rely on the sale of electricity (at the current tariffs) are not profitable; hence they are kept afloat through grants (Cantrell et al., 2008). There were 69,000 manure producing farms in USA with only 157 operational biogas plants that produced 374 GWh in 2009 (Figure 4). There is no operational farm-based biogas plant in the state of North Dakota.



Source: ***http://www.epa.gov/agstar/accomplish.html***.

Figure 4. Distribution of Biogas Plants in USA

2.8 Enhancing Biogas Production in Animal Manure Based AD

Livestock wastes, chiefly dairy manure may be converted into energy in a number of processes classified as biological (biogas, biohydrogen, biomethanol), thermochemical (pyrolysis, gasification) and a combination of biological and thermochemical processes (Cantrell et al., 2008). The single most interest in biogas production is to increase its quantity and quality i.e. the energy content. Several methods have been identified to address quantity and quality of biogas produced. Some of them include longer retention times (Lastella et al., 2002), higher temperatures (Khanal, 2009a), addition of trace elements or enzymes (Ward et al., 2008), pre-treatment (Møller et al., 2004) and co-digestion (Lehtomaki et al., 2007). Among all these methods, anaerobic co-digestion seems to be the most promising due to some of its advantages over other methods which are discussed in the next section.

2.9 Animal Manure Anaerobic Co-digestion

Co-digestion is defined as the simultaneous digestion of a homogeneous mixture of two or more substrates (Braun and Wellinger, 2002). In most cases a major substrate is co-digested with a single or variety of additional substrates such as municipal sludge, food & agriculture processing wastes, energy crops or agricultural residues (Alastriste-Mondragon et al., 2006; Angelidaki and Ellegaard, 2003; Buendía et al., 2009). The major digestion substrate is either an agricultural waste (animal manure) or municipal sourced waste, principally sewage and sludge (Macias-Corral et al., 2008). Dairy manure is the main animal waste for co-digestion. Other possible types of animal manures are swine and poultry. Both swine and poultry manure have restricted use in the co-digestion schemes. Irrespective of some reported success, these two types of manures have been

identified to be more demanding than dairy manure, as they are prone to ammonia inhibition (Chen et al., 2008; Murto et al., 2004; Wu et al., 2010).

Animal manure suffers from low carbon to nitrogen (C/N) ratio. The C/N ratio for dairy manure, swine, and poultry manure are 9:1, 8:1 and 6:1, respectively, as compared to the optimum C/N ratio 15:1 to 45:1 needed for successful AD (Itodo and Awulu, 1999). To increase the C/N to 20:1, agricultural residues such as corn stalks, wheat, and oat straw were added to manure which increased the volumetric methane production by 16 fold (Wu et al., 2010). In addition, the biogas production and methane content in the animal operation may be affected by: the type of animal, manure handling practice, livestock feed, amount of bedding as well as the stage of the animal growth (Møller et al., 2004). In co-digestion, a delicate balance is sought between the co-substrates in terms of ratio of macro- and micro- nutrients, formation of inhibitors, the C/N ratio, alkalinity, formation of toxic compounds and change in biodegradable constituents (Álvarez et al., 2010; Chen et al., 2008; Ward et al., 2008).

Co-digestion is a prime research theme in improving biogas yield (Parawira et al., 2004), especially in Europe where energy crops are being co-digested with manure (Ward et al., 2008). The straw used as bedding in dairy production has been arguably shown to increase the volumetric methane mainly as a result of its high volatile solids (Møller et al., 2004; Ward et al., 2008). But the straw is also recognized as contributing to the recalcitrance of dairy manure (Chen et al., 2008). The selection of a particular co-substrate is largely dependent on local availability and the methane yield potential (Lehtomäki et al., 2007; Parawira et al., 2004). In most cases, the feedstock does not carry a price tag; instead

it attracts some tipping fee and carbon credits, further improving the returns on the co-digestion (Schievano et al., 2009; Tafdrup, 1995).

However, the benefits of co-substrate additions are sometimes eroded by inhibition, in particular food wastes and slaughter house wastes which have been shown to cause excessive foaming, scum formation, and ammonia inhibition (Alvarez and Lidén, 2008; Chen et al., 2008; Murto et al., 2004). Nonetheless, proper mixing and an optimized organic loading are known to reduce scum formation (Lindorfer et al., 2008; Murto et al., 2004).

2.10 Benefits of Anaerobic Co-digestion

Animal manure is known to have high levels of nutrients (nitrogen and phosphorous) but lack of high chemical oxygen demand (COD), while food wastes are known to have high COD but lack the necessary nutrients and the buffering capacity (El-Mashad and Zhang, 2010; Neves et al., 2009a). The co-digestion of these two types of substrates results in better performance (close to 70% CH_4 content) than any individual digestion (Macias-Corral et al., 2008). Substrates that are rich in lipids and/or carbohydrates with high volatile solids (VS) contents are good candidates for co-digestion with manure (Cuetos et al., 2008; Labatut et al., 2011).

Dairy manure co-digested with mustard oil cake (MOC) increased biogas production by 63.44% compared to conventional dairy manure digestion only (Satyanarayan et al., 2008). Alvarez and Lidén (2008) showed that a combined treatment of animal manure and fruit vegetables in the mesophilic range results in the reduction of VS by 50% to 65% and these wastes may not successfully be treated alone. Moreover, co-

digestion of dairy manure with fibrous material presents a rare opportunity of adding value to the fertilizer and manure is known to aid digestion of the fibers (Macias-Corral et al., 2008; Umetsu et al., 2006). The digestate has higher nitrogen, reducing the need of additional nitrogen fertilizer in the affected fields (Lazarus, 2008).

2.11 Disadvantages of Co-digestion

The complexity and the inconsistent nature of the potential co-substrates make it difficult in predicting and anticipating the whole impact of co-digestion (Nielsen and Angelidaki, 2008), including the possibility of inhibitory behavior (Chen et al., 2008). The ratios, composition, physical characteristics and any pre-treatment of the possible co-substrate must be precise for optimum results and methane yield (Labatut et al., 2011; Misi and Forster, 2001; Neves et al., 2009a). Some co-substrates, notably blood irrespective of temperature, are known to cause inhibition and/or toxicity to the digestion process (Alvarez and Lidén, 2008).

Intermittent addition of small quantities of tallow oil has a potential to interrupt a stable digestion process (Nielsen and Angelidaki, 2008). This is due to accumulation of ammonia and the presence of long chain fatty acids (LCFAs) from blood and tallow oil, respectively. In addition, it is possible that co-digested slurries have a higher methane emission in storage than undigested slurry as exemplified by the addition of potato starch in dairy manure and swine manure (Clemens et al., 2006; Kaparaju and Rintala, 2005). Low methane yields in co-digestion are an indication of inhibition that could be caused by accumulation of ammonia and/or volatile fatty acids (VFAs) beyond the threshold levels (Parawira et al., 2004).

2.12 Anaerobic Co-digestion Substrates

Although a diverse number of biological wastes are available, dairy manure co-digestion substrates are mainly food (pre- and post- consumer food remains), energy crops and crop residues, and biofuel processing wastes (Cavinato et al., 2010). Other farm livestock wastes like poultry and swine manure can also be used as either main substrate or co-substrate. It must be noted that liquid biofuels, namely bioethanol and biodiesel have been developing quite rapidly in the last decade, generating increased amount of co-products and wastes that can be used for co-digestion (Schievano et al., 2008).

2.12.1 Food Wastes

Co-digesting processed food with dairy manure increases biogas production and methane yield due to their high biodegradability (Neves et al., 2009a). Furthermore, it provides additional nutrients that promote thriving of the microbial population (Kaparaju and Rintala, 2005). Food wastes are a promising future co-substrate due to the increase of food processing plants and the ban of organic wastes in landfills in some countries (Li et al., 2009b; Murto et al., 2004; Zupancic et al., 2008). Co-digestion of manure and food has an effect of reducing accumulation of volatile fatty acids (VFAs) and other intermediates in the first five days, consequently resulting in higher methane content right from the beginning as compared to digestion of food wastes alone (El-Mashad and Zhang, 2010; Murto et al., 2004). Most fruits and vegetable wastes have high levels of volatile solids, easily biodegradable but suffer from deficiency of total solids (Carucci et al., 2005; Neves et al., 2009b). In most cases, they hydrolyze faster and lead to production of acids, lowering pH, thus causing inhibitions among the methanogens (Ward et al., 2008). Another mechanism in which some food wastes impair dairy manure digestion is via increased

formation of scum, and those that have high salinity are suspected to trigger inhibition (El-Mashad and Zhang, 2010). The suitability of food as a co-digestion substrate is subject to seasonal and composition variation, as well as high heterogeneity associated with the stage of processing/cooking, nutrient content and the particle sizes (Carucci et al., 2005; Neves et al., 2009a). Nonetheless, food that is rich in lipids, and easily biodegradable carbohydrates such as confectionary wastes, used oil, pasta, ice cream, whey etc. has been identified as prime co-substrate (Labatut et al., 2011).

2.12.2 Energy Crops and Agricultural Residues

Most energy crops have high carbon content that balances the low C/N ratio in dairy manure, effectively decreasing the risk of ammonia inhibition (Lehtomäki et al., 2007). For example, a co-digestion of dairy manure with 40% sugar beet tops improved methane production by 150% (Umetsu et al., 2006), while the co-digestion of switch-grass with dairy manure did not show any momentous gains in the biogas yield, but biogas yield improvements were noted with swine manure (Ahn and Smith, 2008). Similarly, the co-digestion of dairy manure with rice husks did not improve the overall biogas production. In some cases, it may be profitable to pre-treat the energy crops, to ease the hydrolysis and consequently reduce on the retention times (Lehtomäki et al., 2007; Ward et al., 2008). However, by-products such as potassium ions resulting from caustic pre-treatment and resins generated in breaking the lignin are likely to add inhibition (Chen et al., 2008). Studies have shown that pre-treatment such as alkaline addition and size reduction of residues permits faster and better hydrolysis (Clemens et al., 2006; Kaparaju and Rintala, 2005; Lindorfer et al., 2008). It is paramount to point out that due to the high carbon content in crop residues, digested material may still have a potential to produce biogas,

leading to methane production during storage and even post-storage (Clemens et al., 2006; Kaparaju and Rintala, 2005; Lindorfer et al., 2008).

Energy crops are slower in adapting to the microbial population (Lindorfer et al., 2008). In addition, energy crops that are lignocellulosic have low digestibility, and required prolonged HRTs (Ward et al., 2008). As the amount of herbaceous biomass is added to the animal manure, there is a threshold beyond which stratification and scum formation is experienced, decreasing the gas space and reducing permeability of the gas. The carbon, crude protein, fats and lignin content of the energy crop have an effect on the methane yield in a co-digestion (Amon et al., 2007; Wu et al., 2010). For energy crops, biomass yield seem to be a primary consideration (Nallathambi Gunaseelan, 1997). The success of co-digestion will be impacted by the costs as detailed in the next section.

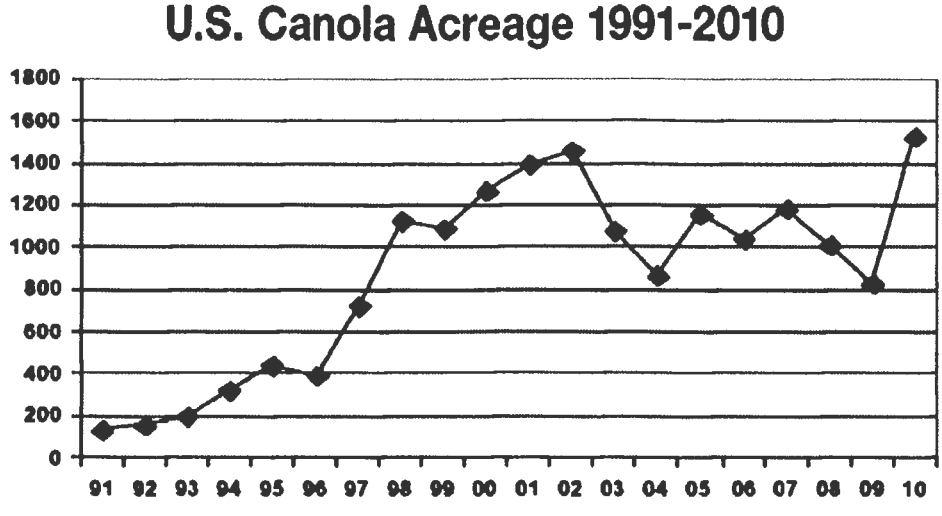
2.13 Economics of Co-digestion

Co-digestion has favorable economic indicators, with a net present value for a medium sized plant (38 m^3/d of liquid manure and 70000 kg/d of maize) with post digestion treatment and biogas used for generation of electricity is pegged at 3-5 years (Cavinato et al., 2010) and a better ratio of energy input to output than mono-substrate digestion (Poeschl et al., 2010). In Europe, cultivation of energy crops for renewable energy attracts a subsidy of 45 Euros/ha (Lantz et al., 2007) further improving on profitability of animal manure co-digestion. Nevertheless, as expected the costs of producing a volume of biogas when energy crops are used are higher as exemplified in analyzed costs of 0.28 Euro/m^3 and 0.20 Euros/m^3 in co-digestion of swine manure with energy crops and organic municipal, respectively (Schievano et al., 2008). Equally, selection of method and reagents for pre-treatment can have an implication on the overall

results, especially digestion inhibition (Hendriks and Zeeman, 2009). In previous studies, more than USD 100,000 might be needed to add co-digestion handling system without storage while of an average size plant would cost USD 5 million in 1995 (Alastriste-Mondragon et al., 2006). Capital and maintenance cost can vary widely depending on biomass, technology and operation philosophy. These factors will affect the choice of co-substrate such as canola meal for dairy manure co-digestion.

2.14 Canola Meal as a Prospective AD Substrate

Canola is a fairly tall plant, about 1.20-1.80 m, with yellow flowers and belongs to the family *Brassicaceae* to which also belong other oil seed plants like rapeseed (Brown et al., 2008). Canola meal is a by-product of oil extraction from canola seed, a small brownish seed that contains 44% oil, and produces an oil cake rich in proteins that is popular as animal feed. The acreage of canola has increased over the last two decades from less than 0.2 million acres in 1991 to more than 1.6 million acres in 2010 as per Figure 5, 90% of the acreage is in North Dakota.



Source: http://www.uscanola.com/crop-production

Figure 5. Growth of Canola Acreage, in 000 from 1991-2010

This acreage is bound to grow as the demand for canola oil increases due to its acclaimed attributes as a vegetable oil as well as a biodiesel feedstock. The vegetable oil from canola seeds is promoted for its potential health benefits (Brown et al., 2008). Canola meal's chemical composition is comparable to other oil meals/cakes that are available (Table 4).

Table 4. Chemical Composition of Various Oil Meals/Cakes

	Oil Cake	DM, %	CP, %	CF, %	Ash, %	Ca, %	P %
1	Canola	90	33.9	9.7	6.2	0.79	1.06
2	Coconut	88.8	25.2	10.8	6.0	0.08	0.67
3	Cotton	94.3	40.3	15.7	6.8	0.31	0.11
4	Groundnut	92.6	49.5	5.3	4.5	0.11	0.74
5	Mustard	89.8	38.5	3.5	9.9	0.05	1.11
6	soybean	84.8	47.5	5.1	6.4	0.13	0.69
7	Sunflower	91	34.1	13.2	6.6	0.30	1.30

DM=Dry matter, CP=Crude Protein, CF= Crude fiber, Ca= Calcium, P= Phosphorous **Source:** Ramachandran et al., 2007

Further the biogas potential from various form of rapeseed, belongs to the same family as canola have been tried, with the meal showing 320 L/kg VS against the seed at 470 L/kg VS and considerably calorific value of 15.8 MJ. It shows that digestion of the rapeseed (same family as canola) is possible. Its digestion is hampered by presence of hemi-cellulose and lignin, structural materials that are more difficult to decompose. Kolesárová et al. (2011) has identified accumulation of VFAs as a probable cause of poor digestion in materials that contain high content of oil, as the fats are known to decompose faster to VFAs than methanogenic bacteria is able to convert them to biogas (Figure 1). Oil cakes are generally rich in nitrogen but lack the necessary carbon. From available literature,

problems associated with use of oil cakes and meals in AD process may be alleviated by application of co-digestion, more especially dairy manure.

Nevertheless, it must be noted that oilcakes and meals have other beneficial uses such as animal feed concentrates, production of enzymes and other bio-products. In addition, oil cakes can be used directly in producing energy by pyrolysis (Ramachandran et al., 2007).

2.15 Justification of the Research

Emission of GHGs and production of odor from the animal husbandry continue to be issues of concern not only in North Dakota but also in most other states in the Northern Plains of USA and Canada. There is no operational farm based biogas plant in North Dakota as per Figure 5. Consequently, this points out the potential for odor nuisance and environmental pollution (Yiridoe et al., 2009). This may be exacerbated by the ever increasing prices of fossil fuels that lead to high heating costs in the dairy barns (Lantz et al., 2007). In response to reducing the energy costs, the department of energy (DOE) in collaboration with USDA, have identified a potential of 1 billion tons of dry biomass that can be put into use in provision of renewable energy where animal manure will contribute about 35-40 million dry tons (Cantrell et al., 2008).

One of the most acceptable forms of converting the animal manure into energy and reducing GHGs emission is via the biogas route (Clemens et al., 2006). Others may include combustion in fluidized beds, pyrolysis into bio-oil, direct liquefaction, biohydrogen production and valorization to bioproducts (Cantrell et al., 2008; Ward et al., 2008). Biogas production, through the well known and proven technology of AD is well developed in

Europe including cold climate countries like Sweden and Denmark (Lantz et al., 2007; Raven and Gregersen, 2007). According to the Great Plains Institute, the Midwest states have a potential to match Europe in producing biogas from agricultural wastes if the technical and policy barriers are removed (Bilek, 2010). The attractiveness of biogas has been hindered by poor publicity as a result of low biogas production rates on manure only based systems. Co-digestion has been identified to enhance biogas production and methane yield (Ward et al., 2008). North Dakota produces over 90% of canola in US, and considerable canola meal that is currently used as an animal feed (Brown et al., 2008). In the future times as canola production grows due to the demand from biodiesel and vegetable oil industries, it is predicted that canola meal will be in surplus, making canola meal a possible co-substrate candidate in dairy manure digestion.

