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**Enhancement of Biogas Production Using Co-Substrates in Anaerobic Digesters for Medium Size
Dairy Farms**

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Enhancement of Biogas Production Using Co-Substrates In Anaerobic Digesters for Medium Size Dairy Farms

Terrence Sauvé

A thesis submitted under the supervision of

Dr. Kevin Kennedy

In partial fulfillment of the requirements for the degree of
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Ottawa Carleton Institute for Environmental Engineering

Department of Civil Engineering

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ABSTRACT

The use of co-substrates can significantly enhance biogas production from manure while helping to treat and dispose of high carbon organic wastes from the food processing industry and municipalities. This thesis will focus on the experimental setup and results collected at the University of Guelph – Campus d'Alfred from a series of Biochemical Methane Potential assays and six 30 L semi-continuous anaerobic digesters operating under mesophilic conditions. The co-substrates investigated during the study include: corn silage, canola meal, whey, glycerine and dissolved air floatation sludge. The use of liquid dairy manure proved to be beneficial for the anaerobic digestion process by supplementing necessary buffering capacity, nitrogen, micro and macro elements for the co-digestion of high carbon organic wastes. Biochemical Methane Potential assays of the co-substrates increased the biogas yield from 5 to 250%. When these co-substrates were elaborated in semi-continuous pilot digesters, volumetric biogas production increased two to three-folds in relation to liquid dairy manure.

RÉSUMÉ

L'introduction de co-substrat augmente considérablement le rendement de biogaz tout en aidant le traitement de rejets riches en matière organique des agro-industries et des rebus municipaux. Cette thèse se concentre sur l'investigation des résultats obtenus au Campus d'Alfred de l'Université de Guelph par une série de test *Biochemical Methane Potential* et de six digesteurs anaérobies de 30 L opérés à température mésophile. Les co-substrats utilisés pendant cette recherche sont l'ensilage de maïs, tourteau de colza, lactosérum de fromage, glycérine issue de la production de biodiesel et boue de flottation à l'air dissous. L'utilisation du lisier liquide de bovin laitier s'est démontrée bénéfique au processus de digestion anaérobie en augmentant la capacité tampon et les éléments nutritifs pour la co-digestion de déchets riches en carbone. Lors de test *Biochemical Methane Potential*, la co-digestion a augmenté la productivité de biogaz de 5 à 250% comparé au lisier liquide de bovin laitier. Pour ce qui est des réacteurs pilotes, la productivité de biogaz a doublé et triplé comparé au lisier liquide de bovin laitier.

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NOMENCLATURE

COD	Chemical Oxygen Demand, mg O ₂ /l
F/M	Food to Mass Ratio, dimensionless
ha	hectares, unit of surface
hr	time, hour
HRT	Hydraulic Retention Time, d
M	Molarity, mole/l
OLR	Organic Loading Rate, gVS/l/d
PA	Partial Alkalinity, g CaCO ₃ /l
pH	Concentration of Hydrogen Ions (H ⁺) in a Solution, dimensionless
pKa	Acid Dissociation Constant, dimensionless
pKb	Base Dissociation Constant, dimensionless
sCOD	Soluble Chemical Oxygen Demand, mg O ₂ /l
S/I	Substrate to Inoculum Ratio, dimensionless
TA	Total Alkalinity g CaCO ₃ /l
TKN	Total Kjeldahl Nitrogen, mg TKN-N/l
TS	Total Solids, weight %
VFA	Volatile Fatty Acids, mg/l
V _r	Volume of reactor, l
VS	Volatile Solids, weight %

LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
ANOVA	Analysis of Variance
ARBC	Anaerobic Rotating Biological Contactor
ASABE	American Society of Agricultural and Biological Engineers
ASBR	Anaerobic Sequencing Batch Reactor
ATP	Adenosine Tri-Phosphate
AU	Animal Units
BMP	Biochemical Methane Potential
CAD	Centralized Anaerobic Digester
CAFO	Confined Animal Feeding Operations
CHP	Combined Heat and Power
CNG	Compressed Natural Gas
C/N	Carbon to Nitrogen
CSTR	Continuously Stirred Tank Reactor
DAF	Dissolved Air Floatation
DFFR	Down-flow Fixed-Film Reactor
DUHR	Down-flow/Up-flow Hybrid Reactor
EGSB	Expanded Granule Sludge Blanket
EU	European Union
FFA	Free Fatty Acids
FFSB	Fixed Film Sludge Blanket
FOG	Fats, Oils and Greases
GC	Gas Chromatograph
GHG	Greenhouse Gas
HC	High Conductivity
HDPE	High Density Polyethylene
kWh	kilowatt per hour
LCFA	Long Chain Fatty Acid
NGO	Non-Government Organization
NMA	<i>Nutrient Management Act O. Reg. 267/03</i>
OBSFAP	Ontario Biogas Systems Financial Assistance Program
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
RESOP	Renewable Energy Standard Offer Program
STP	Standard Temperature and Pressure, 273.15 °K, 1 atmosphere
SSOFMSW	Source Separated Organic Fraction of Municipal Solid Waste
TSMR/MR	Two-stage mixed reactor with Microfiltration membrane
TSUR	Two-Stage Upflow Reactor
UASB	Upflow Anaerobic Sludge Blanket
UFFLR	Up-flow Fixed Film Loop Reactor
USEPA	United States Environmental Protection Agency
WVO	Waste Vegetable Oil
WW	Wastewater

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CHAPTER 1: INTRODUCTION

1.1 Background

The Canadian dairy industry earned \$5.2 billion dollars from 14 660 milking farms across the country in 2007 (Canadian Dairy Information Center, 2009). In the past decades, the Canadian farming sector saw many changes, from having 126 000 farms in 1970 down to 14 660 in 2007, and from an average herd size of 20 heads up to 59 heads in 2001. The average production of milk per farm increased five-fold from 1970 at 631 hectolitres to today's 5375 hectolitres (Werry, 2006). The Canadian dairy industry generates huge amounts of wastes, mainly composed of livestock manure which is handled on the farm. It is considered an underutilized source of biomass for energy, as it is land applied on agricultural fields as a soil amendment of organic matter, nitrogen, phosphorous and potassium. Mismanagement of livestock waste generally leads to bacterial and nutrient contamination of both surface and groundwater sources, accompanied by nuisance odours and possible saturation of phosphorous in certain soils in regions of high livestock density. Anaerobic digestion (AD) of liquid dairy manure contributes to alleviate these problems in many ways; production of electricity and heat, reduction of greenhouse gas production by offsetting electricity consumption from the farm, odour control by reduction of volatile fatty acids, reduction of pathogens, increased availability of nitrogen (as ammonium) to plants and the potential to treat off-farm organic wastes to increase electricity and heat production. Anaerobic digestion can also fraction the digestate in a liquid that contains 75-80% of the nitrogen and in a solid fraction containing 80% of the phosphorous (House et al., 2007). In Canada, it was estimated that a total of 14 million tonnes of dairy manure in 2001 was available for treatment, where Southern and Eastern Ontario and Quebec along the St-Lawrence Seaway, have highly concentrated areas of available dairy manure (Wood and Layzell, 2003). It is estimated that more than 548 million cubic meters of biogas could be produced per year through the anaerobic digestion of this source of available manure instead of land application. Treatment of off-farm organic wastes also enhances biogas production, making biogas plants more economically feasible. Improved digester designs with lower capital and operating costs, and the successful implementation of manure digesters treating off-farm wastes in Europe for

renewable electricity production has justified a new look into digesters for the Ontario agricultural industry. Currently in Eastern Ontario, there is a gap in the information available for design engineers and farmers on the biogas potential of off-farm wastes co-digested with liquid dairy manure.

1.2 Hypothesis

Can regionally available co-substrates, as an additional carbon source, enhance volumetric biogas production when co-digested in anaerobic digesters with liquid dairy manure?

1.3 Research Objectives

1. Select co-substrates available to the region of Eastern Ontario that are suitable for co-digestion with liquid dairy manure
2. Determine the biogas yield and methanogenic inhibition of these co-substrates using Biochemical Methane Potential (BMP) tests
3. Optimize the volumetric biogas production of these co-substrates in semi-continuous pilot reactors with liquid dairy manure

1.4 Thesis Layout

The thesis is divided into five chapters, followed by appendices at the end. In Chapter 2, the literature review consists of AD basics, the present status of AD and co-digestion in Europe and North America and the co-substrates chosen for this co-digestion study. Chapter 3 presents the materials and methods used in this study, including the source of each co-substrate, the operation of batch BMP assays and semi-continuous digesters and analytical methods used for sample analysis. Chapter 4 describes the results from this study and their related discussion, followed by Chapter 5 with the project conclusions and recommendations for future research required.

CHAPTER 2: LITERATURE REVIEW

2.1 Anaerobic Digestion Basics

Anaerobic digestion occurs naturally in the world, for example in bottom lake sediments, peat bogs, marshes, hot springs and in the intestines of ruminants. It also occurs in man-made uncontrolled environments like landfills where organic matter is stabilised. The process of anaerobic digestion is the breakdown of soluble organic matter into methane and carbon dioxide, a mixture of gases called biogas. It is carried out by primarily prokaryotic microorganisms in the absence of oxygen and nitrogen electron acceptors (Grady et al., 1999). Anaerobic digestion can operate from 10 °C up to 60 °C and under a wide range of moisture content. The process has been adapted to degrade a wide range of organic matter, with the advantage of producing less biomass than conventional aerobic treatments (Grady et al., 1999 and Wellinger, 2001).

The basic principal of anaerobic biochemical operations are explained and illustrated in Figure 2.1. For the Bacteria and Archaea to uptake soluble organic matter as electron accepters and donors, particulate organic matter must be broken down and solubilised by hydrolysis reactions. Enzymes produced by the fermentative bacteria, including cellulases, amylases and proteases, are responsible for reaction 1 in Figure 2.1. Acidogenesis is the process where sugars and amino acids are degraded by members of the bacteria domain (reaction 2) into by-products like acetic acid, hydrogen and other volatile fatty acids. There is also hydrogen produced by reaction 3 and 4 (anaerobic oxidation) where most of the hydrogen comes from the oxidation of long chain and volatile fatty acids (LCFA) to acetic acids. Reaction 5 demonstrates that this hydrogen could form acetic acid in combination with carbon dioxide, but since the acetic acid is most preferred by the acetoclastic methanogens, this reaction does not occur. Instead, reaction 6 proceeds by producing methane and carbon dioxide. In reaction 7, the hydrogen oxidizing methanogens reduce carbon dioxide to produce methane and water (Grady et al., 1999).

Hydrolysis

Acidogenesis

Acetogenesis

Methanogenesis

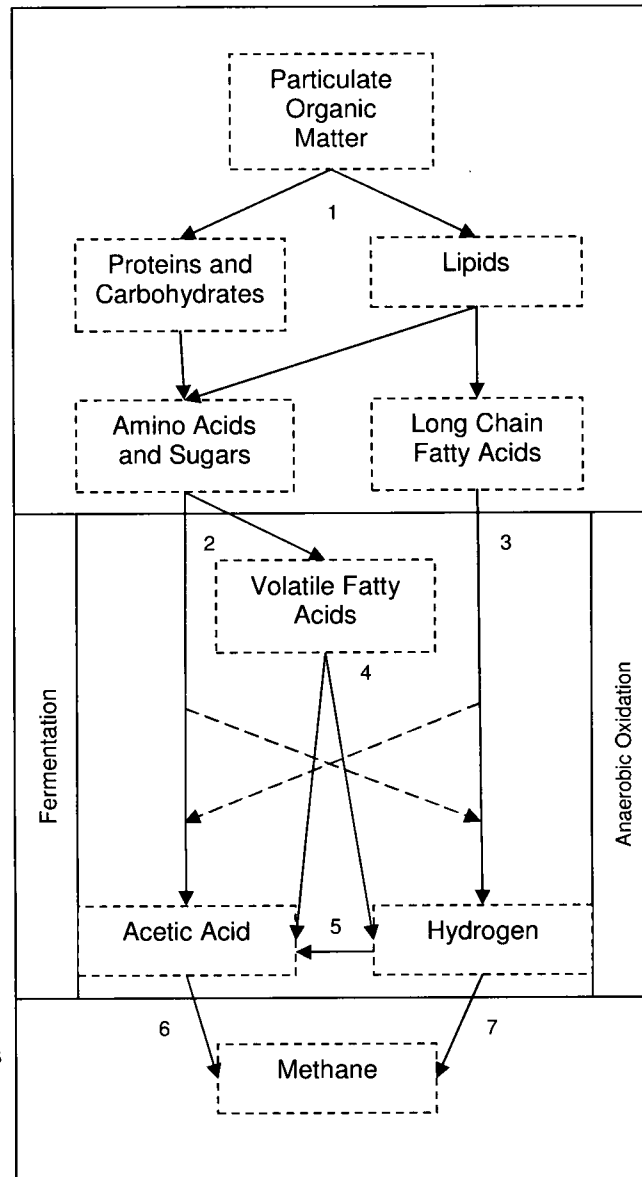


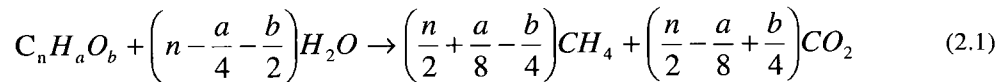
Figure 2.1 Illustration of the Anaerobic Process, adapted from Grady et al. (1999)

2.1.1 Yield, Biomass Growth and Substrate Exchange

As seen briefly Figure 2.1, many Bacteria and Archae consume substrate in different states and transfer their waste down to the next level, which in turn is food for other microbial populations until methane, carbon dioxide and water are produced. Each microbial population has their own independent growth rate and substrate utilisation rate which may be governed by a shortage or excess of substrate in the chain. As an example, overloading of

readily fermentable organic matter leads to an excess of volatile fatty acids (VFA) that shuts down the methanogens and reduces the pH of the growth medium. The low yield of biomass per substrate quantity is demonstrated by the Gibbs energy dissipation per unit of biomass produced, a theoretical method explained in Grady et al. (1999). This method is used to determine the free energy release by substrate oxidation. This method is popular in determining the growth yields of aerobic bacteria using different substrates in the environmental engineering field of wastewater treatment (Droste, 1998).

The biogas yield can be calculated theoretically, based on the chemical composition of the substrate and knowing that it will be degraded to the lowest oxidized form; carbon dioxide (CO₂) and in the most reduced form; methane (CH₄). The ratio of methane to carbon dioxide results from the electron exchange due to the oxidation state of the carbon contained in the waste. The theoretical methane potential yield equation, referred to as Buswell's equation, has been developed in the nineteen thirties:



The specific methane yield ($B_{o,th}$) can be expressed in terms of mass of Chemical Oxygen Demand (COD) or Volatile Solids (VS), as described in

$$B_{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)22.4}{12n + a + 16b} \quad \text{and}$$

$$B_{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)22.4}{\left(n + \frac{a}{4} - \frac{b}{2}\right)32}$$

based on the COD/VS relationship of the substrate in Standard Temperature and Pressure (STP) 1 CH₄ / g VS and STP 1 CH₄ / g COD (Angelidaki and Sanders, 2004).

$$B_{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)22.4}{12n + a + 16b}$$

$$B_{o,th} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)22.4}{\left(n + \frac{a}{4} - \frac{b}{2}\right)32} \quad (2.2)$$

Table 2.1 presents theoretical methane yields of basic organic substrates based on the complete oxidation-reduction to carbon dioxide and methane with water as the external source under anaerobic conditions.

Table 2.1 Theoretical Characteristics of Basic Organic Substrates, adapted from Angelidaki and Sanders (2004)

Substrate	Chemical composition	COD/VS gCOD/gVS	Methane yield l CH ₄ /gVS	Methane yield l CH ₄ /gCOD	CH ₄ content (%)
Carbohydrate	(C ₆ H ₁₀ O ₅) _n	1.19	0.415	0.35	50
Protein	C ₅ H ₇ NO ₂	1.42	0.496	0.35	50
Lipids	C ₅₇ H ₁₀₄ O ₆	2.90	1.014	0.35	70
Ethanol	C ₂ H ₆ O	2.09	0.730	0.35	75
Acetate	C ₂ H ₄ O ₂	1.07	0.373	0.35	50
Propionate	C ₃ H ₆ O ₂	1.51	0.530	0.35	58

Hydrogen in the anaerobic community is a very important electron sink and dictates how much acetic acid can be produced. The partial pressure of hydrogen has to be kept low at all times to assure that acetic acid can be produced; otherwise, fermentation will produce lower energy yielding volatile acids. Moreover, oxidising hydrogen methanogens cannot perform under high partial pressures of hydrogen. It has been the goal of many researchers to model, predict and acquire knowledge of the hydrogen oxidizing methanogens because of their important role in consuming hydrogen to assure steady acetic acid production (Pind et al., 2003). It has been generally agreed upon that about 30% of the methane formed by the methanogens comes from the oxidation of hydrogen and 70% from the decarboxylation of acetic acids for primary sludge in wastewater treatment (Grady et al., 1999).

2.1.2 Parameters Affecting the Anaerobic Processes

Temperature dictates the speed of the anaerobic processes. There are three classes of bacterial activity, based on their optimal biogas rate of production. The psychrophilic

bacteria operates at the lowest of temperature, less than 20 °C; mesophilic in the range of 20 °C to 45 °C with an optimal rate around 35 °C and lastly thermophilic from 45 °C and higher, with optimal biogas production at 55 °C (Membrez, 1993). Below 10 °C, biogas production is hindered. The thermophilic bacteria class has certain advantages over the mesophilic class (Membrez, 1993):

- higher destruction of pathogenic bacteria
- higher degradation rate of many substrates
- higher solubility and transport mechanism
- higher growth rate of the micro-organism
- lower volume requirement to process same amount of substrate

However, these advantages might be offset by:

- larger risk of instability
- higher demand of energy consumption to maintain process
- larger risk of ammonia inhibition and other components

2.1.3 pH and Alkalinity

For the successful operation of anaerobic processes, the pH must be situated in the neutral range, between 6.0 and 8.5. However, methanogenic bacteria efficiently operate at a pH range of 6.5 to 8.5, while the acidogenesis step is optimum within the pH range of 5.0 to 6.5 (Angelidaki et al., 2002). The sudden drop of pH in the reactor commonly occurs or is caused by a sudden increase in volatile fatty acids and depletion or low buffering capacity of the medium. The buffering capacity or alkalinity is commonly expressed in values of mg/L of bicarbonate ions. To sufficiently buffer any drastic changes in volatile acid production, 2500 to 5000 mg/l of bicarbonate should be present in the reactor contents (Angelidaki et al., 2002). It is preferred to monitor alkalinity or VFA frequently for a full-scale operation to properly access the VFAs in the reactor. Since it may take a high amount of VFA to deplete the alkalinity, the pH will remain stable even at high concentration of VFAs. Operators only monitoring pH may suddenly notice a pH drop, but it might be too late to fix the problem because the level of VFAs in the reactor has already exceeded operational limits (Angelidaki et al., 2002). A pH drop can be only noticed when the level of bicarbonate is approximately

lower than 1500 mg/l (Membrez, 1993). Angelidaki et al. (2005) recommended that the VFA concentration in the operation of anaerobic digesters should remain below 1.5 g/l of VFA to maintain a stable operation. Other compounds present in the digestion medium can neutralise the excess of hydrogen ions and add to the value of the buffering capacity, depending on their acid or base dissociation constant pKa/pKb, adapted from van Lier (2004):

- acetate
- phosphate
- ammonium
- sulphide

On the other hand, their excess concentration might pose an operational problem. Angelidaki et al. (2005) have found that most successful full-scale operating digesters had a concentration of ammonium lower than 4 g/l. When process temperatures changed or the digester was fed more substrate than it could handle, these values increased quickly, indicating poor volumetric biogas performance and high risk of process instability. The inhibition by VFA is caused by its unionized form. For example, at pH 7.0, process inhibition of 50% of the methanogens is caused by a concentration of 16 mg COD/l of unionized acetic acid, or 2871 mg COD/l of acetate (van Lier, 2004). Pind et al. (2003) have shown that the monitoring of major VFAs should be measured when reactors are fed different rates of organic loading or have been submitted to temperature drops. It was recommended that not only propionate should be measured for signs of process imbalance but also for increased levels of iso-butyrate, *n*-butyrate, *n*-valerate and iso-valerate. Since propionate degradation was the slowest and most sensitive process, propionate levels must be always kept at low levels to prevent inhibition to acetate degradation.

2.1.4 Essential Nutrients

Membrez (1993) recommended a list of ten basic compounds essential for the successful anaerobic digestion of substrates. These elements must be present at a minimum concentration of around 10^{-4} M in the medium:

- carbon, oxygen, nitrogen, hydrogen (basic compounds of organic matter)

- sulphur for synthesis of amino acids and other cellular building blocks
- phosphorous, potassium, calcium, magnesium, iron for building co-enzymes

Two other elements must be present in quantities less than 10^{-4} M in the medium: cobalt and nickel. Both are important for growth of methanogens. It has been generally accepted that human and animal wastes contain sufficient amounts of the macro and micro elements and supplementation is not necessary (Angelidaki et al., 2002).

2.1.5 Reactor Sizing Parameters

Most Continuous Stirred-Tank Reactor (CSTR) performance and operation are measured using the organic load that enters and exits the reactor. For high solids waste, the mass of total solids, TS, is a measurement that indicates the amounts of solids entering the reactor. For a more precise measurement of the mass of organic matter entering the digester, the mass of volatile solids, VS, represents the available organic matter that could be degraded anaerobically. The volumetric organic loading rate (OLR) is expressed in terms of the mass of volatile solids added per volume of reactor per day (gVS/l/d). It implies that at a certain OLR, there is a flow of organic matter passing through a known and fixed volume of reactor. This leads to the term Hydraulic Retention Time (HRT) or the time the waste stays in the reactor. The degradation and carbon content of the organic matter entering the digester can be expressed in terms of biogas or methane produced per mass of organic matter added or removed in the reactor (l/gVS or $l/gVS_{removed}$), by looking at the mass of organic matter entering and exiting the digester during stable operation while monitoring the biogas production during this time period. Then for a known amount of active anaerobic biomass, the amount of food added to the biomass can be expressed in terms of food to mass ratio (F/M) or in terms of substrate added to active inoculum (S/I) for batch tests (Membrez, 1993; Burke, 2001 and Kaparaju et al., 2002). Another way of measuring reactor performance is the volumetric biogas production. It is reported in volume of biogas produced per volume of reactor for a fixed period of time. The volumetric biogas production is measured in $m^3/m^3/d$ in this thesis.

2.2 Present Status of Anaerobic Digestion on Farms

2.2.1 Ontario

Increasing energy costs and increasing concerns about greenhouse gas emissions, odour and pathogens in manure has led to increased interest in Anaerobic Digestion (AD) for the agricultural industry in Canada. Over the past few years, the adoption of AD in Ontario has been very slow, primarily hindered by high capital costs, past system failures and no feed-in tariff for small energy producers. Improved digester designs with lower capital and operating costs, and the successful implementation of manure digesters in Europe for renewable electricity production has justified a new look into digesters for the Ontario agricultural industry. The interest of building anaerobic digesters on agricultural operations in Ontario has increased since the establishment of the Renewable Energy Standard Offer Program (RESOP) by the Ontario Power Authority and Ontario Energy Board in November 2006. The RESOP has many objectives, one of which is giving an increased tariff in electricity produced from all renewable sources. For non-photovoltaic systems, the program committed a 11¢/kWh (basic) to 14.52¢/kWh (peak power) electrical feed-in tariff with an annual increase at a rate of 20% of the consumer price index per year (Hilborn et al., 2007). In spring 2007, 350 MW of contracts has been issued mainly to wind capacity. Other types of electrical generation include solar, hydro and biomass but few projects have been signed compared to wind power. At the end of 2008, wind power generation produced 59.7% of the RESOP with 12 contracts compared to 121 solar PV projects generating 1.7% of 97046 KW of installed renewable energy production (Ontario Power Authority, 2008). Since this increase in electrical feed-in tariff, the on-farm AD system has attracted even more attention for agricultural producers interested in generating an additional source of revenue along with reduced odours from manure storages and reduced of pathogens in the digestate. In 2007, there were three anaerobic digesters in operation on dairy farms in Ontario. One is located in Cobden, ON at Fepro Farm, the second in St-Eugene, ON at Terryland Farm and the third one at Pinehedge Farm also in St-Eugene, ON. All three farms are dairy operations, while the first two farms have an operating size of between 150 - 250 animal units (AU) and the Pinehedge Farm is milking around 50 cows. The Pinehedge Farm uses the waste heat from

the Combined Heat and Power (CHP) genset to reduce the heat load and electricity necessary for his organic yogurt and kefir plant located on the farm. The Fevro and Terryland farms accept organic waste to improve the biogas production and electrical output of the generator. Fevro Farm has been receiving restaurant grease trap solid residue, fats, oils and greases (FOG), from Organic Resource Management Inc. since July 2007. Under their Certificate of Approval from the Ontario Ministry of the Environment, the organic waste received on farm is pasteurized at 70 °C for one hour before entering the anaerobic digester. Based on the anaerobic digestion of manure alone, the electrical output was 699 ± 80 kWh/d compared to 1213 ± 329 kWh/d using a mixture of 12% by volume of grease trap solid residue and 88% manure (Carruthers, 2008). At Terryland Farm, they have been using various co-substrates without a Certificate of Approval since the start of the operation (no data on manure alone available) but have settled on FOG from a bacon processing plant and grease trap waste. In the summer of 2008, they produced 3684 ± 800 kWh/d of electricity. Throughout Canada in 2007, there were about ten agricultural biogas plants in operation (Goodfellow, 2006 and Goodfellow, 2007).

2.2.2 USA

In the United States, the interest of anaerobic digesters on farms was supported by a national program from the U.S Environmental Protection Agency named AgSTAR. It is a voluntary program jointly sponsored by the USEPA, US Department of Agriculture and the US Department of Energy that encourages the use new technologies like biogas at confined animal feeding operations that manage manures as liquids or slurries. The original aim of the AgSTAR program was not to produce electricity, but rather the environmental benefits (Lazarus, 2008). For the case of the Matlink Dairy farm, odour generation from the barns and manure storage was an issue. The predominant winds carried the odours from the top of the hill where the barn was located down to the village of Clymer, NY in the Chautauqua county. Also, manure was spray irrigated in spring, summer and fall. The local businesses and school had to close their windows and the odour generated from the farm created a wave of issues. The AD system installed in 2001 fixed these issues. Various types of food waste were added to the system with the liquid dairy manure. Biogas was collected and used in a Waukesha

engine generator set and fed back to the grid (Wright et al., 2004). More recently in 2005, the focus on anaerobic digesters for medium size dairy farms was considered in the state of Minnesota by the Non-Government Organization (NGO) Minnesota Project because 95% of the dairy farms in that state are less than 200 head (Goodrich and Bilek, 2005). In early 2009, there were a total of 136 agricultural biogas plants, of which 108 were on dairy farms compared to 88 in November 2007. In terms of electricity production from biogas, 102 facilities were generating 170,000 MWh or approximately representing 19.4 MW_{el} (calculated). In May 2009, there were only 15 operational dairy biogas plants out of 108 that have a herd size of 400 head or less. In total, these dairy biogas plants have only 772 kW_{el} generation capacity installed (USEPA, 2009). On bigger farms, substantially more energy is produced.

2.2.3 EU

The situation in Germany has been much different than in North America. The use of anaerobic digestion on farms was stimulated by the Renewable and Treatment of Wastes Act of 1994 and the Biowaste Ordinance of 1998. Agricultural and municipal biogas plants were then chosen as one of the suitable ways of dealing with organic wastes at a national level, co-digested with liquid manure and sewage sludge. Until year 2000, the number of agricultural biogas plants increased to 850 with reactor volumes between 100 and 4,000 m³ (Weiland, 2000). It was not until the Renewable Energy Sources Act in April 2000 that the number of agricultural biogas plants jumped from 850 to close to 1750 in less than 4 years. Most of these biogas plants were designed to co-digest high energy yield crops like corn and other types of silage. A tour of digesters in Germany organized by the Ontario Large Herd Operator shows a wide picture of the status of AD in 2006. Sixteen on-farm, and cooperative and community digesters were surveyed during this one week trip. Each farm was studied in terms of technology, feedstock digested and energy generated. Systems were operating successfully on farms milking from 100 cows to farms milking 4000 cows. Ten were farm based plants, four were cooperative or community plants and the last two were on research farms (Debruyn et al., 2007). One noteworthy system in Germany was the Bioenergie Ahden Biogas Plant. The feedstock was composed of hog manure from a 2000

head finishing barn as well as the addition of food waste. The operation produced electricity from a 750 kW Jenbacher generator. Additionally, the heat recovered from the generator set was pumped 1 km away through hot water pipes to the local airport and hotel at Paderborn, 1 km away. Sales of hot water provided an additional €0.02/kWh on top of the electricity pricing at that time. The savings on the hot water was 30% less than using natural gas for heating. On a large dairy farm, the Agrarenergie Kaarßen Biogas Plant was expanding from a capacity of 1700 cows to 4000 cows. The biogas plant was receiving all the manure from the dairy but operated from a different ownership. Corn silage was also added to the feedstock mix. The drive for the contract of this biogas plant was to minimize the volume of the manure to be spread due to the confinement and scale of the dairy operation. Digestate from the secondary reactor was run through a centrifuge to remove any solids and the liquid fraction was run through three low pressure evaporators and condensers and heated with the heat from the CHP. The evaporated/condensed water was sent to the local water treatment plant and the concentrated liquid fraction was stored and sold as concentrated liquid fertilizer. Two 1400 kW Jenbacher gas engines were producing electricity from the biogas for a total of 2.8 MW. These systems could not have been possible without the feed-in tariff for the renewable electricity. The feed-in tariff was based on the size of the electrical output of the biogas plants. For farms of less than 500 kW_{el} power installed, the price was set to 10.23 ¢ €/kWh. Due to a revised base price of 11.5 ¢ €/kWh and 9.9 ¢ €/kWh for 150 kW_{el} and 500 kW_{el} power installed in 2004, the number of biogas plants increased to 3500 by the end of 2006 producing a total of 1100 MW_{el}. There is also a bonus tariff based on the technology used to produce energy so that a typical agricultural biogas plant using energy crops and the heat produced from the combined heat and power (CHP) engine would receive 21.5 ¢ €/kWh. For that reason, the surface dedicated to the cultivation of energy crops in 2006 was 203,719 ha compared to 13,621 ha in 2004 (German Biogas Association, 2008). Wellinger (2005) reported that there is not only a strong interest in Germany, but also in many EU countries to promote the construction of biogas plants to produce renewable energy. More biogas plants are present in these countries compared to Canada and North America because there are higher feed-in tariffs for electrical power, more opportunities for district heating, increasing use of compressed/upgraded biogas in CNG vehicles and legislation that targets reducing the landfilling of organic materials.

2.3 Reactor Design of Dairy Biogas Plants

2.3.1 USA

Conventionally, anaerobic digesters in the USA are built on Confined Animal Feeding Operation (CAFO) where the performance of the digester is based solely on the manure as a substrate. Many systems have not taken into consideration off-farm materials as co-substrates to increase the biogas production and revenue from the operation. Co-digestion of organic waste is a growing trend in the USA because of the potential for profitability of the anaerobic digester. On systems already designed to digest manure, only low solids content waste (<15%) may be co-digested, due to design constraints. Amongst these systems, the covered lagoons (18%), horizontal plug-flow (48%) and stirred tank reactors (22%) are the most common. Thirteen anaerobic digesters out of 88 built on farms had less than 400 head of cows (medium size dairies) were 7 plug flow, 3 covered lagoons and 3 were stirred tank reactors in operation (USEPA, 2009). There is very little data on which systems are co-digesting food waste for medium size dairy farmers.