In addition, EPA greenhouse tailoring rule is likely to become more stringent on small sources of GHGs, including animal production units (Bilek, 2010). There is also a need for the animal facilities to cushion themselves against the ever rising prices of energy by being self sufficient. Moreover, biogas production earns carbon credits and reduces net energy consumption for the farmers, ultimately improving their economic returns (Cuéllar and Webber, 2008). The goal of this research is to close the gap on the suitability of canola meal as a co-substrate in enhancing biogas from dairy manure and ultimately making farm based biogas plants in North Dakota profitable.

CHAPTER 3. SUITABILITY OF CANOLA MEAL FOR ANAEROBIC CO-DIGESTION WITH DAIRY MANURE

3.1 Abstract

Due to the renewed interest in the biogas production, co-digestion of livestock manure, especially dairy manure, and other agro-wastes has emerged as an appropriate technology in enhancing the economic viability of anaerobic digestion. In this study, various ratios of canola meal: dairy manure (100:0, 40:60, 20:80, 10:90 and 0:100) by volume basis were co-digested in 0.5 L batch reactors at a temperature of 35 ± 1^0 C for 25 d. Pre- and post-digestion samples were collected and analyzed for nutrients, pH, volatile fatty acids, fibers and chemical oxygen demand (COD). Biogas yield was measured daily using the water displacement method, and gas composition (mainly methane) was analyzed weekly within 72 h of collection using a gas chromatograph with flame ionization detector (FID). Results indicated that 0% canola meal (100% manure) and 100% canola had a specific methane yield of 352 and 83 LCH_4/kg VS, respectively. Addition of canola meal in the dairy manure resulted in decreased cumulative biogas and specific methane yield. This is suspected to be caused by elevated levels of volatile fatty acids (VFAs) of more than 4000 mg/L. It could be necessary to look into ways of overcoming the inhibition caused by elevated VFAs.

Keywords: Biogas, canola meal, co-digestion, dairy manure, methane, volatile fatty acids, anaerobic digestion.

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3.2 Introduction

Large scale confined livestock operations have emerged over the last few years which generate a significant amount of manure and wastewater. Currently, in USA, almost all manure is applied to cropland for disposal as it contains nutrients and organic matter. This is to meet crop nutrient requirements and to improve the physical and biological conditions of the soil. When not properly managed and applied, application of manure on land can pose environmental problems. With the increasing size and regional concentrations of confined animal feeding operations, there is a growing concern of aggravated environmental problems due to increased manure volume and excessive manure application rates on the soil (Larney et al., 2000). Manure may be viewed as a resource for the production of renewable energy and environmental concerns associated with liquid manure storage and land disposal can be overcome through anaerobic digestion (AD), where a large amount of manure can be converted to bio-methane, a renewable energy source.

Anaerobic digestion (AD) is a common practice of organic waste disposal, in which biodegradable material is broken down in the absence of dissolved oxygen or its precursors into biogas; a mixture of methane, carbon dioxide and traces of hydrogen sulfide, ammonia and water vapor (Deublein and Steinhausser, 2008) . According to Kaparaju & Rintala (2011), AD serves three basic purposes: management of odor, provision of energy and reduction of greenhouse gases (GHGs) emission. In addition, AD process reduces water pollution, facilitates better pathogen and weed control (Tafdrup, 1995), and digestate can be used as fertilizer for crops. Biogas is a renewable fuel used in cooking, heating, generating electricity, fuel cells, direct vehicle fuel, and production of chemicals (Cantrell

et al., 2008). These benefits have been offset by the high capital and operating costs of AD installations (Yiridoe et al., 2009), and low biogas production from AD of manure only. Low biogas production per unit mass of dairy manure leads to poor economic performance and a bad reputation of dairy manure based AD processes (Tafdrup, 1995; Zhang et al., 2007). Notwithstanding, there has been a renewed impetus for biogas production for bioenergy and control of GHGs in the last couple of years (Álvarez et al., 2010; Kaparaju and Rintala, 2011). In 2009, operating biogas plants achieved an equivalent of 1.1 million tons of CO_2 avoided GHGs emissions in the USA alone. This is comparable to reducing oil consumption by 2.7 million barrels (USA-EPA, 2010).

To optimize biogas production, co-digestion, the simultaneous digestion of two or more organic substrates has been explored (Lehtomäki et al., 2007; Neves et al., 2009a; Parawira et al., 2008). Anaerobic co-digestion offers a rare balance, synergism in terms of micro- and macro-nutrients, pH, carbon to nitrogen ratio (C/N), and suppresses toxicity (Álvarez et al., 2010). The overall impact is increased biogas production per unit volume of a reactor attributed to increased biodegradable materials. This creates new opportunities for use of various substrates in dairy manure AD processes.

Dairy manure remains the foremost primary substrate for co-digestion, due to its abundance and its unique properties such as high water content, good buffering capacity and presence of almost all the essential nutrients (Li et al., 2009a). The most cited advantages of co-digestion are increased methane yield attributed to additional nutrients from the co-substrates, and maximum benefits on the biogas installations arising from processing many substrates. The carbon to nitrogen (C/N) ratio for dairy manure is 9:1 compared to the optimum C/N ratio 15:1 to 45:1 required for a successful AD (Itodo and

Awulu, 1999). In order to increase the C/N ratio to 20:1 and thus biogas production, agricultural residues such as corn stalks, wheat, and oat straw have been co-digested with dairy manure (Chen et al., 2008; Møller et al., 2004). However, not all co-substrates are suitable for co-digestion and more research is needed to evaluate different substrates that are locally available and considered as waste.

Some of the co-substrates are by-products of food processing such as potato and sugar beet wastes (Parawira et al., 2004), and biofuels processing (Dhanya, 2009; Kolesárová et al., 2011). In addition, there has been an increased exploration of organic residues from various sectors of agriculture and industries over the past decades. Crop residue, such as wheat straw (Møller et al., 2004) is used as a potential raw material in bioprocesses as it provides an excellent substrate for the growth of microorganism supplying the essential nutrients. Co-digestion substrates are selected based on their local availability. The literature review revealed that the use of some co-digestion materials might have both positive and negative impacts on the co-digestion process. The major areas of concern in co-digestion include balance in nutrients, C/N ratio, biodegradability, presence of inhibitors, as well as a favorable pH (Álvarez et al., 2010). Thus, there is a need to explore more suitable materials for the co-digestion process which are locally available and will consequently optimize the biogas production and profitability.

North Dakota is an agricultural state and produces a significant amount of agricultural and food processing wastes which could be co-digested with manure to increase methane production. For example, canola is grown as an oilseed crop in the USA, Canada, and many parts of the world. In USA, the acreage of canola has increased over the last two decades from less than 0.2 M acres in 1991 to more than 1.6 M acres in 2010, of

which more than 90% of the acreage is in North Dakota. This acreage is bound to grow as the demand for canola oil increases due to its acclaimed attributes as a vegetable oil, potential health benefits as well as traits as a biodiesel feedstock (Brown et al., 2008). Canola meal is a by-product of oil extraction from canola seed, a small brownish seed that contains 44% oil, and produces an oil cake rich in proteins that is popular as animal feed (Brown et al., 2008).

Though canola meal is presently used as animal meal, in the future as canola production grows, it is predicted canola meal will be in surplus. Canola meal is heralded as being rich in proteins, and some oil (residual oil from the extraction), making it a suitable substrate for biogas production (Kolesárová et al., 2011). Proteins and oils have a higher specific methane production of 496 and 1014 L CH_4/kg VS, respectively, as compared to carbohydrates at 415 L CH_4/kg VS (Møller et al., 2004). Furthermore, in co-digestion, the addition of oily wastes to dairy manure up to 12 g COD_{oil}/ $L_{reactor}$ has been shown to enhance methane production (Neves et al., 2009a). Thus, for North Dakota, Northern Minnesota and parts of Canada, canola meal might be a potential candidate for use in co-digestion with dairy manure when it is optimised. Limited AD studies have been carried out on rapeseed (the whole seed, the cake and meal), in the same family, indicating some volatile solids reduction improvement in the AD process, but resulted in low biogas production due to accumulation of fatty acids (VFAs) (Kolesárová et al., 2011). No study has been conducted to examine the suitability of canola meal for co-digestion with dairy manure and its biogas potential. Therefore, the objective of this study was to examine the suitability of canola meal for co-digestion with dairy manure, biogas production potential, and methane content.

3.3 Materials and Methods

3.3.1 Substrate and Inocula Preparation

Dairy manure and canola meal were used as the main substrate and co-substrate, respectively. The dairy manure, obtained from the NDSU dairy farm was kept at 4°C for two days prior to starting of digestion while canola meal was obtained from Archer Daniels Midland factory, Velva, North Dakota. This canola processing facility used solvent extraction process for oil extraction that effectively lowers the residual oil in the canola meal to 2.5%. The raw manure was blended for homogeneity. The inocula were collected from an existing biogas plant at American Crystal Sugar (ACS), Moorhead, MN. Sugar beet pulp waste is the substrate in the biogas plant and the operating temperature was 35±2^{0}C with addition of caustic soda to correct the pH. After collecting inocula, they were kept in an incubator (Model 1525, Sheldon manufacturing, Oregon, USA) at 35±1^{0}C for 24 hrs. Sub-samples were collected from substrates and inocula and analyzed for nutrients, solids, pH, volatile fatty acids (VFAs), fibers and chemical oxygen demand (COD). Additionally, canola meal was analyzed for size distribution.

3.3.2 Experimental Set up

In this study, 0.5 L Erlenmeyer flask was used for bioreactor (Figure 6), with the initial volume (substrates and inocula) of 0.35 L and incubated in a water bath (VWR, USA) at 35±2^{0}C for 25 d. The water bath was set up to shake at 70 cycles/min to facilitate mixing and prevent settling. Before adding substrates and inocula, the reactor was flushed with nitrogen (Praxair, Fargo, ND, USA) to expel the oxygen in the reactors. After adding predetermined substrate and inocula in a reactor, it was flushed again with nitrogen and sealed for anaerobic condition. In this study, six bioreactors were set up in three replicates.

Out of six bioreactors, bioreactor R1 had inoculum only; R2, R3, and R4 had canola meal and manure mixture ratio of 10:90, 20:80, and 40:60, respectively, in terms of volume.

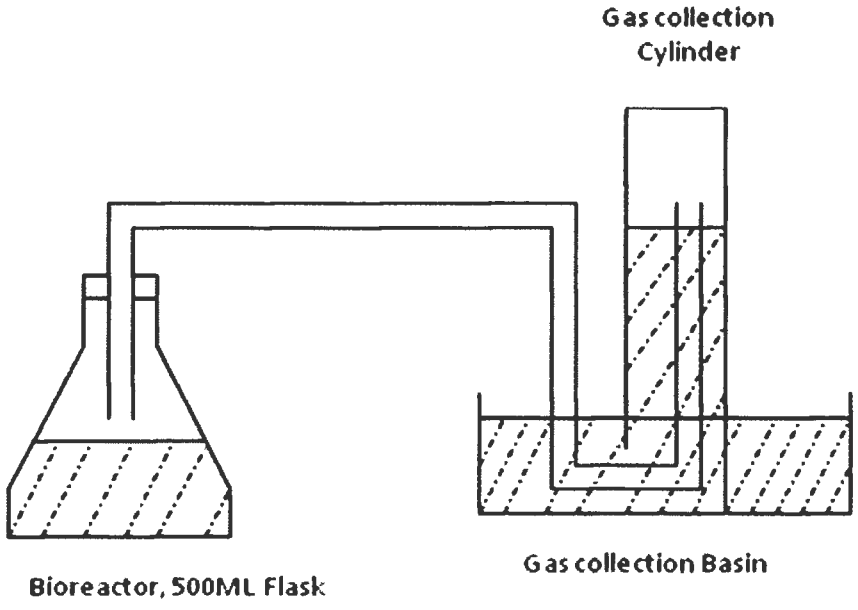


Figure 6. Experimental Set-up

Whereas, R5 had 100% canola meal and R6 had only manure. In each bioreactor, 0.1 L of inoculum was added to maintain inoculum to substrate ratio (ISR) of 2.5 as suggested by Raposo et al. (2008). Both manure and canola meal were diluted to about 7.5±2.0% TS separately before being mixed in the ratios stated above. After set up, the bioreactors were flushed with nitrogen gas for two minutes to induce anaerobic conditions in the headspace. During the course of this study, biogas was measured daily by water displacement method (Figure 6), while the biogas composition was measured weekly from the gas samples collected from the reactor headspace. For each reactor, pre- and post-digested samples were collected and evaluated for pH, VS, nutrients, fibers and COD. In addition, volatile fatty acids (VFAs) were also measured with gas chromatography as per Raposo et al. (2008).

3.3.3 Sample Analysis

Using standard methods (APHA, 2005), substrates (manure and canola meal) and inocula samples were analyzed for nutrients, sediment, pH, and electrical conductivity (EC). Conductivity and pH were analyzed using a hand held Orion pH meter (Model 990). Solids and nutrients were analyzed at Soil Testing Laboratory, North Dakota State University. Chemical oxygen demand (COD) was measured using Hach spectrophotometer (model DR5000) following standard procedure (APHA, 2005). The samples were diluted 100 times to measure COD due to high COD content. Fiber analysis such as cellulose, hemicelluloses and lignin, acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) were analyzed according to the NREL procedure (NREL/TP 510-42618). Hemicellulose content was estimated as the difference between NDF and ADF, the cellulose content was the difference between ADF and ADL, and lignin was the value of ADL. For all samples, canola meal was ground before being extracted with n-hexane solvent as per method described by Haagenson et al.(2010) and proteins were measured from organic nitrogen composition, while the oil content was determined by the soxhlet extraction method.

Biogas was collected from the headspace of reactors using syringes and transferred to 5 mL vials. Gas composition was determined using a gas chromatography (GC) (Hewlett Packard, Model 5890, Agilent, USA) with a super-Q plot column (30 m × 0.53 mm) and flame ionization detector (FID). The oven was set at a maximum temperature of 250^0C. All biogas composition measurements were in triplicates. 5 μL of biogas was injected into the GC column with an inlet temperature of 150^0C and detector temperatures of 250^0C. The

system was calibrated against methane calibration gas (96.8% purity obtained from Praxair Air, Fargo, ND, USA).

3.3.4 Data Analysis

Statistical analysis of variance (ANOVA) on biogas production, methane content, volatile solids destruction, COD consumption and methane content were evaluated using SAS (SAS, Cary, NC) at 5% level of confidence. The biogas production means were compared by LSDs at 95% level of confidence.

3.4 Results and Discussion

3.4.1 Substrate Characteristics

Substrate (fresh manure and canola meal) and inocula characteristics are listed in Table 5. Dairy manure had a higher percentage of volatile solids (90.5 ±1.5%) than canola meal (87 ±3.5%). In terms of solids, canola meal had the highest TS content. In addition, dairy manure had higher COD, VFAs and fiber content as compared to canola meal. The total VFAs in the dairy manure were 4227 ± 43 mg/L, where as it was 514 ± 8 mg/L in canola meal. VFA in manure compromised of acetic acid (2538 ±43), propionic acid (960±12), butyric acids (530±4) and valeric acid (200±3) mg/L. This was explained by the fact that manure had partially hydrolyzed in the gastrointestinal tract of animal. In the case of canola meal, the main VFA was valeric acid (442±27) mg/L which might lead to inhibition of the growth of microbial species and methanogenic process. Canola meal was rich in proteins and nutrients, mainly phosphorous (0.8±0.1 mg/L) and nitrogen (4.5±0.3 mg/L), resulting in C/N of 7.8±0.08. The pH of manure sample was (6.7±0.1), the inoculum was (7.4±0.15), and canola meal was (6.0±0.1). Compared to others, canola meal

was slightly acidic, which might affect the digestion process. The inoculum was slightly alkaline due to the addition of caustic soda in the ACS biogas plant.

Table 5. Substrate Characteristics

Parameter	Units	Dairy manure	Canola	Inocula
COD	g/L	120.0(17.0)	100.0(13.0)	7.1(3.0)
TS	%	12.3(2.5)	90.2(1.2)	4.2(0.7)
VS	%TS	90.5(1.5)	87(3.5)	91(2.5)
pH	-	6.7(0.1)	6.0(0.1)	7.4(0.2)
P	mg/L	0.06	0.8	0.02
N	mg/L	0.6(0.1)	4.5(0.1)	0.05(0.0)
C/N ratio	-	12.6(2.5)	7.8(0.8)	9.8(1.3)
VFAs	mg/L	4227.0(430.0)	514.0(27.0)	78.0(3.0)
Acetic acid	mg/L	2538.0(43.0)	93.0(1.0)	74.0(2.0)
Propionic acid	mg/L	960.0(12.0)	20.0(2.0)	0(0)
Butyric acid	mg/L	530.0(4.0)	0(0)	0(0)
Valeric acid	mg/L	200.0(3.0)	442.0(27.0)	0(0)
Crude protein	%DM	16.7	40.0	13.0
Solubles	%DM	54.0	70.0	90.0
Hemicellulose	%DM	18.0	10.5	6.2
Cellulose	%DM	20.0	11.0	4.3
Lignin	%DM	8.8	7.7	0.6

()=Standard deviation

The high fiber content in manure was the result of the bedding material. However, most of the fiber in manure was hemicellulose (18%) which is known to be more biodegradable than either cellulose or lignin. Canola meal contains higher crude protein (CP) and more soluble matter than dairy manure (Table 5). As a result it could be expected

that canola will have higher potential for biogas production since it contains about 40%TS as crude protein (Møller et al., 2004).