2.3.2 Ontario

On-farm anaerobic digesters in Ontario are mostly located on medium size dairy farms. Most systems use concrete CSTR with a low height to diameter ratio at mesophilic temperature. They are both mixed intermittently with impellers driven by motors mounted on the side walls of the reactor. The biogas is collected and stored on top of the digestate by a flexible dome-shaped High-Density Polyethylene (HDPE) membrane. The anaerobic digesters in Cobden, ON and one in St-Eugène, ON are accepting various types of food waste and are equipped with a pasteurization system. Other systems in Ontario reported by Duke (2009) are currently in development phase or waiting approval by the local electrical distribution company to move forward. It is expected that similar digester designs will be used in these systems, as they are able to process various low solid contents food waste (up to 15% total solids) (Goodfellow, 2007). According to the Ontario Biogas Systems Financial Assistance Program (OBSFAP) from the Ontario Ministry of Agriculture, Food and Rural Affairs

(OMAFRA), 16 digesters have been pre-approved to receive financial assistance of 40% of the cost of construction up to \$400000. The OBSFAP also provided assistance up to 70% of the cost of doing 46 feasibility and design studies for the construction of anaerobic digesters for farmers and agro-food industries in Ontario (Duke, 2009).

2.3.3 EU

The EU countries are the most advanced in terms of anaerobic digester design and implementation. There are two types of systems available to match the farmer's needs. The most common is the vertical single stage CSTR digester at a total solids content of 10-12%TS at a larger HRT. They are usually built in plastic coated concrete up to a certain volume (~1500 m³) and larger systems in glass coated stainless steel tanks. Digestate storage tank is usually covered to capture left over methane potential and to minimize ammonia losses (Braun, 2007). Horizontal cylindrical digesters, usually made of stainless steel with a central paddle-impeller, are used for higher solid content feedstocks (15-35 %TS). Since 2004, in Germany, the new price incentive has driven farmers to use energy crops instead of waste co-substrates due to the regulatory complications and pre-treatments associated for off-farm waste. Their availability also pose a problem due to competition with composting technologies and other organic waste treatment systems (House et al., 2007).

2.4 Anaerobic Co-Digestion

2.4.1 Co-Digestion Basics

The anaerobic digestion process is well known for the single treatment of animal manures, sewage sludge and industrial waste prior to wastewater treatment. It was not until recently that mixing a small amount of co-substrates with manure or sewage sludge in the reactor has become common practice. This feature of anaerobic digestion is called co-digestion, where co-substrates in limited amounts are mixed in. The first co-digestion experiments were started in the nineteen thirties by (Buswell and Lebosquet, 1936). The industry today is well aware of co-digestion and it has become a standard design implication in the EU for agricultural biogas plants and in sewage sludge digesters (Braun and Wellinger, 2003 and

Edelmann et al., 2000). In 2003, only 7% of agricultural biogas plants in Germany were operating with manure as the sole substrate. From 1999 to 2003, more than half of the agricultural biogas plants in Germany were designed for energy crops for co-digestion (Weiland, 2003). In the USA, co-digestion was not standardized in the design of anaerobic digesters for farms and sewage sludge digesters. Some of these already built systems in the USA are now being tested or retrofitted to use co-substrates that have similar properties to dairy and swine manure (Scott and Ma, 2004). The industry is now seeing the advantages of co-digestion and the influence from the EU is now becoming normal practice in North America. Co-digestion is essential to agricultural biogas plants because the biogas production on manure alone does not produce enough income to cover the capital and operating costs of the installation. The addition of co-substrates to the manure attracts the industry mainly because it increased the biogas yield but also because the producer receives tipping fees to process wastes that would be otherwise landfilled, composted or incinerated (Braun and Wellinger, 2003). Co-digestion is also well applied in municipal sewage digesters in wastewater treatment plants since these systems are more often oversized to accommodate future population and organic load. Co-substrate addition can be implemented at reasonably low cost and produce enough biogas to produce electricity and heat for the wastewater treatment plant and reduce treatment costs (Edelmann et al., 2000).

2.4.2 Favorable Characteristics of Co-Substrates

Before using co-substrates, they must be evaluated under a wide range of chemical parameters. The most important chemical parameter is the organic fraction of the co-substrate defined in the amounts of TS, VS, and the ratio of volatile solids to total solids (VS/TS). From these volatile solids, some experimentation must be conducted on the fraction of degradable solids, the speed at which they degrade and if present, the inhibition on the methanogenic process. Other important chemical parameters to evaluate are the ammonia, pH, alkalinity and VFA. Physical parameters of substrate like homogeneity and viscosity behaviour must be considered in the design of the digester to operate properly (Braun, 2002 and Angelidaki et al., 2002). These characteristics and physico-chemical properties of feedstock have been elaborated previously in Chapter 2, section 2.1.2 and 2.1.3.

2.4.3 Biogas Yield of Co-Substrates

The biogas yield of co-substrates is generally expressed in two ways: in terms of volume of biogas per unit of material digested (typically m³ of methane per wet tonne when received on-site) or more suitably in terms of volume of biogas per mass of organic matter (l/gVS) since the co-substrates frequently vary in moisture content. The amount of biogas that can be produced from dairy manure is generally situated between 25 and 36 m³ of biogas per tonne. However, the volume of biogas produced from co-substrate added to the digester is much higher than liquid manure. Organic rich co-substrates that are easily degradable are favoured for their high biogas yield. The biogas yield of co-substrates range from 39 to 800 m³ of biogas per tonne with fat and spent oil wastes having 20 times the biogas yield of manure and higher methane content in the biogas (60 - 70% methane). The major classes of organic waste that are being used in municipal sewage and agricultural biogas plants throughout the EU countries, adapted from Braun et al. (2003) include:

- Food waste residues from restaurants and similar facilities
- Fats, Oils and Greases from restaurants and similar facilities
- Domestic septage
- Source-separated residential organic waste
- Leaf, yard and wood waste
- Pharmaceutical waste
- Biochemical industry waste
- Slaughterhouse waste
- Pulp and paper industry waste
- Cosmetic waste
- Textile organic waste

The use of organic rich wastes is favoured for their initial tipping fee that the farmer receives for accepting them and covers a wide range of food waste generated from food processing plants, food service and markets, greenhouses plant residues, certain slaughterhouse wastes, plant and animal oils wastes and other organic industrial wastes (Baserga, 1998). Figure 2.2

compares the biogas production per tonne of waste compared to the yield of liquid dairy and liquid swine manure.

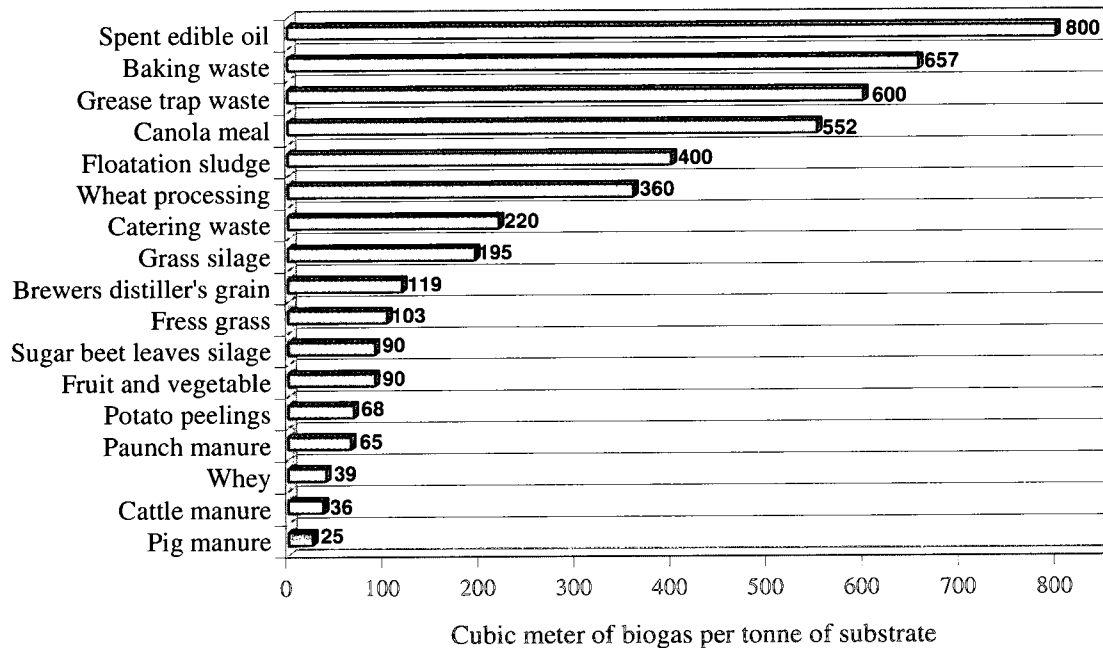


Figure 2.2 Biogas Potential of Available Organic Wastes Compared to Liquid Manure, adapted from Effenberger (2006)

However, these values vary significantly based on the source, location and type of operation. Humidity and organic matter content significantly changes the biogas yield per tonne of fresh mass. To have the correct representation of these biogas yields, Table 2.2 shows what have been studied in the literature for on-farm agricultural biogas plants. For easy consultation, yields of co-substrates have been divided into four classes related to their origin: agricultural, agro-food and biofuels industries, municipal and slaughterhouse. This database has been adapted from three main references on the subject of co-digestion of proteinaceous industrial waste (Braun et al., 2003), waste accepted on agricultural anaerobic digesters in Switzerland (Baserga, 2000 and Amon et al., 2006) and from an older study effectuated for farmers in Germany (Kuhn, 1995).

Table 2.2 Biogas Yields of Various Co-Substrates from the Literature

Class of Material: Agricultural				
Substrate	Biogas Yield (l/gVS)	TS%	VS/TS%	Ref. No.*
Beet leaves	0.40-0.50	15-18	78-80	2
Cattle manure	0.40	6-11	68-85	2
Cattle manure (fresh)	0.25	21.5	82.5	3
Cereals straw	0.25-0.35	85-90	85-89	2
Cereals straw	0.40	15	76	3
Clover	0.50-0.65	20	80	2
Clover	0.45	20	80	3
Corn stover	0.50	86	72	2
Corn stover	0.65	86	72	3
Grass silage	0.50-0.60	26-82	67-98	2
Hay	0.50	86-93	83-93	2
Horse manure	0.30-0.40	28	75	2
Horse manure (fresh)	0.35	28	75	3
Leaves	0.40	85	82	2
Pig manure	0.45	2.5-9.7	60-85	2
Potato leaves	0.55	25	79	3
Poultry manure	0.47	10-29	75-77	2
Poultry manure	0.30	15	77	3
Sheep manure	0.40-0.50	25-30	80	2
Sheep manure (fresh)	0.45	27	80	3
Whole corn	0.65	NR	NR	1
Class of Material: Slaughterhouse				
Substrate	Biogas Yield	TS%	VS/TS%	Ref. No.*
Animal fat	1.00	NR	NR	1
Blood	0.65	NR	NR	1
Blood meal	0.60	90	80	2
Blood plasma	1.36	NR	NR	1
Floatation sludge	1.20	5-24	85-98	2
Grease trap residue	1.30	2-70	70-99	2
Poultry floatation sludge	0.69	NR	NR	1
Rumen contents	0.35	NR	NR	1
Rumen contents	0.30-0.40	11-19	80-90	2
Stomach and gut contents	0.68	NR	NR	1
Stomach and gut contents	0.45-0.55	14	82	2

Table 2.2 (Continued) Biogas Yields of Various Co-Substrates from the Literature

Class of Material: Agri-Food and Biofuels Industries				
Substrate	Biogas Yield	TS%	VS/TS%	Ref. No.*
Apple distillery slops	0.45-0.50	2-3.7	94-95	2
Apple mash	0.35-0.55	25	86	2
Apple slops	0.33	2.5	95	3
Beer distillery slops	0.50	15-21	66-95	2
Canola oil seed residue	0.50-0.60	88	93	2
Corn distillery slops	0.40	21	NR	1
Fermentation slops	0.85	35	NR	1
Fruit mash	0.45-0.50	45-50	30-93	2
Fruit slops	0.40	45	93	3
Glycerine	1.30	NR	NR	4
Medicinal plant waste	0.40	53	55	2
Molasses	0.45	80	95	2
Molasses distillery slops	0.42	14	NR	1
Molasses distillery slops	0.40-0.55	10.5	71	2
Oil seed residue	0.60	92	97	2
Potato chips waste	0.69	45	NR	1
Potato distillery slops	0.47	10	NR	1
Potato distillery slops	0.43-0.50	12-15	90	2
Potato peelings waste	0.90	40	NR	1
Vegetable waste	0.40-0.60	5-20	76-90	2
Wheat distillery slops	0.40-0.50	3-5	96-98	2
Whey	0.80-0.90	4.3-6.5	80-92	2
Class of Material: Municipal				
Substrate	Biogas Yield	TS%	VS/TS%	Ref. No.*
Food leftovers	0.47-1.10	NR	NR	1
Food leftovers from fast food	0.69	NR	NR	1
Food waste from hotels	0.55	13.5	92.5	3
Grass cuttings	0.50	42	90	3
Market waste	0.90	NR	NR	1
Materials from grease separator	0.70	52.5	96	3
Materials from scum residue	0.70	14.5	90.5	3
Restaurant food waste	0.60	9-37	75-98	2
Secondary Sludge	0.20-0.35	NR	NR	1
SSOFMSW	0.40	NR	NR	1
Waste edible oil	1.10	NR	NR	1
(1) Braun et al. (2003); (2) Baserga (2000); (3) Kuhn (1995); (4) Amon et al. (2006); (NR) not reported				

2.4.4 Use of Crops Destined for Animal Consumption

The limited availability of organic waste is not considered sufficient to increase biogas production for a high number of on-farm anaerobic digester. That situation has already been met in Germany where farmers switched to co-digestion of organic waste to energy crop digesters. Yields of energy crops are shown in Figure 2.3 with forage beet as a root crop (light orange) with the highest methane yield of 0.456 l/gVS, corn maize as grain crops (light green) with 0.410 l/gVS and ryegrass or alfalfa as green forage plants (light blue) with 0.410 l/gVS (Weiland, 2003). More importantly, the yield of methane per hectare of cultivated land maximizes the agricultural land use and minimizes the inputs. In Austria, corn silage and pressed beet silage are the top yielding crops generating a methane potential yield of 5897 and 6173 m³/ha/y (Amon et al., 2007). Similarly in Germany, corn silage and forage beet were the top methane yielding crop capable of producing 5780 and 5800 m³/ha/y respectively (Weiland, 2003).

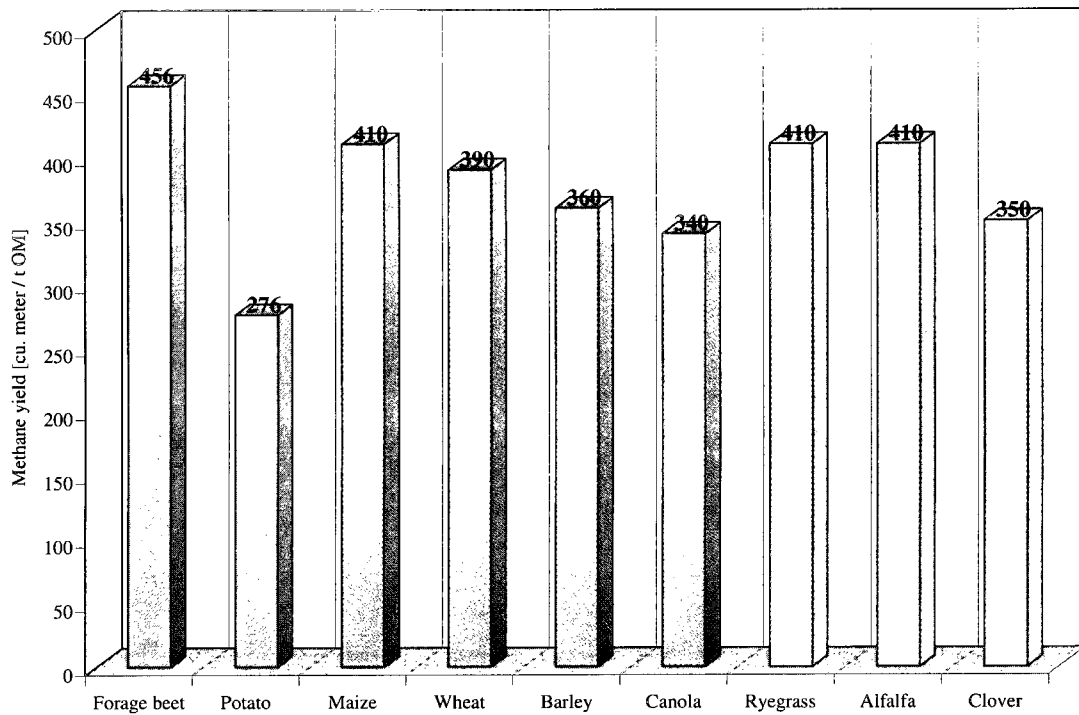


Figure 2.3 Methane Yield of Energy Crops in Germany, adapted from Weiland (2003)

2.4.5 Advantages and Limits of Co-Digestion

There are not only advantages to the addition of co-substrates to anaerobic digesters. Certain types of wastes require high energy demanding pre-treatments and can pose legislative issues. Braun (2002) presented a list of "Merits and Limits" on the advantages and inconveniences of co-digestion for anaerobic digestion:

Merits

- Improved nutrient balance between carbon and nitrogen leads to a more stable process and increase the fertilizer value of the digestate. Typical requirements of nutrients: TOC:N:P = 300:5:1
- Allows the use of organic substrates that otherwise could be problematic to handle in the pure form (particulate matter, aggregate wastes, inhibiting components), as many organic rich feedstocks are easier to handle as co-substrates when mixed with a homogeneous main substrate. Seasonal substrates are also easier to handle in co-digestion
- Compared to normal waste stream treatments, most of the co-substrate must be shipped and treated off-site at a certain price. These wastes could be accepted in biogas plants and revenues increased in the form of tipping fees and improve the return on investment
- The increase in biogas production from the co-substrates greatly improves the payback time of small scale, decentralized anaerobic digesters
- Future legislation or regulation may provide higher volume and quantity of organic waste as seen in the EU countries where different paths of waste handling may come in place; landfilling ban on organic waste, ban on food leftovers used as animal feeds and pilot projects of Source Separated Organic Fraction of Municipal Solid Waste (SSOFMSW)

Limits

- Some organic substrates may be composed of debris and inorganics that will accumulate in the digester and be problematic later on. Sands, metals, stones and precipitates could clog and burst buried pipes. Oily wastes are insoluble and could form a thick scum layer or produce foam in the digester

- Increased storage capacity is necessary when accepting very diluted wastes and may pose a problem in the initial economic assessment. Also, this excess digestate must be treated appropriately by land application; solids composted and liquid irrigated or sent to a wastewater treatment plant
- Risk materials (heavy metals, biological hazardous materials) could be present in organic substrates and must be dealt with accordingly. Thermal sterilization could be costly and require more monitoring of biogas plants
- Economic instability could be an issue when the source of organic rich substrate may no longer be available to a biogas plant. It could be financially difficult to find another source of high yielding co-substrate
- Increased or appropriate mixing of the digester content must be planned when using specific high solid content organic waste
- Mechanical pre-treatment may be required to remove foreign materials (debris and inorganic material) and reduce particle size of the organic waste
- Current legislation or regulation may designate a wide source of organic waste as risk material and anaerobic digestion may not have been proven as an effective means of treatment or costly pre-treatments or post-treatments may be required by local authorities
- Public opinion may cause temporary difficulty in the case of treatment of waste in rural areas or in agricultural biogas plants.
- Other treatment streams may compete with the availability and quantity of organic wastes because these systems may depend on its calorific value, i.e. incineration, gasification/pyrolysis. Whereas, organic wastes used in composting, as soil conditioners and as animal feeds are required primarily for their nutrient value and less so for the energetic value. In general, there are other treatment options that may compete successfully to reduce or negate tipping fees as sources of income for small decentralized biogas plants.

The use of co-substrates may seem problematic after the enumeration of limits and merits of co-digestion, but the increased risk in using them is offset by the many economical and environmental benefits that their use brings in for the biogas plant.

2.4.6 Status of Co-Digestion in North America

Typically in the USA, waste having similar characteristics to manure is preferred for anaerobic digestion since they are available in high amounts and good quality and necessitate low levels of treatment prior to digestion. For that reason, various agro-industrial food wastes (milk and juice processing) with high volatile solids to total solids ratio (VS/TS) have been mixed in low volumes on manure based agricultural digesters in the USA (Scott and Ma, 2004). Few systems have opted for the addition of co-substrates because the excess electricity production has not been valorized as much as the environmental benefits and few states subsidize the price of electricity produced from renewable sources (Lazarus, 2008).

2.4.7 Status of Co-Digestion in Ontario

The use of co-digestion in agricultural biogas plants in Ontario is being promoted by a joint initiative from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the Ontario Ministry of Environment (MOE). To eliminate most of the legislative and regulatory issues from the *Environmental Protection Act Part V* and the *Ontario Water Resource Act*, OMAFRA and MOE Environment agreed on terms amended to the *Nutrient Management Act* (NMA) that sets new ground rules in the operation of two on-farm treatment technologies: on-farm biogas plants to treat off-farm source materials and vegetative filter strips to treat various farm based runoffs. In the context of this research, only a few items of the new regulations have been covered:

- Volume limitations on the amounts of received (off-farm) materials that could be stored at once and processed yearly
- Storage and treatment of received materials
- Types of off-farm and on-farm co-substrates prescribed in Schedule 1, 2 and 3.

2.4.8 Anaerobic Digestion with *Nutrient Management Act* O.Reg. 267/03

The amended *Nutrient Management Act* (NMA) allows the mixing of off-farm source materials less than 25% volume of the digester content. For the reception of off-farm source

materials, there can only be less than 100 m³ stored on site and the operator can only receive up to a total of 5,000 m³ per year of off-farm source materials. If using only farm based substrates, there must be, at all times at least 50% manure by volume in the digester (DeBruyn and Hilborn, 2007). For the digester effluent to be considered equivalent to digested manure when operating under a Certificate of Approval of the MOE, there must be at least 50% of the inputs coming from agricultural sourced materials. Off-farm materials listed below in Schedule 2 need to be further treated at 50°C for 20 hours or at 70°C for 1 hour due to the presence of risk materials. Substrates that do not originate from the production and operation of the agricultural facility and that can be added to a "regulated mixed anaerobic digestion facility" must be included in Schedule 1 and Schedule 2 but not in Schedule 3 in the NMA O.Reg.267/03 :

- Waste products from animal feeds listed in Classes 1, 2, 3, 4 and 5 of Part 1 of Schedule IV to the Feeds Regulation, 1983 (SOR/83-593) made under the *Feeds Act* (Canada) excluding any materials that contain an animal product that has not been denatured (Schedule 1) or including any materials that contain an animal product that has not been denatured (Schedule 2). Materials that previously would have been a product described in this point but are no longer suitable for use in feeding farm animals for reasons that do not include contamination by another material.
- Organic waste matter derived from the drying, cleaning or processing of field crops
- Organic waste matter derived from the production of ethanol or biodiesel
- Aquatic plants
- Organic waste matter derived from food processing at bakeries, confectionary processing facilities, dairies and facilities that process dairy products, fruit and vegetable processing facilities, cereal and grain processing facilities, oil seed processing facilities, snack food manufacturing facilities, breweries and distilleries, wineries and beverage manufacturing facilities
- Fruit and vegetable waste
- Organic waste materials from a greenhouse, nursery, garden centre or flower shop that is not part of an agricultural operation
- Paunch manure (Schedule 2)

Off-farm materials that include the following cannot be processed under NMA on agricultural biogas plants (Schedule 3):

- Solvents, where the solvent is a volatile organic compound that is used as a cleaning agent, diluent, dissolver, thinner, or viscosity reducer or for a similar purpose.
- Petroleum products and hydrocarbon fuels.
- Resins and plastics.
- Waste from food that was presented to a person for consumption but was not consumed, including but not limited to, restaurant waste and airplane food waste.
- Hazardous waste within the meaning of Regulation 347 (General – Waste Management) made under the *Environmental Protection Act*.
- Any material that has a dry matter content of less than 1 per cent.

In 2007, two farms in Ontario were operating with a Certificate of Approval from the MOE to receive off-farm materials not listed in any of the Schedules; grease trap solid residue at the Fepro Farm in Cobden, ON and FOG from a meat processing plant at Terryland Farm in St Eugne, ON. These two farms have chosen not to operate under the NMA and therefore needed to apply for a C of A from the MOE as these co-substrates are managed under the Environmental Protection Act Part V (DeBruyn and Hilborn, 2007).

Similarly in the EU countries, agricultural biogas plants have to apply to their regulating bodies to accept off-farm materials. Agricultural biogas plants in EU countries seldom use co-substrates that contain less than 60% volatile solids because they are rarely considered worthwhile for anaerobic digestion (Braun, 2002). Due to the limited availability of organic rich wastes in Germany, farmers had to turn to energy crops as the main substrate to increase biogas production (Braun and Wellinger, 2003).

2.4.9 Co-Substrate Chosen for this Research

In this research project conducted at the University of Guelph – Campus d’Alfred, four substrates were chosen for evaluation in the co-digestion with liquid dairy manure. The following criteria were used to select the co-substrates:

- locally available;

- presently used or could be used on agricultural biogas plants in Ontario; and
- a selection between agricultural and off-farm materials

The co-substrates chosen for the laboratory evaluation are:

- 1) corn silage – grown in Eastern Ontario
- 2) canola meal – after oil extraction
- 3) glycerine – from biodiesel production using yellow grease
- 4) fats, oils and greases (FOG) – from meat processing waste
- 5) whey – from a cheese processing plant

2.5 Substrate Used in Laboratory Batch and Semi-Continuous Digesters

2.5.1 Liquid Dairy Manure

The Canadian dairy industry generates huge amounts of wastes, mainly composed of livestock manure which is handled on the farm. In Canada, it was estimated that a total of 14 million tonne of dairy manure in 2001 was available for treatment, where Southern Ontario, Eastern Ontario and Quebec along the St-Lawrence Seaway have highly concentrated areas of available dairy manure (Goodfellow, 2007) . It is estimated that more than 548 million cubic meters of biogas could be produced per year through the anaerobic digestion of this source of available manure. The typical manure management practice on dairy farms in Eastern Ontario is the conventional liquid holding lagoon. Goodfellow (2007) summarizes in Table 2.3 the status of the estimated mass of manure produced per day on dairy farms located in the region of Eastern Ontario, with the united counties of Stormount, Dundas and Glengarry, and Prescott and Russell having the highest values. The data is based on the livestock census of Statistics Canada from 2001 and the D384.1 Manure Production and Characteristics report from American Society of Agricultural and Biological Engineers (ASABE) with an average production of manure per cow of 7.68 kg of manure produced per day.

Table 2.3 Estimated Mass of Manure Produced Daily in Eastern Ontario from 2001 Census, adapted from Goodfellow (2007)

Region/County	kg of manure per day
Hastings County	61924
Prince Edward Division	34168
Northumberland County	52017
Peterborough County	42263
Kawartha Lakes Division	33907
Stormount, Dundas and Glengarry County	254285
Prescott and Russell County	202990
Ottawa Division	104724
Leeds and Grenville County	84624
Lanark County	29484
Frontenak County	33976
Lennox and Addington County	30205
Renfrew County	53591

Table 2.4 presents different manure characteristics based on different herd and waste management practices. The changes in characteristics of liquid dairy manure is caused by the different feed composition and ration for the cows, along with seasonal variations and herd management practices.

Table 2.4 Basic Dairy Manure Characteristics

Author	pH	COD (g/L)	TS (%)	VS (%)	N (%)
Amon et al. (2006)	6.66-6.95	-	12.8-15.9	-	-
Chen et al. (2008)	-	-	12.8-14.1	10.2-12.0	6.2
Ghaly (1996)	7.00	74.9	6.59	5.4	12.6
Lehtomaki et al. (2007)	-	-	6.5	5.3	4.2
Wright et al. (2004)	5.64-7.45	72.1-134.7	8.9-12.5	7.1-10.9	3.4-4.9
Kirk et al. (2004)	8.99	123.8	10.5	7.4	-

The diet of the cows also greatly affects the biogas yield of the anaerobic digestion of manure shown in Austria (Amon, 2006), New York State (Wright et al., 2004) and in Denmark (Hartmann et al., 2000). General figures show that biogas yield from cattle manure is between 350 to 400 l/gVS in Switzerland (Baserga, 2000), but batch laboratory experiments of six weeks indicate biogas yields to be 208 and 268 l/gVS for dairy cattle manure in

Austria fed different rations of minerals, hay, grass silage and corn silage. It was confirmed that the lignin and cellulose content of the manure greatly influenced the biogas yield which hovers from 140 to 266 l/gVS from previous literature reviewed (Amon et al., 2007).

To further increase the biogas yield of manure Hartmann et al. (2000) completed a detailed study of mechanical maceration for centralized anaerobic digesters (CAD) in Denmark at the lab and full scales. Since the initial interest for AD in Denmark was energy production, supply of high yielding biogas organic wastes was becoming scarce as the number of CAD plants increased. This situation pushed the industry to obtain the maximum yield out of the manure collected from farmers. It was found that pre-treating manure increased the biogas potential from 5% to 25% in CADs (higher than 20% in 3 out of the 7 plants monitored). The increase of biogas potential from manure was not significantly related to the shorter length of fibre due to the maceration but more to the composition and fraction size of the manure after maceration. Higher biogas yields of the fibre fraction did not significantly increase the biogas yield of manure but was more related to the shearing effect from other particulate material present in manure. Three out of the seven CAD monitored did not notice an increase in the biogas yield of manure when using mechanical maceration because of the low fibre and particulate content of the substrate fed. The biogas yield of these plants was more related to the non-particulate degradable material of the feed. It was deemed economical to use mechanical maceration only if the feed contained a high amount of manure and an expected 20% increase in biogas yield could be obtained. At the CAD scale of anaerobic digestion, a minimum increase of 4% in the biogas yield justifies the purchase and operation of mechanical maceration (Hartmann et al., 2000). Laboratory tests were further investigated in another set of batch tests and in two thermophilic CSTR fed with macerated and unmacerated manure (Angelidaki and Ahring, 2000). It was found that for cattle manure in batch tests, mechanical maceration increased the methane potential by 20% if particle size was reduced below 0.35mm, but a more representative maceration of 1-2 mm increased the biogas potential by 16% from the control of 230 l/kgVS. For the CSTR experiments, the reactor receiving macerated manure produced 17% more biogas than the control. It was later confirmed in the experiment that the lower VFA concentration in the reactor fed untreated manure was due to the lower hydrolysis of biofibers (Angelidaki and Ahring, 2000).

Liquid dairy manure is a favourable substrate because of its low TS content that is suitable to mix with other rich organic wastes, sufficient ammonia, alkalinity and trace elements to support methanogenic bacteria, medium pH and low initial VFAs concentration. For the region of Eastern Ontario, it is clearly demonstrated in Table 2.3 that the intensity of milking farms present in the area is enough to foster a strong anaerobic digestion market. Additionally, this area is located between 2 large urban centers, Montreal and Ottawa, which would produce sufficient organic wastes suitable for blending with liquid dairy manure.

2.5.2 Corn Silage

Corn silage is a high yielding crop and is used to feed livestock for meat and milk production. Its use in anaerobic digesters is not recent in Germany, where off-farm materials suitable as co-substrates are not as available as in North America. In Central Europe, corn is the most dominating crop for biogas production and is considered to have the highest yield potential of crops grown in the region (Amon et al., 2007). Average data from Central Europe shows that crop yields of corn, can be as high as 20 tonne of TS per ha. Since one tonne of corn on a TS basis, can produce 400 to 600 l CH₄/kgTS, one hectare of corn silage could generate 13,000 to 20,000 kWh of electricity in Central Europe (Braun and Wellinger, 2003).

Due to its widespread use in agriculture as livestock feed, the adoption of corn silage as an energy crop did not necessitate an intensive change in the farmer's current crop production. The expertise farmers have in selecting suitable seed, growing and maintaining the crop, choosing the optimal harvest time and storing the crop with minimal losses are already acquired. Its high carbon content and VS/TS ratio made corn silage a suitable co-substrate as shown in Table 2.5 (Weiland, 2003).

Table 2.5 Various Characteristics of Corn Silage Based on Harvest Period, adapted from Amon et al. (2007a)

Corn Variety (n=4)	Harvest Stage	%TS	%VS	Crude Protein (%TS)	C/N	Methane Yield (l/kgVS)
Tonale PRG13	Milk	18.0 - 19.4	17.2 - 18.4	7.8 - 10.1	24.2 - 43.5	313 - 366
	Wax	29.0 - 30.2	26.9 - 28.6	6.7 - 7.9	33.5 - 44.1	283 - 326
Tixxus LZM600	Full	43.1 - 52.9	41.4 - 50.7	5.9 - 7.2	42.2 - 52.1	268 - 313

Further research is needed in using this crop in anaerobic digesters to optimize biogas production and increase the economical viability of the installation (Amon et al., 2007a and Amon, 2006).

Previous research studies have looked at the methane yield and crop yield based on the different parts of the plants, and harvest time of different varieties of corn grown in Austria (Amon et al., 2007a and Amon, 2006). The experiments were conducted in 1 litre batch tests at 37 °C. The measurement of methane was based on the average measured value of 58.5% CH₄ / vol. at standard atmospheric conditions. It was shown that the digestion of the whole plant harvested in Austria (variety Benicia FAO#300) generated the highest specific methane yield (326 ± 6.6 l/kgVS) compared to the corn cob mix (316 ± 7.5 l/kgVS). From the corn kernels only, a specific methane yield of 309 ± 7.1 l/kgVS was observed compared to the lowest specific yield of 274 l/kgVS for the corn plant without the cobs and kernels (Amon et al., 2007a).

The Benicia variety showed a methane yield per hectare of 8788 ± 233 m³ CH₄/ha when harvested at wax ripeness compared to yields of 7226 m³ CH₄/ha for cultivars PR 34G13 to 9039 m³ CH₄/ha and cultivar LZM 600, respectively, harvested at full ripeness. Only 30% of the energy came from the digestion of the corn kernels compared to the digestion of whole maize crops. Amon et al. (2007a) then specified that whole corn crop should be harvested for energy crop production. As the specific methane yield dropped as the plant aged, the biomass harvested per ha increased. For milky ripeness, specific methane yields ranged from 312 to 365 l/kgVS and for full ripeness from 268 to 286 l/kgVS. For early to medium ripening varieties, harvest at 35-39% TS content was optimal at the end of wax ripeness. For late

ripeness varieties, harvest at full ripeness at a TS content of 44% was optimal. At milky ripeness the VS yield observed was between 17.2 Mg VS/ha and 20.2 Mg VS/ha. At the wax ripeness VS yields were between 21.9 to 26.7 Mg VS/ha compared to higher yields at full ripeness of 22.3 to 31.4 Mg VS/ha.

In a latter study from (Amon et al., 2007b), the biomass and specific methane yields of seven other corn crops grown in the same location in Austria showed similar results. The seven corn varieties were also harvested at different times: milky, wax and late ripening. The average specific methane yield of the seven crops was 398 ± 23 l/kgVS with a range of 359 to 422 l/kgVS. It was deemed that there were no significant differences in specific methane yields between varieties and the time of harvest. However, the biomass yield per hectare of medium ripening varieties was higher than late ripening varieties compared to the previous study (Amon et al., 2007a). For that reason, the variety Baxter FAO#380 had the highest methane yield per hectare ($12390 \text{ m}^3 \text{ CH}_4/\text{ha}$) compared to the conservative figure obtainable in Austria from corn silage of 7500 to $10000 \text{ m}^3 \text{ CH}_4/\text{ha}$.

Due to the increased anaerobic stabilisation of corn silage during storage, the methane yield is much higher than corn digested shortly after harvest. Amon et al. (2007a) reported an increase of 25% of specific methane yield but the loss of biomass during storage and the use of silage additives were not assessed in this paper.

Erguder et al. (2001) recommended that the corn silage must be pre-treated to reduce particle size for ease of mixing and pumping in the digester. Crust formation is inevitable as the ligno-cellulosic fraction of corn and other energy crops floats to the surface of the digester contents.

2.5.3 Canola Meal

Canola is the most produced oilseed crop grown in Canada with 12.6 million tonne produced in 2008 (Canola Council of Canada, 2008). It is a plant developed during the 1970's from the common rapeseed to have a content of less than 2% erucic acid and less than 30 μmoles

glucosinolates (Canola Council of Canada, 2008). The average oil content of canola exceeds 40%w/w. The total acreage of harvested canola in Canada has surpassed 16 million acres, where the province of Saskatchewan has notably increased its surface use by 30.5% since 2004 at 5.9 million acres to 7.7 million acres in 2008 (Canola Council of Canada, 2008). Annual crop exports of canola are the most valuable for Canada, with \$2.8 billion in average revenue from the seed, oil and meal exports. The canola industry contributes more than \$13.8 billion and supports 52000 farmers nationally (Canola Council of Canada, 2008).

The oilseed crushing, edible oil refining and food oil packaging industry of Canada greatly profits from the high amounts of canola seed produced in Canada. This industry is controlled by 4 major companies: ADM Agri-Industries, Bunge Canada, Cargill and Richardson Oilseed. They operate 14 large size plants that crushed a total of 5.4 million tonne of seed, with 3.8 million tonne destined to produce 1.6 million tonne of oil and 2.2 million tonne of canola meal in 2007 (Canadian Oilseed Processors Association, 2008).

Canola meal is the solid residue from the oilseed extraction process. Due to its high protein content, canola meal is the second most important traded source of animal feed proteins worldwide, soybeans being the first. An estimated 1.59 million tonne (71% of the total production) were exported, where 97% was shipped to the United States. Domestic use and export of canola meal accounted for \$680 million dollars in 2007 (Canadian Oilseed Processors Association, 2008). It is expected that the drive from the federal government through bill C-33 to blend biodiesel (B2) in transportation fuel by 2012 (Environment Canada, 2008) may increase canola oil production, generating more canola meal. Canola will mainly be used, as soybean oil production in Ontario is too low (most is imported from the United States) and that tallow and yellow grease cannot fulfil the commitment of biodiesel production (Riley, 2004). It was also verified if off-grade, frost damaged canola seeds, which represents up to 5% annual production and sold at 50% of the cost of the #1 grade seed, could be used for biodiesel production. If all off-grade canola seed should be used for biodiesel production, only 1% of the 25 billion litres of diesel fuel consumed in Canada could be replaced. Also, the off-grade canola is already processed in different blends by the oilseed processing industry and the price would increase because of the added demand on the off-

grade seeds (Riley, 2004). Based on this assessment 630 thousand tonne of off-grade canola seeds could be used as a co-substrate in the Prairies.

Canola meal characteristics can vary on the type of extraction, quality of the seed and location grown. For a small scale cold-press canola grown in Idaho, the dry matter content is 93%, with a crude protein percentage of 37.5% and 16.9% fat (Aarke et al., 2008). According to the Canola Council of Canada (2008), canola meal issued from large-scale solvent based oil extraction plant contains on average 90% total solids, 35% crude protein, 3.5% fat and 12% crude fibre. The canola meal from the large-scale process also contains more ash (6.1%) than the cold-press operation (5.1%), as there is 4 times less fat (Aarke et al., 2008).

The use of canola meal in this research was studied to fill this gap in the anaerobic digestion of oilseed residue. For example, its energetic potential could be compared with biodiesel production. It was also chosen since the interest in oilseed crops have resurrected in Northern Ontario. Although canola meal has a high biogas yield, it should not be cultivated as an energy crop similar to the situation in Germany with corn silage. The methane yield reported for whole rapeseed is 0.34 l/gVS but only 1190 m³/ha/yr compared to 5780 m³/ha/yr for corn silage (Weiland, 2003). Due to its high value associated as an animal feed, there is a limited amount of work being done on its use as a co-substrate for digestion. Baserga (2000) reported a biogas yield of 0.50-0.60 l/gVS for canola flour extraction residue, with an 88 %TS and 93 %VS/TS ratio and a C/N ratio of 8. Oilseed residue resulted in a biogas yield of 0.60 l/gVS with the oilseed residue containing 92%TS and 97 %VS/TS, and a much higher C/N ratio of 41.

It was found in batch experiments by Amon et al. (2006) that the co-digestion of 10 %w/w rapeseed meal with 6%w/w of glycerine with a basic mixture of corn silage (31%w/w), corn grains (15%w/w) and pig manure (54%w/w) obtained a specific biogas and methane yield of 0.701 l/gVS and 0.432 l CH₄/gVS compared to the digestion of the basic mixture alone that yielded 0.569 l/gVS of biogas or 0.335 l CH₄/gVS of methane. In a older report from Baserga (1998), a hog farmer in Switzerland co-digested cereals-mill processing residues in a 100 m³ horizontal digester. For the last part of this full-scale trial run, unreported amounts of wheat

mill residues and canola meal were co-digested with 8m³ of pig manure and the reactor produced 350 m³/d of biogas, which represented a volumetric biogas production of 3.5 m³/m³/d.

2.5.4 Glycerine

Glycerine, also known as glycerol, is a sugar based alcohol which is a by-product of saponification and trans-esterification reactions of fats, oils and greases. It is used by the pharmaceutical, chemical, cosmetic and food industry to produce nitro-glycerine, propylene glycol, de-icing fluids, sweeteners and emulsifiers, soaps and detergents to name the few. Glycerine is also the principal by-product of the Fatty Acid Methyl Ester reaction used for biodiesel production. For each US gallon of biodiesel produced, approximately 0.3 kg of crude glycerol remains, depending on the amount of catalyst used (Reaney et al., 2006). Reaney et al. (2006) also reported that the production of one litre of biodiesel results in 0.079 kg of glycerine. Crude glycerine contains large amounts of impurities and would require costly processing steps to purify the glycerine to commercial grades. For small or medium-size biodiesel plants, the purification of crude glycerol is too costly and therefore can only supply the market with low cost glycerine (Thompson and He, 2006). Due to the Canadian government's commitment to Bill C-33, diesel and heating fuels must contain 2% biodiesel by 2012 (Environment Canada, 2008). Most of this production is assumed to be from large-scale facilities that can afford treatment and purification of crude glycerine. It is expected that there will be an excess supply of glycerol, inevitably effecting the cost of manufacturing biodiesel (Reaney et al., 2006). Therefore, low-value crude glycerol could be introduced into other processes or end up at biogas systems within the concept of an integrated bio-refinery complex or transported to agricultural biogas systems that can accept off-farm materials (Goodfellow, 2007).

Due to the wide range of feedstock available to biodiesel production, crude glycerol compositions will also vary. Thompson and He (2006) verified the composition of crude glycerol produced from 6 types of seed oil (IdaGold, PacGold, rapeseed, canola, soybean, crambe) and waste vegetable oil (WVO) in a batch laboratory tests. The crude glycerol yield

of the oilseed ranged from 8.8 to 12.3 g per 100 g of input oil using a 6:1 molar ratio of methanol, but the WVO crude glycerol yield was 22g per 100g of input oil due to higher soap and impurity content. Table 2.6 shows the different characteristics of crude glycerol produced from seed oil and WVO. Crude glycerol from WVO also contained a much higher fat and carbon content due to residual glycerides, ester and biodiesel present in the layer, since the WVO free fatty acids (4.4%) were not properly neutralized before the reaction. Chen et al. (2008) reported a total solids concentration of 87.75% and high volatile solids content of 80.9 % for glycerine used in BMP experiments issued from a biodiesel plant in California.

Table 2.6 Nutrient and Elements Analysis in %w/w of Crude Glycerine, adapted from Thompson and He (2007)

Type	Carbon	Nitrogen	Sodium	Fat	Carbohydrates	Proteins	Ash
WVO Glycerine	76.3	0.25	1.4	60.1	26.9	0.23	5.50
Oilseed Glycerine	77.0-77.7	0.17-0.22	1.06-1.20	2.03-13.1	75.2-83.8	0.05-0.44	0.25-2.80

Glycerine's high carbon content makes it an ideal substrate for co-digestion with dairy manure and other organic materials to increase biogas yields. Glycerine is a high yielding biogas substrate generating a potential of 1.295 l/gVS or methane potential of 0.750 l/gVS reported by Amon et al. (2006). Batch tests were also conducted by Chen et al. (2008) and reported a biogas yield of 0.67 l/gVS with 80% of the biogas produced during the first 8 days of digestion. It is suitable for co-digestion because it is an easy co-substrate to handle on the farm and contains little debris from the manufacturing of biodiesel (Amon et al., 2006). Recent experiments dealing with the new supply of crude glycerine from the biodiesel industry have shown successful results in the increasing of biogas yields. Amon et al. (2006), which was also referred to in section 2.5.3 for the use of canola meal as a co-substrate, have successfully co-digested 6 %w/w glycerine with rapeseed meal and a basic mixture composed of corn silage, corn grains and pig manure. They obtained a specific biogas and methane yield of 0.701 l/gVS (0.432 l CH₄/gVS) with this mixture compared to the digestion of the basic mixture, without rapeseed meal, yielding 0.569 l/gVS (0.335 l CH₄/gVS). With 6%w/w glycerine co-digested with 94% pig manure, the biogas and methane yield was 1.114 l/gVS (0.617 l CH₄/gVS) compared to a biogas yield of 0.412 l/gVS (0.216 l CH₄/gVS) with

pig manure alone. Chen et al. (2008) also conducted a series of laboratory scale digestion experiments of glycerine and dairy manure. The mixture used was 45% glycerine and 55% manure based on VS with a C/N ratio of 15. The biogas yield ranged from 0.47 to 0.61 l/gVS, with OLR ranging from 1 to 6 gVS/l/d. The VS reductions ranged from 57.6 to 66.5 %VS, while the volumetric biogas production rate increased from 0.47 to 3.78 m³/m³/d at the end of the 160 day trial with a fixed HRT of 20 days. Holm-Nielsen et al. (2008) spiked three thermophilic 5 litre CSTRs with glycerine to monitor VFA accumulation and inhibition of the methanogens by means of near infrared spectroscopy. It was found that concentrations of glycerine exceeding 5g/l in the reactor lead to accumulation of VFA's up to 10g/l and ultimately reactor failure. Stable addition of glycerine in the reactor was found to be situated from 3 to 5 g/l. Co-digestion of glycerine was also tested in a different setup, this one using potato processing wastewater in a UASB reactor operating under mesophilic temperature, a retention time of 20 hr and a duration of 54 days (Ma et al., 2008). Three types of glycerine were tested, pure, crude and high conductivity (HC) glycerol. The COD concentrations of the glycerine types tested were 1200, 1120 and 925 gCOD/l, respectively. When pure glycerine was used, the reactor was operated at a OLR of 7.6 gCOD/l/d, compared to the control reactor digesting potato processing wastewater alone and having a loading rate of 4.9 gCOD/l/d. The addition of 79% (as COD) of pure glycerine (2 ml/l of feed) yielded the highest methane potential of 0.48 l CH₄/l of pure glycerine. When testing crude and HC glycerine at 23% and 13% (as COD) of the feed, respectively, the methane yields observed were 0.44 l CH₄/l crude glycerine and 0.30 l CH₄/l of HC glycerine. Due to the varying COD of the potato processing wastewater supplied, the reactor only reached low COD removal efficiencies of 73 to 85%. Loading rates varied from 2 to 14.1 gCOD/l/d and the HRT had to be reduced in the last phase when testing HC glycerine to prevent overloading down to 40h.

2.5.5 Fats Oils and Greases

Fats, oils and greases are produced by the food processing and preparation sectors. FOG may be discharged to sanitary sewers, incinerated in cement kilns, disposed in landfills or further processed for different types of industries. Municipalities throughout Canada have banned the discharge of FOG into the sewer system due to clogging problems in the piping system

and at the wastewater treatment facility. Few municipalities have banned disposal in sanitary landfills, but as organic waste diversion plans becomes more frequent in Canada, other suitable ways of treatment like composting or anaerobic digestion may take place and be enforced by municipalities. In Ontario alone, it is approximated that 45000 tonne of FOG are available for such treatments (Rashid and Voroney, 2004). Riley (2004) indicated that animal fat production in Canada is situated around 500000 tonne annually and yellow grease produced from the food service sector in British Columbia was situated at 21.4 million litres. Similar figures were addressed in a report by Tampier et al. (2004) stating that 160000 tonne of yellow grease, 200000 tonne of beef tallow and 70000 tonne of pork lard was available in Canada. The availability by province is listed in Table 2.7.

Table 2.7 Canadian Provinces 2001 Annual Production of Various FOG Wastes in Million Litres, adapted from Tampier et al. (2004)

FOG Type	BC	AB	SK	MB	ON	QC	Maritimes	Canada
Yellow Grease	24	18	6	7	70	43	13	180
Beef Tallow	12	90	41	22	31	20	4	221
Pork Lard	1	11	7	15	20	24	2	79

FOG varies greatly in terms of quality. Major characteristics of FOG are its water content, presence of other inorganic contaminants, hydrophobicity and floatability in aqueous solutions and inhibition of the methanogenic bacteria by long-chain fatty acids (LCFAs). A detailed Swiss study conducted by Membrez and Fruteau de Laclos (2002) on the co-digestion of FOG in anaerobic digesters has grouped FOG into five classes:

1. Floatation Sludge

Slaughterhouses commonly use floatation sludge systems to treat colloidal and suspended matter in their wastewater. The water content varies and the FOG composed mainly of lipids and proteins. Depending on the type of production or processing, the ammonia and scum layer may present a problem for anaerobic treatment.

2. Grease Trap Residue

These residues are separated solids and FOG from the wash waters of restaurants, food preparation and transformation industries. Grease traps are used so that the heavy organic load from these solids and FOG do not end up in the sewers and consequently in the

wastewater treatment plant. These solids also contain wide amounts of grit, sand and proteinaceous organic matter.

3. Industrial Oil-Pressing Wastewater

This stream of wastewater contains diluted amounts of organic matter present in forms of fats, starches, sugars, polyalcohols, pectins, tannins and polyphenols with other trace components that are problematic to aerobic and anaerobic degradation. Principal countries that have developed anaerobic treatments of this wastewater are Mediterranean countries due to their intense vegetable oil production, particularly olive oil processing. Physical-chemical treatments are required before anaerobic digestion of this stream of wastewater.

4. FOG Fraction of SSOFMSW

Generally, from 6 to 10% of the separated household waste contains fats on a dry matter basis. Other components of this waste include hemi-cellulose, cellulose, proteins and lignin. Their content varies due to the age of the waste, water content, cuisine traditions, separation treatments and biodegradability.

5. Waste Edible Oil

These lipids come from large scale food preparation industries and restaurants. Low levels of contaminants are present when source separation is practiced. Further filtration and processing renders this waste favourable to other markets as well. Vegetable sourced lipids are composed of triglycerides that are unsaturated and are in liquid form at room temperature compared to animal fats that contain mostly saturated lipids and in solid form. The chemical composition of the oil presented in Table 2.8 changes due to the high temperature frying of foods. Depending on the number of uses, humidity, type of food and temperature of frying, short chain fatty acids and LCFA are produced along with other organic compounds. Table 2.8 includes a list of the characteristics that make FOG a suitable co-substrate with liquid dairy manure.

Table 2.8 Various Characteristics of the 5 Types of FOG

Ref No.	Fats, Oils and Greases type	Total Solids (%TS)	Volatile Solids (%VS/TS)	COD (g/l or g/kg*)	Lipids (%TS or %VS/TS*)	Protein (%TS)	Total N (%TS)	NH ₄ -N (%TN or g/l*)	P ₂ O ₅ (%TS)	K ₂ O (%TS)
1	Olive oil wash water	0.6	86	8-220	11.1*	-	-	-	-	-
	Fat fraction of SSOFMSW	93.7	73	-	6-37*	-	1.54	-	0.19	-
	Grease trap residue high TS	25-70	96	600-800*	>15-96.5	-	0.5-3.6	15-20	0.1-0.6	0.1-0.5
	Grease trap residue low TS	5	84	116	28.6	-	1.2	-	-	-
	Grease trap residue	6.7-13.7	90.8-93.8	180	21	21	3.3	-	0.2	0.75
2	Floataion sludge	5-24	83-98	95-400*	17.7-44	20-54.8	1-8	10-30	0.9-3	0.05-0.2
	Waste edible oil	99.9	99	2420	99	-	<0.05	-	-	-
	Floataion sludge (Slaughterhouse)	5-24	85-98	-	-	-	3-9	0.2-0.6*	1-3	0.06-0.2
3	Grease trap residue	2-70	70-99	-	-	-	0.1-3.6	15-43	0.1-0.6	0.1-0.5
	Poultry slaughterhouse floataion sludge	12	96	125	-	-	3.5	0.4*	-	-

1. Membrez and Fruteau de Lacios (2002); 2. Baserga (2000); 3. Braun et al. (2003)

All classes of FOG are suitable for co-digestion with liquid dairy manure. They all have high %VS/TS content and rich in carbon. Kuhn (1995) and Baserga (2000) reported a range of biogas yield for grease trap residue of 0.7 and 1.3 l/gVS, respectively. For floatation sludge from slaughterhouse waste, Baserga (2000) indicated a biogas yield of 1.2 l/gVS. On the other hand, Braun et al. (2003) completed batch digestion studies based on the standard #DIN 38414 S6 of various proteinaceous wastes, including animal fat, floatation sludge and waste edible oil and found biogas yields of 1.0, 0.69 and 1.104 l/gVS, respectively.

Cirne et al. (2007) conducted BMP tests on synthetic lipid rich waste to verify the influence on hydrolysis and methane conversion due to inhibition of LCFA. The BMP vials contained a mix of starch, whey protein and cellulose along with different concentrations of triolein ranging from 5-47 %COD w/w incubated at 37 °C. Methane production rates were similar for the 5, 10 and 18 %COD w/w lipid concentration. A strong inhibition was observed for the 31, 40 and 47 %COD w/w lipid concentration bottles. It was concluded that the VFA's were not the major cause of inhibition of the gas production since all bottles, except the 47% lipid, showed similar VFA's profiles but the 31 and 40% lipid bottles showed inhibition. Lipid loading rates of 1 and 1.34 gCOD-LCFA/gVS (31 and 40 %COD-LCFA w/w) were inhibitory to the methanogens, with high stable levels of palmitate. Biogas yields for 10, 18 and 40% lipids ranged from 0.575 to 0.700 l/gVS.

Various configurations of reactors and other anaerobic processes have been tested at laboratory and pilot scales due to the physico-chemical characteristics of FOG, co-substrates used and organic loading rates applied to the reactor (Membrez and Fruteau de Laclos, 2002). This particular study mentions CSTR to be mostly used for testing the digestion of FOG due to the homogenization from the mixing, and prolonged contact between the biomass compared to sludge blankets and fixed film reactors. Also, the thermophilic temperature of the digestion was deemed suitable to kill pathogens in the substrate with longer retention times and liquefy FOG still in a solid state at mesophilic temperature. Presented in Table 2.9 are co-digestion results of FOG done in a continuous state with other substrates.

Table 2.9 Literature of FOG Anaerobic Digestion

Ref No.	Fats, Oils and Greases type	Co-Digested with	Reactor	HRT (d)	FOG Fraction of Feed (% w/w or v/v*)	OLR COD or VS* (g/l/d)	Removal Rate (%COD or %VS*)	Biogas Yield (l/gVS or l/gCOD*)	Methane Yield (l/gVS or l/gCOD*)
1	LCFA (Oleate)	-	EGSB	1	-	8	97	-	0.6
	Olive Oil WW	-	CSTR	15	-	3.5	90	-	0.41
	Fat Fraction of SSOFMSW	-	CSTR	-	37 %VS	3.8-6*	72-77*	0.8-0.83	-
	Fat Fraction of SSOFMSW	-	CSTR	20	6 %TS	2.7*	59*	0.35*	0.22
	Grease and Flootation Sludge	Manure	CSTR	12-25	20*	-	-	0.41	-
	Grease Separators	Manure	CSTR	-	70-80*	3-11.5*	89*	1.07	0.74
	Grease Separators	Manure	CSTR	27	68*	-	-	0.73	-
	Flootation Sludge	Sewage Sludge	CSTR	10-60	20*	-	-	0.75	-
	Waste Edible Oil	Manure and Slaughterhouse Waste	CSTR	14.5	58 %TS	2.6*	-	0.8	-
	Waste Edible Oil	Manure	CSTR	-	-	-	-	-	0.8-1.038
2	Waste Edible Oil	Paunch Manure	CSTR	24	45 %VS	2.8	-	0.46-1.2	0.29-0.84
	Waste Edible Oil	Grease Trap Residue	CSTR	30-45	21-41 %TS	3.0-6.4	56-86*	0.32-0.68	0.15-0.30*
	Grease Trap Residue	Manure, Slaughterhouse and Industrial Waste	CSTR	28-36	15 %w/w	2.6-3.7*	-	0.8-1.0	0.55-0.69
3	Grease Trap Residue	Manure, Restaurant and Pharmaceutical Waste, Animal By-Products	CSTR	22-75	7-51 %v/v Grease Trap Residue	1-5*	-	0.8-1.2	-
	Flootation Sludge	Sewage Sludge	-	20-30	5-20 % Flootation Sludge	-	59.9-70.8	0.35-0.60*	-

1. Membrez et Fruteau de Laclos (2002); 2. Murto et al. (2004); 3. Edstrom et al. (2003); 4. Braun et al. (2003)

The continuous laboratory scale studies identified in Table 2.9 clearly show that anaerobic digestion of the various types of FOG is problematic due to rapid VFA increase and accumulation of LCFAs, which are both inhibitory to methanogens. To demonstrate the inhibitory effect of VFAs and LCFAs on methanogens, Cirne et al. (2007) tested the use of enzyme additions with lipase (80,000 LU/g) to increase FOG degradation. The study showed that at higher concentrations of lipase added to the BMP bottles, the higher was the inhibition of methanogens, measured as methane production. At low levels of lipids, 10 and 18% w/w triolene, the enzyme increased the rate of methane production, but at 40 %w/w of triolene, the three tested levels of enzyme added (3.6, 61.0 and 120.8 IU/gVS) showed slower methane production than the controls. It was concluded that high levels of palmitate in these bottles caused the inhibition, since VFA profiles were similar for the 10, 18 and 40 %w/w of lipids. It was also shown in Membrez and Fruteau de Laclos (2002) during their laboratory trials that the addition of PEG glyceryl ricinoleate (5 %v/v of FOG) lead to rapid degradation of the accumulated FOG in the reactor. There was an increase in acetic acid up to 9 g/l in only two weeks of operation at 14 %v/v of waste edible oil and grease trap residue at an OLR of 3.2 gCOD/l/d. It was first verified whether the emulsifier was inhibitory to methanogens directly, but it was deduced that the emulsifier had increased the LCFA concentration by hydrolysis, thus increasing the contact of the LCFA with the methanogens at concentrations exceeding the inhibitory limit of 300-500 mg LCFA/l. The automatic addition of lime to maintain a neutral pH in the reactor did not prevent reactor failure by inhibition of VFA, since the acetic acid concentration greatly exceeded the theoretical limit of 2871 mg COD/l of acetate (van Lier, 2004). The addition of waste edible oil conducted in this laboratory trial was higher than 15 %TS co-digested with grease trap residue and was found to drastically reduce drastically reduce the biogas yield from 0.586 down to 0.488 l/gVS, even at a low OLR of 3 gCOD/l/d. It was also recommended that the FOG fraction of the feed not exceed 40%TS, which also corresponded to half of the COD contained in the feed. The optimal operation of the reactor was reached at 15%TS addition of waste edible oil at an OLR of 3.1 gCOD/l/d with a HRT of 34 days. The methane yield obtained was 0.252 l CH₄/gCOD which represented 72% of the maximum theoretical methane yield observed at a volatile solids removal of 83 %VS (Membrez and Fruteau de Laclos, 2002). Murto et al. (2004) tested the co-digestion of 15% w/w grease trap

residue with various mixtures described in Table 2.10 of industrial waste, pig manure, slaughterhouse wastewater, restaurant, fruit and vegetable waste.

Table 2.10 Composition of Industrial Waste in %w/w from Murto et al. (2004)

Grease trap residue	87
Confectionary waste	7
Dairy product waste	2
Bakery waste	3
Fodder/mill waste	1

Three CSTR at mesophilic temperature were used to test each type of homogenized and sanitized feedstock at 70 °C for 1h prior to feeding, described in Table 2.11.

Table 2.11 Composition of Feedstocks Co-Digested in Three Reactors (%v/v) from Murto et al. (2004)

Reactor	A	B	C
Industrial Waste Mixture	17	17	17
Pig Manure	83	71	66
Slaughterhouse Waste	-	12	12
Restaurant, Fruit and Vegetable Waste	-	-	5

The total solids content of the industrial waste, mainly composed of grease trap residue (representing 15% w/w of the feed) was 19%TS with a 93% VS/TS content of organic matter. The total solids concentration of the three feedstocks were situated between 9.7 and 10.3 %TS with a C/N ratio of 8 to 11. The HRT was set at 50 days for the reactor start-up and slowly decreased to 30 days. Reactor C failed and showed high levels of foaming and the OLR had to be reduced from 3.7 down to 2.6 gVS/l/d at a HRT of 36 days. Reactor A and B reached stable conditions at 2.6 and 3.1 gVS/l/d, respectively. The biogas yields reported were 0.8, 0.9 and 1.0 l/gVS for reactors A, B and C, respectively, with an average methane concentration of 70 %. Braun et al. (2003) co-digested poultry slaughterhouse floatation sludge with sewage sludge in a 4.4 m³ pilot digester. The floatation sludge was 12%TS, with an organic fraction of 96% VS/TS, and contained a high fraction of lipids and blood from the poultry slaughtering. The waste was co-digested in fractions of 5, 10 and 20% for a 160 day trial. The initial volumetric biogas productivity of the sewage sludge digester was 0.47 m³/m³/d and increased from 0.9 to 1.5 m³/m³/d when using 5% and 20% floatation sludge. The specific biogas yield ranged from 0.35

to 0.60 l/gCOD, with a COD removal of 59.9 to 70.8% with the maximum values reached when the HRT was increased from 20 to 30 days.