Ingestate (initial) characteristics of bioreactors are listed in Table 6. The C/N ratio in the R5, the bioreactor containing 100% canola was lowest (7.8), while all other reactors had a ratio of more than 12.5, except inoculum (9.8). The low C/N ratio in the R5 can be attributed to high nitrogen content since canola meal contains 40% crude protein. Based on the low C/N ratio in canola meal, co-digestion with dairy manure was aimed at improving the C/N ratio of the canola meal. The R5 had the highest total solids (92.8 ± 1.4 g/L), while R6 and R2 had the lowest TS (68.9 ± 0.5 and 67.1±4.1 g/L, respectively). Although the aim was to have uniform initial total solids, this was not achieved due to the non-homogeneity in the substrates.

Table 6. Ingestate Characteristics

Brct	TS (g/L)	VS(g/L)	COD(g/L)	pH	C/N Ratio	VFA (mg/L)
R1	33.4(7.5)	26.4(3.3)	5.0(2.0)	7.4(0.1)	9.8(1.3)	78.0(3.0)
R2	67.1(4.1)	57.5(0.9)	85.7(15.3)	6.6(0.1)	15.4(2.3)	3977.0(35.0)
R3	78.7(6.8)	72.7(0.8)	82.3(9.6)	6.6(0.0)	15.2(3.1)	3633.0(59.0)
R4	72.3(5.7)	60.4(0.4)	81.4(13.0)	6.4(0.1)	13.1(1.7)	3032.0(49.0)
R5	92.8(1.4)	76.6(1)	72.9(8.7)	6.0(0.1)	7.8(0.8)	555.0(27.0)
R6	68.9(.5)	63.5(0.6)	87.2(6.8)	6.7(0.0)	12.6(2.5)	4227.0(43.0)

% Canola R1=Inoculum, R2=10%, R3=20%, R4=40%, R5=100%, R6=0% (100% manure)
()=Standard deviation

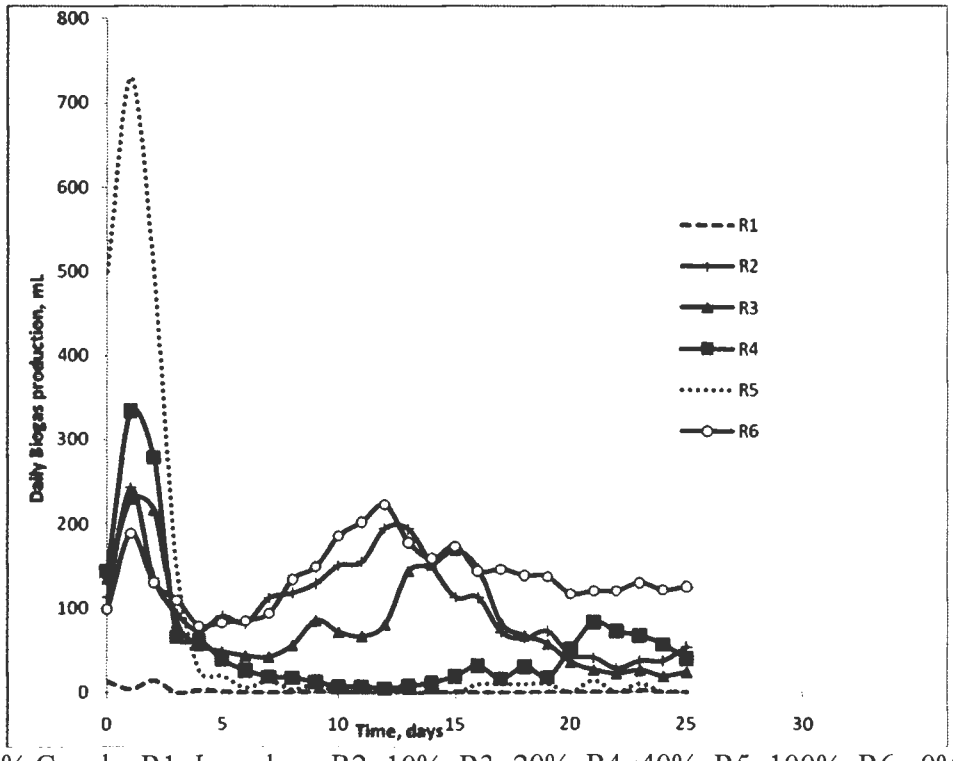
Averages of initial total VFAs in ingestate are listed in Table 6. Among the bioreactors, R6 (100% manure) had the highest initial VFAs concentration (4227±43 mg/L), while R5 (100% canola meal) had the least concentration, excluding R1 (555±27 mg/L) (Table 6). Among individual constituents of VFAs, the most prominent VFA in R6

was acetic acid (2538±43 mg/L); while in R5 it was valeric acid (442±27 mg/L). It must be noted that the total initial VFAs decreased as canola meal ratio in the bioreactor increased (Table 6). The trend was as follows: R2 (3977±35 mg/L), R3 (3633±59 mg/L) and R4 (3032±49 mg/L).

The fiber content in R6, in terms of hemicellulose, cellulose and lignin were 18, 20, and 8.8% of TS, respectively, and these numbers in R5 were 10.5, 11.0, and 7.7% of TS, respectively. It shows that dairy manure had a higher percentage of fiber than canola meal. For the remaining bioreactors (R2, R3 and R4) hemicellulose content slightly decreased with an increase in canola meal content (15.7%TS in R2, and 13.6% TS in R4). There was no clearly discernible trend for both cellulose and lignin content.

3.4.2 Biogas Production and Methane Yield

The average daily biogas production is as shown in Figure 7. In this experiment, two distinct double peaks of biogas production were observed for the manure bioreactor (R6) and low ratios of canola bioreactors (R2, R3, and R4). The first peak appeared approximately on the second day, which is in agreement with other researchers (El-Mashad and Zhang, 2010) and the second peak was observed on 12^{th} day. All six reactors, except R1 (inoculum only) showed high biogas production rates during the first 5 days. Thereafter, a sharp drop in biogas production was observed, followed by a gradual increase in biogas production until the 12^{th} day, when a gradual drop off started until the 25^{th} d (Figure 7). In R5 (100% canola meal), following a sharp peak during 2-3 d, biogas production dropped near to zero and remained steady in the remaining experimental period (25 d). This was likely due to rapid hydrolysis of soluble fractions of feedstock in R5. A similar trend was noticed for the R4 reactor, apart from for a small peak after the 20^{th} d.

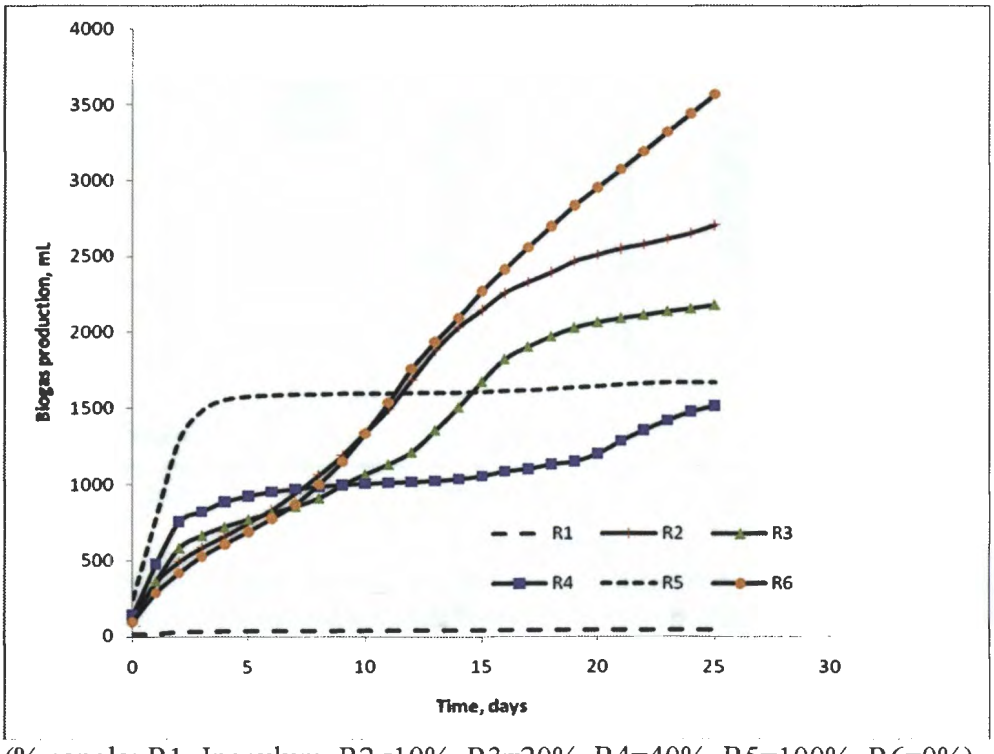


% Canola: R1=Inoculum, R2=10%, R3=20%, R4=40%, R5=100%, R6= 0%

Figure 7. Average Daily Biogas Production

Overall, R6 had the highest total biogas production (3.6±0.7 L) while R4 had the lowest cumulative biogas production, excluding R1 (1.5±0.7 L) (Figure 8). With 100% mono-substrates (R5 and R6), a clear contrast was observed in terms of biogas production profile. From Figure 8, it showed that in the R6 reactor (i.e.100% manure) cumulative biogas production increased steadily as experiment was progressing and produced highest biogas production. In the R5 reactor (i.e., 100% canola meal), the maximum biogas was produced during first five days, thereafter biogas production halted. The biogas production rate in 100% manure (R6) remained steady at about 110-120 mL/d from the 16th day to 25th day (Figure 8). Among canola and manure mixture, 10:90 canola meal: manure mixture reactor (R2) produced the highest biogas production as compared to 20:80 (R3), and 40:60 (R4) reactors. However, in all canola meal: manure mixture bioreactors, the cumulative

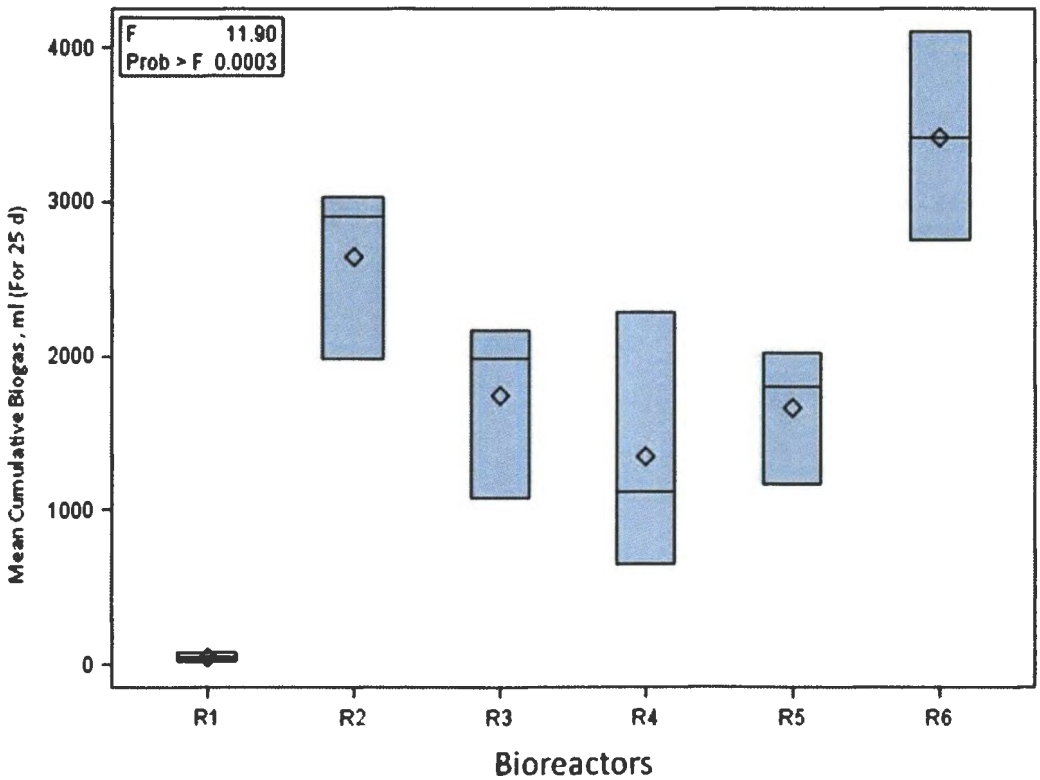
biogas production were less than that of R6 reactor (i.e.100% manure). This implies that irrespective of the percentage of canola meal added to the co-digestion with manure, canola meal has no beneficial impact on biogas production in this study.



(% canola: R1=Inoculum, R2=10%, R3=20%, R4=40%, R5=100%, R6=0%)

Figure 8. Cumulative Biogas Production after 25d

Addition of canola meal to the dairy manure had a significant impact. Based on the cumulative biogas (at 95% level of confidence), the bioreactors can be categorized in four groups: Group1 – R6 & R2, Group 2 - R2, R3, R5, & R4, Group 3 – R3, R5, & R4 and group 4- R1 (Figure 9). This means that R6 reactor produced statistically significantly higher biogas as compared to other reactors, except the R2 reactor. The R2 reactor (10% canola meal) produced higher biogas compared to other canola meals, but differences were not statistically significant. Averages of biogas production rates are presented in the box plot Figure 9.



% Canola: R1=Inoculum, R2= 10%, R3=20% R4= 40% R5= 100% canola R6=0 (100% Manure)
Note: ♦= Mean cumulative biogas, middle line=Median, bottom of the box=25th percentile and top is the 75th percentile.

Figure 9. Box Plot for Total Cumulative Biogas Production

The average methane content in the biogas is given in Figure 10. The bioreactor with 100% manure (R6) had the highest methane content (64.5±1.0%) and R5 had the least methane content (54.8±1.7%) in the biogas. As the canola fraction increased in the reactors R2-R4, methane content in the biogas decreased linearly. Similarly, the specific methane yield was the highest in the R6 (352±55 L/kg VS) and the lowest was in the R5 (83±7 L/kg VS). For R6 (100% manure), these values were comparable with values reported by El-Mashad and Zhang (2010), where they digested manure for 30 d and found specific methane yield of 436, 404 and 366 L/kg VS for screened, fine and course manure, respectively.

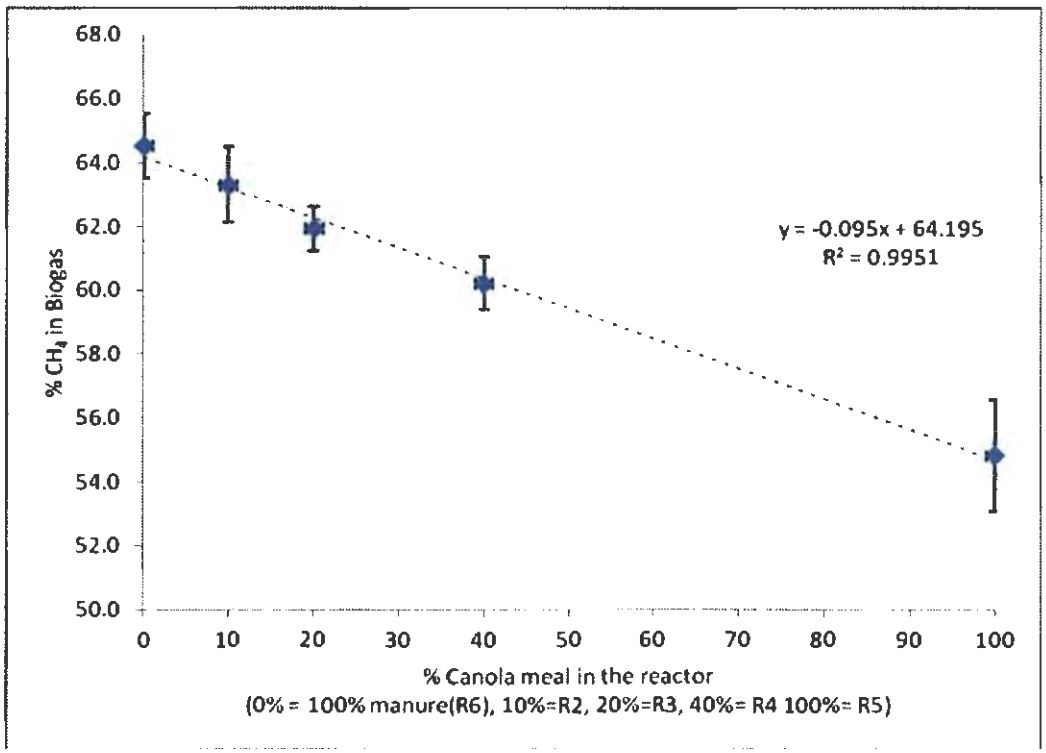


Figure 10. Methane Content, % Biogas After 25 d

3.4.3 Digestate

At the end of digestion process, samples were collected and analyzed for different parameters as listed in Table 7. All four reactors with canola meal (R2, R3, R4 and R5) had VFA values of more than 4000 mg/L, the threshold value for biogas production inhibition as presented by Siegert and Banks (2005). This might have inhibited biogas production compared to the manure only bioreactor, R6 with VFA value of 541±8 mg/L (Table 8). The greatest VFAs accumulation was in the R5, in which it changed from 555±27 to 4629±47 mg/L, more than 8 fold increments.

In spite of higher VFAs in the digestate in R2, R3 and R4 bioreactors, the biogas production rates were higher than R5, since initial VFAs was lower than the threshold values. This could be explained by the fact that bioreactors R2 and R3 had propionic acid,

while R4 and R5 had butyric and valeric acids that might have influenced biogas production. It was noted that, despite of high initial VFA in R6 (4227±43 mg/L), after digestion it decreased considerably to 514±8 mg/L. In R6, VFAs were mostly acetic and butyric acid forms. These are even numbered carbon and known to degrade easily.