2.5.6 Whey

Cheese whey is a by-product of cheese making process. It is a pale yellow liquid that is left over from the coagulation of milk. It comprises close to half of the nutrient value of raw milk, mainly consisting of carbohydrates, proteins, minerals and vitamins. Cheese whey is typically further processed to produce whey protein concentrate. They are used in the food processing sector as a high source of proteins, thickening agents, food binder and as animal feed (Agriculture and Agri-Food Canada, 2009). Other treatments include whey powder, crystallized lactose and ethanol production via fermentation (Fruteau de Laclos and Membrez, 2004). Cheese whey in Canada is produced in some of the 445 federally and provincially regulated dairy processing plants. The dairy manufacturing sector in Canada represents 15% of the market value of the food and beverage sector with 11.6 billion dollars in 2007 (Canadian Dairy Information Center, 2009). The major portion of whey protein concentrate and whey powder marketed for national and international markets is produced in large plants regulated at the national level. They are produced in 14 plants owned by the four major players in cheese processing; Agropur, Kraft, Parmalat and Saputo. Cheese production in 2006 was valued at 359000 tonne, compared to 4328000 tonne for U.S.A, 1858000 tonne for France, 1995000 tonne for Germany and 1154000 tonne for Italy (Canadian Dairy Information Center, 2009). The Canadian cheese production closely resembles those of Australia, Denmark, U.K, Russia and Spain (Canadian Dairy Information Center, 2009). The composition of whey varies from the type of coagulation used. Sweet whey is obtained from proteolytic enzyme coagulation, commonly used for cheddar. Acid whey comes from the coagulation of milk by acids, mainly from lactic acid fermentation of lactose used in cottage cheese production (Handajani, 2004). In general, whey contains from 4.3 to 6.5 %TS, 80 – 90 %VS/TS, C:N ratio of 27, 4.5 to 5 %w/w of lactose, traces of proteins (0.7 %w/v), fat (0.5 %w/v) and minerals (0.5 %w/v) (Baserga, 2000 and Goblos et al., 2008). Characteristics of raw whey are presented in Table 2.12.

Table 2.12 Various Whey Characteristics

References	pH	TS (%)	VS (%)	Ash (%)	TSS (g/L)	COD (g/L)	Proteins (%)	Fat (%)	Lactose (%)	TKN (g/L)	NH ₃ - N (g/L)	TP (g/L)	Notes
Saddoud et al. (2007)	4.9	5.93	5.61	0.31	1.35	68.6	0.27	0.94	4.6	1.12	-	0.5	Tunisia
Gellegenis et al. (2007)	3.5	7.80	4.80	2.80	-	74.9	0.60	0.70	3.6	1.02	0.06	0.3	Greece
Handajani (2004)	-	6.35	5.85	0.50	-	-	0.80	0.50	4.9	-	-	-	Sweet whey
	-	6.50	5.70	0.80	-	-	0.75	0.40	4.9	-	-	-	Acid whey
Ghaly (1996)	4.5	6.59	4.73	1.87	-	72.2	-	-	-	1.15	0.03	-	Canada
Najafpour et al. (2008)	5.5	6.00	4.90	1.10	-	60.0	0.22	-	5.5	-	-	0.6	Iran
Erguder et al. (2001)	3.9	-	-	-	9.38	75.5	-	-	-	0.14	-	0.1	Turkey
Wildenauer and Winter (1985)	4.5	6.80	6.10	0.70	-	79.0	0.82	-	4.5	2.00	0.07	-	Acid whey Germany
Malaspina et al. (1995)	-	3.37	3.19	0.18	1.30	68.8	-	-	-	1.46	0.06	0.4	Italy

The high organic content of cheese whey renders conventional aerobic treatment difficult due to the cost of oxygen supplement, excess sludge production and its final disposal (Grady et al., 1999 and Wildenauer and Winter, 1985). In a typical activated sludge wastewater treatment plant, BOD load of 11% with whey was found problematic in Germany (Handajani, 2004). For these reasons, anaerobic treatment is the favoured disposal method if the whey is not further processed (Ghaly, 1996). Two advantages to using anaerobic treatment is the substantial generation of energy through biogas production and little sludge production (Grady et al., 1999). However, full-scale anaerobic treatment of pure whey is seldom reported due to the lack of alkalinity in whey which would lead to a rapid acidification in the digester and ultimately acid inhibition (Fruteau de Laclos and Membrez, 2004).

On the contrary, anaerobic digestion of whey has been widely reported at laboratory and pilot scales. Whey used as a co-substrate with liquid dairy manure is more practical for digesters planning to use manure as a sole substrate. As shown in Table 2.12, whey has the same %TS as liquid dairy manure and most of the COD is lactose, which is a great source of carbon. Batch tests have demonstrated yields of 30 to 55 m³/tonne of whey and methane yield of 0.4 l CH₄ /gCOD and a biogas yield of 0.800-0.900 l/gVS (Wildenauer and Winter, 1985; Weiland, 2000; Erguder et al., 2001 and Baserga, 2000). Erguder et al. (2001) completed BMP experiments on various concentrations of whey with and without basal medium support, similar in composition as Owen (1976) for 68 days. At an influent concentration of 5.5 gCOD/l of whey, the BMP bottle with basal medium produced 90% of the cumulative biogas in 16 days, compared to 35 days required for the BMP bottles without basal medium. Similar results have been reported for higher concentrations of influent COD in other bottle tests. Erguder et al. (2001) concluded that the basal medium was necessary to reach maximum theoretical biogas due to the nutrient, trace metal and alkalinity supplementation. In this study, the anaerobic methane generation for cheese whey was found to be 0.424 l CH₄/gCOD or 23.4 l CH₄/l whey. Table 2.13 includes past studies that treated whey and diluted whey using high and low rate reactors.

Table 2.13 Lab Scale Anaerobic Digestion of Whey

References	Comments	Reactor *	OLR (gCOD/l/d)	HRT (d)	Duration of Trials	Influent OLR	Removal Efficiency	Methane Yield (Biogas*)	Volumetric Biogas Production (m ³ /m ³ /d)
Erguder et al. (2001)	Screened undiluted whey	UASB	10.4-24.6	5-2	51 days	42.7-55.1 gCOD/l	92-97 %COD	-	-
Goblos et al. (2008)	Centrifuged undiluted acid whey	ASBR	1.6-12.8	40-5	-	-	91-95 %COD	122-276 l/kg COD	0.5-2.5
Fruteau de Lacos et Membrez (2004)	Centrifuged undiluted whey	DFFR	5.2-19.6	21.2-4.8	7 months	71 gCOD/l	91 %COD	309-339 l/kg COD	2.3-10.3
Saddoud et al. (2007)	Diluted whey	TSMR w/ MF	3-19.8	4	45 days	10-70 gCOD/l	79-98.5 %COD w/ MF	300 l/kg COD	0.7-6.3
Najafpour et al. (2008)	Diluted pH adjusted whey	UFFLR	5-45.4	3-4	65 days	7-60 gCOD/l	80-97.5 %COD	-	3.6-3.75
Wildenauer and Winter (1985)	Undiluted acid whey	UFFLR	max 14.0	5	15 weeks	79 gCOD/l	max 95 %COD	400 l/kg COD	max 5.6
Malaspina et al. (1995)	Undiluted whey	DUHR	2.0-10.0	-	260 days	68.8 gCOD/l	75-98.4 %COD	330 l/kg COD	4.0-10.0
Ghaly (1996)	Undiluted whey	TSUR	3.3-6.6	10-20	20-60 days	65.8 gCOD/l	38.7-55.9 %COD	214-343 l/kg COD	0.81-1.01

Most of the studies on anaerobic whey treatment described in Table 2.13 used high rate anaerobic reactors at short retention times (2 - 5 days) and high influent concentrations (40 - 70 gCOD/l) and high loading rates (5 – 25 gCOD/l/d). All of these studies reported high removal efficiencies (80 – 98 % COD) (Erguder et al., 2001; Fruteau de Laclos and Membrez 2004; Saddoud et al., 2007; Najafpour et al., 2008 and Wildenauer and Winter, 1985). Wildenauer and Winter (1985) reported the highest methane yield for acid whey at approximately 0.40 l/gCOD, while Fruteau de Laclos and Membrez (2004) and Malaspina et al. (1996) reported methane yields of 0.31 – 0.34 l /gCOD. Under stable conditions, volumetric biogas production of high-rate anaerobic reactors ranged from 5 to 10 m³/m³/d (Saddoud et al., 2007; Malaspina et al., 1996; Fruteau de Laclos and Membrez, 2004 and Wildenauer and Winter, 1985).

This situation is not followed for the co-digestion of manure and whey. Little literature was found on the co-digestion of manure with whey, compared to the numerous articles available on anaerobic digestion of whey digested as a single substrate. All papers found on co-digestion of whey with poultry and dairy manure concluded that the co-digestion provided the necessary nutrients and buffering capacity, while adjusting the C:N ratio in the reactor to produce methane more efficiently than the digestion of whey digested alone (Desai et al., 1994; Gelegenis et al., 2007 and Lo et al., 1988). Table 2.14 gives a brief summary of the various experiments conducted on the co-digestion of whey and manure.

Table 2.14 Whey Co-Digested with Manure

References	Comments	Reactor	OLR	HRT (d)	Influent Characteristics	Removal Efficiency	Methane Yield (l/gVS)	Volumetric Biogas Production (m ³ /m ³ /d)
Desai et al. (1994)	Dried and screened poultry and cattle manure with whey	CSTR	1.9-19.9 gTS/l/d	3-30	6.0 %TS	50-71 %COD	-	2.5-3.5
Gellegenis et al. (2007)	Diluted and homogenized poultry manure with whey	CSTR	4.45-4.94 gCOD/l/d	20	89 - 99 gCOD/l, 6.5 - 7.0 %TS	70-77 %COD	0.5-0.7 (biogas)	-
	Diluted and homogenized poultry manure with whey	Pilot reactor, type N/A	5 gCOD/l/d	20	91 gCOD/l	-	-	1.5-2.2
Lo et al. (1988)	Screened dairy manure and undiluted whey	ARBC	5.2-16.4 gVS/l/d	2-5	36.0-67.5 gCOD/l, 3.12-6.6 %TS	46.3-62.6 %COD, 48.7-58.7 %VS	0.193-0.273	0.28-0.75 (methane)
	Screened dairy manure and diluted whey	ARBC	6.7-13.9 gVS/l/d	2-4	32.7-33.5 gCOD/l, 2.05-3.22 %TS	50.7-67.5 %COD, 56.1-72.2 %VS	0.227-0.334	0.44-0.63 (methane)

One significant difference observed from the experiments outlined in Table 2.14 is that various types of reactor configurations were used for the co-digestion. Desai et al. (1994) and Gelegenis et al. (2007) used a CSTR system, however, this reactor type cannot be organically loaded as high as conventional fixed-film or sludge blanket reactors. The average loading rates and volumetric biogas productions were lower and the retention time is longer compared to the conventional high rate reactors, except for Lo et al. (1988) since they were using a high-rate rotating biological contact reactor. It is also noted that the removal efficiency of COD or VS ranged in between 55-75 % regardless of reactor technology. We have seen previously in section 2.5.1 that Hartmann et al. (2000) demonstrated that a certain fraction of the VS of manure are not converted into biogas depending on its lingo-cellulose and cellulose fraction. This could explain the limit of COD or VS destruction observed for any types of reactor treating manure. Lo et al. (1988) investigated the treatment efficiency of different mixtures of dairy manure with cheddar cheese whey in a 5.0 litre ARBC reactor. Two sets of feedstock were prepared. In the first set, the cheese whey, at an original 5.7 %TS, was diluted with tap water to give a similar VS concentration of the diluted screened dairy manure. Before mixing with the whey, the dairy manure was diluted in a 1:1 mixture with tap water and passed in a vibrating solid-liquid separator with a 2.0 mm screen. Then a 3:1 mixture of diluted cheese whey and diluted screened dairy manure was tested in an ARBC reactor at a temperature of 36°C and an HRT of 4 days with an OLR of 6.7 gVS/l/d. In this set of experiments the volatile solids concentration was set at 2.70 %VS, the influent COD was in the range of 32.7 – 35.7 gCOD/l and the total solids concentration was in the range of 2.05 – 3.28 %TS. In a second set of experiments, the HRT in this reactor was reduced to 2 days with an OLR of 13.9 gVS/l/d. For the second set of feedstock, one volume of the undiluted manure was mixed with two volumes of cheese whey then passed in the 2.0mm screen. The influent characteristics were 4.92 – 5.63 %VS and for the influent COD 62.6 – 65.7 gCOD/l with a total solids concentration of 6.11 – 6.66 %TS which resulted in a higher OLR from 10.2 – 16.4 gVS/l/d. The maximum methane yield observed when using undiluted whey was 0.273 l CH₄/gVS. Although this reactor is considered as high rate, the volumetric biogas production did not exceeded 0.75 m³/m³/d for the diluted feedstock and 0.63 m³/m³/d for the pure mixture. Gelegenis et al. (2007) had a setup of 4 lab scale CSTR, in which each reactor had a separate fraction of diluted poultry manure replaced by whey. All reactors were fed with diluted poultry manure at start-up (for 30 days) and then whey was

added in the feed mixture step-wise by replacing the same volume of poultry manure with whey to keep the HRT relatively constant and avoid reactor instability. Predetermined levels of whey had been set at 15%, 25%, 35% and 50% whey by volume. The biogas yield obtained during the 35% and 50% whey by volume ranged from 0.5 to 0.7 l/gVS. Throughout the experiment, volatile solids removal of 70-77%VS was reported for a HRT of 20 days. Desai et al. (1994) prepared their feedstock differently and used dried and powdered poultry and cattle manure with whey in 19 different ratios on a dry weight basis. The manures were dried at 60°C and powdered to a 50 size mesh. The average total solids concentration for all 19 ratios was 6 %TS, retention time set at 10 days with a corresponding loading rate of 6 gTS/l/d digested at a temperature of 40 °C. Methane yields were not reported, but the volumetric biogas production of the reactor ranged from 2.5 and 3.5 m³/m³/d and was significantly higher than the other experiments compared of Gelegenis et al. (2007) and Lo et al. (1988).

CHAPTER 3: MATERIALS AND METHODS

3.1 Origins of the Substrates Tested

3.1.1 Source of Liquid Dairy Manure

The liquid dairy manure obtained for the batch and semi-continuous reactors was collected throughout the testing period from the organic dairy research farm at the University of Guelph, Campus d'Alfred. The farm currently milks approximately 45 head of Holstein cows and has about 15 in gestation. During the summer months, the cows are put to pasture for grazing. They receive their ration of feed inside the barn. Liquid manure was pumped from the in-barn storage pit in sealed pails and stored inside the garage of the research station until May. Due to the large volume of manure required to be stored during the summer, while the cows were at pasture, the liquid dairy manure was stored in a 1 m³ polyethylene tote (Figure 3.1.) The liquid dairy manure used for this study was taken directly from the in-barn manure pit.

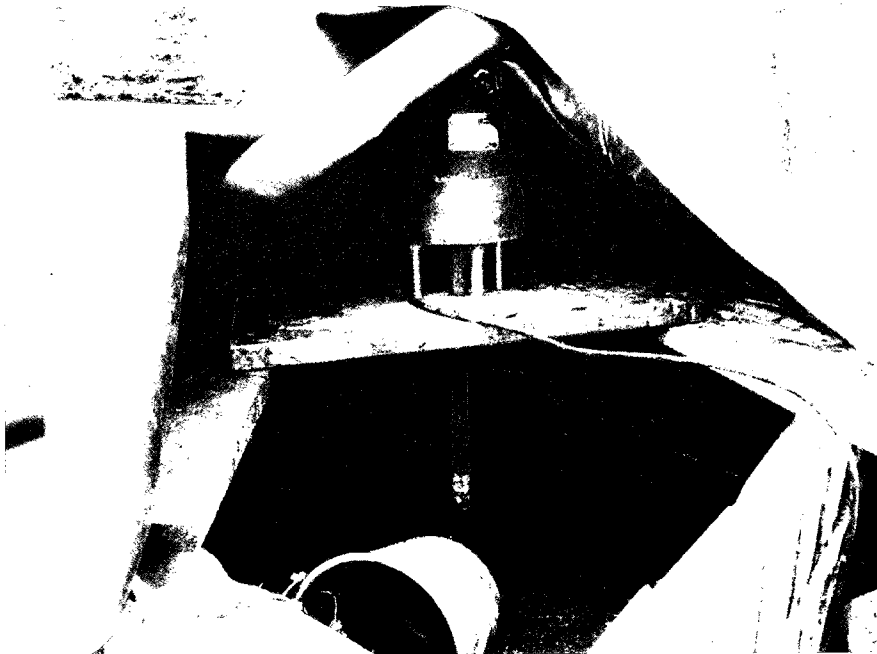


Figure 3.1 Liquid Dairy Manure Storage Container

Straw and sawdust present in the liquid dairy manure were screened with a 2.5 cm mesh from the tote to minimise crust formation inside the semi-continuous digesters. A modified bulk tank mixer was mounted to the top of the tote to make sure manure was homogeneous, where the liquid manure was mixed at 25 rpm 15 minutes prior to use. Liquid manure was collected from the barn at the beginning of the semi-continuous study, on August 12th, September 30th and October 20th of 2008. The cows were housed in the barn for a week when the last sample was collected on October 20.

3.1.2 Source of Corn Silage

Corn silage is a high energy source and is the main component of feed rations supplied to dairy cows in eastern Ontario. The corn silage obtained for this study was sampled twice at Terryland Farm (St. Eugene, ON) about 110 km east of Ottawa. The corn silage collected for the fall 2007 BMP experiments was from the crop of the year 2006. The corn silage sample was taken just before the 2007 crop was ensiled in the bunker silos. For this study, it was preferred to use corn silage that had already been anaerobically stabilised (2006 crop) as opposed to fresh un-ensiled corn silage (crop 2007). The second sample of corn silage taken at the farm and used for semi-continuous runs was in spring 2008, which was the ensiled 2007 crop. The farm used a mix of seeds mainly composed of Mycogen BMR and Mycogen TMF. Yields of 20 to 22 tonne per acre of fresh corn silage were reported by the farmer. The thermal units are 2800 and the area is located in the 4b plant hardiness zone on clay soil. The farmer applies each year 68 kg of “starter” fertilizer and 22700 litre of manure per acre to farm fields growing corn each year. The corn silage is stored in side-walled, conventional cement bunker silos and without the use of additives to preserve the crop. The samples were stored in three sealed pails at room temperature in the garage of the Campus d’Alfred research station. The head space was gassed with nitrogen each time a sample was taken for further processing. A hand driven meat grinder was used to chop the corn silage prior to mixing in the feed bottles to reduce crust formation in the digester.

3.1.3 Source of Canola Meal

The canola meal collected for the trials came from a contractor and distributor of mill equipment in Saint-Isidore, ON. The meal was issued from a cold-press oil extractor model Oleane20 (MBU20) from the manufacturer Mécanique Moderne (Figure 3.2).

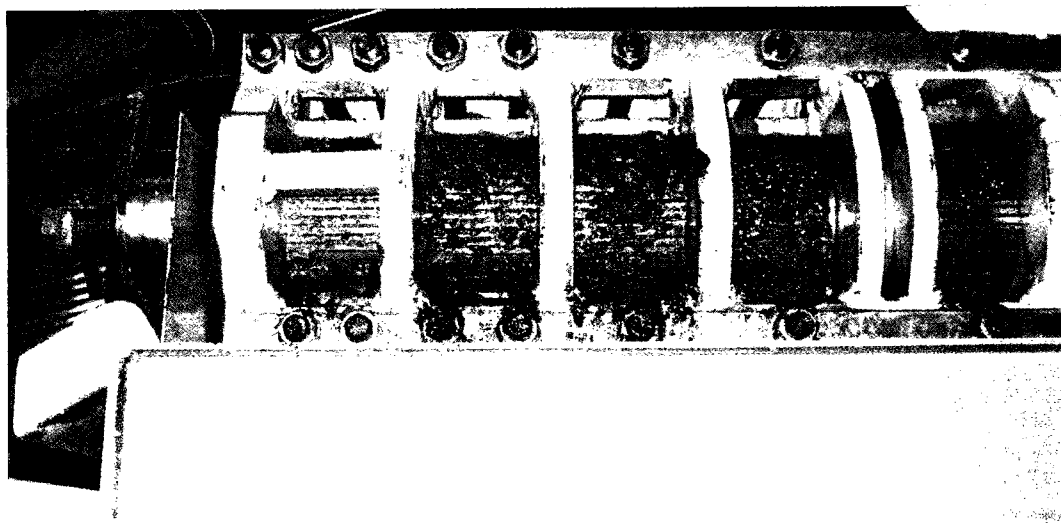


Figure 3.2 Canola Seed Oil Extractor

The canola seeds originated from a farmer in Northern Ontario for yield and equipment testing. After processing, the meal was stored in open large feed bags. The samples collected in September 2007 were further stored in 15 litre sealed pails at room temperature

3.1.4 Source of Glycerine

The glycerine used in this study was obtained from the biodiesel plant of Rothsay-Laurenco in St. Catherine, QC, on the south shore of Montreal, QC. The plant is owned by Maple Leaf Foods and produced approximately 35 million litres of biodiesel in 2008 (Caparella, 2009). Their feedstock is made from rendered materials such as grease trap waste from restaurants, slaughterhouse waste and waste from meat processing plants (Caparella, 2009). The glycerine was collected in one of the storage silos in the yard on June 4, 2008 and stored in 20 litres sealed plastic pails. The operator indicated that the methanol used for biodiesel is recycled and the glycerine also contains wastes from pre-treatment of feedstocks, which

includes water, fryer residues, soaps and other residues from the centrifugation stage after the trans-esterification step.

3.1.5 Source of Fats, Oils and Greases

The FOG waste used for the lab trials was obtained on May 24, 2008 from Terryland Farm, at which time this waste was the main co-substrate being fed to the full scale digester at the farm. The owner indicated that the FOG waste was dissolved air floatation sludge that came from a bacon processing plant in the province of Québec. The samples were taken after pasteurization at 70 °C for one hour in the pasteurizer tank. They were stored in 20 litre sealed plastic pails at room temperature in the garage of the Campus d'Alfred research station. The FOG was taken after the pasteurization process because it became more liquefied and homogeneous due to the high temperature treatment.

3.1.6 Source of Cheese Whey

The whey used in this study was obtained from the Skotidakis cheese factory in St. Eugène, ON, which processes goat and cow milk. Skotidakis raises its own goats and processes the goat milk with cow milk purchased. The milk processing plant uses a combined milk quantity of about 200000 litres of milk per week (Personal communication, 2008). This averages roughly to 400000 litres of whey and washwaters produced per week. The whey was fed to a harrow finishing barn and the rest was applied on a portion of the farm land as an organic soil amendment. However, since production has gone up in the recent years, most of the whey is being trucked away to other hog farmers in the region, also being used as a feed supplement for hog production. The whey was sampled right after ricotta production directly from a tank that contained no washwaters in November 2007 and in May 2008. The whey was frozen at (-20°C) for the duration of the study and kept in 20 litre sealed plastic pails.

3.1.7 Source of Inoculum

The inoculum for this study was collected from the Terryland Farm's mesophilic anaerobic digester. The inoculum was put in sealed containers (Figure 3.3) designed with a water seal to prevent expansion of the containers from residual biogas produced by the inoculum. During transportation, the plastic containers were placed in a cooler that was warmed in the incubator chamber prior to the sampling to prevent thermal shock to the inoculum. The same procedure was used to transport the inoculum for the semi-continuous reactors. For the batch tests, the inoculum container was put back in the incubator chamber at 37°C and kept active by feeding intermittently with the same co-substrate fed at the farm's digester until used for the Biochemical Methane Potential (BMP) tests.

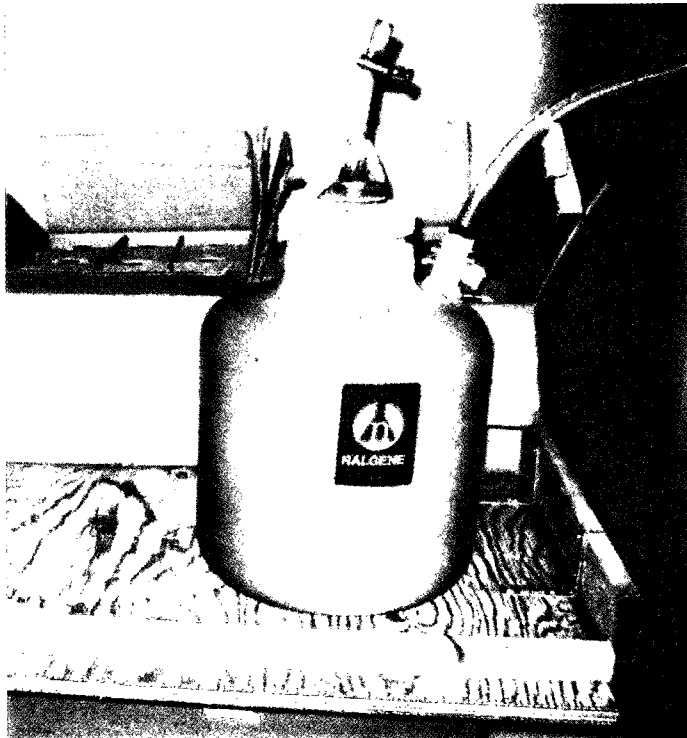


Figure 3.3 Inoculum Transport and Storage Container

3.2 Experimental Protocols

3.2.1 Biochemical Methane Potential Assays

The first set of BMP trials commenced on December 12, 2007 and were completed on March 12, 2008. The design of the first BMP assay was set so that the amount of volatile solids from

the substrate in each bottle is known. This method has been chosen to facilitate the biogas yield calculations (volume of biogas per unit of VS added), to remove the impact of the variability of waste characteristics and to add a known amount of "food" to a known amount of "active" inoculum (F/M ratio). The first batch (corn silage, canola meal, whey and grease trap) contained 0.68 gVS of substrate with 100 g of inoculum and the second batch (glycerine and DAF sludge) contained 0.42 gVS of substrate with 100 g of inoculum per BMP assay. It is asserted that the inoculum VS does not count as food but rather as a mass of active acid and methane formers. The activity was assured by feeding the inoculum reactor small amounts of co-substrate and verified with a tipping bucket. The F/M ratio was set at 2 gVS Substrate: 1 gVS Inoculum.

The main objective was to determine if there was any difference in biogas yield for different mixes of co-substrate volatile solids and manure. The mixtures chosen were representative of what would be used in the semi-continuous laboratory reactors. Table 3.1 summarizes the contents of each BMP bottle. For solid co-substrates (corn silage and canola meal), the mixtures used in the BMP trials were set to obtain a maximum TS concentration of the influent that would be fed to semi-continuous pilot reactors (set at 12%TS). This limit was chosen to respect operation limits of CSTR. The mixtures using liquid co-substrates (grease trap waste, glycerine and whey) in the BMP trials were set so that no more than 70% of the VS come from the liquid co-substrates.

Table 3.1 Mixes of Co-Substrate VS and Manure for BMP Tests at a 2 gVS Substrate: 1 gVS Inoculum Ratio

Substrate	BMP Label	% VS of Liquid Dairy Manure	% VS of Co-Substrate	% TS of Mixture	Mass of VS in BMP (g)
Manure	M1	100	0	5.5	0.68
Corn Silage	M7S3	70	30	7.2	0.68
	M5S5	50	50	9.1	0.68
Grease Trap	M7G3	70	30	6.9	0.68
	M4G6	40	60	9.3	0.68
Canola Meal	M8C2	80	20	6.8	0.68
	M6C4	60	40	8.5	0.68
DAF Sludge	DAF4	60	40	7.3	0.42
	DAF6	40	60	11.0	0.42
Raw Glycerine	GLY4	60	40	8.4	0.42
	GLY6	40	60	11.7	0.42
Cheese Whey	M7W3	70	30	5.6	0.68
	M3W7	30	70	5.8	0.68

BMP trials were conducted following a modified version of Angelidaki and Sanders (2004) using 500ml Wheaton borosilicate glass bottles with a 43mm diameter. The respective feedstock quantities based on the VS mixes chosen in Table 3.1 and the results of the TS and VS tests, were placed in each bottle. The substrates were weighted in disposable plastic dishes on an electronic Denver Instrument scale model S-4002. Any residual feedstock left in the dishes was rinsed with distilled water to ensure that all the weighted feedstock was added to the bottles. A new plastic dish was used for each BMP bottle. Sodium bicarbonate (FisherBrand, reagent grade) was added at 0.3 g to each bottle for alkalinity. Measured amount of distilled water that was previously gassed for one hour with nitrogen gas was added to each bottle to reach a volume of 200 ml. Next, 100 ml of inoculum measured with a 100 ml graduated cylinder was added to three bottles at a time. The bottles were de-gassed with nitrogen gas for 60 seconds and capped with black halobutyl stoppers. The inoculum stored in a 1 l beaker covered with parafilm, was de-gassed using nitrogen gas throughout the preparation of the BMP bottles. A manometer apparatus was then used to equalize the pressure to atmospheric inside the bottles after they were placed for 30 minutes in the incubator-shaker. This was done to allow the contents of the bottles to reach the incubation temperature and release the pressure gained during the dilatation of the headspace. After the pressure inside the bottles were set back at atmospheric, they were put back in the New-Brunswick model G25 incubator (Figure 3.4), maintained at 37 °C and a rotation speed of 100 rpm. BMP test were done in duplicates in succession and inoculum test bottles in quadruplets.

The second set of BMP experiments were conducted on October 21, 2008 and the last biogas measurements were taken on December 1, 2008. The second set of BMP experiments were conducted in two parts. The first part consisted of repeating the same procedure for preparation of the bottles as in the first batch but for raw glycerine and dissolved air floatation sludge in 250ml Wheaton borosilicate glass bottles at the same F/M ratio. The second part consisted of preparing BMP bottles based on a second design to determine different kinetic rates of degradation based on VS ratios for canola meal and inoculum that were different for each bottle. The different design used VS ratios shown in Table 3.2. The mass added to each bottles was determined by TS and VS tests and is shown in Table 4.6, the

details of the design is further explained in section 4.1.2, following the conclusions observed for the digestion of canola meal.

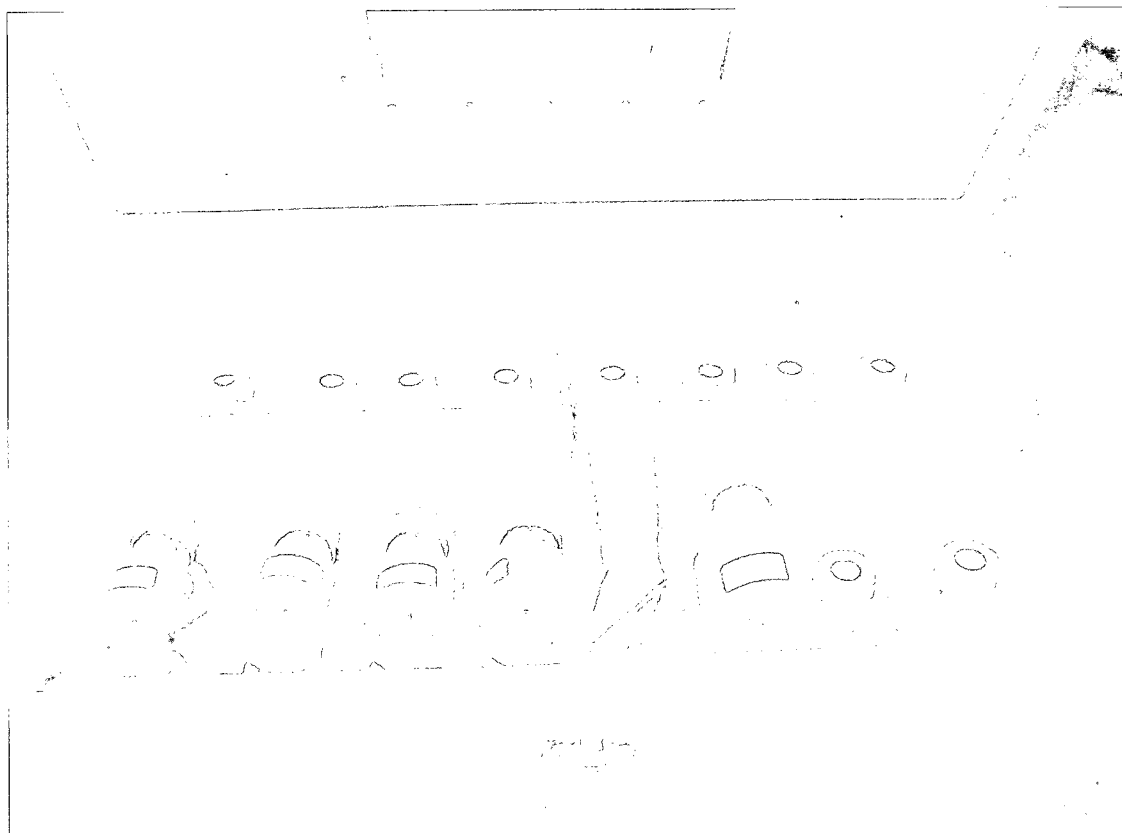


Figure 3.4 BMP Bottles in G25 Incubator

Table 3.2 Ratio of gVS of Substrate, gVS Co-Substrate and gVS Inoculum for the Second Design of BMP

Substrate	Bottle ID	Canola Meal gVS / Inoculum gVS	Liquid Dairy Manure gVS / Inoculum gVS	Substrate gVS / Inoculum gVS	Mass of VS in BMP (g)
Canola Meal	C1	0.25	-	0.25	0.09
	C2	0.5	-	0.5	0.18
	C3	0.75	-	0.75	0.27
	C4	1	-	1	0.36
	C5	2	-	2	0.72
Canola Meal with Liquid Dairy Manure	CM1	0.25	0.75	1	0.27
	CM2	0.5	0.75	1.25	0.36
	CM3	0.75	0.75	1.5	0.45
	CM4	1	0.75	1.75	0.54
	CM5	2	0.75	2.75	0.90

These bottles were setup to have 5 different substrate levels compared to inoculum with and without a set amount of VS from manure. The 5 bottles containing manure had the same VS fraction of co-substrate to inoculum (Table 3.2). This would then enable the possibility of determining the benefits of co-digestion with manure and the changes of biogas production and kinetic rates of degradation. These BMP were also conducted in 250ml Wheaton borosilicate glass bottles and the same procedure to prepare the first set of BMP was followed, but sodium bicarbonate was not added to BMP assays for the second design experiment. Sodium bicarbonate was not added to test the benefits of co-digestion of manure, based on its ability to supply alkalinity (Braun and Wellinger, 2003).