Table 7. Digestate Characteristics

Brct (%C)	TS (g/L)	VS(g/L)	COD (g/L)	pH	C/N Ratio	VFA (mg/L)
R1(Ino)	32.4(7.6)	-	-	7.1(0.4)	8.7(1.1)	74(2.0)
R2 (10%)	53.8(5.3)	40.5(2.0)	-	7.4(0.1)	7.2(1.3)	4261(86)
R3 (20%)	58.6(4.7)	41.8(2.6)	73.4(8.3)	7.2(0.1)	11.5(1.9)	4791(90)
R4 (40%)	62.3(5.5)	43.7(0.5)	77.7(28)	7.1(0.3)	9.7(0.9)	4751(43)
R5 (100%)	66.7(5.5)	35.5(0.4)	-	5.9(0.6)	12.0(1.0)	4629(47)
R6, (0%)	53.3(2.9)	43.7(2.3)	38.0(0)	7.4(0)	12.0(1.4)	514(8.0)

- = Missing values, Brct=Bioreactor, %C= % canola meal, Ino=Inoculum
()=Standard deviations

The C/N ratio decreased in all the reactors except R5, where there was a notable increase from 7.8±0.8 to 12±1.0. Although, COD was not comprehensively measured, from the partial results, there was high consumption in R6.

3.4.4 pH Changes

Changes in pH in reactors are shown in Figure 11. At the end of the digestion process, pH increased slightly compared to initial pH, except R5 where pH decreased slightly and this value was much lower than the optimum pH required for the methanogens (Figure 10). The accumulation of VFAs in the bioreactor R5 resulted in a drop of pH from 6.0±0.1 to 5.9±0.6 (Table 7). In most AD processes, the optimum pH is limited to about 6.5-8.2, and operations outside this range could result in process imbalance. Methanogens are particularly sensitive to pH below 6.0, as they grow quite slow and the associated

system recovery is painstakingly long (Speece, 1996). In addition, low pH can also be associated with high carbon dioxide in the reactor as observed in R5.

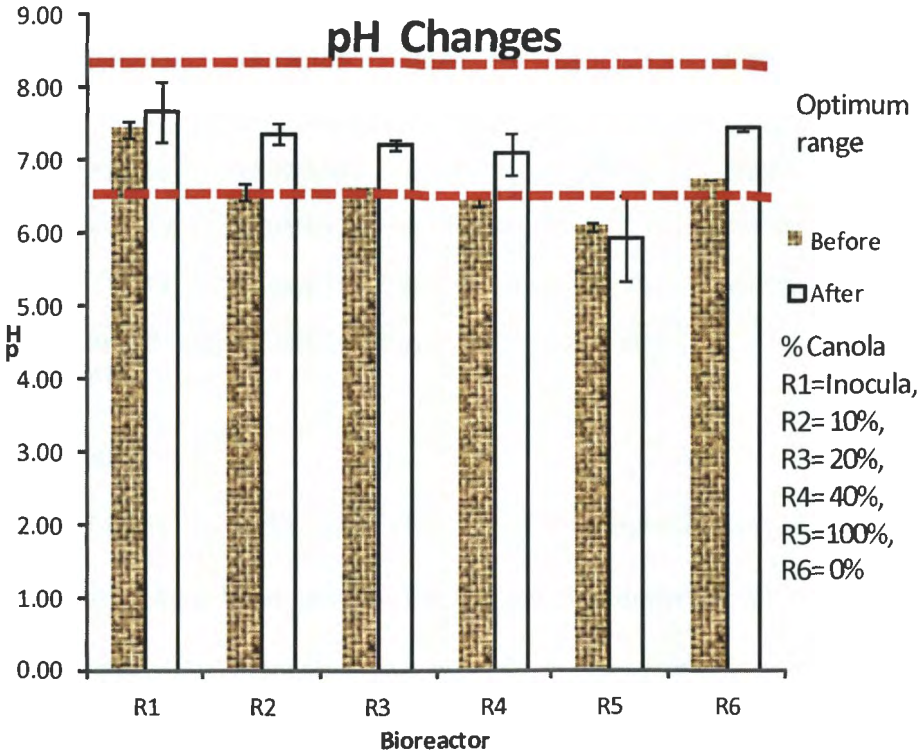


Figure 11. Initial and Final pH in the Bioreactors

3.4.5 Volatile Solids Destruction

The performance of the bioreactor was evaluated in terms of both biogas production and methane content per volatile solids destroyed as per Table 8. The highest specific methane production was noted in R6, followed by R2 and R4 while R3 and R5 had dismal performance. R5 had only 87±7 $LCH_4/kg\ VS_{destroyed}$ and R3 had 127±16 $LCH_4/kg\ VS_{destroyed}$. In spite of an increase of volatile solids in the canola rations (Table 6), as a result of addition of manure, there was no associated improvement in biogas production. The percentage of $VS_{destroyed}$ was the highest in the R5 (54%), followed by: R3 (43%), R6 (32%), R2 (30%), and R4 (28%).

Table 8. Specific Biogas and Methane Production

Brct (%C)	Cum. Biogas (L)	$TS_{destr.}$ (g/L)	$VS_{destr.}$(g/L)	CH_4 (%)	Specific CH_4(L/kg TS)	Specific CH_4(L/kg VS)
R1(Ino)	0.05(0.01)	0.94(0.22)	-	-	-	-
R2 (10%)	2.70(0.63)	13.33(5.0)	16.99(2.86)	63.3(1.2)	525(115)	329(75)
R3 (20%)	2.18(0.12)	20.16(6.29)	30.95(1.81)	61.9(0.7)	203(27)	127(16)
R4 (40%)	1.52(0.66)	10.07(3.94)	16.77(0.13)	60.2(0.8)	292(54)	183(86)
R5 (100%)	1.67(0.44)	26.04(6.92)	41.11(0.57)	54.8(1.7)	134(12)	83(7)
R6 (0%)	3.57(0.74)	15.64(2.34)	20.33(1.68)	64.5(1.0)	562(94)	352(55)

-=not determined, Brct=Bioreactor, destr.=Destroyed, %C= %canola meal
()=Standard deviations

3.4.6 VFAs Changes

Changes in individual VFAs (acetic, propionic, n-butyric, i-butyric, n-valeric and i-valeric) have been listed in Table 9. Accumulation of VFAs in the bioreactor results in reduction of pH, making the process unstable and eventually leading into reduction of biogas production. Though it appears that towards the end the bioreactors recovered from low pH. In all the bioreactors, only R1 and R6 had a negative change in total VFAs. The most common VFAs, acetic acid was noted to be reduced in five of the six bioreactors. Bioreactor R5 indicated an accumulation of acetic acid (1543±50 mg/L) at the end of 25 days. Bioreactor, R6 had the highest reduction in VFA from 4227±43 mg/L to 514±8 mg/L, an 88% reduction. In addition to acetic acid, the other major VFA was propionic acid. Bioreactor, R2 had the highest accumulation of propionic acid (3128±84 mg/L) and the lowest was in R6 (76±3 mg/L). This slightly exceeded the threshold value of 3000 mg/L of propionate suggested for stable process (Asinari Di San Marzano et al., 1981). Propionate, which is formed from the decomposition of odd-numbered carbon molecules, is slower to decompose to acetate, thus the probable reason for the accumulation

(Wang et al., 1999). The increase of propionic acid in R2, R3, R4 and R5 (bioreactors with fractions of canola) may be explained by the degradation of n-valeric and i-valeric acids originally in the canola.

Table 9. VFA Changes

Bioreactor		Individual volatile fatty acids (VFAs), mg/L						
		Acetate	Propionate	i-butyrate	Butyrate	i-valerate	Valerate	Total
R1	Initial	78(3)	0	0	0	0	0	78(3)
	Final	74(2)	0	0	0	0	0	74(2)
R2	Initial	2215(32)	998(31)	61(5)	542(27)	78(6)	83(2)	3977(35)
	Final	158(1)	3128(84)	333(14)	12(1)	598(17)	33(2)	4261(86)
R3	Initial	2022(54)	892(29)	53(1)	475(5)	69(1)	123(4)	3633(59)
	Final	1490(11)	1840(56)	292(8)	216(8)	474(47)	480(44)	4791(90)
R4	Initial	1660(49)	726(6)	42(2)	371(15)	51(5)	181(3)	3032(49)
	Final	1323(41)	1067(11)	180(2)	961(14)	352(16)	868(25)	4751(43)
R5	Initial	93(1)	20(2)	0	0	0	442(27)	555(27)
	Final	1543(50)	648(16)	146(3)	1774(43)	298(5)	221(4)	4629(43)
R6	Initial	2538(43)	958(12)	74(4)	455(3)	145(3)	57(1)	4227(43)
	Final	209(2)	76(3)	17(1)	183(8)	30(6)	0	514(8)

()=Standard deviations

The four carbon VFAs, i-butyric and n-butyric acid accumulation was noted to be highest at about 1920±46 mg/L in R5 and lowest at 199±14 mg/L in R6. This was consistent with previous studies where they noticed inhibition due to butyric acid degradation to acetate acid in the range of 1500 mg/L (Pind et al., 2003). Due to their low concentration, and thus low microbial population adapted to their degradation, both n-butyric and i-butyric acids have low affinity kinetically to degrade to propionic and acetic acids (Aguilar et al., 1995).

3.4.7 Discussion

Surprisingly, addition of canola meal in the anaerobic digestion of manure is not very promising as per current study. The amount of biogas and methane content produced is closely tied to VFAs level in the digestate and variation in the compositions of the ratios. Accumulation of VFAs leads to a decrease in pH, resulting in an overall impact of poor quality gas characterized by high CO_2 content (Boe et al., 2010). In addition VFAs could result in reduced ATP production and more VFAs are produced as the hydrogen ions are diverted from the acetate metabolic pathway (Stafford, 1982). Accumulation of VFAs affects process stability, causing methane formation to lag behind (Speece, 1996). An initial concentration of VFA (as acetate) of 1000 mg/L could stimulate biogas production to two fold but the other types of VFAs are suspected to cause inhibition (Stafford, 1982). In agreement with previous findings (Pind et al., 2003), the R6 had a good performance since it had a high initial acetate level.

The accumulation of acetic and propionic acids may only cause oscillation in gas production but the overall stability remains good. Low levels of acetic and propionic acids are an indication of quick production of methane from the intermediates (Raposo et al., 2008). This can be inferred of R6 while there was build up of acetic and propionic acids in R2, R3, R4 and R5. This build up of acetic and propionic acids in the reactor will eventually lead to accumulation of i-buytric acids and i-valeric acids, by inhibiting their degradation, further depleting the buffering capacity, resulting in an immediate drop of pH and sharp decrease of biogas production (Asinari Di San Marzano et al., 1981). There was no continuous monitoring of pH, thus it is not clear from this study how the pH profile changed.

In 100% canola meal (R5), there was accumulation of i-butyric and n-butyric acids, which may have caused low gas production. Before being degraded to methane, n-butyric and propionic acids are converted into acetate and hydrogen by obligate acetogenic bacteria while the acetate and hydrogen are converted into methane by methanogens (Deublein and Steinhausser, 2008). Low pH is known to affect the methanogenic bacteria that convert acetate to CH_4 and CO_2. In the case of R5, the methanogenic microbial activity is overwhelmed by VFAs caused by the rapid hydrolysis of complex matter, and lags behind in removing them as they are formed (Parawira et al., 2004). This becomes more of concern as their concentration goes beyond 4000-4500 mg/L (Aguilar et al., 1995; Siegert and Banks, 2005).

In the canola meal hydrolysis, as the oil components are hydrolyzed faster, depositing VFAs that depress the pH, inhibiting the continuation of the digestion. In the presence of high concentration of acetate, the degradation of propionate and n-butyric acid would only take place in low concentration of partial hydrogen pressures due to the positive values of Gibbs energy involved (Wang et al., 1999). Aguilar et al. (1995) contends that proper choice of microbial population can significantly impact on the degradation of VFAs. The limitation on VFAs levels does not count in a two stage continuous operation due to increased buffering, and a clear separation of acidification and methanogenesis (Li et al., 2010). As long as the methanogenic phase is held at optimum pH, drop of the acidification phase to pH of as low as 3.2 did not affect the biogas production rate.

In addition to the VFAs, canola meal co-digestion may be impacted negatively by the presence of high ammonia-nitrogen beyond 4000 mg/L and toxicity due to

glucosinolates (Kolesárová et al., 2011), although none of these variables were measured in this study. In R5, the ammonia-nitrogen accumulation approached to 3294 mg/L, being the highest against 1656 mg/L in R6 at the lower end. Though, there exists remote possibility of ammonia inhibition, these figures point otherwise. There is also a possibility of toxicity due to glucosinolates. The level of glucosinolates in the canola meal was not tested, and therefore this risk was not assessed.

The results obtained in this study are in contrast to those obtained by Satyanarayan et al. (2008) in a co-digestion of mustard seed oil cake (MOC), which even though proven to be highly acidic (pH=4.9-5.2), showed improved AD performance with about 13% better reduction of volatile solids (VS). This was accompanied by high biogas production rates and increased methane content. This was expected as the pH in the various ratios of MOC, was in the range of 6.7-7.7, which promotes the methanogenic bacteria activity. In addition, the canola meal used in the present study had only 2.5% oil as compared to 12.8% reported in MOC studies. Another difference in the result would be based on the crude fiber in which MOC has only 10.2% as compared to 29.2% in canola meal. The MOC study neither spells out the portioning of the fiber nor identifies the changes of the fiber content in digestion.

In a different study, sunflower oil cakes and meals were digested at mesophilic temperatures, at different inoculum to substrate ratios, ISR (Raposo et al., 2008). An increase of COD was observed as a consequence of accumulation of VFAs at lower ISR, impacting the methanogenic process. Just like the current findings, sunflower oil cake at low levels of ISRs is prone to suffer from process instability due to build up of VFAs.

3.5 Conclusions

In the present study, it can be inferred that canola meal from chemically extracted seeds is not a good choice for co-digestion with dairy manure. However, due to the expected abundance of the meal, it could be necessary to look into ways of overcoming the inhibition caused by elevated VFAs level such as pretreatment, two stage digestion, lowering the initial organic loading, and operating the reactor at thermophilic temperature range.

CHAPTER 4. ORGANIC LOADING AND CANOLA MEAL OIL CONTENT IMPACTS IN DAIRY MANURE CO-DIGESTION

4.1 Abstract

Two different canola meals, high oil content (HOC) and low oil content (LOC) were co-digested with dairy manure at two levels of organic loading, low organic loading (LOL) and high organic loading (HOL) had approximately 4.5% and 7.5% total solids (TS), respectively. In this experiment, three sets of canola meal: dairy manure (10:90, 20:80, 40:60), two sets of mono-substrate (100% canola meal and 100% manure) were digested at a temperature of 35 ± 2^0C (mesophilic). Biogas production rate was measured by water displacement and methane (CH_4) content was measured by gas chromatography. Other parameters measured before and after digestion included, total solids (TS), volatile solids, nutrients, volatile fatty acids and pH. The results showed that at HOL, canola meal had a less desirable impact on dairy manure digestion attributed to accumulation of VFAs beyond threshold value (4000 mg/L). However, at LOL, both 10% and 20% HOC resulted in increased specific methane of 535 L/kg VS and 445 L/kg VS, respectively. Organic loading, fractions of canola meal and oil content in the canola meal have an impact on biogas production and specific methane. Low organic loading resulted in better performance.

Keywords: Biogas, Co-digestion, Dairy manure, Methane, Volatile fatty acids, Canola meal.

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4.2 Introduction

Emission of greenhouse gases (GHGs) and odor are concerns for the growing confined animal feeding operations (CAFOs). The manure and waste water generated from these operations, when not properly handled can be an environmental bane. In addition, CAFOs are concerned with the ever increasing prices of fossil fuels that lead to high heating costs (Lantz et al., 2007). In response to reducing the energy costs, the department of energy (DOE) in collaboration with USDA, have identified a potential of one billion tons of dry biomass that can be put into use in provision of renewable energy, and animal manure will contribute about 35-40 million dry tons (Cantrell et al., 2008). One of the most acceptable forms of converting the animal manure into energy and reduce GHGs emissions is via the biogas route (Clemens et al., 2006). Others may include combustion in fluidized beds, pyrolysis into bio-oil, direct liquefaction, bio-hydrogen production and valorization to bio-products (Cantrell et al., 2008; Ward et al., 2008). In addition, biogas production through AD process results into digestate that is rich in minerals that can be applied as a fertilizer.

Biogas production, through the well known and proven technology of anaerobic digestion (AD) is well developed in Europe in countries like Germany, Denmark and Sweden (Lantz et al., 2007; Raven and Gregersen, 2007). According to the Great Plains Institute, the US Midwest states have a potential to match Europe in producing biogas from agricultural wastes if the technical and policy barriers are removed (Bilek, 2010). One of the technical issues that have been prominently identified is low biogas production from dairy manure digestion only. Co-digestion has been identified to enhance biogas production and methane yield (Ward et al., 2008). Anaerobic co-digestion is the simultaneous

digestion of more than one substrate. Dairy manure is the most prominent primary substrate as it offers better buffering capacity, and has almost all the essential nutrients (El-Mashad and Zhang, 2010). Co-digestion has favorable economic indicators, with a payback period for a medium sized plant whose biogas is used for generation of electricity pegged at 3-5 years (Cavinato et al., 2010).