Tests of TS and VS were done according to section 3.3, two weeks prior to the first BMP assays and one week prior to the second set of BMP assays. After the first set of BMP assays, the glass bottles were stored in a refrigerator at 4°C and tests of TS and VS were done one week after the end of the experiment. For the second set of BMP assays, the tests of TS and VS were done two weeks after the end of the experiment. VFAs and gas composition were measured once during the second set of BMP assays according to the methods explained in section 3.3 for the bottles using gas chromatography at University of Ottawa. Gas composition was measured occasionally with a near infra-red (NIR) detector in the first set of BMP assays when the manometer tube was full (biogas sample exceeded 400 ml). The NIR sensor was rented for the duration of the experiment from Enviro-Mittner Technologies in North Hatley, QC. The sensor was manufactured by Serwin, model SR2-DO and equipped with CH₄ (0-100 %/v), CO₂ (0-100 %/v) and H₂S (2000 ppm max) sensors. Biogas production was measured twice on the first day and every day after with the manometer for both BMP runs. When biogas production tapered off at the end of the experiment, measurements for these bottles were taken every 2 or 4 days. The BMP bottles were shaken prior to take a reading.

3.2.2 Semi-Continuous Laboratory Reactors

Following the BMP assays, the feedstocks were further tested in six semi-continuous laboratory reactors. The reactors were constructed in Plexiglas by Canus Plastics in Ottawa, ON. The overall dimensions of the reactor are 40.64 cm high and 30.48 cm diameter for an

approximate total volume of 30 l. The reactors were enclosed in an insulated room made of 2 inch polyurethane foam encapsulated in thin sheet metal. The room was heated to 37 ± 1.5 °C by a 1200W baseboard heater. A column fan was fixed under the bottom of the heater to circulate the air in the room. The temperature in the heated room was monitored daily with two Checktemp model C dipstick thermometers. Hydrogen sulphide concentration in the heat room was monitored with a portable Honeywell B&W electrochemical sensor with a range of 0-100 ppm. The reactors were fed once every day with a custom made Plexiglas syringe fixed on the feeding valve. The feed for every reactor was prepared once a week and stored in individual 2 l wide mouth HDPE bottles in a refrigerator at 4°C. Feed mixtures were allowed to come to the heated room temperature before feeding. Manure was collected from the storage tank 15 minutes after the bulk tank mixer was activated. Volumes of liquid dairy manure and whey were measured using a 1 ± 0.05 l plastic graduated cylinder. Corn silage and canola meal were weighed using a Denver Instrument scale model S-4002. Glycerine was measured with a calibrated 60 ml plastic BD syringe and added to the contents of the feed bottles. A container of DAF sludge was stored in the incubator room to liquefy the contents. It was found that the DAF sludge would not liquefy in the feed bottle and would stick to the wall of the feed bottle. Instead, using a calibrated 60 ml plastic BD syringe, the DAF sludge was added directly in the feed valve and then the manure was added with the Plexiglas syringe. The mixing intervals were programmed with a FisherBrand electronic timer. Every two hours, the reactors were mixed for 20 minutes. Mixing was done with a top mounted custom modified Cole-Parmer Masterflex 7553 peristaltic motor and controller by Enviro-Mittner Technologies in North Hatley, QC. A modified shaft was welded to the gear in the peristaltic motor so that a flexible shaft adapter could be installed. On the mixing shaft, a custom made impeller similar to a boat engine propeller with three paddles of 3 inches in diameter was installed in a 60° angle and fixed 4 inches from the bottom of the reactor.

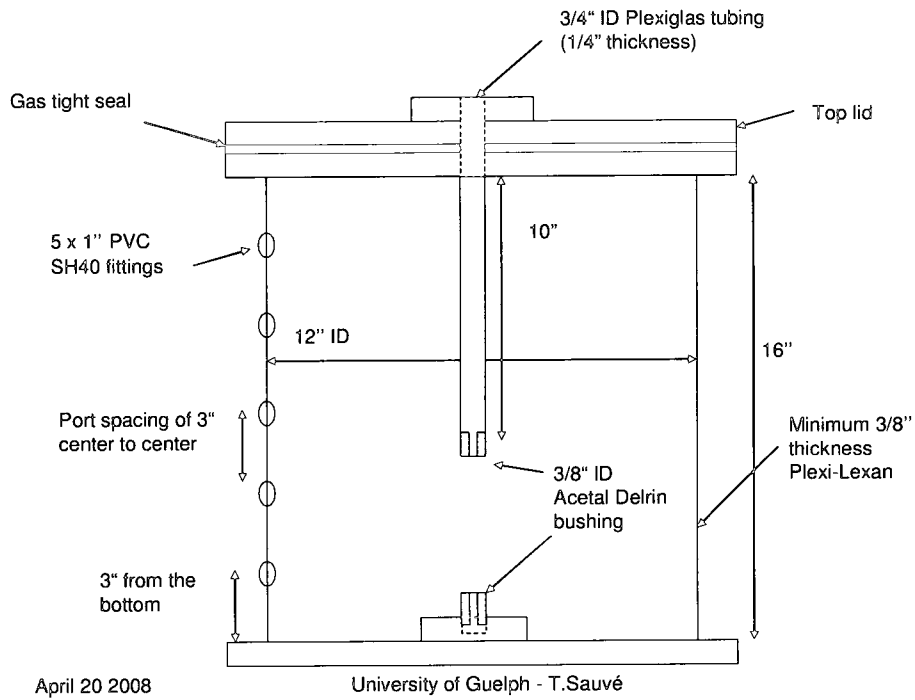


Figure 3.5 Sketch of the Cross-Sectional View of Pilot Scale Reactors

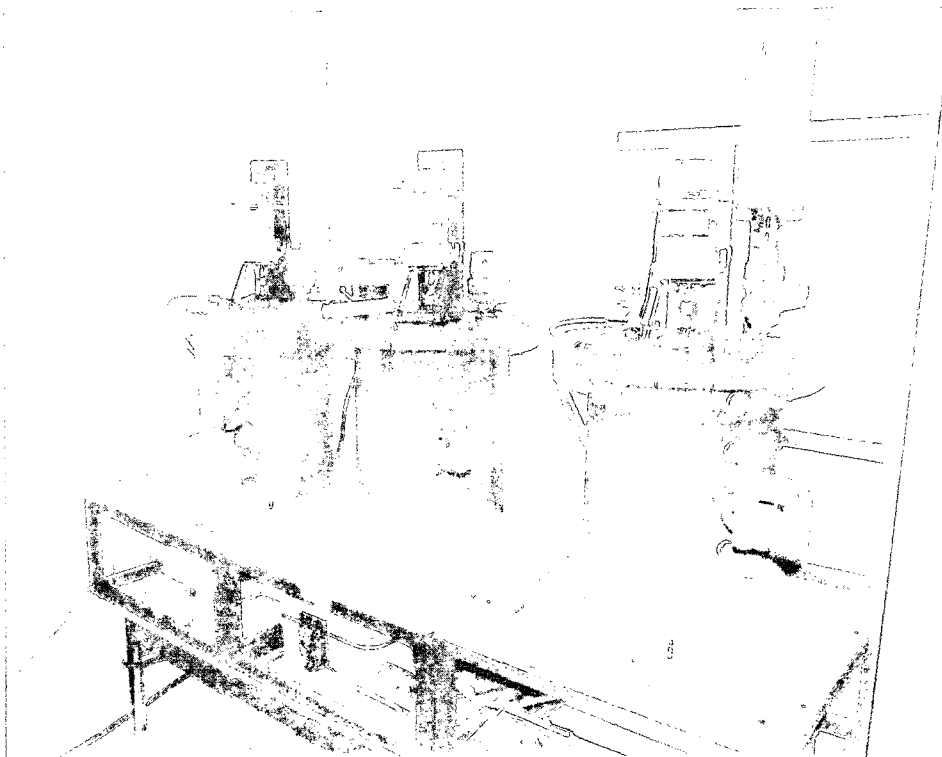


Figure 3.6 Pilot Scale Reactors Installed in the Heated Room

3.3 Analytical Methods

3.3.1 Partial Alkalinity, Total Alkalinity and pH

In addition to alkalinity, partial alkalinity is also useful in the evaluation of parameters for the monitoring of co-digestion process. The method was used by Bjornsson et al. (2000) and Bjornsson et al. (2001). The titration points are pH 5.75 for partial alkalinity and pH 4.3 for total alkalinity. Effluent samples were centrifuged at 3900 rpm in an IEC model Centra7 for 20 minutes. Twenty five 25 ml of centrifuged sample was added to a 100 ml beaker and stirred using a Hanna magnetic stirrer running at 75% speed. The pH was measured with an Accumet pH probe on a Sper Scientific field meter with the ATC probe. The pH field meter was calibrated with FisherBrand standard solutions of pH 4 and pH 7. Between each measurement, the temperature and pH probes were rinsed with distilled water and dried with Kimwipes®. A standard solution of 0.1 M HCl was prepared by adding 9.9163g of HCl to a 1l volumetric flask containing approximately 500 ml of distilled water. Distilled water was then topped up to the demarcation line and the content of the solution was stored in a 750 ml amber bottle for future use. The solutions were kept at room temperature and used within two months.

3.3.2 Dissolved Ammonia

Standard Method 4500D was used to determine the dissolved ammonia ($\text{NH}_3_{(aq)} + \text{NH}_4^+$) concentration of effluent and influent samples from the anaerobic digesters (APHA, 1995). Samples were centrifuged at 3900 rpm in an IEC model Centra7 for 20 minutes in 15 ml Fisherbrand HDPE centrifuge tubes. An Orion ammonia electrode model 95-12 and Fisher Accumet model 750 were used for the measurement of dissolved ammonia. Calibration curves using standard concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mg $\text{NH}_3\text{-N/l}$ diluted from a Fisherbrand Ammonia stock solution of 1000 mg $\text{NH}_3\text{-N/l}$ were done before each set of measurements. Ten millilitres of sample from the centrifuge tube was diluted with 10 ml of distilled water in a 50 ml beaker. Samples were left to come to room temperature for one hour while the calibration curve was setup. To ensure a pH greater than 11, 0.5 ml of 10N NaOH was added to the sample prior to taking a mV reading with the electrode. The beakers were placed on a Thermix model 120MR stirrer at 75% speed. Between each measurement, the ammonia probe and the stir bar were rinsed with distilled

water and dried with Kimwipes®. The ammonia probe was stored in a 1000 mg NH₃-N/l between each measurement and for long-term storage.

3.3.3 Biogas Composition

Biogas samples collected for gas analysis from the semi-continuous reactors were collected by removing the biogas line attached to the tipping bucket and fixing it to a Tedlar bag model TB002-0909 from Canadian Life Sciences in Guelph, ON. The air in the Tedlar bag was removed by air suction with a peristaltic pump. The GC was turned on following the lab instructions and warmed up for two hours prior to measuring the first sample. Using a 1 ml BD syringe, a sample was removed from the Tedlar bag and half the sample was wasted prior insertion in the injection port of the gas chromatograph. The GC used is a Hewlett Packard model 5710A equipped with a thermal conductivity detector that used helium as carrier gas and an integrator model 3380A. National Instruments LabView version 6.0 is used to calculate % volume of nitrogen, methane and carbon dioxide gases. The values were recorded directly from the computer monitor. Biogas composition was measured with a near infra-red (NIR) detector in the first set of BMP assays when the manometer tube was full (biogas sample exceeded 400ml). The sensors was manufactured by Serwin, model SR2-DO and equipped with CH₄ (0-100 %/v) and CO₂ (0-100 %/v) NIR sensor and H₂S electrochemical sensor (0-2000 ppm). A Milipore 50mm diameter hydrophobic filter was used to pre-treat the sample for moisture. Since the H₂S sensor was expired, the hydrogen sulphide readings for this sensor were not recorded.

3.3.4 Biogas Production

A water displacement method was used to measure the biogas production from BMP bottles. The manometer consisted of a Tygon ¾ inch (1.90 cm) ID tube fixed to a plywood support. A ruler strip in metal graduated to 1 mm was fixed next to the section of the pipe used to measure the water displacement. One end of the tube was opened to atmosphere and the other end was fixed to a 3-way valve. The manometer was calibrated manually with a BD plastic syringe of 60 ml. Biogas production was measured by inserting a ¾ inch 21 gage BD (21G¾) needle in the black halobutyl stopper. The change in water level measured on the metal ruler was recorded and multiplied by the horizontal cross-sectional area of the Tygon tube to obtain the biogas volume vented from the bottle. For the semi-continuous reactors, the biogas production was measured using a wet tip meter. A wet tip meter was installed in the

incubator room for each semi-continuous reactor. Each tip of the bucket represented a volume of biogas and actuated an Orion self-powered electronic counter. The value of the counters was recorded daily before feeding each reactor.

3.3.5 Total Chemical Oxygen Demand

Total chemical oxygen demand was measured using the closed reflux colorimetric method described in Standard Method 5220D (APHA, 1995). Pre-treatment of particulate samples was necessary. Homogenization of the samples was done with a Brinkman Polytron 3000. In a 250 ml borosilicate Kimax bottle, 10 ml of sample measured in a 50 ml graduated cylinder and 90 ml of distilled water was mixed. The Kimax bottle was placed in a Pyrex beaker #3140 filled with ice. The shaft of the homogenizer was placed $\frac{3}{4}$ inch from the bottom of the Kimax bottle. The homogenizer was turned on for 3 minutes at 25000 rpm. The temperature of the sample was monitored and did not exceed 26 °C. Further sample dilution was carried out in 100 ml plastic dilution bottles and then transferred in 16 x 125 mm vials with a Teflon cap. Calibration curves were set up with the following standard COD concentrations: 0, 100, 200, 300, 400, two 500 (one used as a check for the second batch), 600, 700, two 800 (one used as a check for the second batch), 900 and 1000 mg O₂/l. The standards were prepared by making dilutions of a potassium biphthalate Fisherbrand stock solution with a concentration of 1000 mg O₂/l. All Fisherbrand stock COD solutions were discarded prior to the expiry date, typically three months. The procedures to prepare the acid and digestion solutions can be found in the Standard Method 5220D (APHA, 1995). The COD vials were prepared for digestion by mixing 2.5ml of sample, 1.5 ml of digestion solution and 3.5 ml of sulphuric acid reagent using 5 ml Brinkman automatic dispensers. The vials were capped, vortexed with a Fisher Genie vortex and placed in a Fisherbrand convection oven model 771 at 150°C for two hours. After reflux, the vials were cooled to room temperature in the absence of light for one hour. The absorbance was measured using in Fisher 21D spectrophotometer set at a wavelength of 600nm.

3.3.6 Total and Volatile Solids

Standard Method 2540G was used to determine total and volatile solids (APHA, 1995). Porcelain crucibles were scrubbed in soapy water, rinsed, left overnight in 2.5% HCl acid bath and dried in a Fisher Isotemp 600 series convection oven at 105 °C for 2 hours. After drying the crucibles, they were placed in desiccators for a minimum of 30 minutes before

being weighed. The empty crucibles were weighted on a table-top Denver S402 digital scale. The scale was calibrated daily using a standard certified 200 g weight. A volume of sample (25 to 35 ml) was added to the crucible and placed in the oven to be dried overnight (at least 12 hours) The weight of the empty dish and approximately 25 to 35 ml of the sample was added to the dish and recorded before leaving the dishes overnight (typically more than 12 hours) in a Fisher Isotemp 600 series convection oven at 105 °C. After drying, the samples were allowed to cool in desiccators for at least 30 minutes and then weighed. Two crucibles at a time were ignited in a Barnstead Thermolyne muffle furnace for 45 minutes at 550°C. These crucibles were first cooled on lab counters for 5 minutes before being placed in desiccators, for at least 30 minutes to be cooled to room temperature. The weight of the cooled crucible with ashed sample was recorded. Percent total solids and volatile solids were calculated based on the equations in Standard Method 2540G (APHA, 1995).

3.3.7 Volatile Fatty Acids

Acetic, butyric and propionic acids were determined by a standard method of Ackman (1972), using a Hewlett-Packard 5840A GC equipped with a flame ionization detector, an auto sampler, a 5840 model integrator and a Chromosorb 101 packed column. The oven temperature was 180°C, the injector temperature was 250°C and the detector temperature was maintained at 350°C. Helium saturated with formic acid flowing at 15 ml/min was the carrier gas. The GC was calibrated by the technician of the Environmental Engineering laboratory using a solution of 0.5 ml of an internal standard with 2000 mg/l of isobutyric acid and 0.5 ml of VFA standard mixture containing 2000 mg/l of acetic, butyric and propionic acid. Effluent and influent samples were centrifuged in an Eppendorf model 5415 at 14000 rpm for 30 minutes in 1.5 ml centrifuge plastic cups. Equal amounts of supernatant and 2000 mg/l isobutyric acid internal standard (0.5 ml) was added to vials, capped with Parafilm® and stored in the refrigerator until further processing. The samples were placed in the auto-sampler and 1 ml of sample was injected in the column. The printed results of the samples were then recorded and sent to the University of Guelph, Campus d'Alfred research station. Total VFA was calculated in section 4.2 as the average of the sum of acetic, propionic and butyric acids. If the sum was less than 30 mg/l, it was considered below the minimum detection limit, since it was 10 mg/l for each individual acid.

3.4 Sample Preservation

After the end of the BMP assays and to store original samples of manure and inoculum, sludge samples were stored in one bottle for further tests of TS and VS. Samples of the effluent from the pilot reactors were stored in two different bottles; a raw sample for Alkalinity, TS and VS and an acidified sample that could be kept for longer storage to analyse VFAs, Ammonia and COD. Table 3.3 summarizes how samples were preserved for the various analyses. All bottles were rinsed with tap water, washed with soap, soaked in 2.5% HCl acid bath water and rinsed twice again with tap water.

Table 3.3 Preservation Methods for Sludge Samples according to APHA (1995)

Sample Preservation for BMP				
Analyses Required	Bottle Size	Material Type	Preservation	Max. Storage Time
TS	500ml	Borosilicate	Refrigerated	2 weeks
VS				
Sample Preservation for Semi-Continuous Reactors				
Analyses Required	Bottle Size	Material Type	Preservation	Max. Storage Time
Alkalinity	500ml	HDPE	Refrigerated	1 week
TS				2 weeks
VS				2 months
sCOD	250ml	HDPE	Added 1ml of H ₂ SO ₄ , refrigerated	1 week
VFA				3 weeks
Ammonia				2 months
COD				

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterization of Inoculum and Substrates

The liquid dairy manure from the organic milking barn at the University of Guelph, Campus d'Alfred was mixed with 6 co-substrates in different ratios of volatile solids. The co-substrates used in this study were divided into two groups: liquid (1-18%TS) which included glycerine, grease trap waste, DAF sludge and whey, and solid (18%TS and up) which included corn silage and canola meal. The solids characteristics of the substrates are summarized in Table 4.1. COD for these solid substrates were not done for these materials because of their particulate nature requiring very high dilution ratios (1 in 100000) that would render the analysis with the current equipment unreliable. For the liquid substrates, their COD values are presented in Table 4.2. Results of nitrogen, carbon and feed analyses are presented in Table 4.3. These parameters were analysed by A&L Canada Laboratories and University of Guelph Lab Services both located in Guelph, ON. The DAF sludge sample was sent to the food division of Maxxam Analytics for fatty acids analysis and the results are presented in Table 4.4.

Table 4.1 Total Solids (TS) and Volatile Solids (VS) Characteristics of Substrates used in Laboratory Studies

Substrate	Total Solids (g TS/g wet wt.)		Volatile Solids (g VS/g wet wt.)		% VS of total solids	n =
	Mean	STDEV	Mean	STDEV		
Liquid Dairy Manure	0.053	0.013	0.042	0.012	79.6	24
Manure Inoculum	0.048	0.003	0.034	0.002	71.2	5
Corn Silage	0.358	0.029	0.337	0.026	94.5	8
Canola Meal	0.900	0.011	0.834	0.008	93.5	6
Cheese Whey	0.061	0.007	0.049	0.006	80.0	8
FOG - Grease Trap Waste	0.149	0.005	0.143	0.005	96.2	4
FOG - DAF Sludge	0.766	0.199	0.745	0.211	96.8	5
Raw Glycerine	0.879	0.009	0.857	0.012	97.2	5

Table 4.2 COD Characteristics of Liquid Substrates Used in Laboratory Study

Substrates	Total COD (mg/l of COD)			Soluble COD (mg/l of sCOD)		
	Mean	STDEV	n =	Mean	STDEV	n =
Liquid Dairy Manure	75209	10998	18	6927	3252	21
Cheese Whey	72534	695	2	70938	868	2
Raw Glycerine	934117	34497	3	-	-	-
DAF Sludge	52729	6540	3	-	-	-
Grease Trap	129542	72409	9	-	-	-

Table 4.3 Chemical Characteristics of Substrates Used in Laboratory Study

Analysis	Corn Silage	Canola Meal	DAF Sludge	Glycerine	Liquid Dairy Manure	Whey
Crude Protein (%TS)	8.19	36.00	0.31	1.38	15.25	13.00
Total Fat (%TS)	2.67	17.30	55.90	15.00	2.64	0.08
Total Sugar (%TS)	47.30	12.80	-	-	-	60.10
Crude Fibre (%TS)	18.28	13.26	-	-	-	-
BTU/lb	-	-	7282	7551	7012	-
Total Nitrogen (TKN-N %w/w)	0.42	4.62	<0.06	<0.06	0.13	0.14
Total Carbon (%TS)	45.7	50.5	70.1	35.7	39.1	44.9
Inorganic Carbon (%TS)	<0.05	0.46	<0.05	<0.05	0.74	0.11
Organic Carbon (%TS)	45.7	50.0	70.1	35.7	38.4	44.8

Table 4.4 Fatty Acids Analysis for DAF Sludge in g/100g of Sample

C16 Palmitic Acid	14.6
C18 Stearic Acid	5.49
C18:1 Oleic Acid	26.3
C18:2 Linoleic Acid	18.7
Fat (GC/FID)	75.1
Saturated Fatty Acids	23.4

The solids concentration of the Campus d'Alfred dairy manure were similar to values obtained by Ghaly (1996) and Lehtomaki et al. (2007), where the %TS values ranged from 6.5 to 6.6 % and VS values ranged from 5.3 to 5.4 %. However, Wright et al. (2004) and Amon et al. (2006) reported much higher solids values for dairy manure with TS values ranging from 8.9 to 15.9 %. These differences in solids concentration is due to varying farm management, primarily with animal feed and handling of milkhouse waste (Amon et al.,

2006). The average COD concentration (Table 4.2) for the Campus d'Alfred dairy manure was also similar to literature, where Ghaly (1996) reported a dairy manure COD of 74.9 g/l. The VS content of the liquid dairy manure varied slightly during the laboratory trials. For the second batch of BMP tests, the VS content was 6.2%VS as opposed to 4.6%VS for the first batch. The variation on the VS content of the liquid dairy manure at the campus barn is explained in details in section 4.2.1.

The corn silage obtained from Terryland Farm was found to be in between wax and full ripeness stage of harvest with measured TS values of 36 % (Table 4.1). Literature reported similar solids values for corn silage with TS values ranging from 29 to 53 % and VS values ranging from 27 to 51 % (Amon et al., 2007).

Indifferent from being issued by solvent based extraction or cold-pressed, the cold-pressed canola meal obtained from Mercier Mill Services was measured to have TS and VS values of 90% and 83% (Table 4.1), respectively. These values agree with the TS range reported of 90 to 93 % TS (Aarke et al., 2008; Canola Council of Canada, 2008). Fat and protein content match the reported amount for cold pressed canola meal reported by Aarke et al. (2008)

The cheese whey collected at Skotidakis Goat Farm was measured to have a COD of 72.5 g/l and 71 g/l of sCOD. TS content of cheese whey was 6% with a TS/VS of 80%. As reported in the literature for cheese whey, values of COD, %TS and %VS, TKN-N, sugars and proteins used in our laboratory studies did not differ significantly from values published in the literature (Baserga, 2000; Goblos et al. 2008; Handajani, 2004; Ghaly, 1996 and Wildenauer and Winter, 1985).

COD values obtained for grease trap waste and DAF sludge were taken from a previous interim report by Crolla and Kinsley (2008.). TS analyses reported a 77%TS for DAF sludge and 15% for grease trap waste. Values reported in the literature for DAF sludge were higher than the range reported of 5 to 24 % TS which might be indicative of the origin of the waste (Baserga, 2000; Kuhn 1995; Braun et al. 2003 and Membrez and Fruteau de Laclos, 2002). The DAF sludge obtained in this laboratory trial was from a meat processing plant, rather than a slaughterhouse as reported in the literature. On the other hand, TS values measured for

grease trap waste were lower than reported (25-70 %TS) from the same source. From Table 4.4, the high amount of saturated fatty acids renders this waste undesirable for biodiesel production and must be blended with other oils or fats with lower content of saturated fatty acids (Chhetri et al., 2008). DAF sludge also has a low nitrogen content which might represent a possible problem for anaerobic bacteria growth if this waste was treated as a single substrate in anaerobic digestion.

The TS and VS values obtained for the raw glycerine used in this study were 88 %TS and 87 %VS (Table 4.1), respectively, and these values corresponds with the values obtained from biodiesel production in California which reported TS of 87.8 % and VS values of 80.9 % (Chen et al., 2008). The glycerine also contained a 15.0 % crude fat (Table 4.3) which is considerably lower than the 60% fat in the glycerol from a biodiesel laboratory trial using Waste Vegetable Oil (WVO) in a study conducted by Thompson and He, 2006. The study indicated that a high amount of soaps and unreacted glycerides were present in the crude glycerol, produced from waste vegetable oil because of the Free Fatty Acids (FFA) content of 4.4%. Moreover, they did not neutralise that acidity of the WVO before the transesterification process, which led to the formation of soaps and unreacted glycerides, lower yield and higher viscosity of the biodiesel (Chhetri et al., 2008). Glycerine also has a low nitrogen content which might represent a possible problem for anaerobic bacteria growth if this waste was treated as a single substrate in anaerobic digestion.

4.1 Biochemical Methane Potential Assays

4.1.1 Biogas Production, Yield and Solids Removal from the First Design of BMP Assays

Mesophilic digestion of the co-substrates studied was carried out for approximately 70 days for the first batch and 40 days for the second batch in duplicates. Table 3.1 refers to the different mixes of volatile solids tested in the BMP experiments at an F/M ratio of 2 VS substrate: 1 VS inoculum with addition of alkalinity. The first batch (corn silage, canola meal, whey and grease trap) contained 0.68 gVS of substrate and the second batch (glycerine and DAF sludge) contained 0.42 gVS of substrate per BMP assay.

Biogas composition was measured during peak biogas production for certain bottles that achieved a daily biogas production exceeding 400 ml. These measurements were completed using the Serwin SR2-DO meter. Methane concentrations ranged from 55 to 83 %/vol. with an average of 72 %/vol. from 19 measurements. For the second batch of BMP experiments, two samples were measured for the biogas composition on October 28; one week after the BMP assay was prepared. One bottle of DAF Sludge 60% VS had a composition of 4.6 %/vol. of N₂, 62.5 %/vol. of CH₄ and 32.9 %/vol. of CO₂. One of the Manure (Control) bottles had a biogas composition of 9.7% /vol. of N₂, 45.8 %/vol. of CH₄ and 44.5 %/vol. of CO₂.

The criteria to select the optimum VS mixtures for the various co-substrate was that 80% of the cumulative biogas production occurred in the first 30 days of digestion and that no signs of lag or inhibition was present throughout the digestion. Since the ratio of VS substrate: VS inoculum (F/M ratio) and the ratio of VS co-substrate: VS liquid dairy manure is fixed by the design of the BMP, the amount of VS from each substrate was calculated using Equation 4.1 and 4.2. From these VS ratio, the quantities of fresh matter are summarized in Table 4.5 and Table 4.6. Certain values from Table 4.1 cannot be used directly with Equation 4.1 and Table 4.5 to determine the fresh mass of feedstock to add since the measurements of TS and VS varied throughout the season as explained in section 4.1.

Table 4.5 Feedstocks by Mass Added to First Batch of the First Design of BMP with 0.68 gVS of Substrate

Bottle ID	Liquid Dairy Manure (g)	Corn Silage (g)	Grease Trap Residue (g)	Cheese Whey (g)	Canola Meal (g)	Inoculum (g)
M1	159.3					100.0
S1		21.0				100.0
G1			47.8			100.0
C1					8.1	100.0
W1				123.6		100.0
M7S3	111.5	6.3				100.0
M5S5	79.6	10.5				100.0
M7G3	111.5		14.4			100.0
M4G6	63.7		28.7			100.0
M8C2	127.4				1.6	100.0
M6C4	95.6				3.2	100.0
M7W3	111.5			37.1		100.0
M3W7	47.8			86.5		100.0
I1						100.0

Table 4.6 Feedstocks by Mass Added to Second Batch of the First Design of BMP with 0.42 gVS of Substrate

Bottle ID	Liquid Manure (g)	DAF Sludge (g)	Raw Glycerine (g)	Inoculum (g)
M6DAF4	47.4	1.6		100.0
M4DAF6	31.6	2.3		100.0
DAF1		7.8		100.0
M6GLY4	47.4		1.7	100.0
M4GLY6	31.6		2.6	100.0
GLY1			8.5	100.0
MS1	80.0			100.0
IS1				100.0

Equation 4.1 is used to calculate the amount of VS from the substrate that needs to be added to the bottle.

$$MassVS_{substrate} = 100g \times VS_{inoculum} \times (F / M) \quad (4.1)$$

where $MassVS_{substrate}$ is the mass of volatile solids from the substrate, $VS_{inoculum}$, the volatile solids concentration of inoculum, and F/M , the food to mass ratio, here expressed as 2 VS substrate : 1 VS inoculum. To calculate the VS of the co-substrate present in the bottle, the amount of VS of the co-substrate is determined by Equation 4.2.

$$MassV_{S_{co-substrate}} = MassV_{S_{substrate}} \times \left(\frac{V_{S_{co-substrate}}}{V_{S_{substrate}}} \right) \quad (4.2)$$

where $MassV_{S_{co-substrate}}$ is the mass of volatile solids from the co-substrate and $V_{S_{co-substrate}}$ the volatile solids concentration of co-substrate. From Equation 4.1 and Equation 4.2, the amount of volatile solids of each constituent is known. The following section shows the biogas produced by the volatile solids added to the BMP assays

In Figure 4.1 and Figure 4.2, biogas production of the substrates digested alone with a 2 VS substrate: 1 VS inoculum is shown compared to manure and inoculum.

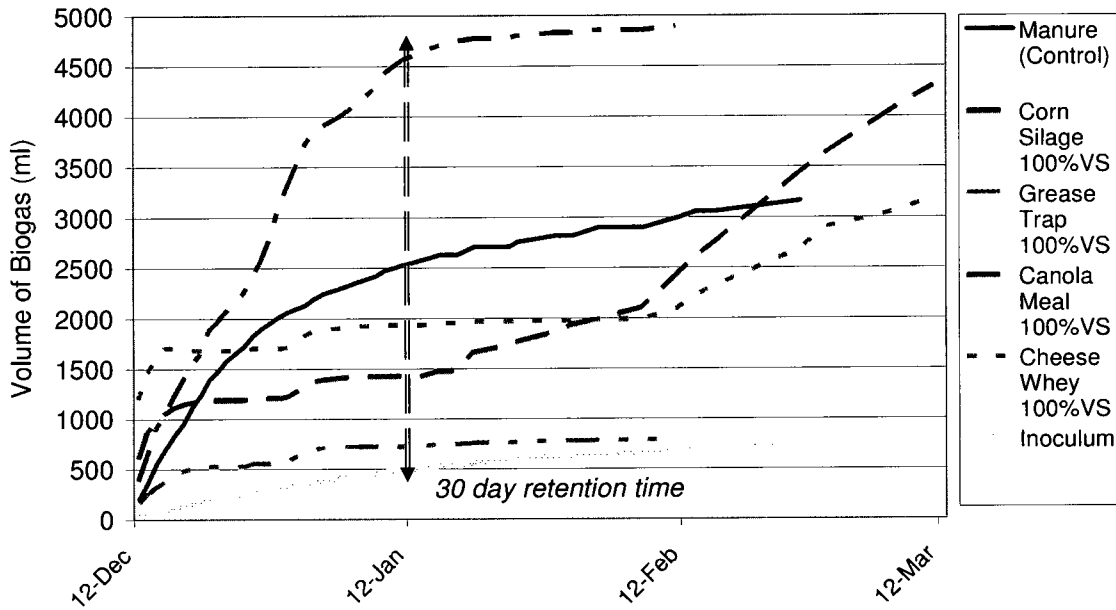


Figure 4.1 Cumulative Biogas Production from Co-Substrates Without Addition of Liquid Dairy Manure in the First Batch of BMP at a Fixed Organic Loading of 0.68 gVS per Assay

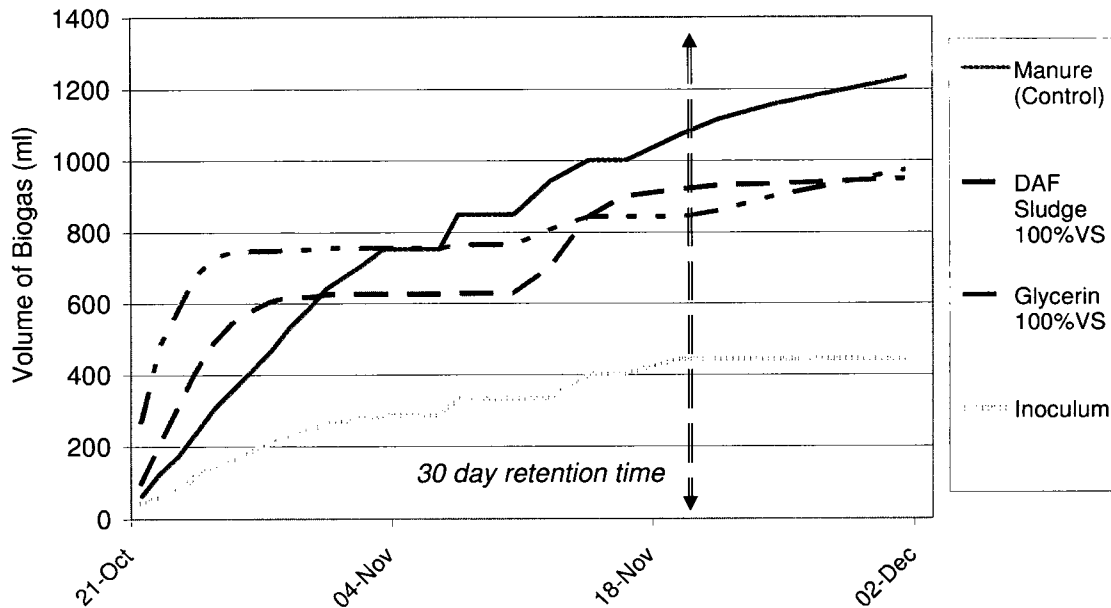


Figure 4.2 Cumulative Biogas Production from Co-Substrates Without Addition of Liquid Dairy Manure in the Second Batch of BMP at a Fixed Organic Loading of 0.42 gVS per Assay

It can be noted from Figure 4.1 and Figure 4.2 that the DAF sludge, glycerine and grease trap waste do not digest without liquid dairy manure at a 2 VS substrate: 1 VS inoculum ratio with addition of alkalinity based on the selection criteria of best mixes. Their cumulative biogas production (calculated with the average cumulative biogas production from the inoculum removed) after was significantly lower (DAF 691 ml and glycerine 713 ml for 40 days; grease trap 192 ml for 60 days) than liquid dairy manure. Although the cumulative biogas production of corn silage and cheese whey (4300 and 3195 ml) exceeded liquid dairy manure (3151 ml), there were signs of inhibition and lag at the same F/M ratio and most of the biogas produced did not occur before 30 days of digestion. DAF Sludge 100%VS and Glycerine 100%VS bottles showed a much higher biogas production rate in the first week of digestion, but tapered off after 8 days due to accumulation of VFAs. The rapid degradation of these substrates led to the accumulation of VFAs and was suspected to have caused the inhibition of the digestion process (Pind et al., 2003). On the other hand, digestion of canola meal with inoculum did not lead to the accumulation of VFAs and biogas was produced at a faster rate and had a higher cumulative biogas production after 60 days (4214 ml) than digestion of liquid dairy manure (3151 ml). In this case, due to the particulate nature of

canola meal, it is suspected that hydrolysis was the rate limiting step in the biogas production (Grady et al., 1999). Six of the twelve best mixtures of co-substrates to manure VS are shown in Figure 4.3 and Figure 4.4. In Figure 4.3, the first BMP batch ran for 70 days, while the second batch ran for 40 days. Other mixes of co-substrate and manure, along with the measurements taken using the manometer are included in Appendix A.

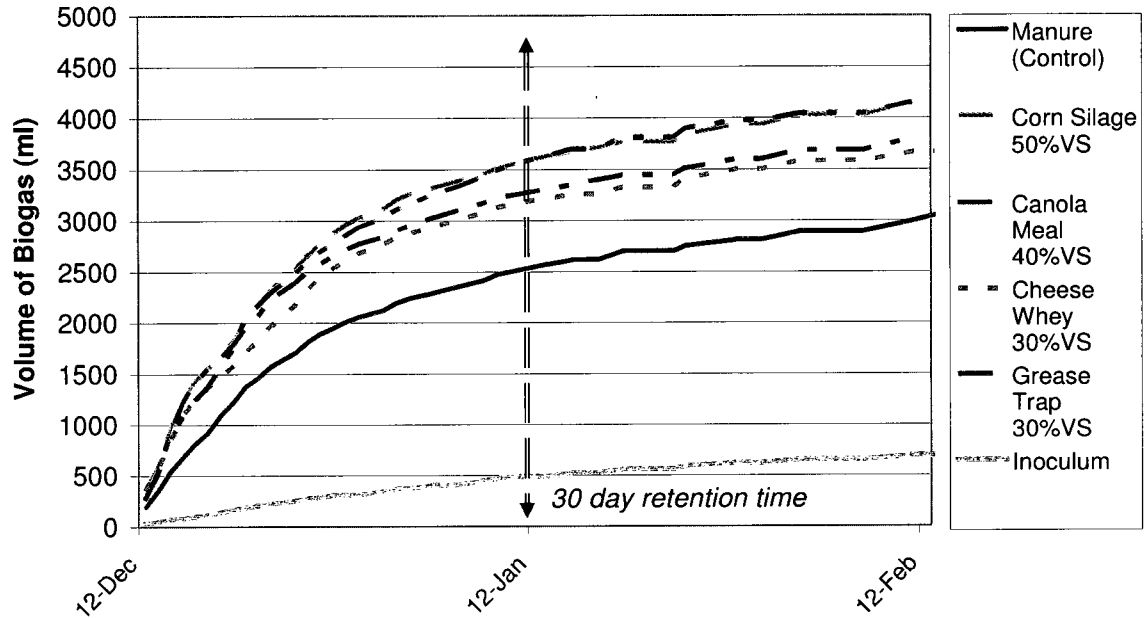


Figure 4.3 Cumulative Biogas Production for Best Mixes of Co-Substrates and Liquid Dairy Manure in the First Batch of BMP at a Fixed Organic Loading of 0.68 gVS per Assay

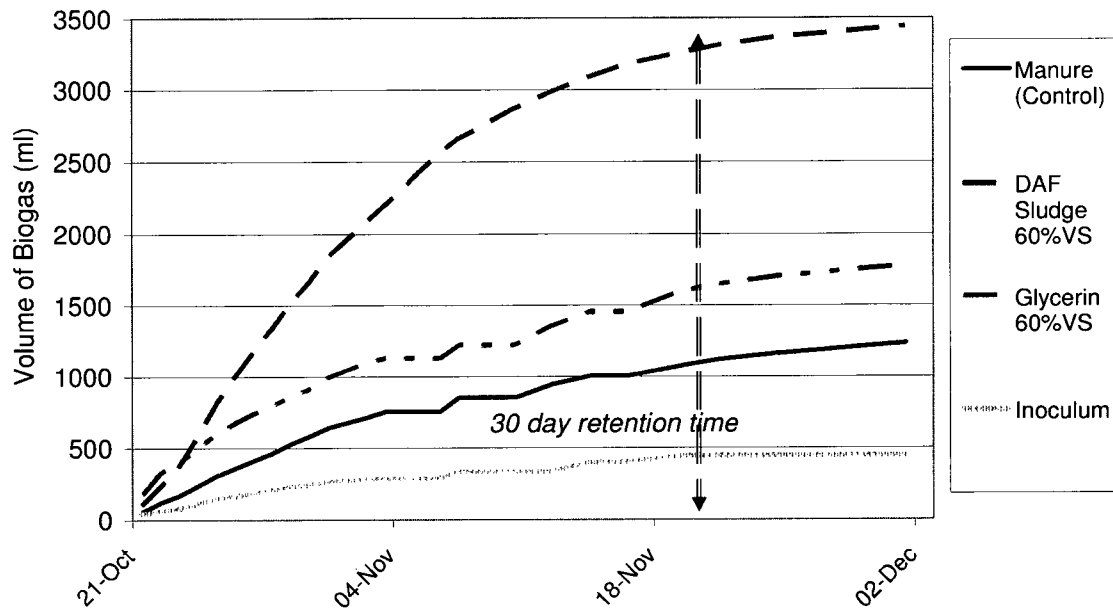


Figure 4.4 Cumulative Biogas Production for Best Mixes of Co-Substrates and Liquid Dairy Manure in the Second Batch of BMP at a Fixed Organic Loading of 0.42 gVS per Assay

In the case of the DAF 60 %VS, Glycerine 60 %VS, Corn Silage 50 %VS, Canola Meal 40 %VS, Grease Trap 30 %VS and Cheese Whey 30 %VS, 80% of their cumulative biogas production occurred within 30 days of digestion and did not show signs of methanogenic inhibition. Braun et al. (2003) indicated that 12 days for digestion of DAF sludge and 33 days for digestion of animal fat would be the minimum retention time for continuous digestion. From Figure 4.4, the DAF 60%VS cumulative biogas production was not completed within 12 days of digestion. Due to the nature of the waste (meat processing plant), the recommended minimum retention time of 33 days should be required as opposed to the 12 days recommended by Braun et al. (2003). As recommended by Membrez and Fruteau de Laclos (2002), high amounts of LCFA in the DAF sludge shown in Table 4.4 caused inhibition. Figure 4.5 illustrates all mixtures of VS of DAF sludge with liquid dairy manure. Both the DAF 40 %VS and 60 %VS produced the most cumulative biogas, 328% and 244% greater than the liquid dairy manure control bottle of the second batch of BMP assays.

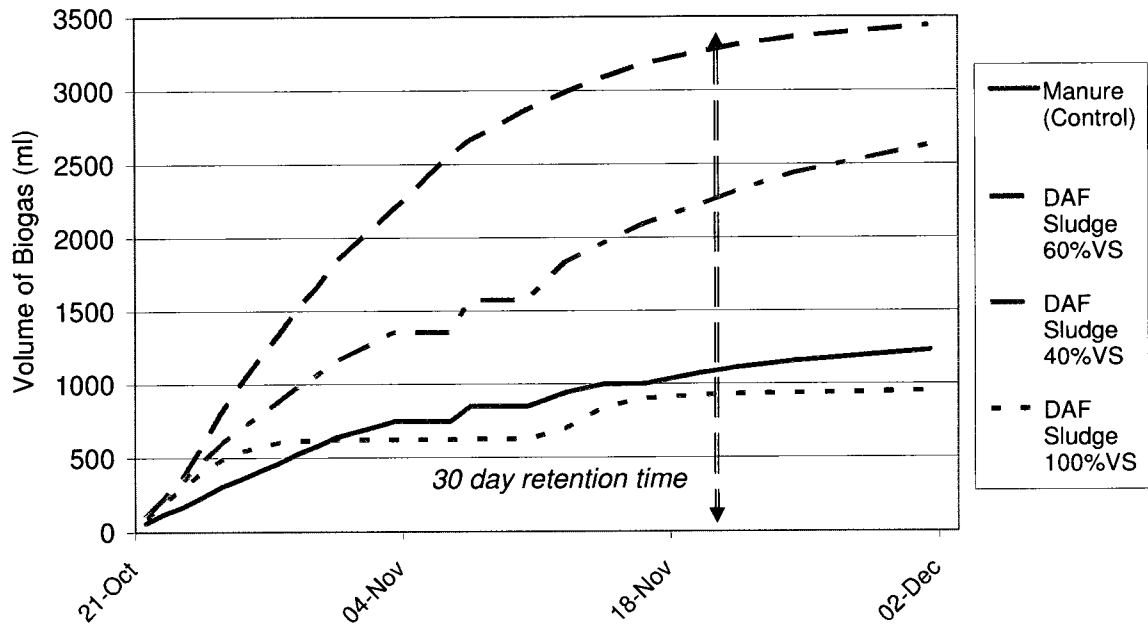


Figure 4.5 Cumulative Biogas Production of Mixes of Liquid Dairy Manure with DAF Sludge in the Second Batch of BMP at a Fixed Organic Loading of 0.42 gVS per Assay

Although Grease Trap 60 %VS mixture did not meet the criteria at a 2 VS substrate: 1 VS inoculum ratio with addition of alkalinity, it did produce more cumulative biogas (3247 ml) than the Grease Trap 30 %VS assay (2329 ml) after 70 days of digestion. Braun et al. (2003) indicated that food leftover from fast food restaurants would require a minimum of 33 and 35 days of continuous digestion. Figure 4.6 clearly demonstrates that the Grease Trap 60 %VS had a lag that approximately lasted 3 weeks due to acidification and did not produced 80% of its cumulative biogas before the 30 day retention mark.

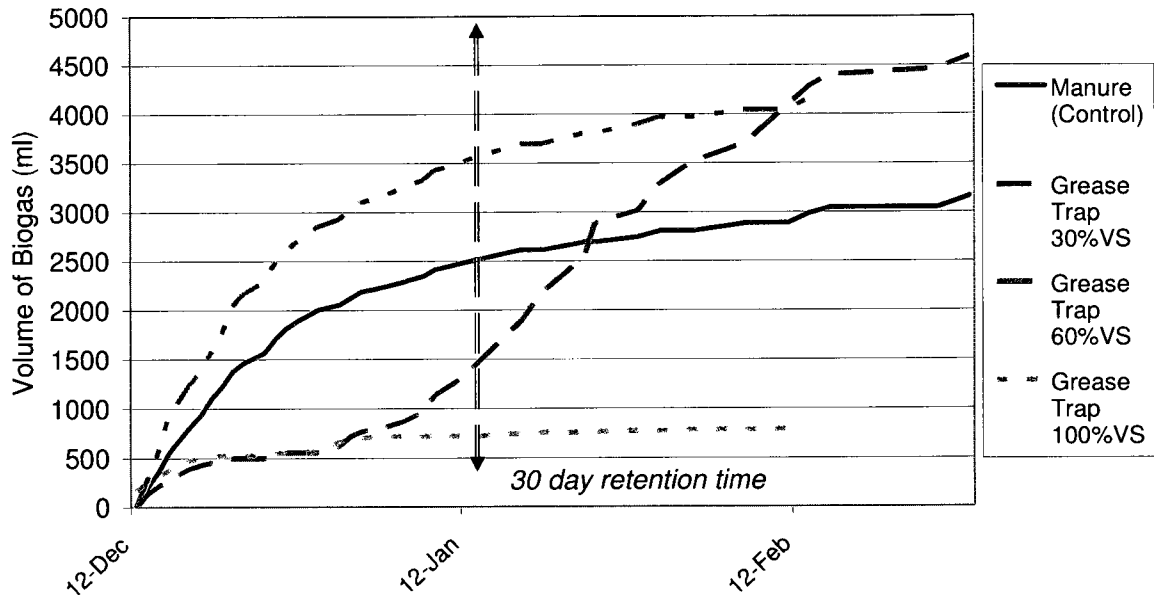


Figure 4.6 Cumulative Biogas Production from Mixes of Liquid Dairy Manure and Grease Trap Waste at a Fixed Organic Loading of 0.68 gVS per Assay

A similar situation occurred with the digestion of whey in Figure 4.7. The Cheese Whey 70 %VS also produced more biogas than Cheese Whey 30 %VS, but the Cheese Whey 70 %VS produced 80% of its cumulative biogas after the 30 day retention mark and clearly showed signs of methanogenic inhibition due to high accumulation of VFAs. The three mixes also had an initial biogas production rate higher than manure, mainly due to the rapid degradation of carbohydrates present in cheese whey (Gelegenis et al., 2007).

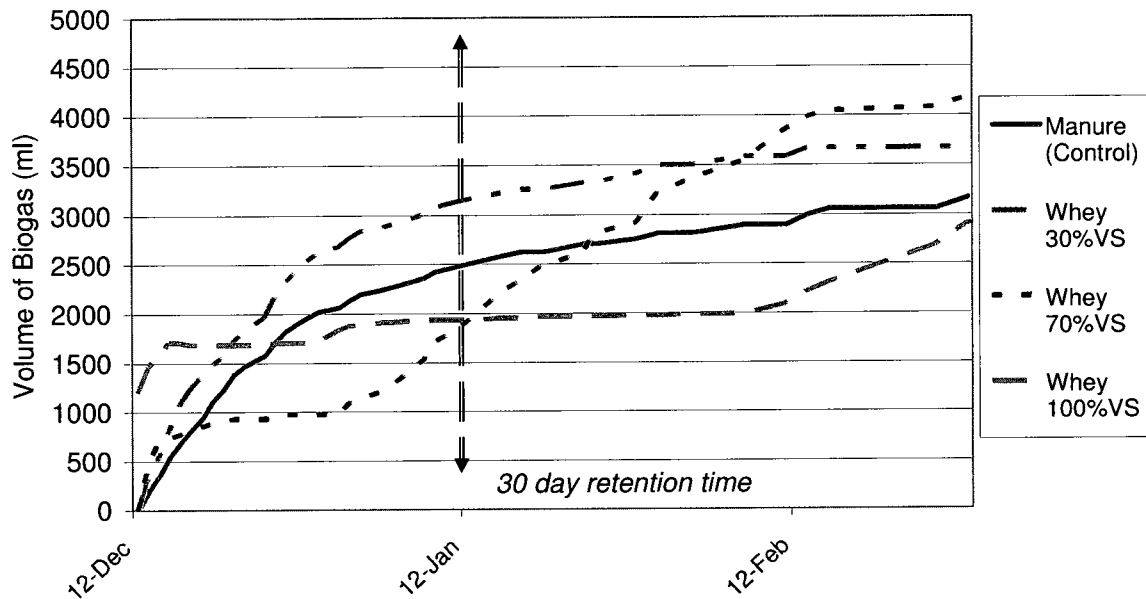


Figure 4.7 Cumulative Biogas Production from Mixes of Liquid Dairy Manure and Cheese Whey at a Fixed Organic Loading of 0.68 gVS per Assay

The cumulative biogas production of all canola meal VS mixes produced more cumulative biogas and occurred at a higher rate than the Manure (Control). As shown in Figure 4.8, they also produced 80% of their biogas before the 30 day retention time. It is also important to note that both Canola Meal 100 %VS and Canola Meal 40 %VS exhibited the same biogas production rate until December 27. After this point, the Canola Meal 100 %VS biogas production rate unexpectedly increased. It could be expected that the anaerobic culture acclimated after this initial 3 weeks of digestion to the un-acclimated degradation of canola meal, a similar situation was exhibited later for glycerine by Chen et al. (2008).

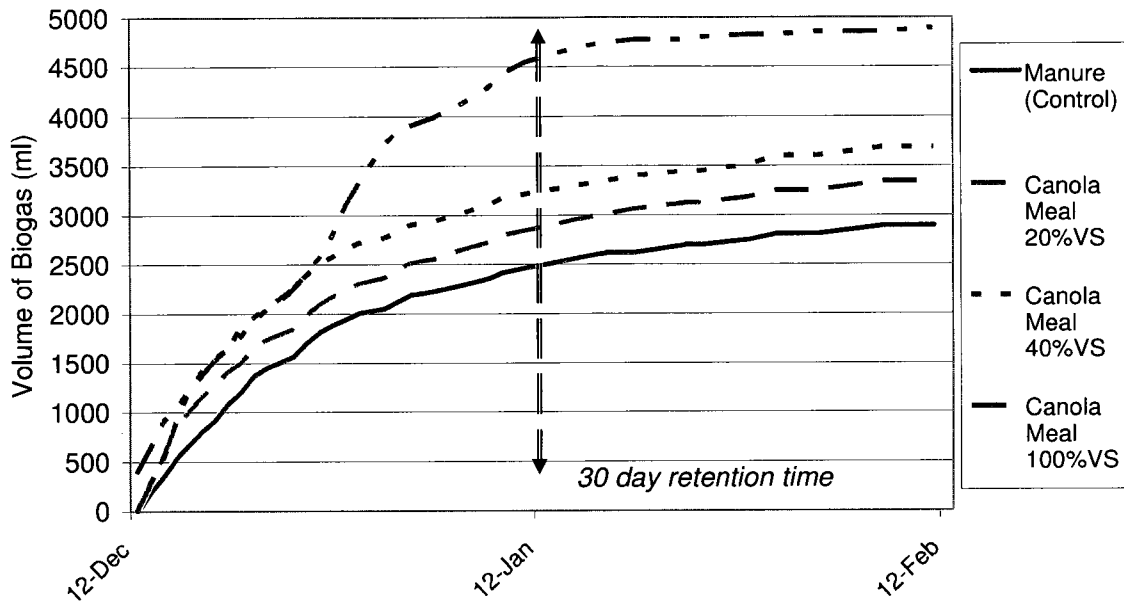


Figure 4.8 Cumulative Biogas Production from Mixes of Liquid Dairy Manure and Canola Meal at a Fixed Organic Loading of 0.68 gVS per Assay

As for the digestion of corn silage, only the pure substrate (Corn Silage 100 %VS) digested at a 2 VS substrate: 1 VS inoculum ratio, with addition of alkalinity, did not meet the criteria of producing most of its biogas within the first 30 days of digestion. This fact emphasises the importance of the buffering capacity of manure and sensitivity of single substrate digesters widely reported in the literature (Braun and Wellinger, 2003; Baserga, 1998). As shown in Figure 4.9, both mixes of corn silage and manure had the same biogas production rate until December 27. After this date, Corn Silage 50 %VS biogas production rate slightly increased until digestion was complete.

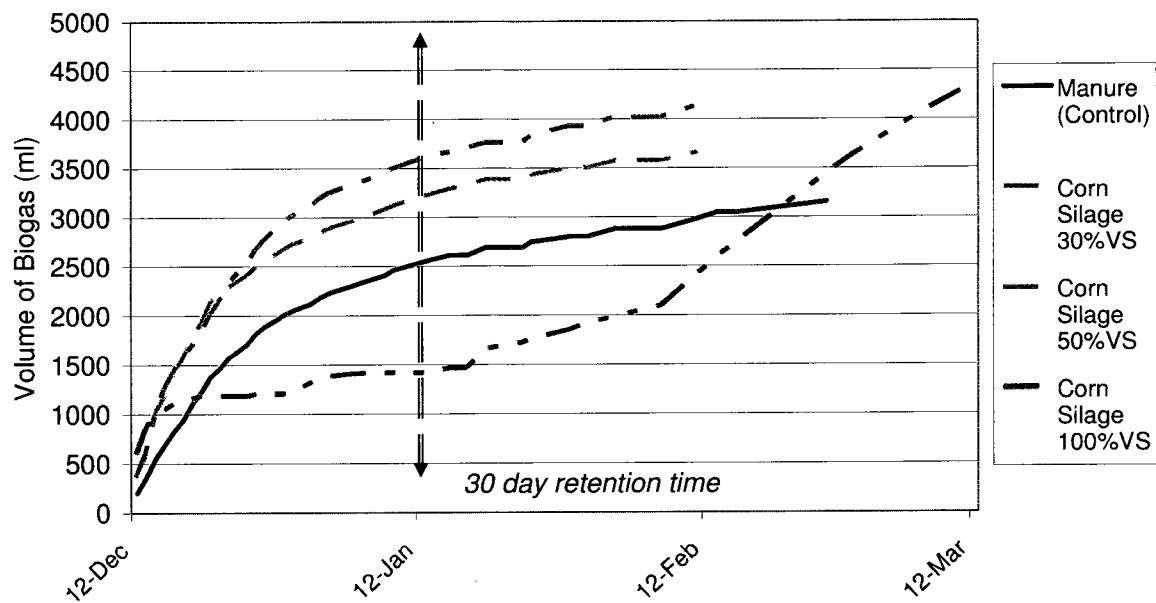


Figure 4.9 Cumulative Biogas Production from Mixes of Liquid Dairy Manure and Corn Silage at a Fixed Organic Loading of 0.68 gVS per Assay

The same observation for the corn silage BMP assays was also seen with the BMP assays of glycerine in Figure 4.10 where the rate of biogas production of Glycerine 60 %VS and Glycerine 40 %VS were similar for the first three weeks (19 days), then increased slightly on November 12, 2008 until complete digestion. It should be noted that these BMP assays were not conducted at the same time (and possibly not exposed to the same environmental and experimental conditions) as the BMP assays of canola meal and corn silage, where these two co-substrates saw an increase in biogas production rate after three weeks of digestion on December 27, 2007 during the first batch of BMP. No other explanation than acclimation of the inoculum to the substrate would explain the lag observed in those 3 assays done on two different batches.

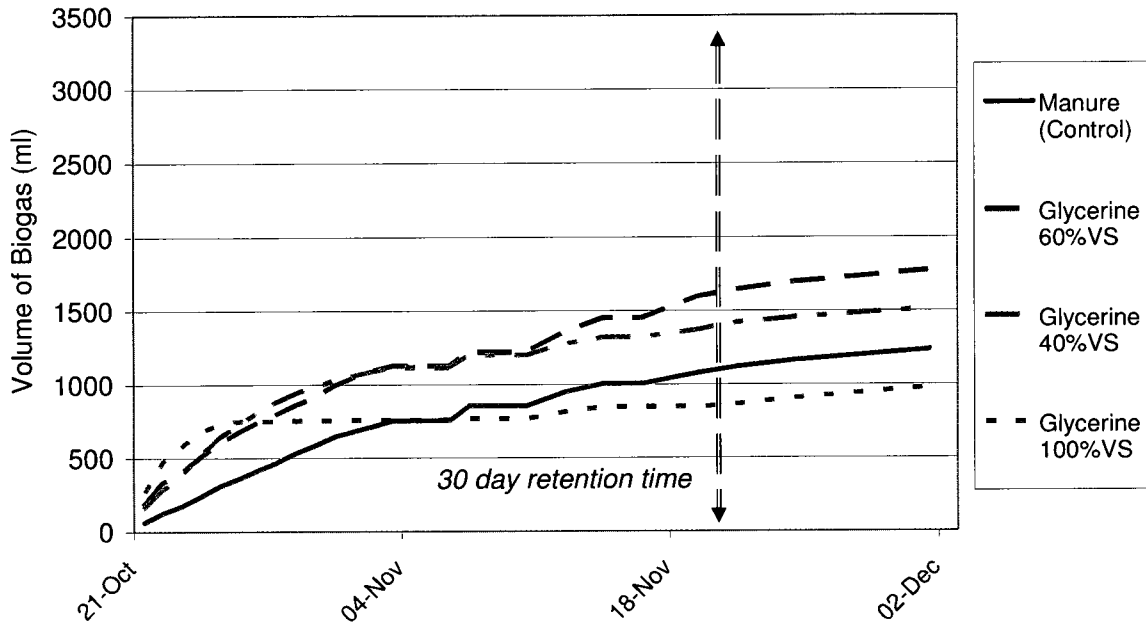


Figure 4.10 Cumulative Biogas Production from Mixes of Liquid Dairy Manure and Glycerine at a Fixed Organic Loading of 0.42 gVS per Assay

Although a small time lag during batch tests was observed in Chen et al. (2008), there is no indication that would explain the low biogas production from all glycerine BMP assays. All BMP assays produced less biogas than expected (1507 ml for the 40%VS Glycerine and 1772 ml for the 60%VS Glycerine assay). Acclimation to the glycerine by the bacteria would have led to a sudden increase in cumulative biogas production and acidification of the media would have stopped completely biogas production.

Inoculum biogas production for the first batch of BMP (n=4) during the 60 days of digestion produced an average of 726 ± 26 ml of biogas for 100 ml of active inoculum. To compare the activity of the inoculum in the second batch of BMP, the cumulative biogas production for the same time span must be used. After 40 days of digestion, the inoculum produced an average of 560 ± 37 ml of biogas for the first batch. At the end of the second batch, inoculum biogas production (n=2) was 258 ± 39 ml of biogas. The inoculum in the second batch produced 46% less biogas than in the first batch. This could be due to the fact that the inoculum in the first batch was fed two days before the BMP assays were conducted, as

opposed to the second batch, where the inoculum was fed 5 days before. For the liquid dairy manure used in the first batch of the BMP, the cumulative biogas production was significantly higher after 40 days of digestion (2133 ml) than the manure used in the second batch (971 ml). This difference in cumulative biogas production could be attributed to the difference in ration of the cows as explained previously in section 4.1. During the first batch of BMP, the dairy cows were housed all day inside the barn. For the second batch, the cows were still grazing and only came in the barn for milking and supplement feeding.