Normally, the substrates have a synergistic effect on each other whose overall impact is more microbial activity, and higher conversion of the organic compounds to biogas. The synergistic effect may be in terms of nutrients, pH, and suppressing toxicity/inhibition. In recent years, biofuels by-products mainly oil cakes and oil seed meals have become attractive (Dhanya, 2009; Kolesárová et al., 2011; Ramachandran et al., 2007). Oil meals and cakes are thought to have high lipids and proteins that promote biogas production (Chandra et al., 2011). One of the oilseeds in USA is canola, which is in the same family as rapeseed. In our previous studies, the co-digestion of canola meal with dairy manure had a non-beneficial impact. Canola meal proved to be a challenging co-substrate. It was noted that canola meal led into accumulation of volatile fatty acids (VFAs), suppressing the pH, and consequently resulting in low biogas production due to reduced microbial activity.

In low buffering system, organic loading (OL) has been identified as a critical parameter in AD processes, as it results in accumulation of VFAs (Alvarez and Liden, 2008). Accumulation of VFAs beyond the threshold levels (4000 mg/L) may be reduced in a batch bioreactor by reduction of total solids (TS) (Carucci et al., 2005). Very limited information is available on the impact of organic loading and oil content of canola meal on biogas production when co-digested with dairy manure. Therefore, the objective of this

study was to compare the biogas production at high organic loading (HOL) and low organic loading (LOL) using high oil content (HOC) and low oil content (LOC) canola meal.

4.3 Materials and Methods

The dairy manure used in the study was obtained from the NDSU dairy barn, while the canola meals were obtained from two sources; NDSU Pilot Plant, Fargo, ND and Archer Daniels Midland (ADM) canola oil processing facility, Velva, North Dakota, USA. The manure was kept at 4^0C before being used. The dairy manure was blended to increase homogeneity. The canola meal obtained from ADM was different from the NDSU mainly on the residual oil content, the percentage oil left in canola meal after extraction. ADM uses solvent extraction process, which results in 2.5% oil content canola meal (LOC) while NDSU used double mechanical screw press, which resulted in 8% residual oil (HOC). In both cases, the canola meals were analyzed for oil content by a method described by Haagenson et al., (2010). The inoculum seed was collected from American Crystal Sugar (ACS), Moorhead, MN. ACS' biogas plant used sugar beet pulp as substrate and its operating temperature was 35 ± 2^0C. The plant uses $NAHCO_3$ to adjust the pH. The inocula were kept in an incubator (Sheldon Manufacturing, Oregon, USA, Model 1525) for 48 h at 35 ± 1^0C before being used in the experiment.

Samples of manure, canola meal and inoculum were analyzed for nutrients, total solids (TS), total suspended solids (TSS), volatile solids (VS), fiber content, volatile fatty acids (VFAs) and chemical oxygen demand (COD). The physico-chemical characterization of the substrates and inocula are summarized as shown in Table 10.

Table 10. Physico-chemical Characterization of Substrate

Parameter	Units	Dairy manure	LOC	HOC	Inocula
COD	g/L	120(17)	100(13)	-	7.1(3)
TS	%	12.3(2.5)	90.2(1.2)	93.7(1.1)	4.2(0.7)
VS	%TS	90.5(1.5)	87(3.5)	78.9(1.0)	91(2.5)
pH	-	6.7(0.1)	6.0(0.1)	6.4(0.1)	7.4(0.15)
P	mg/L	0.06	0.8	1.0	0.02
N	mg/L	0.6(0.05)	4.5(0.08)	4.6(0.1)	0.05(0.01)
C/N ratio	-	12.6(2.5)	7.8(0.8)	8(0.2)	9.8(1.3)
Oil content	%	-	2.5	8.0	-
VFAs	mg/L	4227(43)	514(27)	604(52)	78(3)
Crude protein	%DM	16.7	40	39	13
Solubles	%DM	54	70	73	90
Hemicellulose	%DM	18	10.5	7	6.2
Cellulose	%DM	20	11	12	4.3
Lignin	%DM	8.8	7.7	9	0.6

LOC= Low oil content canola meal; HOC=High oil content canola meal
()=Standard deviations

4.4 Experimental Set up and Procedure

In this study, the two canola meals namely high oil content (HOC) and low oil content (LOC) were co-digested with dairy manure at two levels of organic loading: high organic loading (HOL) and low organic loading (LOL). The details will be described in the following section. The batch bioreactors were made out of 0.5 L Erlenmeyer flasks with a working volume of 0.35 L. A rubber stopper with two holes was used to seal the bioreactors. One of the holes was used for gas yield measurement, while the other hole was used for sampling the headspace gas. The experiments were completed at two levels of organic loading as described below.

4.4.1 Co-digestion of Canola Meals at High Organic Loading (HOL)

In this level, the objective was to adjust the resultant total solids (TS) in each bioreactor to about 7.5±2%, thereafter referred as high organic loading (HOL). Two sets of investigation were done, the first one using canola meal from solvent extraction and mechanical screw press (sourced from NDSU), herein referred to as high oil content canola (HOC), and the second was based on canola from solvent extraction (ADM canola meal), herein referred to as low oil content canola (LOC). In the two sets, the bioreactors were labeled as follows. First, for the LOC, the two non-canola containing bioreactors were coded as R1 (only inoculum) and R6 (100% dairy manure) while the rest were characterized as fraction of TS that was canola meal by volume basis: R2 (10%), R3 (20%), R4 (40%), R5 (100%). Second, for HOC, the non-canola containing bioreactors were labeled as RA1 (only inoculums) and RA6 (only manure), while the canola containing fractions as volume basis were RA2 (10%), RA3 (20%), RA4 (40%) and RA5 (100%).

4.4.2 Co-digestion of Canola Meal at Low Organic Loading (LOL)

In this level, the TS in each of the bioreactors were lowered to about 4.5±2%. Only HOC was used for this set of experiments at mesophilic temperature. The labeling was as follows and percentage canola meals are shown in brackets: LR1 (only inoculums), LR2 (10%), LR3 (20%), LR4 (40%), LR5 (100%), LR6 (only manure). The design of the experiment and procedure was as in HOL.

In both levels bioreactors were incubated in a water bath (VWR, USA), maintained at 35±2^{0}C and oscillated at 70 cycles/min to prevent settling (Figure. 12). For each bioreactor except reactors containing only inoculum, 0.1 L of inocula was blended for 2-3 min with the appropriate canola meal fraction and manure as described above. The

bioreactors were flushed with nitrogen twice; before and after the introduction of the substrates. This was to expel oxygen, and to quickly induce anaerobic condition. The biogas yield was measured by water displacement method, in which gas from the bioreactors was delivered into an inverted 250 mL graduated cylinders on a water basin (Figure 12). In order to limit the dissolution of CO_2, the water used in the graduated cylinders was saline. The graduated cylinders were emptied and reset after recording the volume of gas on a daily basis for a period of 25 d.

Figure 12. Experimental Set-up

On a weekly basis, headspace gas was collected for analyzing methane content. From each bioreactor a feedstock sample was collected and analyzed for nutrients, fiber content, VFAs, pH, TS, VS, and COD content. In all bioreactors, biogas production rate and methane content was recorded.

4.4.3 Analytical Methods

Standard methods were used for the analysis of nutrients (TKN, ammonium-nitrogen, phosphorous), total solids (TS), volatile solids (VS), and chemical oxygen

demand (COD) (APHA, 2005). Fiber analysis such as cellulose, hemicelluloses and lignin, acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) were determined according to the NREL procedure (NREL/TP 510-42618). Hemicellulose content was estimated as the difference between NDF and ADF, the cellulose content was the difference between ADF and ADL, and lignin was the value of ADL.

For determination of volatile fatty acids (VFAs), the samples were mixed in 25 mL centrifuge tubes and centrifuged for 10 min at 2000 rpm. Five milligrams of the resultant supernatant was filtered through 0.45 μm filter, then reacted with 25% meta-phosphoric acid (w/v) and allowed to stand for 30 min. The samples were again centrifuged at 1000 rpm for 10 min, before being transferred to 2 mL vials, which were in turn injected in volumes of 1.0 μL to the gas chromatography machine (Agilent 6890, USA) using a flame ionization detector (FID) and capillary column (Supelco 0.53 mm ID fused silica). The temperature in the oven was programmed to increase from 124^0C to 190^0C at a rate of 70^0 C/minute. The injector temperatures and the FID were set at 250^0C at 260^0C, respectively. Helium was used as the carrier gas and it was supplied at 35 mL/min.

Biogas was collected from the headspace of reactors using syringes and transferred to 5 mL vials and gas composition was determined using a gas chromatography machine (Hewlett Packard, Model 5890, Agilent, USA) with a super-Q plot column (30 m × 0.53 mm) and flame ionization detector (FID). The oven was set at a maximum temperature of 250^0C. All biogas composition measurements were done in triplicates. Five (5) μL of biogas was injected into the GC column with an inlet temperature of 150^0C and detector temperatures of 250^0C. The system was calibrated against methane calibration gas (96.8% purity obtained from Praxair Air, Fargo, ND, USA).

4.4.4 Data Analysis

Statistical analysis of variance (ANOVA) on biogas production, methane content, volatile solids destruction, COD consumption and methane content were done using SAS (SAS, Cary, NC) at 5% level of confidence. The biogas production means were compared by LSDs at 95% level of confidence.

4.5 Results and Discussion

4.5.1 Substrate Characterization

Based on results from Table 10, there were physico-chemical differences between HOC and LOC are noted. Previously, the oil content in canola meal has been reported as 4.5% (Kolesarova, 2011) and 3.5% (Luo et al., 2011). However, in this study LOC had 2.5% oil content, while HOC had 8.0% oil content. This difference, being the most noticeable between the two types of canola meal was a result of the extraction method. Solvent extraction method is more effective than mechanical screw press in removing most of the oil from the canola seed. The TS value was slightly higher than previously reported for rapeseed at 85.6±1.55%, but the VS was comparable, 79.6±1.28% (Luo et al., 2011). Other properties did not differ between HOC and LOC canola meal include the carbon/nitrogen (C/N) ratio, total volatile fatty acids (VFAs), nitrogen, phosphorous and the fiber content. Canola meal is comparable to the rapeseed meal, except for the residual oil content that depends on the extraction method (Kolesarova, 2011).

4.5.2 Co-digestion of Canola Meals at HOL

4.5.2.1 Ingestate Characterization

The properties of ingestate (initial feedstock) are given in Table 11.

Table 11. Ingestate Characterization

Reactor (%C)	TS (g/L)	VS(g/L)	pH	C/N Ratio	VFA (mg/L)
HOL, LOC canola meal					
R1 (Ino)	33.4(7.5)	26.4(3.3)	7.4(0.1)	9.8(1.3)	78.0(3)
R2 (10%)	67.1(4.1)	57.5(0.9)	6.6(0.1)	15.4(2.3)	3977.0(35)
R3 (20%)	78.7(6.8)	72.7(0.8)	6.6(0.0)	15.2(3.1)	3633(59)
R4 (40%)	72.3(5.7)	60.4(0.4)	6.4(0.1)	13.1(1.7)	3032(49)
R5 (100%)	92.8(1.4)	76.6(1.0)	6.0(0.1)	7.8(0.8)	555(27)
R6 (0%)	68.9(.5)	63.5(0.6)	6.7(0.0)	12.6(2.5)	4227(43)
HOL, HOC canola meal					
RA1 (Ino)	23.8(2.6)	17.5(1.7)	7.1(0.2)	10(1.4)	155(3)
RA2 (10%)	102.1(5.2)	82.3(2.8)	6.5(0.2)	8.0(0.1)	4575(25)
RA3 (20%)	84.6(2.1)	73.3(1.3)	6.6(0.2)	8.0(0.5)	4288(51)
RA4 (40%)	76.4(1.2)	69.0(1.0)	6.6(0.1)	7.0(0.7)	3878(76)
RA5 (100%)	93.8(1.1)	78.9(1.0)	6.4(0.1)	8.0(0.2)	604(52)
RA6 (0%)	104.1(2.2)	92.5(0.1)	6.6(0.1)	13.0(0.5)	3726(48)
LOL, HOC canola meal					
LR1 (Ino)	17.4(0.9)	6.7(0.8)	7.5(0.2)	6(0.4)	0
LR2 (10%)	44.3(0.7)	33.1(0.6)	7.0(0.1)	9(0.1)	5255(127)
LR3 (20%)	53.0(0.8)	40.2(0.9)	6.9(0.2)	10(0)	5498(59)
LR4 (40%)	48.1(0.7)	39.2(0.8)	6.7(0.1)	9(0.1)	5554(29)
LR5 (100%)	46.5(0.7)	40.2(0.3)	6.5(0.2)	7(0.3)	5353(54)
LR6 (0%)	60.1(1.7)	48.9(0.9)	7.0(0.2)	11(0.3)	5121(112)

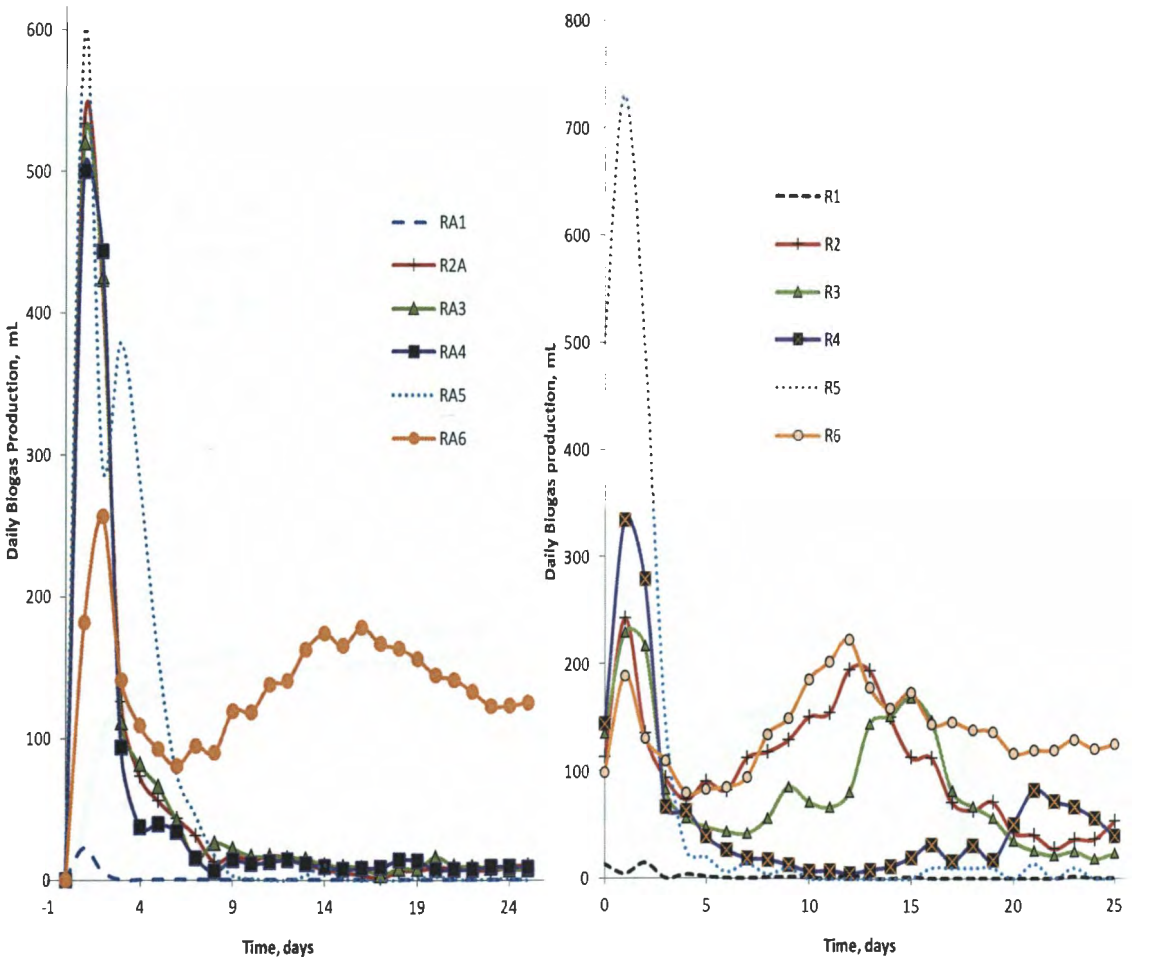
% C= % Canola in the bioreactor
TS= Total solids, VS= Volatile solids, ()=standard deviations

Generally, HOC bioreactors (RA2, RA3 and RA4) had higher values in terms of TS, VS, VFAs and C/N ratios than the LOC bioreactors (R2, R3, and R4). There was a slight increase in pH for the canola only reactors for 6.0 in LOC (R5) to 6.4 in HOC (RA5), but the properties of manure only bioreactors (RA6 and R6) were similar. Even though, the

difference in pH between HOC and LOC was small, the difference in C/N ratio was more pronounced in canola fractions (RA2 vs R2, R3 vs RA3, R4 vs RA4).

4.5.2.2 Biogas Production and Methane Content

Daily biogas production for the HOC and LOC are shown in Figure 13. For HOC, biogas production was rapid for the first five days, thereafter, biogas production reduced to less than 20 mL/d for all the bioreactors except RA6 (100% manure) (Figure 13a).



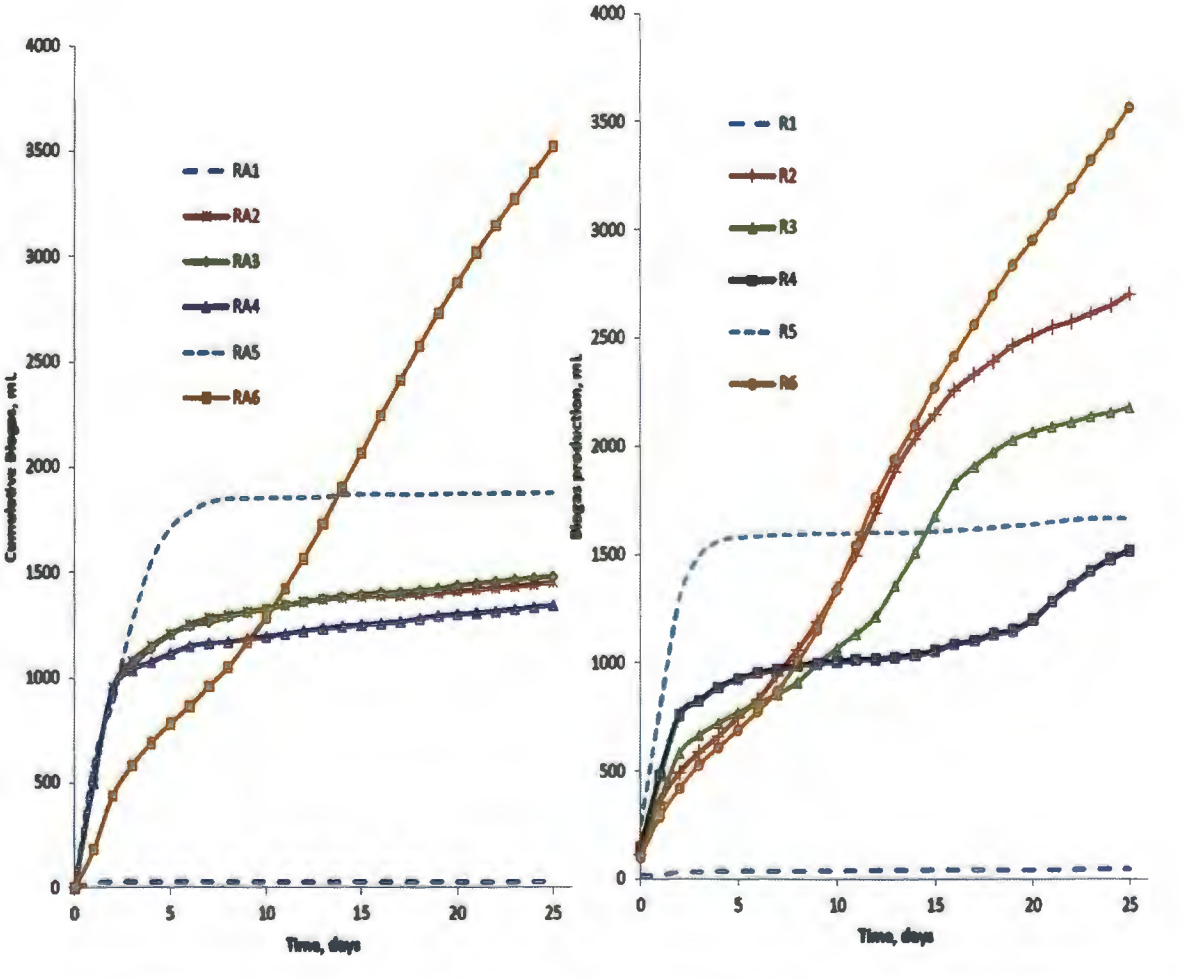
a) Daily biogas production rate for high oil content canola

b) Daily biogas production rate for low oil content canola

% Canola in bioreactor: ending in 1=Inoculum only, 2=10%, 3=20%, 4=40%, 5=100%, 6=0% =100% manure

Figure 13. Daily Biogas Production for (a) HOC and (b) LOC

Similarly for the LOC, there was rapid production of biogas during the first five days but the trend was slightly different thereafter (Figure 13b). There was a double peak for R6, R2, R3 and R4 on the 11th, 12th, 15th and 14th d, respectively. In spite of the highest peak for the R5 and RA5 bioreactors (both 100% canola meal), biogas production in these two bioreactors halted after six days. RA6 and R6 had a similar trend in both cases, exhibiting a fairly stable process. In addition to the difference in daily biogas production rates, the cumulative biogas production for the whole period is shown in Figure 14.



a) Cumulative biogas production rate for high oil content canola b) Cumulative biogas production rate for low oil content canola

(% Canola in bioreactor: ending in 1=Inoculum only, 2=10%, 3=20%, 4=40%, 5=100%, 6=0% =100% manure).

Figure 14. Cumulative Biogas Production (a) HOC and (b) LOC

Overall, there was high cumulative biogas production in the LOC fractions bioreactors (R2, R3, and R4) as opposed to the HOC fractions (RA2, RA3 and RA4). For 100% canola meal (RA5 and R5), cumulative biogas production was slightly different between HOC (1896 mL) and LOC (1668 mL). For 100% manure (R6 and RA6), the cumulative biogas production was 3567 and 3520 mL, which was not any different for the two sets of study. On one hand, 10% and 20% LOC bioreactors (R2 and R3) performed better than their counterpart in HOC bioreactors. On the other hand, 40% and 100% canola were comparable in either oil content (i.e. R4 vs. RA4 and R5 vs. RA5).

In terms of specific methane production (L CH_4/kg VS), as shown on Table 12, there were differences between the HOC and LOC. The methane content (%CH_4) is shown to decrease linearly with an increase of canola meal in the bioreactors, with a bigger impact on HOC than LOC. The mono-substrate bioreactor (100% manure and 100% canola) had extreme output, 64.5% for RA6 and 49.7% for RA5. In terms of specific methane output, the manure only bioreactors, R6 and RA6 had 352±55 L CH_4/ kg VS and 324±72 L CH_4/ kg VS, respectively.

The slight difference in specific methane from manure only bioreactors (R6 and RA6) may be attributed to the difference in initial TS (Table 11). For canola only bioreactors, R5 and RA5 had methane production of 83±7 and 127±17 L CH_4/kg VS, respectively. This is less than one third of the methane yield (378±21 and 385±29 L/kg VS for one stage and two stage, respectively) as reported by (Luo et al., 2011), where co-digestion was conducted with rapeseed oil cake operated at 6.8%TS. Unlike the current study, Luo et al. (2011) adjusted the pH to 7.5 to maximize on the methanogenic activity. Control of pH to about 6.5-8.2 is beneficial for the methanogenic bacterial (Khanal 2009c).

Table 12. Specific Methane Production

Reactor (%C)	Cumulative Biogas (L)	$TS_{destroyed}$ (g/L)	$VS_{destroyed}$(g)	CH_4 (%)	Specific CH_4(L/kg TS)	Specific CH_4(L/kg VS)
HOL, LOC Canola meal						
R1 (Ino)	0.05(0.01)	0.94(0.22)	-	-	-	-
R2 (10%)	2.70(0.63)	13.33(5.0)	16.99(2.86)	63.3(1.2)	525(115)	329(75)
R3 (20%)	2.18(0.12)	20.16(6.29)	30.95(1.81)	61.9(0.7)	203(27)	127(16)
R4 (40%)	1.52(0.66)	10.07(3.94)	16.77(0.13)	60.2(0.8)	292(54)	183(86)
R5 (100%)	1.67(0.44)	26.04(6.92)	41.11(0.57)	54.8(1.7)	134(12)	83(7)
R6 (0%)	3.57(0.74)	15.64(2.34)	20.33(1.68)	64.5(1.0)	562(94)	352(55)
HOL, HOC Canola meal						
RA1 (Ino)	0.03(0.00)	7.22(0.50)	9.33(1.24)	64.7(0.6)	6.9(0.1)	5.4(1.1)
RA2 (10%)	1.45(0.03)	24.39(6.1)	21.51(5.02)	62.0(1.0)	106(26)	124(27)
RA3 (20%)	1.48(0.06)	12.82(1.60)	13.93(0.71)	55.3(1.5)	187(9)	172(22)
RA4 (40%)	1.34(0.07)	10.43(1.85)	13.89(1.38)	56.7(1.5)	216(25)	161(6)
RA5 (100%)	1.87(0.05)	27.98(0.1)	21.01(2.59)	49.7(2.1)	94(0.50)	127(17)
RA6 (0%)	3.57(0.34)	25.70(0.40)	19.24(2.15)	64.0(1.0)	324(72)	324(72)
LOL, HOC canola meal						
LR1 (Ino)	0.05(0.004)	1.4(0.20	1.4(0.5)	64.5(0.7)	71(4)	78(26)
LR2 (10%)	3.6(0.06)	8.14(1.3)	12.16(1.6)	63.5(0.7)	800(120)	535(75)
LR3 (20%)	3.42(0.04)	11.6(2.14)	12.18(0.1)	60.0(1.4)	479(111)	445(24)
LR4 (40%)	2.1(0.04)	15.8(0.1)	13.4(1.2)	56.5(0.7)	214(14)	254(7)
LR5 (100%)	0.86(0.0)	10.97(0.5)	14.25(0.1)	49.5(0.7)	109(7)	84(1)
LR6 (0%)	3.39(0.08)	22.3(1.4)	20.7(1.3)	65.5(2.1)	278(16)	300(19)

-=not determined, %C= % Canola in the bioreactor, ()=Standard deviations

4.5.2.3 Digestate Characterization

The characteristics of the digestate (resultant effluent) from the various bioreactors are given in Table 13. In all cases, the main parameters of interest such as TS, VS, VFAs were higher in HOC than LOC canola meal fractions.

Table 13. Digestate Characteristics

Reactor	TS (g/L)	VS(g/L)	pH	C/N Ratio	VFA (mg/L)
HOL, LOC canola meal					
R1	32.4(7.6)	-	7.1(0.4)	8.7(1.1)	74(2)
R2	53.8(5.3)	40.5(2.0)	7.4(0.1)	7.2(1.3)	4261(86)
R3	58.6(4.7)	41.8(2.6)	7.2(0.1)	11.5(1.9)	4791(90)
R4	62.3(5.5)	43.7(0.5)	7.1(0.3)	9.7(0.9)	4751(43)
R5	66.7(5.5)	35.5(0.4)	5.9(0.6)	12.0(1.0)	4629(47)
R6	53.3(2.9)	43.7(2.3)	7.4(0)	12.0(1.4)	514(8.0)
HOL, HOC canola meal					
RA1	16.6(2.1)	8.1(2.9)	7.2(0.1)	5.0(0.2)	175(5)
RA2	77.7(0.9)	60.8(2.2)	6.4(0.1)	20.0(0.8)	6838
RA3	71.8(0.5)	59.3(0.6)	6.3(0.2)	19.0(0.52)	6011
RA4	66.0(0.7)	55.2(0.4)	5.9(0.1)	17.0(0.7)	5745
RA5	65.8(1.0)	57.9(1.6)	5.7(0.1)	9.0(0.5)	6178
RA6	78.4(1.8)	73.3(2.3)	6.7(0.2)	14.0(0.8)	2857
LOL, HOC canola meal					
LR1	16.6(0.8)	5.4(0.2)	8.3(0.2)	5(0.2)	0
LR2	36.7(0.1)	24.0(0.8)	7.4(0.1)	20(0.8)	1618(107)
LR3	41.4(1.4)	28.0(1.0)	7.6(0.20	19(0.6)	3845(9)
LR4	32.2(0.6)	25.9(0.4)	7.1(0.1)	17(0.7)	5898(65)
LR5	35.6(1.2)	25.9(0.4)	5.4(0.2)	9(0.5)	5672(45)
LR6	37.8(0.3)	28.2(0.5)	7.4(0.2)	14(0.8)	30(5)

()=Standard deviations

In all HOC bioreactors, the total VFAs was higher (RA2=6838, RA3=6011, RA4=5745 and RA5=6178 mg/L) than LOC bioreactors (R2=4261, R3=4791, R4=4751, and R5= 4629 mg/L). The differences in VFAs explains the slightly low pH in HOC only bioreactor (RA5). For the manure only bioreactor, the digestate VFA was higher in RA6

than R6 (Table 13), due to the difference of the ingestate TS levels in the bioreactors (Table 11). At the completion of the 25 d, it must be noted that there was better VS destruction for LOC meal than for HOC (Table 12). That explains the higher values of VFAs in HOC than LOC. At the same level of canola meal addition, there was a low digestate pH in HOC than HOC (e.g. RA5 vs. R5).

4.5.2.4 Volatile Fatty Acids (VFAs) Changes

The change in volatile fatty acids (VFAs) is shown in Tables 14 and 15. The HOC canola meal only bioreactor (RA5) had high initial VFAs content as well as the greatest change, from 604±52 mg/L to 6179±107 mg/L, a ten folds increase. There was a difference between manure only bioreactors (R6 and RA6), which was attributed to the difference in the starting initial TS. All HOC bioreactors had higher VFAs values (RA2=4575±82, RA3=4288±97and RA4=3878±42 mg/L) at the beginning, which was higher than the threshold value (4000 mg/L), while LOC fractions had lower initial VFAs values (R2= 3977±35, R3= 3633±59, and R4=3032±49 mg/L) and less than 4000 mg/L. This led to higher VFAs values for HOC than the LOC bioreactors towards the end. The HOC bioreactors were suspected to have suffered from organic overloading. All the canola containing bioreactors had final VFAs of more than 6000 mg/L.

For the canola meal mono-substrate bioreactors, the VFAs content in HOC canola only (RA5) and LOC canola only (R5) had 459 ±30 and 442±27 mg/L, respectively, implying that HOC had slightly higher values of n-valeric acids. In addition, RA5 had n-butyric acid (20±3 mg/L) that was not detected in R5. At the end of 25 d, in both RA5 had more of n-valerate (1467 mg/L), propionate (1627 mg/L) and acetate (1056 mg/L) while R5 had n-butyrate (1774 mg/L) and acetate (1543 mg/L).

Table 14. Volatile Fatty Acids (VFAs) Changes at High Organic Loading

Bioreactor		Individual volatile fatty acids, mg/L, standard deviation in brackets						
		Acetate	Propionate	i-butyrate	Butyrate	i-valerate	Valerate	Total
HOL, LOC Canola meal								
R1	Initial	78(3)	0	0	0	0	0	78(3)
	Final	74(2)	0	0	0	0	0	74(2)
R2	Initial	2215(32)	998(31)	61(5)	542(27)	78(6)	83(2)	3977(35)
	Final	158(1)	3128(84)	333(14)	12(1)	598(17)	33(2)	4261(86)
R3	Initial	2022(54)	892(29)	53(1)	475(5)	69(1)	123(4)	3633(59)
	Final	1490(11)	1840(56)	292(8)	216(8)	474(47)	480(44)	4791(90)
R4	Initial	1660(49)	726(6)	42(2)	371(15)	51(5)	181(3)	3032(49)
	Final	1323(41)	1067(11)	180(2)	961(14)	352(16)	868(25)	4751(43)
R5	Initial	93(1)	20(2)	0	0	0	442(27)	555(27)
	Final	1543(50)	648(16)	146(3)	1774(43)	298(5)	221(4)	4629(43)
R6	Initial	2538(43)	958(12)	74(4)	455(3)	145(3)	57(1)	4227(43)
	Final	209(2)	76(3)	17(1)	183(8)	30(6)	0	514(8)
HOL, HOC Canola meal								
RA1	Initial	59(3)	28(1)	0	68(3)	0	0	155(7)
	Final	68(4)	56(3)	52(1)	1(0)	0	0	176(9)
RA2	Initial	2199(51)	1235(13)	91(2)	814(8)	135(5)	101(3)	4575(82)
	Final	1911(46)	1801(53)	214(6)	1525(78)	441(9)	946(27)	6839(219)
RA3	Initial	2046(43)	1124(33)	78(1)	697(16)	112(1)	232(3)	4288(97)
	Final	1670(17)	1386(4)	199(3)	1388(3)	410(1)	959(2)	6011(28)
RA4	Initial	1825(6)	983(10)	66(3)	586(10)	91(2)	326(11)	3878(42)
	Final	1509(31)	1410(36)	201(10)	1306(55)	396(10)	924(23)	5745(165)
RA5	Initial	103(2)	22(1)	0	20(3)	0	459(30)	604(36)
	Final	1056(8)	1627(10)	288(7)	1073(11)	667(16)	1467(28)	6179(78)
RA6	Initial	2036(28)	1156(14)	260(2)	214(4)	61(11)	0	3726(59)
	Final	955(9)	1693(32)	200(5)	10(42)	0	0	2857(88)

Table 15. Changes in VFA at Low Organic Loading

Bioreactor		Individual volatile fatty acids, mg/L, standard deviation in parenthesis						
		Acetate	Propionate	i-butyrate	Butyrate	i-valerate	Valerate	Total
HOC canola meal								
LR1	Initial	0	0	0	0	0	0	0
	Final	0	0	0	0	0	0	0
LR2	Initial	1875(42)	1566(23)	209(7)	1137(32)	337(17)	131(5)	5255(127)
	Final	154(25)	513.7(3)	0	0	951(80)	0	1618(107)
LR3	Initial	1927(33)	1837(18)	158(2)	1222(4)	271(2)	84(0)	5498(59)
	Final	41(1)	3160(10)	47(2)	0	597(5)	0	3845(9)
LR4	Initial	1499(4)	2059(13)	155(1)	1506(10)	280(1)	55(0)	5554(29)
	Final	982(15)	2741(34)	543(8)	80(1)	1146(5)	405(2)	5898(65)
LR5	Initial	298(2)	683(5)	0	4372(47)	0	0	5553(54)
	Final	1165(28)	1148(11)	281(1)	1317(3)	698(2)	1062(2)	5672(45)
LR6	Initial	2237(51)	1161(20)	157(3)	1103(26)	265(7)	200(5)	5121(112)
	Final	20(2)	10(3)	0	0	0	0	30(5)

4.5.3 Co-digestion of Canola Meal at LOL

4.5.3.1 Ingestate Characterization

The low organic loading (LOL) bioreactors ingestate characterization is as shown in the Table 11. For the canola containing bioreactors, the pH ranged from 6.5 (LR5) to 7.0 (LR2). An optimum pH of 6.5-8.5 is required for successful AD process (Khanal, 2009a). In this case, LR5 and LR2 reactors had pH lower than the recommended pH. All the bioreactors had high VFAs, with LR3 having the highest at 5498 mg/L. The C/N ratio was relatively low, ranging from 7.0 in canola meal only bioreactors (LR5) to 11.0 in dairy manure only bioreactor (LR6). This was lower than the optimum C/N ratio of 15-45 expected for AD (Itodo and Awulu, 1999), affecting biogas production (Figure 15).