From this point, all necessary information is available to calculate the biogas yield. The biogas yield is expressed in volume of biogas produced per unit of volatile solids added to the BMP assay. Since the amount of volatile solids from the substrate and co-substrate is known, the biogas yield of the volatile solid mixture can be calculated by Equation 4.3

$$BiogasYield_BMP = \left(\frac{(Cum.BiogasBMP - Cum.BiogasInoc)}{MassVSsubstrate} \right) \quad (4.3)$$

Where *BiogasYield_BMP* is the biogas yield of the BMP in l/gVS_{added}, *Cum.BiogasBMP* is the cumulative biogas produced from the BMP assay, and *Cum.BiogasInoc* the average cumulative biogas produced from the inoculum. To determine the biogas yield of the co-substrate, an assertion has been set: the yield of biogas produced from liquid dairy manure (LDM) is the same in all BMP assays and is proportional to the amount of volatile solids present in the bottle, according to the VS co-substrate : VS liquid dairy manure ratio. In our case, two different batches of BMP were done and their respective liquid dairy manure biogas yield was asserted to be proportional to the amount of VS added. That being stated, the biogas yield of the co-substrate can be calculated by "removing" the biogas yield of liquid dairy manure present in the BMP assay depending on the VS co-substrate: VS liquid dairy manure with Equation 4.4.

$$BYield_Co-s = \left(\frac{BiogasYield_BMP - (BYield_LDM \times r_VS_LDM)}{r_VS_Co-s} \right) \times r_VS_Co-s \quad (4.4)$$

Where $BYield_{Co-s}$ is the biogas yield of the co-substrate, $BYield_{LDM}$ is the average biogas yield of liquid dairy manure, $r_{VS_{LDM}}$ the fraction of VS in the substrate from liquid dairy manure and $r_{VS_{Co-s}}$ the fraction of VS in the substrate from the co-substrate. Table 4.7 shows the average biogas yield of the two batches of BMP assays. The different mixes used for the BMP trials are summarized in Table 3.1.

Table 4.7 Biogas Yield of BMP Assays of Individual Substrates and Co-Substrates Digested with Liquid Dairy Manure

Substrate Digested Individually at 2 VS substrate: 1 VS inoculum	Mean Biogas Yield* of BMP Assay l/gVS _{added}	Mean Biogas Yield* of Co-Substrate l/gVS _{added}	Met Criteria of "Good" BMP **
Liquid Dairy Manure (LDM) First Batch	0.32	-	Yes
Liquid Dairy Manure (LDM) Second Batch	0.20*	-	Yes
Canola Meal (CC100)	0.62	-	Yes
Corn Silage (CS100)	0.17	-	No
Dissolved Air Flootation Sludge (DAF100)	0.10	-	No
Glycerine (GLY100)	0.10	-	No
Grease Trap Waste (FOG100)	0.03	-	No
Whey (W100)	0.21	-	No
Inoculum - First Batch	0.17	-	-
Inoculum - Second Batch	0.08	-	-
Mixes of VS (% based on VS of substrate, refer to Table 3.1)			
80% LDM + 20% CC	0.38	0.63	Yes
60% LDM + 40% CC	0.43	0.59	Yes
70% LDM + 30% CS	0.42	0.66	Yes
50% LDM + 50% CS	0.47	0.63	Yes
60% LDM + 40% DAF	0.54*	1.24	Yes
40% LDM + 60% DAF	0.78*	1.30	Yes
60% LDM + 40% GLY	0.29*	0.47	Yes
40% LDM + 60% GLY	0.37*	0.53	Yes
70% LDM + 30% FOG	0.48	0.86	Yes
40% LDM + 60% FOG	0.34	0.36	No
70% LDM + 30% W	0.41	0.63	Yes
30% LDM + 70% W	0.33	0.34	No
* Biogas yield reported for 40 days of digestion			
** 80% of cumulative biogas produced before 30 days and no signs of methanogenic inhibition			

The co-digestion of wastes with liquid dairy manure increased the biogas yield from 5 to 52% in the first batch and from 46 to 254% in the second batch. To compare the biogas yields, a one-way analysis of variance (ANOVA) was setup under the assumption that the data is continuous, approximately normally distributed and that the variance of each group is homogeneous. The null hypothesis is that there is no difference between the mean biogas yields of every group. The Minitab limited licence, student edition, version 15.1.0 (State College PA, USA) was used with the “ANOVA – One-way” function with a P-value of 0.05. All BMP assays were entered first to find if the null hypothesis has been rejected. The analysis of variance ($F_{22,22}=196.07$, $P<0.000$) showed that there was a significant difference between the biogas yields. Now that the null hypothesis has been rejected, post hoc testing can be used to compare the yield of mixes of co-substrate VS to the yield obtained by the liquid dairy manure control. The data sets have been divided in two because they were completed in two different batches. In the first verification, the first batch of BMP assay and their yields will be compared to the yield of liquid dairy manure control (mixes of corn silage, canola meal, grease trap waste and whey with liquid dairy manure). The second verification includes the second batch of BMP assay (mixes of DAF sludge and glycerine with liquid dairy manure) and will be compared to the yield of liquid dairy manure of the second batch. Analysis of variance showed that the biogas yield of the mixtures of VS for the first batch have a significantly higher biogas yield than the liquid dairy manure control BMP (LDMCONTROL-BATCH1) ($F_{7,8}=67.17$, $P<0.000$):

- LDM70% + CS30%; 0.42 and 0.42 l/gVS
- LDM50% + CS50%; 0.48 and 0.46 l/gVS
- LDM70% + FOG30%; 0.48 and 0.48 l/gVS
- LDM80% + CC20%; 0.38 and 0.37 l/gVS
- LDM60% + CC40%; 0.43 and 0.42 l/gVS

For the second batch of BMP assays, the Least Significant Difference (LSD) method excluded these mixes of VS co-substrate from the null hypothesis such that these bottles have a significantly higher biogas yield than the liquid dairy manure control in the second batch (LDMCONTROL-BATCH2) ($F_{7,8}=187.39$, $P<0.000$):

- LDM60% + DAF40%; 0.55 and 0.54 l/gVS
- LDM40% + DAF60%; 0.79 and 0.77 l/gVS
- LDM40% + GLY60%; 0.38 and 0.37 l/gVS

Only BMP assay using 40 %VS liquid dairy manure and 40 %VS glycerine did not have a significantly higher biogas yield than the liquid dairy manure control. The BMP LDM40% + GLY 40% had a biogas yield of 0.32 and 0.25 l/gVS.

One-way ANOVA and LSD have also been used to compare the co-substrates biogas yield when digested individually and in mixes of volatile solids with liquid dairy manure. Analysis of variance showed that the biogas yield of corn silage when digested individually (0.17 l/gVS) was significantly lower than the two other yields obtained from the mixes of 30 % corn silage (0.42 l/gVS) and 50% corn silage (0.47 l/gVS) on a VS basis with liquid dairy manure ($F_{2,3}=110.01$, $P<0.002$). Similarly, the biogas yield of DAF sludge (0.10 l/gVS) and whey (0.21 l/gVS) digested individually (DAF100% and W100%) were significantly lower than the mixes of 60% and 40% DAF and of 70% and 30% whey on a VS basis with liquid dairy manure ($F_{2,2}=968.54$, $P<0.001$) ($F_{2,3}=632.72$, $P<0.001$) respectively. All three BMP assays digested individually did not meet the criteria of a good BMP. The co-substrate biogas yield of the 60% and 40% DAF sludge (1.30 and 1.24 l/gVS) were not different. The co-substrate biogas yield in the BMP assay LDM70% + W30% (0.63 l/gVS) was higher than W100% (0.21 l/gVS) and LDM30% + W70% (0.34 l/gVS). For grease trap waste, the co-substrate biogas yields in LDM40% + FOG60% (0.36 l/gVS) and FOG100% (0.03 l/gVS) were significantly lower than the biogas yield obtained in the BMP assay LDM70% + FOG 30% ($F_{2,3}=573.39$, $P<0.001$) with the second highest co-substrate biogas yield in this trial (0.87 l/gVS). This difference can be explained since the bottles LDM40% + FOG60% and FOG100% acidified and did not meet the criteria of a good BMP.

A one-way ANOVA with LSD for canola meal was possible between the substrate digested individually (CC100%) and in the two BMP assays of 20% and 40% VS co-substrate

digested with liquid dairy manure. Analysis of variance showed that there was no significant difference in co-substrate biogas yield for canola meal ($F_{2,3}=2.79$, $P<0.109$). The average co-substrate biogas yield for canola meal in all BMP assays was 0.62 l/gVS. For glycerine, due to the high variability between duplicates, analysis of variance showed that there was no significant difference in biogas yield for glycerine, although the GLY100% assay acidified ($F_{2,2}=6.11$, $P<0.141$).

The TS and VS removal during both batches of BMP assay was calculated by taking into account the remaining VS contributed by the inoculum based with the assertion that the final volume of the BMP assay did not changed during the digestion. This was calculated using Equation 4.5.

$$TS_{.removal} = \frac{(TS.S_1 \times Vol.S) - [(TS.BMP_2 \times Vol.BMP) - (TS.I_2 \times Vol.I)]}{(TS.S_1 \times Vol.S)} \times 100 \quad (4.5)$$

where $TS.S_1$ is the initial total solids concentration of the substrate, $Vol.S$ is the volume of substrate, $TS.BMP_2$ the final TS concentration at the end of the BMP assay, $Vol.BMP$ the volume of the BMP assay, $TS.I_2$ the average TS concentration of the inoculum, $Vol.I$ the volume of inoculum in the BMP assay. The similar equation is used to calculate the VS removal and was calculated using Equation 4.6 and the similar description used for the variable above, but for volatile solids instead of total solids.

$$VS_{.removal} = \frac{(VS.S_1 \times Vol.S) - [(VS.BMP_2 \times Vol.BMP) - (VS.I_2 \times Vol.I)]}{(VS.S_1 \times Vol.S)} \times 100 \quad (4.6)$$

The biogas yield based on the volatile solids removal was calculated according to Equation 4.7 and is presented in Table 4.8.

$$BiogasY_{.removed} = \frac{Cum.BiogasBMP - Cum.BiogasInoc.}{VS_{.removal} \times MassVS_{substrate}} \quad (4.7)$$

where *VS.removal* was obtained from Equation 4.6 and *Cum.BiogasInoc.* is the cumulative biogas produced from the inoculum.

Table 4.8 Solids Removal and Biogas Yield Based on VS Removal for the First Set of BMP Assays

Substrate Digested Individually at 2 VS substrate: 1 VS inoculum	Mean %TS Removal	Mean %VS Removal	Mean Biogas Yield l/gVS _{removed}	Met Criteria of "Good" BMP **
Liquid Dairy Manure (LDM) First Batch	34.2%	45.5%	0.79	Yes
Liquid Dairy Manure (LDM) Second Batch	20.6%	25.2%	0.78*	Yes
Canola Meal (CC)	58.1%	70.3%	0.88	Yes
Corn Silage (CS)	50.6%	66.1%	0.80	No
Dissolved Air Flootation Sludge (DAF)	5.8%	2.1%	4.58*	No
Glycerine (GLY)	76.4%	79.7%	0.13*	No
Grease Trap Waste (FOG)	9.7%	19.5%	0.07	No
Whey (W)	51.0%	75.4%	0.48	No
Inoculum - First Batch	15.6%	32.1%	0.60	-
Inoculum - Second Batch	19.1%	22.5%	0.36*	-
Mixes of VS (% based on VS of substrate, refer to Table 3.1)				
80% LDM + 20% CC	18.5%	31.0%	1.32	Yes
60% LDM + 40% CC	35.6%	47.5%	0.97	Yes
70% LDM + 30% CS	41.4%	51.2%	0.86	Yes
50% LDM + 50% CS	46.5%	55.4%	0.92	Yes
60% LDM + 40% DAF	59.7%	61.1%	0.88*	Yes
40% LDM + 60% DAF	69.1%	70.4%	1.11*	Yes
60% LDM + 40% GLY	56.9%	61.8%	0.47*	Yes
40% LDM + 60% GLY	65.6%	67.0%	0.55*	Yes
70% LDM + 30% FOG	46.9%	53.8%	0.95	Yes
40% LDM + 60% FOG	39.6%	54.5%	1.05	No
70% LDM + 30% W	32.2%	45.2%	0.97	Yes
30% LDM + 70% W	62.2%	80.5%	0.63	No
* Biogas yield and solids removal for 40 days of digestion (second batch of BMP assays)				
** 80% of cumulative biogas produced before 30 days and no signs of methanogenic inhibition				

Liquid dairy manure BMP assays yielded in both batches approximately the same values (0.79 and 0.78 l/gVS), indifferent of their respective volatile solids removal of 45.5% and 25.2%. This could be explained by the difference in fibre composition based on the fact that

the manure used in the first batch was sampled when the milking cows were inside the barn, as opposed to the second batch, when the cows were grazing most of the day outside.

From the literature, liquid dairy manure, corn silage, canola meal, FOG and glycerine did not have biogas yields reported in terms of VS removal. As for cheese whey, the biogas yields that were reported were measured mostly $l/gCOD_{removed}$ rather than $l/gVS_{removed}$ (Wildenauer and Winter, 1985; Goblos et al., 2008; Saddoud et al., 2007 and Membrez et Fruteau de Laclos, 2004). Lo et al. (1988) reported only methane yields in terms of VS removed for co-digestion of cheese whey with dairy manure. The biogas yield could have been calculated if the methane content of the biogas was reported. This gap could be explained by a common standard municipal wastewater research has been reporting biogas or methane yields from digestion of municipal sludge or highly soluble wastewaters. As explained briefly in section 4.1, solid substrates did not have their COD measured because of their particulate nature requiring very high dilution ratios. From this practicality standpoint, reporting biogas yields in terms of organic load added to the reactor has been the accepted standard when using high solids or particulate materials (Braun and Wellinger, 2003). Similarly, co-digestion of cheese whey with manure (Desai et al., 1994; Gellegenesis et al., 2007; and Lo et al., 1988) and glycerine with manure and potato processing wastewater (Chen et al., 2008; Holm-Neilson et al., 2008; Ma et al., 2008) did not report their biogas yields in terms of VS removed, which are both highly soluble co-substrates.

4.1.2 Biogas Production, Yield and Solids Removal from the Second Design of BMP Assays

The second design of BMP experiments were conducted for 40 days at mesophilic temperature in duplicates. Canola meal was added in 5 different VS Canola / VS Inoculum ratios (F/M ratio), as presented in Table 3.2. Those bottles were replicated with the addition of manure in a 0.75 VS Liquid Dairy Manure / VS Inoculum ratio to observe the changes on biogas production and kinetic rates of degradation.

Fresh mass of canola meal, liquid dairy manure and inoculum were calculated according to Equation 4.1 and Equation 4.2 and presented in Table 4.9. The same amount of inoculum (100 g) was used in the BMP assays, as in the first design.

Table 4.9 Feedstocks by Mass Added to the Second Design of BMP Assays

Bottle ID	Liquid Dairy Manure (g)	Canola Meal (g)	Inoculum (g)
C1		1.1	100.0
C2		2.2	100.0
C3		3.3	100.0
C4		4.4	100.0
C5		8.8	100.0
CLDM1	40.0	1.1	100.0
CLDM2	40.0	2.2	100.0
CLDM3	40.0	3.3	100.0
CLDM4	40.0	4.4	100.0
CLDM5	40.0	8.8	100.0
LDM	80.0		100.0
Inoculum			100.0

Biogas composition was measured on October 28; one week after the BMP assays were started. The assay C2.B had a composition of 6.8 %/vol. of N₂, 52.5 %/vol. of CH₄ and 40.7 %/vol. of CO₂. The assay CLDM3.A had a composition of 4.8 %/vol. of N₂, 66.5 %/vol. of CH₄ and 28.7 %/vol. of CO₂ as opposed to CLDM4.B which had produced less biogas with a composition of 42.6 %/vol. of N₂, 57.4 %/vol. of CH₄ and 0.0 %/vol. of CO₂. One of the Manure (Control) bottle had a biogas composition of 9.7%/vol. of N₂, 45.8 %/vol. of CH₄ and 44.5 %/vol. CO₂. The CLDM4.B analysis showed a CO₂ composition of 0.0 %/vol., which might indicate an improper sampling procedure or an equipment fault. The biogas composition could not be repeated to verify the biogas composition of CLDM4.B.

Maximum biogas production rate (*Max.Biogas.P.R*) was calculated according to the following equation:

$$Max.Biogas.P.R = \frac{Daily.Biogas.prod.BMP - Daily.Biogas.prod.Inoculum}{MassVSSubstrate} \quad (4.4)$$

Where *Daily.Biogas.prod.BMP* is the biogas produced during that day by the assay and *Daily.Biogas.prod.Inoculum* is the biogas produced by the inoculum for that day. The average correlation coefficient in between daily measurements of biogas production in duplicates is R²=0.9790 with a STDEV of 0.0254 for all 11 BMP assays. The minimum correlation coefficient between daily measurements of biogas production in duplicates was

$R^2=0.9406$ for the CLDM4 assay. Average cumulative biogas production and maximum biogas production rate from the assays of canola meal and inoculum are presented in Figure 4.11 and Figure 4.12.

The assays of canola meal co-digested with liquid dairy manure are shown in Figure 4.13 for the cumulative biogas production and in Figure 4.14 for the average maximum biogas production rate.

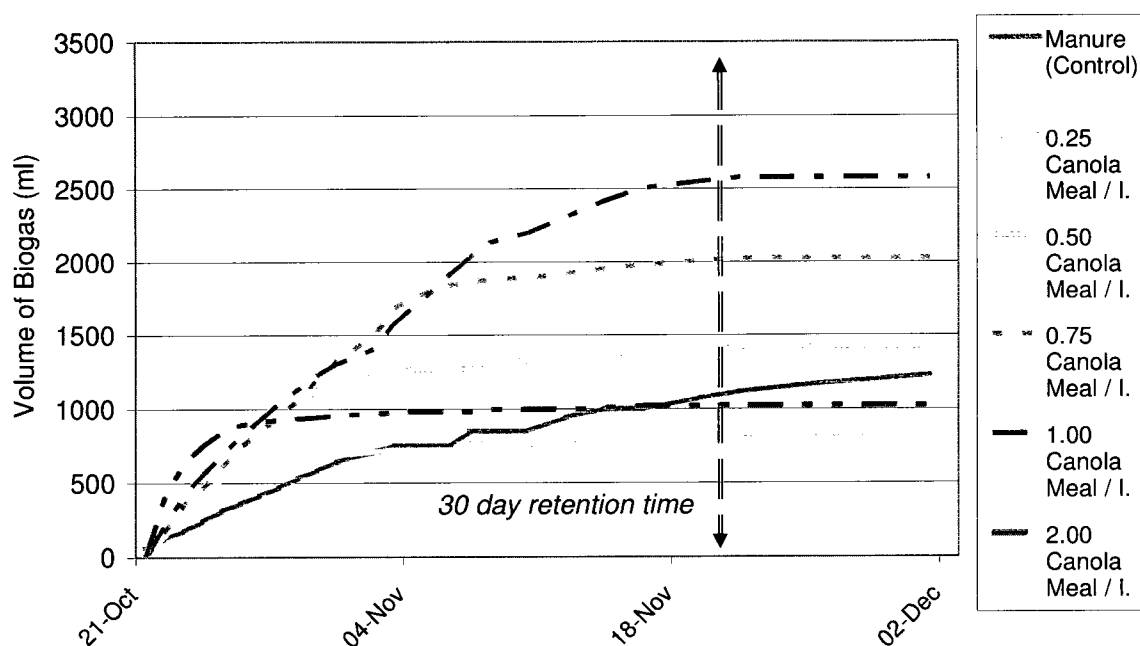


Figure 4.11 Average Cumulative Biogas Production of Canola Meal in 5 Mixes of VS Canola / VS Inoculum Ratio

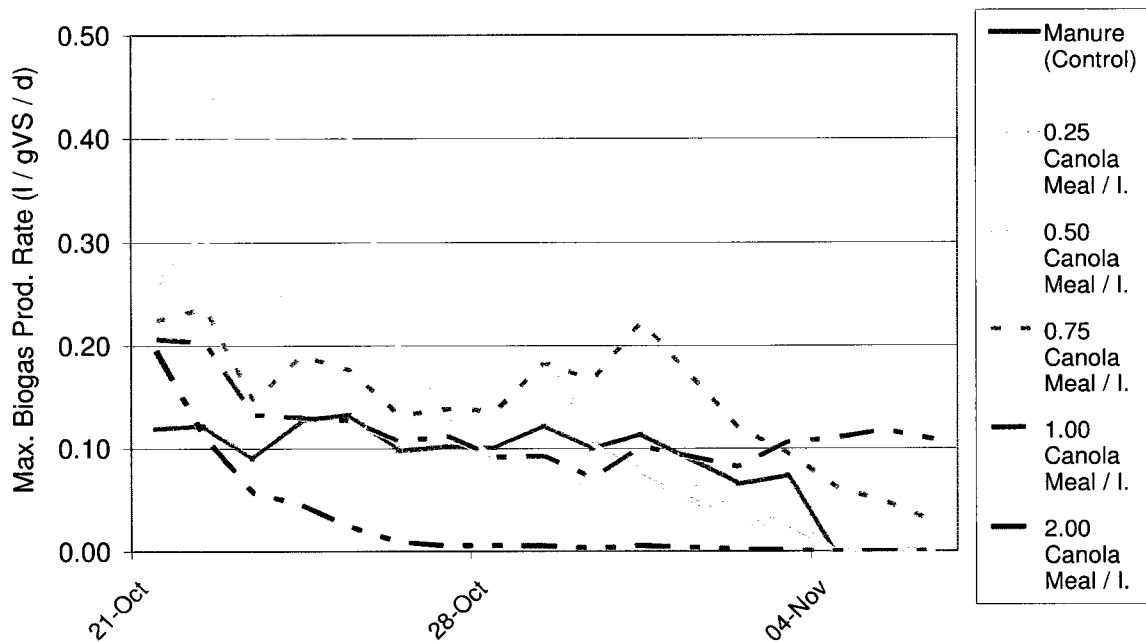


Figure 4.12 Average Maximum Biogas Production Rate of Canola Meal Digested Alone

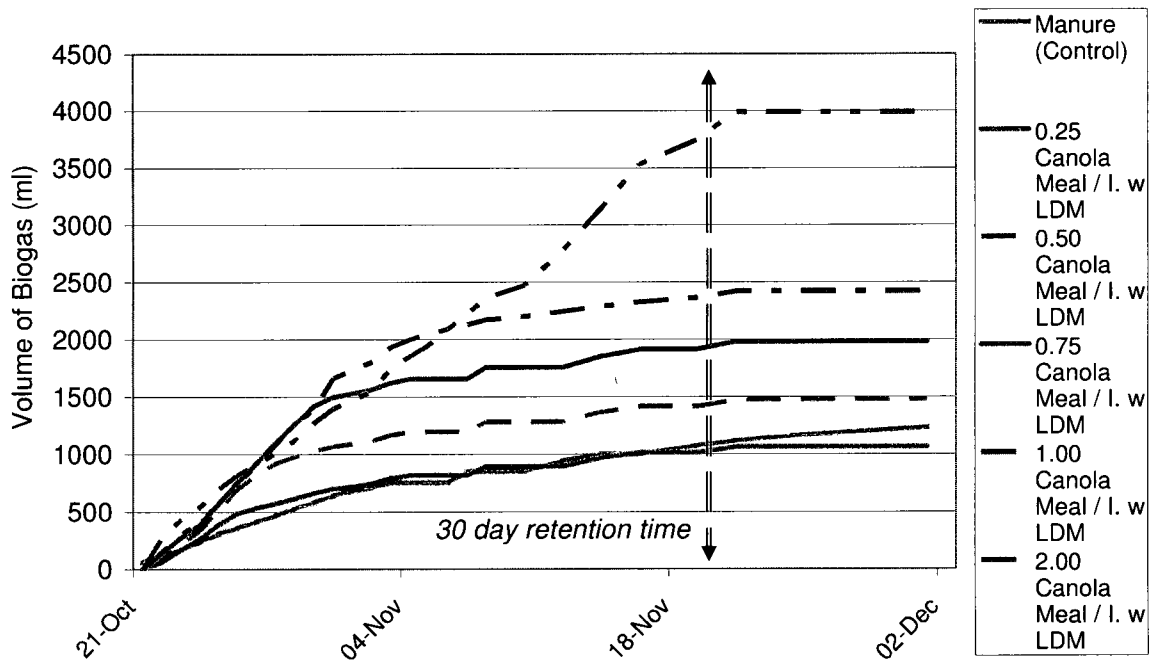


Figure 4.13 Average Cumulative Biogas Production of Canola Meal Co-Digested with Liquid Dairy Manure in 5 Mixes of VS Canola / VS Inoculum Ratio

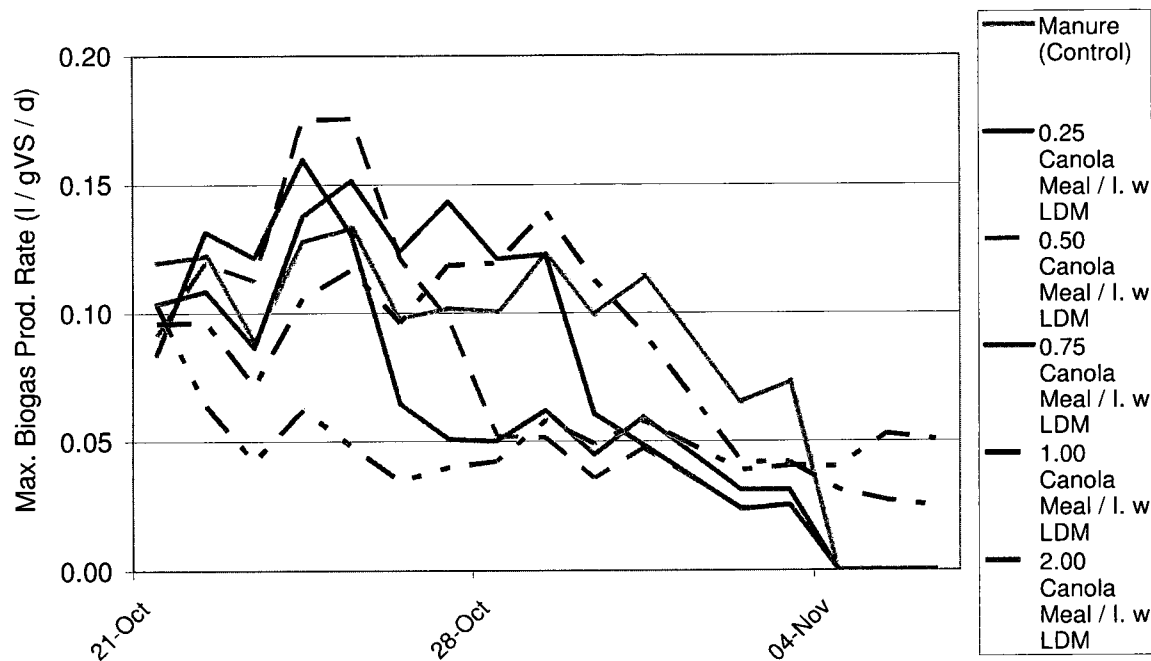


Figure 4.14 Average Maximum Biogas Production Rate of Canola Meal Co-Digested with Liquid Dairy Manure

Solids removal and biogas yields are shown in Table 4.10 using Equation 4.3.

Table 4.10 Average Biogas Yields and Solids Removal for the Second Design of BMP Assays

BMP ID – VS Canola Meal / VS Inoculum	Mean Biogas Yield (l/gVS _{added})	Mean %TS Removal	Mean %VS Removal	Mean Biogas Yield (l/gVS _{removed})
Liquid Dairy Manure	0.20	20.6%	25.2%	0.78
Inoculum	0.08	19.1%	22.5%	0.36
C1 - 0.25	0.92	85.7%	80.8%	1.13
C2 - 0.50	0.79	66.1%	68.1%	1.16
C3 - 0.75	0.75	71.6%	72.1%	1.04
C4 - 1.00	0.72	68.9%	73.3%	0.98
C5 - 2.00	0.14	43.1%	43.8%	0.32
BMP ID - VS Canola Meal / VS Inoculum with 0.75 VS LDM / VS Inoculum				
CLDM1 - 0.25	0.32	52.8%	52.8%	0.60
CLDM2 - 0.50	0.35	52.3%	57.5%	0.60
CLDM3 - 0.75	0.38	53.0%	56.7%	0.68
CLDM4 - 1.00	0.40	59.3%	63.0%	0.63
CLDM5 - 2.00	0.41	53.6%	58.8%	0.70

From the assays C1 to C4, the cumulative biogas production increased successively to reach a maximum for C4 at 2570 ml of biogas. Thus, the BMP assays that did not acidify were obtained between the 1.00 and 2.00 VS C. / VS I. ratio where the BMP assay C5 (2.00 VS C. / VS I) acidified with a mean biogas yield of 0.14 l/gVS. Rapid biogas production rate was observed for the first 3 days of digestion and reached a maximum on the second day for C1, C2 and C3 assays with daily biogas yields reaching 0.46, 0.31 and 0.24 l/gVS/d. C4 and C5 assays reached their maximum biogas production rate on the 3rd day of digestion with lower biogas daily yields of 0.21 and 0.20 l/gVS/d compared to the assays containing less volatile solids of canola meal. Mean %TS and %VS removal were similar for C2, C3 and C4 having a range of 66.1 to 71.6 % for TS and 68.1% to 73.3% for VS removal.

It can be noted that in Figure 4.13 the benefit of co-digesting canola meal with liquid dairy manure is beneficial in terms of cumulative biogas produced and meeting the criteria as opposed to digestion of assays alone with inoculum (C1 to C5). All assays from CLDM1 to CLDM5 produced more cumulative biogas and showed no signs of inhibition (seen in C5) than the same assays with the same VS Canola Meal / VS Inoculum ratio without co-digestion of liquid dairy manure (C1 to C5 assays) in Figure 4.11. Maximum biogas production rate were reached for all bottles including liquid dairy manure on the fourth day of digestion. This initial time span could be attributed to an initial time lag or acclimation as explained by Chen et al. (2008) for their BMP assay on glycerine. Assay CLDM2 daily biogas yield reached 0.18 l/gVS/d as opposed to CLDM5 with a value of 0.10 l/gVS/d. It shows that as the amounts of canola meal are added to a fixed amount of liquid dairy manure, the daily biogas yield lowered considerably. This could be explained by the VS fraction of liquid dairy manure which is more recalcitrant to anaerobic degradation (20.6 %TS and 25.5% VS removal) compared to canola meal (68.9%TS and 71.2%VS removal). In comparison with C5 and CLDM5, the addition of 0.75 VS LDM / VS Inoculum to the assay prevented inhibition measured by observing the biogas production during digestion. The assay produced an average of 3986 ml of cumulative biogas compared to 1021 ml, for C5 and 971 ml for LDM. The cumulative biogas produced by CLDM5 represented a 390% increase over C5 and 411% over LDM. The co-digestion of canola meal with liquid dairy manure at a 2.00 VS Canola Meal / VS Inoculum also increased %TS and %VS removal

from 10% and 15 % respectively and increased biogas yields both in terms of VS added and removed.