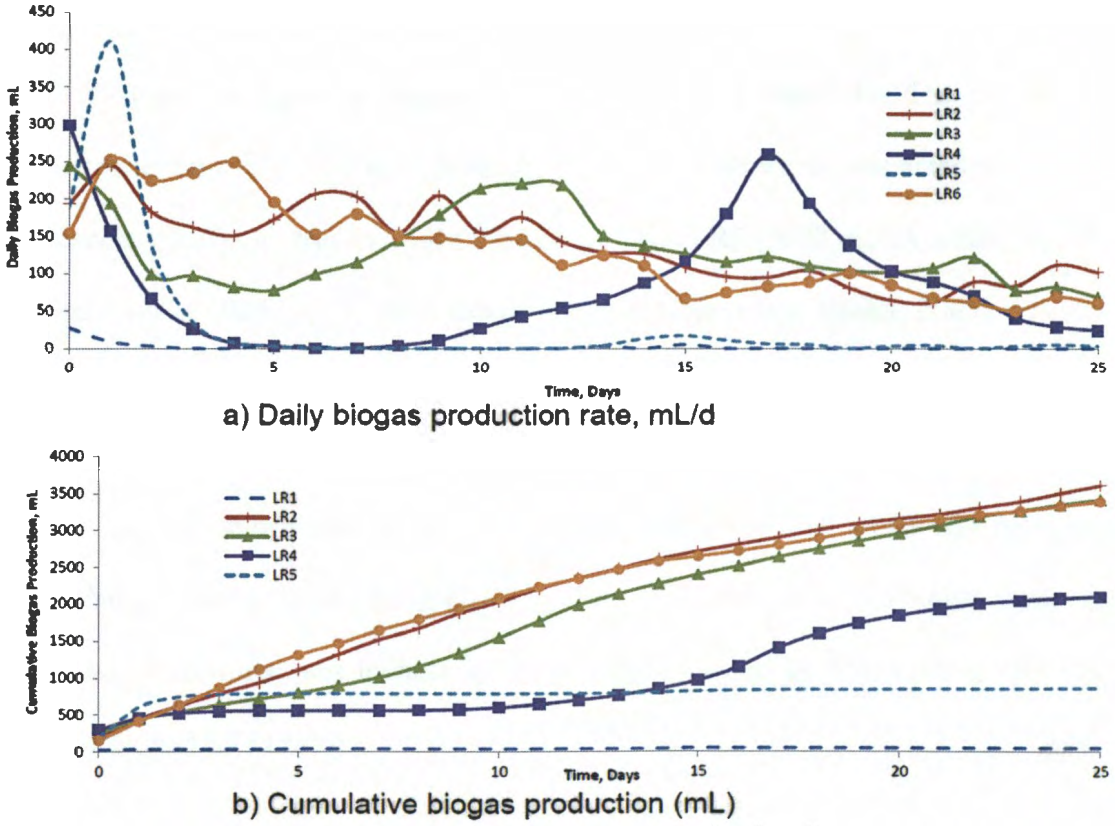


Figure 15. a) Daily b) Cumulative Biogas Production for HOC at LOL

4.5.3.2 Biogas Production and Methane Content

The daily biogas production rates and the cumulative biogas for 25 d are shown in Figure 15. There was similarity among three bioreactors, LR2, LR3 and LR6, in terms of cumulative biogas production and the biogas production profile. Each of them had an initial peak after the 2nd day and another after the 12th day. However, high canola ratio bioreactors, LR4 and LR5, seemed to halt biogas production after the 5th day. LR4 recovered to peak on the 22nd day. In general, it shows that high canola meal ratios had less cumulative biogas production. In Table 12, an increase of canola meal (HOC) to the dairy manure showed decreased methane quantity, with 100% HOC bioreactor having a final methane of 49.5%. Biogas is only combustible when the methane content is more than 45% (Deublein and Steinhausser, 2008).

4.5.3.3 Digestate Characterization

Changes in digestate characteristics are shown in Table 13. The pH in all the bioreactors except LR5 (5.4) was close to neutral. The final VFAs was noted highest in the high canola fraction bioreactors, LR4 (5898 mg/L) and LR5 (5672 mg/L), which was above the threshold of 4000 mg/L for a stable process (Siegert and Banks, 2005). These two bioreactors had the lowest cumulative biogas production as well as low pH. It can be deduced that increased VFAs concentration suppresses pH and results in low methanogenic activity, thus less biogas production. The lowest quantity of final VFAs was noted in the LR6 (30 mg/L) and LR2 (1618 mg/L) bioreactors, all of them had high biogas yield (Table 12). The VS reduction was highest in LR6 (42%), followed by LR5 (36%), LR4 (34%), LR2 (34%), and LR3 (30%).

4.5.3.4 pH and VFA Changes

The initial and final pH of bioreactors is shown in Table 11 and 13, respectively, while changes in VFAs are listed in Table 14 and 15. In spite of high initial levels of VFAs in canola containing LOL bioreactors, the pH was near neutral, promoting better degradation. Only two bioreactors had increased VFAs such as LR4 VFA changes from 5554±29 mg/L to 5898±65 mg/L and LR6 from 5353±54 to 5672±45 mg/L. The high VFAs notwithstanding, LR4 had higher biogas than LR5, mainly attributed to a neutral pH in LR4 (7.1), compared to LR5 (5.4). The manure only bioreactor had the highest reduction of VFAs, from 5121 to 30 mg/L. Unlike LOC, HOC canola meal bioreactor (LR5) had higher initial VFAs (5353 mg/L), which produced only 0.86 L biogas mainly compromising of n-butyrate acid, which degrades slowly. After 25 d, most of the n-butyrate had been converted and final value was 1317 mg/L. However, there was

appearance of valerate (1760 mg/L), probably formed from the degradation of long chain fatty acids. Other VFAs present include acetate (1165 mg/L) and propionate (1148 mg/L). Although not determined in this study, closely tied to VFAs and pH, is the amount of free ammonia in the reactor. At a pH equal to 7.4, a rare balance is achieved and VFAs are re-used as well as any effect of ammonia inhibition is curtailed (Chen et al., 2008). This explains the high cumulative biogas production in LR2 (3.6 L) and LR6 (3.4 L) (Table 12).

4.5.4 Effect of Oil Content, Organic Loading (OL) and Canola Fraction

Based on the results obtained from the three sets of experiments, the biogas production analysis of variance (ANOVA) was done in SAS. From the interaction plot (Figure 16), there are interactions between the various levels of treatment.

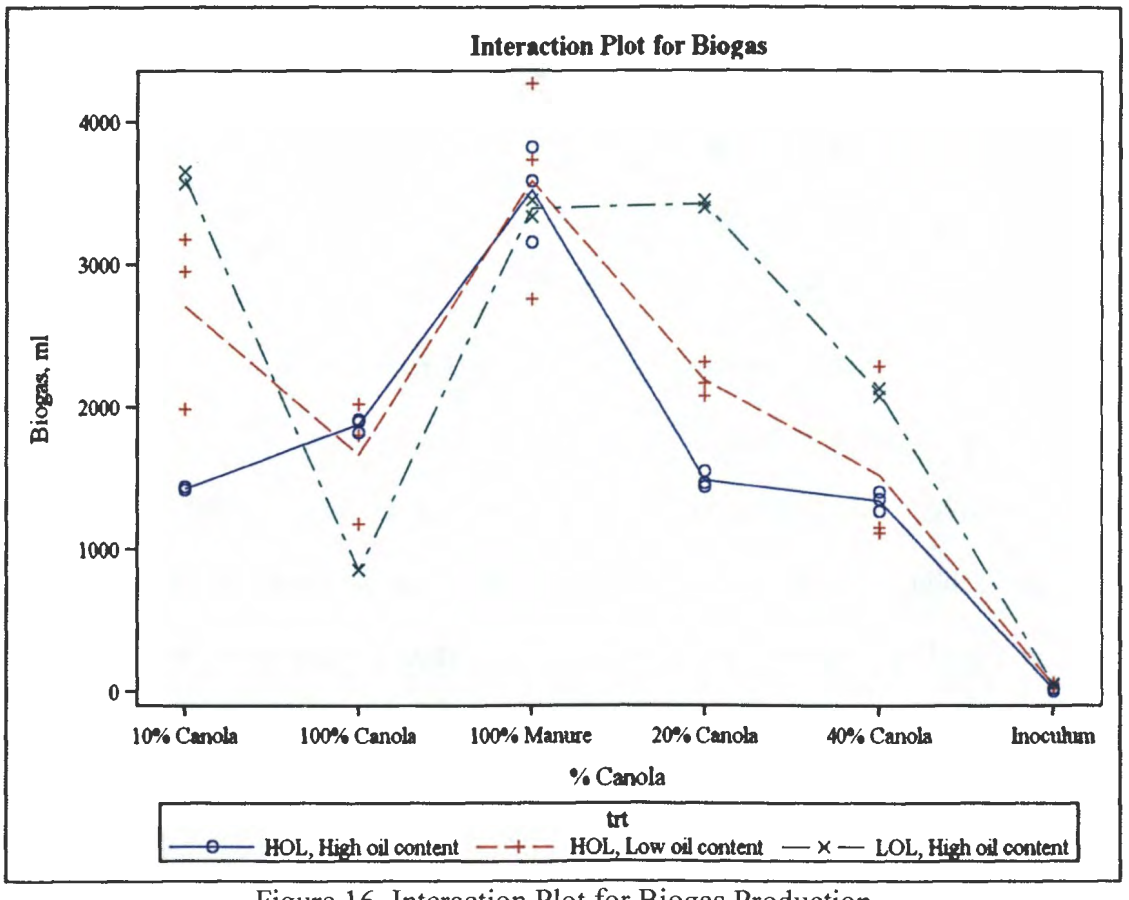


Figure 16. Interaction Plot for Biogas Production

The biogas production rate for the various canola containing bioreactors at three levels of treatment were found to be significantly different ($p<0.0001$). Within each canola containing bioreactor, there was significant difference in terms of oil content as well as the organic loading rate ($p<0.0006$). The mean separation by LSDs shows that among the canola containing bioreactors, 10% and 20% canola meal are not significantly different. Also, 40% and 100% canola are not significantly different. For the levels of treatment, at about 7.5% TS (HOL), LOC and HOC are not significantly different but there is a significant difference between them at HOL and LOL. The differences and similarities are expounded in the following section.

4.6 Discussion

4.6.1 Comparison between Canola Meals Fractions and Dairy Manure

From the current study, it appears that the accumulation of VFAs plays a leading role in the co-digestion of canola meal with dairy manure. Six types of dissociated VFAs were identified: acetate, propionate, n-butyrate, i-butyrate, n-valerate and i-valerate. For HOL, bioreactors with high initial acetate (R6=2538±43, RA6=2036±28, R2=2215±32, RA2= 2199±51 mg/L) had better degradation than bioreactors with low initial acetate (R5=93±1, RA5=103±2 mg/L). Unlike butyrate and propionate that degrade in days and do not necessarily follow the Michaelis-Menten kinetics, acetate degrades in a matter of hours following Michaelis-Menten kinetics for acetate pre-grown culture (Aguilar et al., 1995). The inocula used in the study, mainly had acetate values of 78±3 and 59±3 mg/L in R1 and RA1, respectively at the beginning. This is suspected to have had microbial population that was better adapted for the acetate degradation. As the canola fraction in bioreactor increased, there was gradual decrease of acetate, but for both propionate and butyrate

gradually increased. Proteins take longer time to decompose, in the range of days as compared to carbohydrates and lipids that break down in hours (Kolesárová et al., 2011). Just like studies carried out on sunflower oil (Raposo et al., 2008), a high protein substrate, n-valerate and i-valerate were observed in canola meal fractions.

Due to the slow degradation of propionate and butyrate, accumulation of VFAs is closely tied with the initial values of these two. VFAs play an important role as intermediate products for the methanogenic step (Ahring, 2003). However, they also lead into inhibition as they cause a drop in pH, which has a strong impact on the growth of the microbial population (Khanal, 2009a). A neutral pH value of 7.0 is known to be the best for biogas production since it allows for fast dissociation of acetate (Khanal, 2009b). In the current study, accumulation of VFAs didn't result into large drops in pH due to buffering from dairy manure. This explains the reasons for better performance for the bioreactors whose pH was closer to neutral (R6=6.6±0.1 and RA6=6.7±0.1).

In addition to VFAs, some of the commonly identified acetate degradation inhibitors include NH_3, H_2, H_2S and CO_2. As the pH decreases, H_2S and CO_2 concentration increase. Only CO_2 in the gas phase was determined, which was consistent with low pH bioreactors resulting in minimal biogas production. Typically as the pH drops to 6.0, methane production is halted and only hydrogen gas is detected (Luo et al., 2011), but H_2 was not monitored in this study along with VFAs. Propionate and n-butyrate have positive Gibbs energy, and their degradation is only thermodynamically feasible at low levels of H_2 and acetate (Wang et al., 1999). The high levels of acetate, propionate and n-butyrate in canola fractions (R2, RA2, R3, RA3, R4, RA4) may have resulted in less biogas production than in dairy manure only (R6, RA6).

Canola meal is a challenging substrate for AD process; it leads into souring of the bioreactor within days, halting the production of biogas (Figure 14 and 15). Canola meal mono-substrate bioreactors had the lowest biogas production, with R5 (1.67 L) being slightly higher than RA5 (1.87 L) (Table 13). This is attributed to the accumulation, and type of VFAs present. Although the changes in VFAs level was not monitored in the course of the experiment, canola meal bioreactors (R5 and RA5) showed the greatest accumulation in the course of the run. This was accompanied by a drop in pH. For HOC, most of the accumulation was propionate (1627±10 mg/ L), followed by n-valerate (1467±28 mg/L). In LOC meal, n-butyrate and acetate were 1774±43 and 1543±50 mg/L (Table 14), respectively. For the HOC meal, propionate values beyond 900 mg/L may have caused inhibition of the methanogenic step (Wang et al., 1999). The LOC meal has high concentrations of butyrate (2072±48 mg/L), which is known to be slow in degrading (Aguilar et al., 1995).

In addition, there was minimal difference in fiber composition; HOC meal had less of hemicelluloses (7%) and more of cellulose and lignin (21%) as compared to LOC meal which had more of hemicelluloses (10.5%) and less of cellulose and lignin (18%) (Table10). Hemicelluloses are more readily hydrolysable than cellulose or lignin, explaining the slight difference in biogas production between the two canola meal mono-substrate bioreactors.

4.6.2 Comparison of HOC and LOC Meals

In previous studies, oil cakes/meals have been touted as adding value in co-digestion with dairy manure. Oil cakes/meals are attractive as they contain highly digestible materials (Kolesárová et al., 2011). The cumulative biogas production comparison between

HOC (RA5=1.87 L) and LOC (R5=1.67 L) shows a slight difference. The theoretical specific methane from rapeseed is estimated at 461 L/kg VS (Luo et al., 2011). The values of VFAs initially in each of the bioreactors are proportional to the amount of oil meal/cake added (Raposo et al., 2008). Thus, there were higher initial VFAs in HOC bioreactors than LOC bioreactors (Table 14). This trend is observed in terms of accumulation of VFAs, in which HOC had 5575 mg/L while LOC had 4074 mg/L. In terms of individual VFAs, the greatest change was observed in propionate (R2=2130, RA5= 1605, R3= 948 mg/L), n-butyrate (RA2=711, RA3=691, RA4=720, R5=1774 mg/L) and valerate (R4=687 mg/L) (Table 14). It can be inferred that the greatest accumulation in LOC meal was propionate and n-butyrate in HOC meal. In spite of high concentration of propionate in R2, it had high biogas production (2.7 L), mainly due to falling concentration of acetate (Table 14 and 15). High propionates are not necessarily inhibitory (Pullammanappallil et al., 2001). Another aspect will be changes in pH.

The variation of pH values has an influence on the overall degradation. At a pH of 7.0, the highest breakdown of acetic acid, the most prominent VFA, producing about 75% of the methane, is expected (Aguilar et al., 1995). Dairy manure has a buffering capacity to keep the pH approximately constant, thus in spite of high levels of VFAs, pH does not drop immediately but the impact on microorganism is immediate. There are three types of microorganism; for hydrolyzing, fermenting and production of methane. The fermenting microbes are inhibited by fermentation products, thus they rely on how quickly the methanogenes convert intermediate products into biogas. As VFAs accumulate, the methanogens are overwhelmed, lagging behind the fermenting bacteria.

On the basis of this study, for the case of co-digestion of canola meal and dairy manure, the methanogenic step is believed to be the rate limiting, defining the overall kinetics. Enriching the bioreactors with better adapted bacterial population can result into rapid conversion of the VFAs, thus improved process stability (Aguilar et al., 1995).

4.6.3 Comparison between HOL and LOL

Based on the biogas production rate and specific methane, all the canola meal fractions had better performance in low organic loading (LOL), about 4.5% TS. This is consistent with findings done on rapeseed oil cake at different loadings 2.5, 5 and 10 g VS/L, in which there was low methane production at higher loading (Luo et al., 2011). At LOL, there is high specific methane production in 10% and 20% HOC canola of 535 L/kgVS and 445 L/kg VS, respectively. Manure only bioreactor (LR6) had a specific methane of 300 L/kg VS (Table 12). When the organic loading is lowered from HOL to LOL for 10% and 20% HOC canola, there was a four and 2.5 folds increase, respectively. This showed that canola when added to 20% can impact the specific biogas production. At HOL, HOC canola seems to cause process instability as a result of overloading.

In spite of comparable HOC canola's VFAs levels in HOL and LOL, there was better performance for the LOL. This may be attributed to a favorable pH, which suppressed inhibition due to VFAs. In the past, studies showed that VFA inhibition is dependent on pH (Siegert and Banks, 2005). At a pH of 7.4, AD process remains stable even at high concentrations of propionate, up to 6000 mg/L (Gourdon and Vermande, 1987). However, for canola only bioreactors, the cumulative biogas production dropped from 1.87 L in RA5 to 0.86 L in LR5, a significant drop. This could be explained by the

low pH of 5.4 in LR5, which is suspected to have halted the methanogenic bacteria activity. At a pH lower than 6.2, there is a higher possibility of toxicity (Chen et al., 1980).

4.7 Conclusions

Based on the current findings, canola meal has a less desirable impact on dairy manure co-digestion at HOL. However, it must be noted that addition of canola meal (about 10 and 20%) to manure greatly improves dairy manure AD at LOL. This has a net impact of increasing biogas production per unit volume. However, at high canola meal fractions (more than 20%), these benefits are eroded as the biogas volume and specific methane production decreases. This is suspected to be caused by accumulation of VFAs. Accumulation of VFAs leads to low pH and consequently halting the methanogenic process. From this study, it is possible to control the accumulation of VFAs by two factors, namely the organic loading and the oil content. Though it is not explicit on the levels, low organic loading may result in better performance. Other approaches that may limit the accumulation of VFA include: use of separate fermentation and methanogenis (two stage digestion) and/or raise the temperature to thermophilic.

CHAPTER 5. PRELIMINARY AND FUTURE RESEARCH RECOMMENDATIONS

5.1 Summary

Based on research findings in chapter 3, use of canola meal for anaerobic co-digestion with dairy manure did not show significant improvement in biogas production and methane yield. In order to address this, a preliminary study based on pre-treatment of the canola meal and raising the temperature to thermophilic range was implemented. In the first study, 10g of canola meal was pretreated with 0, 0.5 and 1.0 g sodium hydroxide (NaOH) with the total solids (TS) adjusted to 10%. In addition, 10 % NaOH solution by w/w was also used for pretreatment. For a period of 12 d, changes in its pH were monitored every 3 d. For each treatment, the fiber content was measured at the start and the end of the 12 d period. In the second study, co-digestion of low oil canola meal with dairy manure was investigated at thermophilic temperatures, 60^0C. The canola meal-to- dairy manure ratios were 0:100, 10:90, 20:80 and 40:60. In each of the bioreactors, the daily cumulative biogas production was measured by water displacement method.

Results from the study showed that addition of 5% NaOH by w/w (0.5 g NaOH in 10 g canola meal) adjusted the pH to optimum levels of 6.5 to 8.2. There was 30% better degradation of the fibers (hemicellulose, cellulose and lignin) and more than 10% increase in soluble by pre-treating with a 10% NaOH solution. The preliminary results on thermophilic co-digestion showed a more than 30% decrease in the daily biogas production (compared to chapter 3). More research will be needed to optimize the preliminary results, and enhance the biogas production from canola meal.

5.2 Introduction

Based on the findings from chapter 3 and 4, it is evident that canola meal is not a suitable candidate for co-digestion. It did not have a positive impact on dairy manure co-digestion biogas production rates and methane content. In all the cases, canola meal co-digested with dairy manure had low biogas production as well as the quality of methane, pointing to some inhibition. In all the runs (chapter 3 and chapter 4), biogas production and methane content from100% canola meal trailed the others. Canola meal alone proved to be a challenging AD substrate, with an initial rapid production of poor quality gas for 5 days, and then gas production halted. Similarly, canola meal and dairy manure mixtures produced less gas compared to manure only.

From chapter 3, the main reason given for the failure of canola meal in enhancing biogas production rate was the accumulation of volatile fatty acids (VFAs). During anaerobic co-digestion, the complex organic matter is broken down to intermediate products. VFAs and other intermediary products (mainly alcohols) are produced as the hydrolyzing and fermenting microorganism break down complex organic matter (Ahring, 2003). In a well balanced AD process, about 20-30% of the organic matter will be transformed into methane via this route. While it is recognized that VFAs are important in the AD process, their accumulation resulted in drop of pH, and consequently causing inhibition.

From chapter 4, the accumulation of VFAs is tied to the level of residual oil as well as the proportion of canola added. A threshold value of 4000 mg/L is given for total VFAs in the bioreactor before inhibition starts (Siegert and Banks, 2005). In addition to the total VFAs value, individual concentrations are also important. For instance, the ratio of acetate

to propionate has been suggested to no less than 4:1 (Khanal, 2009b). The oil content in the canola is directly related to the organic loading. The higher the oil content, the higher the organic loading rate and the higher the propensity for accumulation of VFAs. In all cases, 100% canola meal is shown to have the highest accumulation of VFAs and consequently the lowest resultant pH.

Khanal (2009b) proposes that in order to reduce the accumulation of VFAs, a reduction of the volumetric organic loading coupled with addition of chemicals to adjust the pH, and more recently dosing with oxygen is necessary. In addition, organic wastes with high nitrogen content such as canola meal contribute to increased alkalinity. A VFA/alkalinity ratio of 0.1-0.25 is optimum for acidification. In order to control the quantities of VFAs, future research is needed to focus on pre-treatment, thermophilic co-digestion, and two stage continuous digestion. In a limited manner, trials for the co-digestion of canola meal and dairy manure were done with pre-treatment and at high temperatures. The objective of the trials was to establish the impact of pretreatment and high temperature on canola meal digestion.

5.3 Materials, Methods and Experimental Procedure

5.3.1 Caustic Pretreatment

Low oil canola (LOC) as previously described in Chapter 3, was pretreated at three levels of alkaline hydrolysis using sodium hydroxide (NaOH). The composition of the three levels was as follows: level I (0 g NaOH + 10 g canola meal + 90 g water), level II (0.5 g NaOH + 10 g canola meal + 89.5 g water), level III (1 g NaOH + 10 g canola meal + 89 g water). Each of the treatment was monitored for a period of 12 d and evolution of its pH every three days. For purposes of monitoring the changes in fiber content, an additional

treatment of 10% NaOH by v/v was included to the previous treatment. The experiments were carried out at ambient conditions, with daily stirring.

5.3.2 Thermophilic Co-digestion

The preparation, experimental procedure and methods were exactly the same as described in chapter 3, except for the temperature, which was elevated from 35^0C to 60 ± 3^0C. Only low oil content canola (LOC) was used. The data and sample analysis remained as described previously in chapter 3.

5.4 Results and Discussion

5.4.1 Pre-treatment

Preliminary result of treating canola meal with caustic (NaOH) showed greater degradation of fibers to more hydrolysable state, readily absorbed by the microbial population. The pre-treatment improves the biodegradability of the canola meal. The impact of addition of caustic to canola meal was investigated in two parameters: pH and fiber changes (Figure 17 and 18).

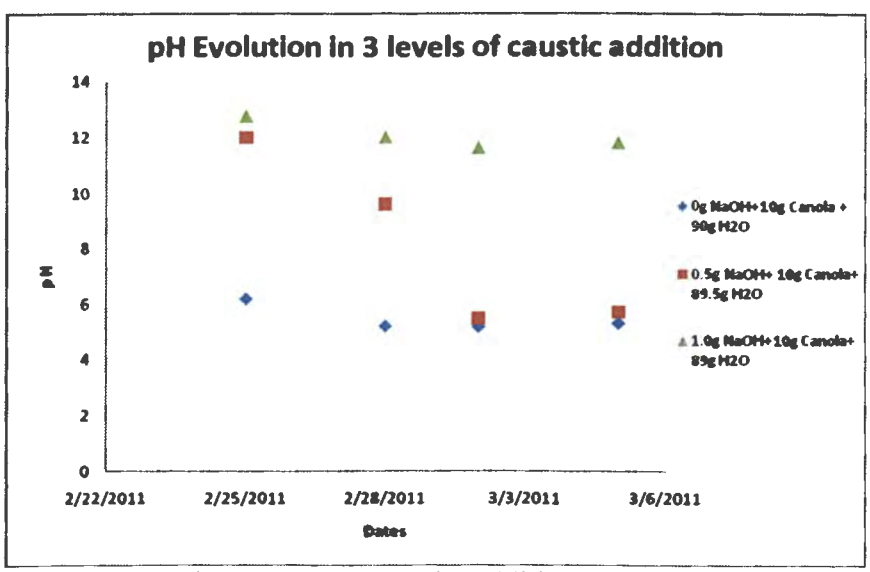


Figure 17. pH Changes After Caustic Addition on Canola Meal (LOC)

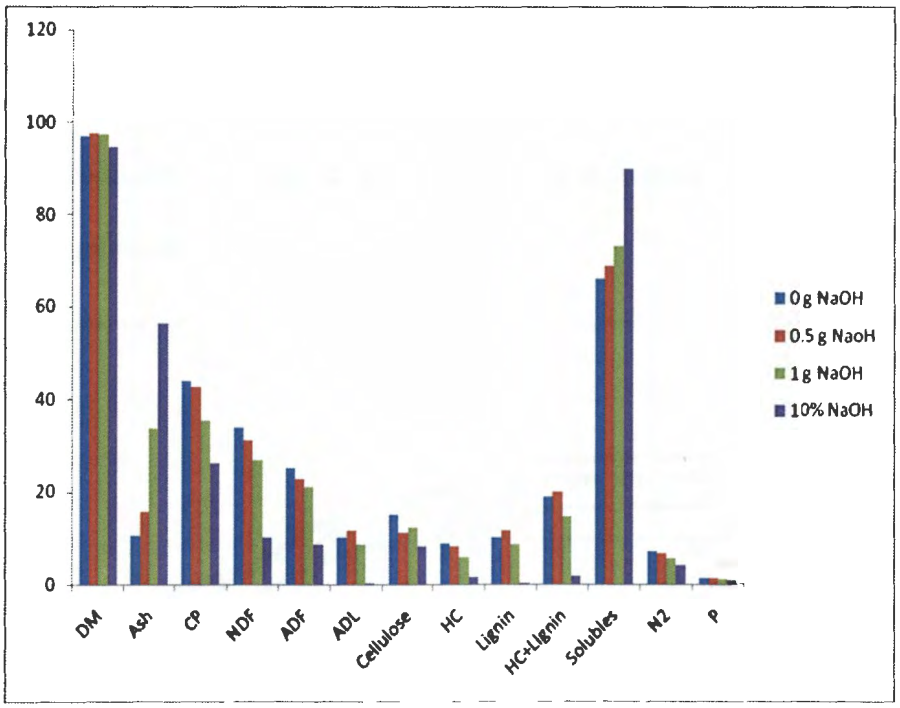


Figure 18. Fiber Changes After Addition of Caustic on Canola Meal (LOC), as % TS

10% NaOH pre-treatment changes the composition of canola meal, reducing the amount of fibers, especially hemicelluloses and lignin. This is consistent with previous caustic pre-treatment done on lignocellulosic organic matter (Pang et al., 2008). Use of alkaline hydrolysis pre-treatment generates less toxic products and/or inhibition in the ensuing processes (Neves et al., 2006). However, NaOH pre-treatment results in high pH as shown in Figure 19. This will require correction either before or during the AD process. This is another area where further research is needed.

5.4.2 Thermophilic Co-digestion

Biogas production experiments carried out at high temperatures have shown improved biogas production due to significant shift of microbial strains (Ahring, 2003). A larger part of the carbon is channeled directly into acetate as opposed via VFA route. Thus,

less of propionate and butyrate are produced. Trials done at thermophilic temperatures did not show any significant improvement (Figure 19), instead there was a drop in all bioreactors. This was attributed to less adaptation of the mesophilic micro-organisms to thermophilic conditions. It must be noted that it takes a long time to establish a true thermophilic population.

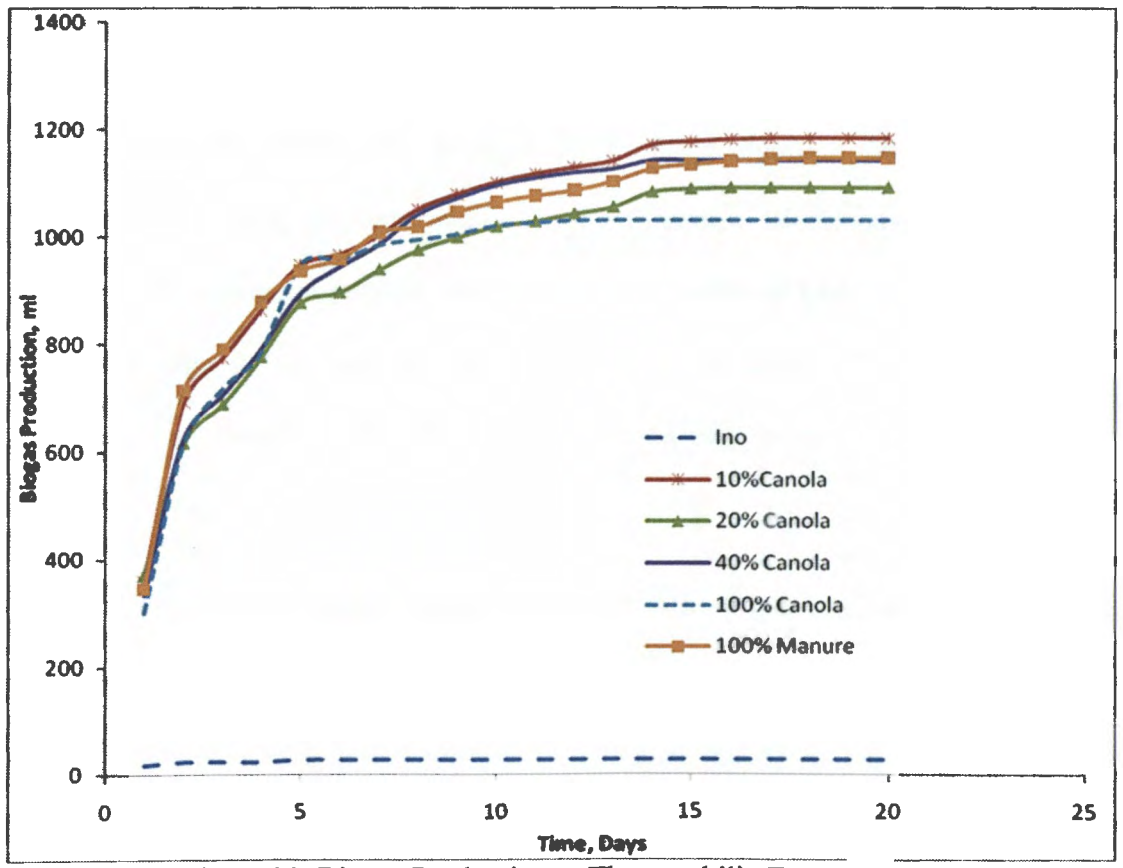


Figure 19. Biogas Production at Thermophilic Temperatures

At high temperatures, there is less formation of VFAs in the digestion of dairy manure as opposed to their quantities in raw wastes (Ghaly, 1996). In treating thin stillage, similar to canola meal due to its high protein content, thermophilic AD treatment proved valuable (Khanal, 2009a). More studies are recommended for canola meal and dairy manure at these temperatures.

5.5 Future Research Recommendation

The accumulation of volatile fatty acids stipulated in chapter 3, results in a drop in pH. Methanogenic bacteria are more sensitive to changes in pH, impacting both the biogas production rate as well as the methane content in biogas (Ghaly, 1996). In AD process, the existing anaerobes in the bioreactor may be classified into two pH groups: acidogens with optimum pH of 5.5-6.5 and methanogens with optimum 7.8-8.2 (Khanal, 2009a). In practice, a one stage bioreactor maintains the pH near neutral point. Thus, neither the acidogens nor methanogens are optimized. In two stage digestion the two processes are separated, thus creating optimum conditions for both groups (Blonskaja et al., 2003). In one experiment, the separation resulted into about 50% reduction of total VFA, from 3.5 mM in the second stage to 1.8 mM in one stage (Liu et al., 2006). Consequently for this experiment, since most of the VFAs are converted into biogas, 21% higher methane production was demonstrated.

In treating high organic content distillery wastes, a two stage approach was found successful (Blonskaja et al., 2003). Another advantage of two stage is the possibility of operating the bioreactor at high organic loading rate (12.6 g VS/L) that is infeasible in one stage, resulting in cost savings as the volume is reduced (Demirer and Chen, 2005). The inhibition by VFAs beyond 4 g/L is the most improbable in continuous systems, and the digesters are only inhibited as VFAs rise beyond 10 g/L (Aguilar et al., 1995). In order to overcome limitations in one stage AD, two-stage process is recommended for canola meal and dairy co-digestion.

CHAPTER 6. GENERAL CONCLUSIONS

Dairy manure AD had the highest biogas production as well the best biogas quality (high methane content). In co-digesting dairy manure with canola meal, there was overall improvement on biogas production and methane content as compared to canola meal alone. Canola meal co-digestion bioreactors produced less biogas and low quality gas when compared to dairy manure only. Therefore, based on the current study, canola meal co-digested with dairy manure does not improve biogas production.

Nevertheless, from this study, two factors have been identified as affecting the biogas production and methane content in the co-digestion of canola meal and dairy manure. These factors are: the oil content and fraction of canola meal in the co-digestion. These factors affect the biogas production and methane content by influencing the rate of accumulation of volatile fatty acids (VFAs). High residual oil is found in mechanically pressed canola and low oil content is found in solvent extraction process. As the canola fraction in the co-digestion increases, the less desirable impacts are more pronounced. Statistically, 10% and 20% canola meal did not have any significant difference in terms of biogas production. 40% and 100% canola produced less gas in all cases. Therefore, canola meal can only be added to about 20% on weight basis.

In spite of the identification of the main factors affecting canola meal co-digestion with dairy manure, this study did not optimize them. There is a need to clearly demonstrate the impact of the two factors. In addition, other ways of improving biogas and methane content can be explored such as pre-treatment of canola meal, continuous two-stage AD and thermophilic co-digestion.

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