An analysis of variance of the biogas yields, with respects to VS removed, obtained in the second design of BMP assays for canola meal revealed that the biogas yields are significantly different ($F_{10,11}=36.79$, $P<0.000$). An LSD for biogas yields of BMP assays C1 to C5 showed that the biogas yield of assay C5 was significantly lower from assays C1 to C4, which in return were statistically similar ($F_{4,5}=42.32$, $P<0.000$). For the LSD of BMP assays from CLDM1 to CLDM5, analysis of variance showed that biogas yields based on VS removal were not significantly different from each other ($F_{4,5}=2.11$, $P<0.218$). The biogas yields observed in this study are much higher than what Baserga (2000) observed for canola meal residue. A general biogas yield of 0.60 l/gVS for oilseed residues at a 92%TS and 97 %VS/TS with a higher C/N ratio of 41 was reported as opposed to 0.92 to 0.72 l/gVS/d for bottles C1 to C4.

It was observed from the first design of the batch test that liquid dairy manure provided buffer and nutrients for bacteria growth and increased the cumulative biogas production for 0.68 gVS per assay for the first batch and for 0.42 gVS per assay for the second batch at these VS Co-Substrate: VS Liquid Dairy Manure ratios:

- LDM50% + CS50% (Corn silage)
- LDM70% + FOG30% (Grease trap)
- LDM70% + W30% (Whey)
- LDM60% + CC40% (Canola meal)
- LDM40% + DAF60% (DAF sludge)
- LDM40% + GLY60% (Glycerine)

4.2 Semi-Continuous Pilot Scale Reactors

The six 30l lab-scale anaerobic reactors were inoculated with 10 l of digestate from Terryland Farm digester and 5 l of effluent from the Ottawa ROPEC digester in the first week of February 2008. Three days after inoculation, they were subsequently fed increasing amounts of fresh liquid dairy manure from the Alfred Campus dairy barn until reaching the

20l demarcation line. Equal amounts of influent were fed and effluent removed from the reactor until co-substrate addition on the first week of June, as per the following schedule:

- 0.5 l at 5 days a week until third week of March;
- 1 l at 7 days a week until June 7.

In May, the average daily biogas production was 17.5 l/d at an HRT of 20 days. The reactors were fed liquid dairy manure at 4.5 to 5.5 %TS and the average biogas yield was 0.350 to 0.357 l/gVS.

In the first week of June, co-substrate addition of corn silage, canola oilseed meal and whey started. In the second week of June glycerine and DAF sludge were added to the last two reactors, while one reactor was kept as a control with liquid dairy manure loading.

Increasing fractions of the co-substrate (as VS) were added to the reactors under three OLRs as following:

- Period A: From first week of June to second week of July, the reactors were fed 20% VS co-substrate at an OLR of 2.5 gVS/l/d (except for DAF sludge and glycerine reactors that started on the second week of June);
- Period B: From second week of July to second week of August, the reactors were fed with 30% VS co-substrate at an OLR of 2.5 gVS/l/d;
- Period C: From second week of August to mid-September, the reactors were fed with 30% VS co-substrate at an OLR of 3.0 gVS/l/d;
- Period D: From mid-September to third week of October, the reactors were fed with 30% VS co-substrate at an OLR of 3.5 gVS/l/d;
- Period E: From third week of October to mid-November, the DAF sludge, Glycerine and Canola Meal reactors were fed with 40% VS co-substrate at an OLR of 4.0 gVS/l/d and the whey and corn silage reactors, they were fed with 30% VS co-substrate at an OLR of 4.0 gVS/l/d;
- Period F: From mid-November to first week of December, the DAF sludge, Glycerine and Canola Meal reactors were fed with 50% VS co-substrate at an OLR of 4.0 gVS/l/d and for the whey and corn silage reactors, they were fed with 40% VS co-substrate at an ORL of 4.0 gVS/l/d;

From the information of successful batch test conducted with liquid dairy manure and co-substrates, the co-substrate VS fraction were determined so that the influent %TS concentration did not exceed 10-12 %TS. This was done to prevent reactor failure, decreased biogas yields (due to incomplete digestion of fresh materials “wasted” as opposed to a higher volumetric biogas production) and to achieve an HRT > 10 days for the normal operation of a CSTR. The organic loading rate of 4.0 gVS/l/d was selected since most completely stirred tank reactors digesting particulate substrates in Western Europe operate in this range for operational stability of the biological process (Weiland, 2003). The amount of the VS from the co-substrate was limited from 40-50% based on the VS limits experimented in Lehtomaki et al. (2007) with co-digestion of cow manure and grass silage. The same approach was used for the co-digestion of canola meal, using the knowledge gained from the BMP experiments, where the hydrolysis was the rate limiting step in the production of biogas and the maximum VS co-substrate was set at 50%. For the co-digestion of whey, Gelegenis et al. (2007) discovered that using a mixture of 50% vol. of whey with poultry manure led to reactor failure in a mesophilic semi-continuous digester at an HRT of 20 days. The limit of VS from glycerine was set at 50%, based on the failure of a mesophilic semi-continuous digester at a 60 %VS glycerine and 40 %VS liquid dairy manure influent mixture at a HRT of 20 days reported by Chen et al. (2008). Similarly for the co-digestion of DAF sludge, the total fat content of the influent was limited at 40% VS based on the experiments carried out by Membrez and Fruteau de Laclos (2002) on grease trap waste and WVO.

The amount of fresh material to be fed to each digester was calculated with the same equations used in the BMP assays. A spreadsheet was set up with a series of input cells on which the information of the reactor was added and shown in the Appendix B:

- Desired organic loading rate (OLR) in terms of mass of VS added / volume of reactor / time
- The volume of reactor in litres (20 l)
- A table (similar to Table 3.1) containing updated characterization of substrates to calculate new recipes

This spreadsheet contained a section for each reactor where the different VS co-substrate / VS liquid dairy manure could be chosen. The output required to operate the reactors were the fresh mass of co-substrate and liquid dairy manure to be added daily. It also displayed the HRT, %TS of influent, %Vol. and %Mass of co-substrate added in the mixture for each VS co-substrate / VS liquid dairy manure ratio selected.

Each reactor has been labelled as follows:

- R1 – Reactor co-digesting chopped corn silage
- R2 – Reactor co-digesting canola meal
- R3 – Reactor co-digesting cheese whey
- R4 – reactor co-digesting raw glycerine
- R5 – Reactor co-digesting DAF sludge
- R6 – Control reactor digesting liquid dairy manure alone

The results of each recipe for OLR 2.5, 3.0, 3.5 and 4.0 gVS/l/d are shown in Appendix A. The volumetric biogas production was measured in m³ of biogas produced per m³ of reactor per day. Readings from the tipping buckets monitoring biogas production were taken daily and the daily biogas production was normalized for 24 hours. The volume of biogas produced daily was calculated according to Equation 4.5.

$$Daily.Biogas.Production = \frac{(TipCounter_2 - TipCounter_1) \times VolumeTip}{(TimeTipCounter_2 - TimeTipCounter_1)} \quad (4.5)$$

where $TipCounter_1$ is the reading on the digital counter from the previous day reading and $TimeTipCounter_1$ the time when that reading occurred in the format hh:mm, $TipCounter_2$ is the reading on the digital counter from the current reading and $TimeTipCounter_2$ the time when that reading occurred and $VolumeTip$ is the calibrated and corrected volume of the tipping bucket in ml biogas per tip at STP. Volumetric biogas production was calculated by dividing the daily biogas production by the volume of the reactor. The biogas yield was calculated by using the average daily biogas production in the respective period divided by the volatile solids added per day.

The results of the semi-continuous reactors first start with the data and analysis of the control reactor (R6) digesting liquid dairy manure. Each reactor, first from R6, then from R1 to R5, has their own section presenting the data and analysis as the following:

- Graph of volumetric biogas production measured in $\text{m}^3/\text{m}^3/\text{d}$;
- One table with biogas yield, methane content and methane yield shown for each OLR and %VS co-substrate ratio;
- A second table presenting pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio. Some certain feedstock and digestate COD values were not done for reasons explained in the end of section 4.1.1.

4.2.1 R6 – Liquid Dairy Manure, Control Reactor

In Figure 4.15, the volumetric biogas production of liquid dairy manure is shown from May 22, 2008 through December 4, 2008.

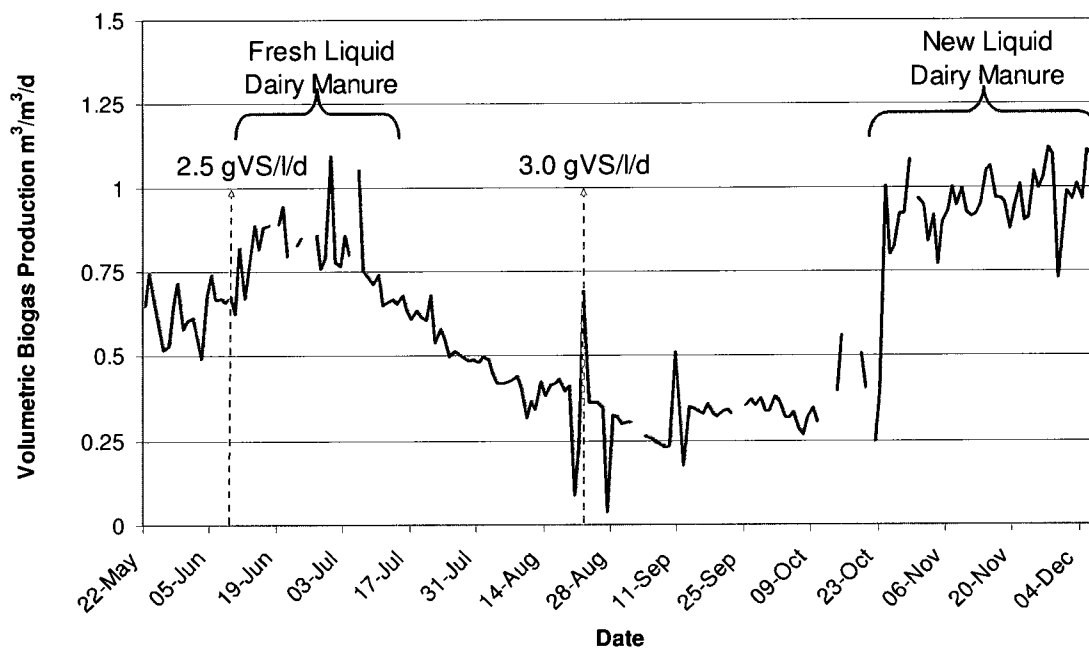


Figure 4.15 Daily Volumetric Biogas Production of R6 Liquid Dairy Manure (Control) Reactor

From Figure 4.15, average volumetric biogas production of liquid dairy manure varied considerably throughout the experiment. This fact strengthens the requirement to have one reactor used as a control to monitor the quality of the main substrate chosen in co-digestion experiments. As explained previously, external factors due to large requirements of storage for liquid dairy manure and also because of the manure management system of the organic dairy farm on campus caused the decrease in biogas production seen from mid-July to the end of October. Nonetheless, the effects of co-substrate addition on biogas production can still be shown because of this control reactor. When fresh liquid dairy manure was collected from the pre-pit in the barn at the end of October, the volumetric biogas production was quite similar to the beginning of the experiment when the heifers and calves were housed all day in the barn. Table 4.12 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the LDM reactor.

Table 4.11 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R6 – Liquid Dairy Manure Control (Standard Deviation in Parenthesis)

Feeding Regime	Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS/d)	Methane Content (%Vol.)	Methane Yield (l/gVS/d)
Control Period - May 7 to June 6 OLR 2.2 gVS/l/d	0.63 (0.08)	0.29 (0.04)		
Period A - June 7 to July 16 OLR 2.5 gVS/l/d	0.78 (0.12)	0.31 (0.05)		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d	0.52 (0.08)	0.21 (0.03)		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d	0.34 (0.12)	0.11 (0.04)	45.1 (5.8)	0.05
Period D - September 10 to October 23 OLR 3.0 gVS/l/d	0.36 (0.09)	0.12 (0.03)	46.7 (6.1)	0.06
Period E - October 23 to November 14 OLR 3.0 gVS/l/d	0.93 (0.08)	0.31 (0.03)	54.5 (0.9)	0.17
Period F - November 14 to December 4 OLR 3.0 gVS/l/d	0.99 (0.09)	0.33 (0.03)	56.0 (3.9)	0.18

Table 4.13 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio for the LDM reactor.

Table 4.12 Feedstock and Digestate Properties for R6 – Liquid Dairy Manure Control

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.0	3.0	3.0
HRT (d)	19	16	16	13	13	13	13
Duration of HRT (d)	30	41	23	32	44	22	20
Feedstock							
%TS	6.0	4.9	5.1	4.9	4.7	4.7	4.8
%VS	3.92	3.9	4.0	3.9	3.8	3.9	3.9
COD (g/l)	76.1	69.6	74.9	85.3	86.8	76.5	72.0
Digestate							
Mean pH	7.7	7.8	7.8	7.6	7.9	7.7	7.5
Mean PA (g CaCO ₃ /l)	6.25	5.77	5.60	5.23	5.02	4.28	4.11
Mean TA (g CaCO ₃ /l)	0.00	7.10	6.93	6.40	6.03	5.33	5.14
Mean Ammonia (g NH ₃ -N/l)	1.5	1.1	1.0	1.0	0.6	0.7	1.2
Mean TVFAs (mg/l)	58	<30	50	<30	129	69	68
Mean %TS	3.29	3.16	4.18	3.66	3.20	5.41	5.51
Mean %VS	2.38	2.36	3.28	2.81	2.33	3.84	3.86
Mean COD (g/l)	45.6	52.7	55.1	53.7	70.7	64.5	56.9
Removal							
Mean %TS	45.2	35.4	18.1	25.3	31.9	-15.1*	-14.8*
Mean %VS	39.2	39.7	17.9	28.4	38.7	1.9	1.4
Mean %COD	40.1	24.3	26.5	37.0	18.5	15.7	21.0
* Mean %TS increase: TS of effluent was higher than the influent during period E and F							

From period C to period D, the OLR for the LDM reactor was fixed at 3.0 gVS/l/d to assure a minimum retention time of 10 days required for stability of the anaerobic bacteria. During that time, the biogas yield ranged from 0.12 to 0.33 l/gVS because of the quality of the LDM stored in the plastic tote. The biogas yield jumped from 0.12 to 0.31 l/gVS when fresh LDM was collected at the campus barn at the end of October. According to Amon et al. (2007), the biogas yields for liquid dairy manure ranged from 0.14 to 0.27 l/gVS, which is in agreement with the observations of this study. However, Baserga (2000) reported significantly higher biogas yields ranging from 350 to 400 l/gVS. The mean pH in this LDM reactor ranged from 7.5 to 7.9, while the PA and TA lowered slightly, from their initial levels in period A of 5.77 and 7.10 g CaCO₃/l down to 4.11 and 5.14 g CaCO₃/l in period F. The mean ammonia

concentration in the digestate ranged from 1.0 to 1.2 g NH₃-N /l except in period D and E when the mean ammonia levels of LDM were 0.6 g NH₃-N /l (from September 10 to November 14, n=14). When the new LDM was collected at the end of October from the campus barn, the reactor had accumulated a high volume of crust formed by fibres (bedding, straw). The quantity of fibres in the effluent was much higher than during the rest of the experiment, since all the fibres were accumulating in the crust and were not eliminated through the effluent port. During period E, parts of the crust layer started to leave the reactor through the effluent port when feeding the reactor. This explains the negative TS removal during period E and F of -15.1 and -14.8 %TS accounted as an increase in TS rather than removal. In the beginning of the trial, TS, VS and COD removal ranged from 18.1 to 45.2 %TS, 17.9 to 39.7 %VS and 18.5 to 40.1 %COD removal, respectively. Desai et al. (1994) operated a CSTR with dairy manure at 6 gTS/l/d at 10 HRT and achieved a 50% reduction in COD with a volumetric biogas production of 0.83 m³/m³/d which was not significantly different from the results obtained during the control period and period A, B, E and F for R6. Higher volumetric biogas productions was reported by Ghaly (1996) in a two stage reactor with values ranging from 0.92 to 1.22 m³/m³/d with dairy manure and having a HRT of 10 to 20 days at mesophilic temperature. TS and VS removal ranged from 51.1 to 57.8 %TS and 52.6 to 60.1 %VS while COD removal ranged from 43.1 to 55.9 %COD.

4.2.2 R1 – Corn Silage Reactor

In Figure 4.16, the volumetric biogas production of corn silage as the co-substrate is shown from May 22, 2008 through December 4, 2008 with the volumetric biogas production of liquid dairy manure (Figure 4.15) represented by the green dotted line.

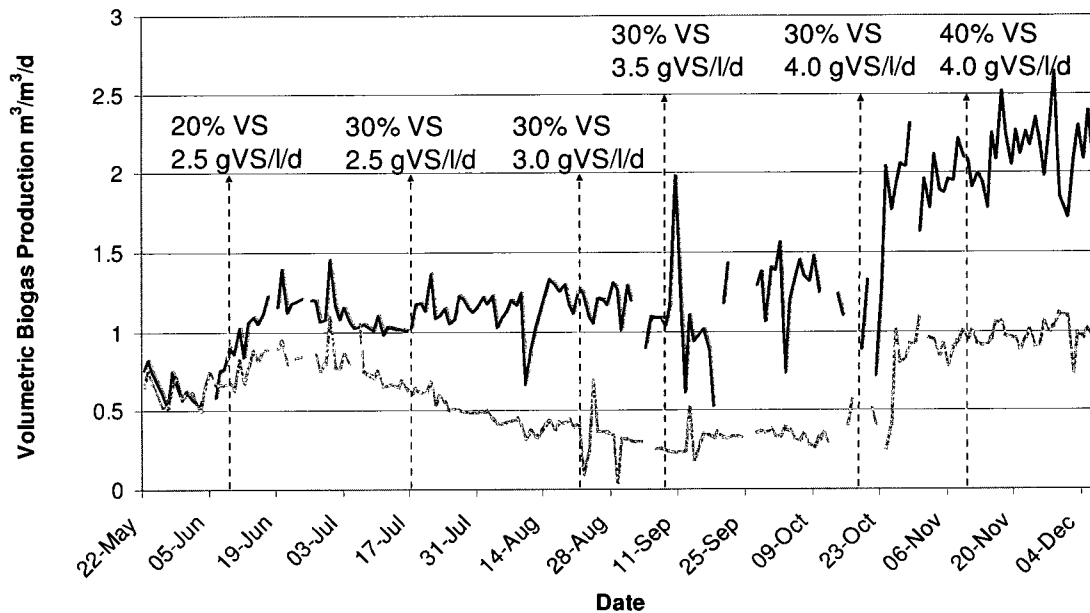


Figure 4.16 Daily Volumetric Biogas Production of R1 Corn Silage Reactor

A few days following co-substrate addition on June 7, 2008, the volumetric biogas production increased. As the co-substrate VS and OLR were increased, the volumetric biogas production stayed relatively constant until the fresh liquid dairy manure was added to the feed in late October. Nonetheless, the effects of co-substrate addition can be clearly seen, more than doubling the volumetric biogas production when the loading rate reached 4.0 gVS/l/d at a 40% co-substrate VS fraction. Table 4.13 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the corn silage reactor.

Table 4.13 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R1 – Corn Silage (Standard Deviation in Parenthesis)

Feeding Regime	Volumetric Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS)	Co-Substrate Biogas Yield (l/gVS)	Methane Content (%Vol.)	Methane Yield (l/gVS)
Control Period - May 7 to June 6 OLR 2.2 gVS/l/d Liquid Dairy Manure	0.64 (0.10)	0.29 (0.05)			
Period A - June 7 to July 16 OLR 2.5 gVS/l/d 20 %VS Co-Substrate	1.07 (0.14)	0.43 (0.06)	0.90		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d 30 %VS Co-Substrate	1.14 (0.08)	0.46 (0.03)	1.04		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d 30 %VS Co-Substrate	1.18 (0.21)	0.39 (0.07)	1.05	45.4 (3.1)	0.18
Period D - September 10 to October 23 OLR 3.5 gVS/l/d 30 %VS Co-Substrate	1.19 (0.31)	0.34 (0.09)	0.86	45.2 (0.2)	0.15
Period E - October 23 to November 14 OLR 4.0 gVS/l/d 30 %VS Co-Substrate	1.97 (0.16)	0.49 (0.04)	0.77	47.0 (2.1)	0.23
Period F - November 14 to December 4 OLR 4.0 gVS/l/d 40 %VS Co-Substrate	2.19 (0.21)	0.55 (0.05)	0.77	48.9 (1.7)	0.27

Table 4.14 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio for the corn silage reactor.

Table 4.14 Feedstock and Digestate Properties for R1 – Corn Silage

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.5	4.0	4.0
HRT (d)	19	21	22	17	15	13	15
% VS Co-Substrate	0	20	30	30	30	30	40
%w/w. Co-Substrate	0.0	3.3	5.0	4.8	4.8	4.8	7.3
Duration of HRT (d)	30	41	23	32	44	22	20
Feedstock							
%TS	6.0	6.4	6.5	6.1	6.1	6.1	6.9
%VS	3.92	4.9	5.4	5.3	5.3	5.3	6.0
COD (g/l)	76.1	-	-	-	-	-	-
Digestate							
Mean pH	7.70	7.76	7.55	7.52	7.67	7.61	7.21
Mean PA (g CaCO ₃ /l)	-	6.4	5.5	5.3	5.1	4.4	3.9
Mean TA (g CaCO ₃ /l)	-	8.0	6.8	6.6	6.0	5.4	5.3
Mean Ammonia (g NH ₃ -N /l)	1.7	1.2	1.0	0.8	0.6	0.7	0.9
Mean TVFAs (mg/l)	<30	<30	314	<30	<30	57	67
Mean %TS	3.61	3.65	4.14	3.86	4.16	4.97	5.16
Mean %VS	2.63	2.73	3.23	2.97	3.10	3.73	3.84
Mean COD (g/l)	36.9	-	-	-	-	-	-
Removal							
Mean %TS	39.9	43.4	35.9	36.7	31.8	18.3	25.7
Mean %VS	33.0	43.8	39.5	43.6	41.1	29.2	36.0
Mean %COD	51.5	-	-	-	-	-	-

The volumetric biogas production from co-digestion of corn silage was increased from 0.64 m³/m³/d to 1.07 m³/m³/d with the first loading rate of 3.3%w/w corn silage and then doubled to 2.19 m³/m³/d using a 40% VS ratio of corn silage (7.3 %w/w) with liquid dairy manure at a OLR of 4.0 gVS/l/d. The highest biogas and methane yield was observed at the maximum OLR and VS co-substrate ratio at a value of 0.55 and 0.27 l/gVS, respectively. The methane content observed ranged from 45.2 to 48.9 %Vol.. Compared to an extensive batch study completed in Austria by Amon et al. (2007), methane yield ranged between 0.268 to 0.366 l/gVS for chopped and ensiled corn silage and the methane composition of the biogas ranged from 55 to 62% as opposed to a lower value recorded for semi-continuous trials.

The co-substrate biogas yield was significantly higher during period B and C. This observation recorded during these two feeding regimes coincides with the lower quality of the liquid dairy manure stored in the plastic tote. From period D to F, the biogas co-substrate yield ranged between 0.77 to 0.86 l/gVS, which is higher than the average biogas yield of corn silage reported by Braun and Wellinger (2003) of 0.40 to 0.60 l/gTS (0.421 to 0.631 l/gVS at 95 %TS/VS). The average pH ranged from 7.21 to 7.76. The lowest pH observed was recorded during the highest loading rate and co-substrate VS ratio (period F). TA and PA dropped considerably during the experiment, starting at values of 6.4 and 8.0 g CaCO₃/l down to 3.9 and 5.3 g CaCO₃/l. During the co-digestion experiment, the reactor ammonia concentration never exceeded 1.2 g NH₃-N /l. The lowest solids removal was observed at period E when the HRT was 13 days. Mean TS and VS removal were 18.3 %TS and 29.2 %VS, respectively. The highest solids removal was observed at the lowest OLR in period A with values of 43.4 %TS and 43.8 %VS. The %TS of the influent never exceeded the target of 10-12% TS. The highest %TS of the influent was 6.9 %TS during the last and highest OLR and VS co-substrate ratio in period F.

Based on the average harvest yield of corn silage in Ontario of 45 t/ha/yr (Hilborn, 2007) and according to the biogas yield obtained in this co-digestion trial (0.75 to 0.90 l/gVS), the methane yield per hectare of ensiled and pre-treated corn silage is situated between 5500 to 6600 m³/ha/yr when co-digested with liquid dairy manure using the characteristics obtained during period F of the trial. As indicated by Amon et al. (2007), these yields can be increased by optimizing cultivar, harvesting period and storage conditions.

4.2.3 R2 – Canola Meal Reactor

In Figure 4.17, the volumetric biogas production of canola meal as the co-substrate is shown from May 22, 2008 through December 4, 2008 with the volumetric biogas production of liquid dairy manure (Figure 4.15) represented by the green dotted line.

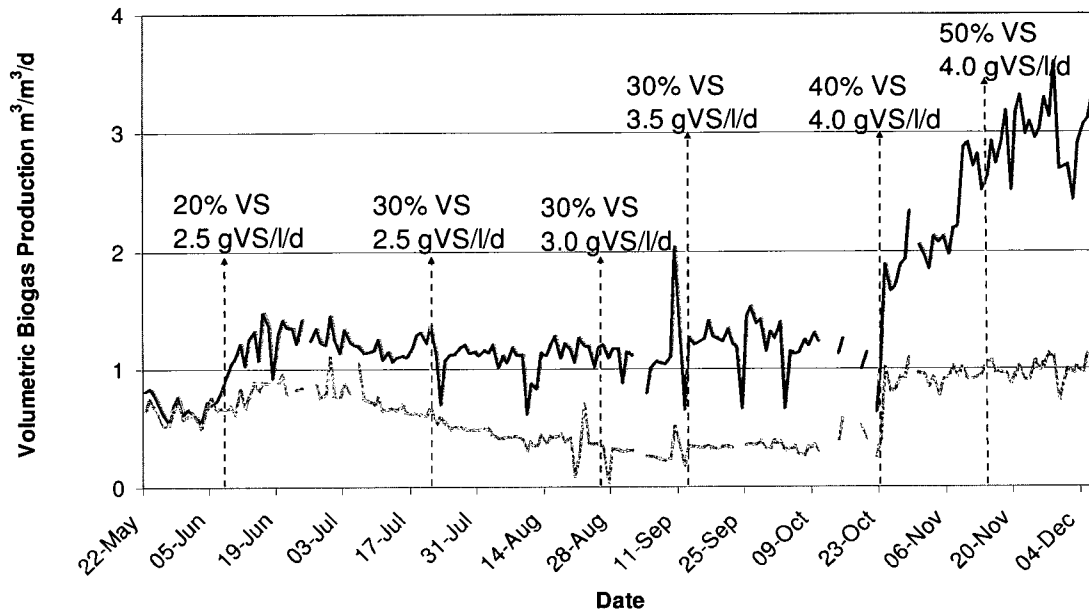


Figure 4.17 Daily Volumetric Biogas Production of R2 Canola Meal Reactor

The volumetric biogas production of canola meal when the fresh liquid dairy manure was added at the end of October did not stabilise during the loading rate of 4.0 gVS/l/d at a 40% co-substrate VS ratio. However, the reactor was quick to respond to the addition of canola meal, where the daily volumetric biogas production jumped to average daily levels of period A after two days. Table 4.15 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the canola meal reactor.

Table 4.15 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R2 – Canola Meal (Standard Deviation in Parenthesis)

Feeding Regime	Volumetric Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS)	Co-Substrate Biogas Yield (l/gVS)	Methane Content (%Vol.)	Methane Yield (l/gVS)
Control Period - May 7 to June 6 OLR 2.2 gVS/l/d Liquid Dairy Manure	0.69 (0.09)	0.31 (0.04)			
Period A - June 7 to July 16 OLR 2.5 gVS/l/d 20 %VS Co-Substrate	1.20 (0.15)	0.48 (0.06)	1.14		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d 30 %VS Co-Substrate	1.14 (0.12)	0.46 (0.05)	1.03		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d 30 %VS Co-Substrate	1.11 (0.22)	0.37 (0.07)	0.97	52.4 (3.1)	0.19
Period D - September 10 to October 23 OLR 3.5 gVS/l/d 30 %VS Co-Substrate	1.21 (0.27)	0.35 (0.08)	0.87	53.5 (2.1)	0.18
Period E - October 23 to November 14 OLR 4.0 gVS/l/d 40 %VS Co-Substrate	2.20 (0.39)	0.55 (0.10)	0.91	54.9 (1.6)	0.30
Period F - November 14 to December 4 OLR 4.0 gVS/l/d 50 %VS Co-Substrate	2.99 (0.28)	0.75 (0.07)	1.17	57.3 (0.8)	0.43

Table 4.16 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio for the canola meal reactor.

Table 4.16 Feedstock and Digestate Properties for R2 – Canola Meal

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.5	4.0	4.0
HRT (d)	19	22	23	18	15	15	19
% VS Co-Substrate	0	20	30	30	30	40	50
%w/w. Co-Substrate	0.0	1.3	1.9	1.9	1.9	2.9	4.4
Duration of HRT (d)	30	41	23	32	44	22	20
Feedstock							
%TS	6.0	6.6	6.7	6.3	6.3	7.2	8.6
%VS	3.92	5.0	5.5	5.4	5.5	6.2	7.4
COD (g/l)	76.1	-	-	-	-	-	-
Digestate							
Mean pH	7.93	7.91	7.87	7.78	7.92	7.75	7.53
Mean PA (g CaCO ₃ /l)	6.0	6.9	7.1	7.6	6.6	6.6	7.1
Mean TA (g CaCO ₃ /l)	-	8.6	8.9	9.6	7.9	8.1	9.2
Mean Ammonia (g NH ₃ -N/l)	1.7	1.3	1.5	1.6	1.8	2.1	2.3
Mean TVFAs (mg/l)	<30	38	52	82	79	383	263
Mean %TS	3.48	3.81	4.29	4.13	4.74	5.48	5.70
Mean %VS	2.53	2.87	3.34	3.17	3.55	4.09	4.22
Mean COD (g/l)	42.2	-	-	-	-	-	-
Removal							
Mean %TS	42.0	42.3	36.2	34.7	25.0	24.0	33.5
Mean %VS	35.6	42.0	39.5	41.5	35.6	34.4	43.1
Mean %COD	44.5	-	-	-	-	-	-

For the canola meal reactor, the change in the co-substrate biogas yield throughout the various periods (0.87 to 1.17 l/gVS) was not significantly different compared to the variation observed in biogas yields for corn silage (0.77 to 1.05 l/gVS). The volumetric biogas production almost tripled between the period A (OLR 2.5 gVS/l/d; 20 %VS Co-Substrate) and period F (OLR 4.0 gVS/l/d; 50 %VS Co-Substrate) from 1.20 to 2.99 m³/m³/d. Similarly, the highest biogas and methane yield was observed during period F with values of 0.75 and 0.43 l/gVS, respectively, and an average biogas composition with 57.3 %Vol. methane. The canola meal reactor was the only reactor with increasing mean ammonia concentrations during the co-digestion experiment. The mean ammonia concentration during period A was 1.3 g NH₃-N/l and reached 2.3 g NH₃-N/l in period F. Ammonia levels did not exceed the

recommendations in Pind et al. (2003) of 4 g NH₃-N/l. Ammonia also contributed to the increase of PA and TA during the co-digestion as explained in Membrez (1993) and van Lier (2004). During period A, mean PA and TA was 6.9 and 8.0 g CaCO₃/l. The values of PA and TA dropped during periods D and E (at HRT of 15 days) to be restored at values of 7.1 and 9.2 g CaCO₃/l at a HRT of 19 days. The influent %TS reached 8.6 % which was the highest level recorded for all 5 reactors tested with co-substrates during period F. The maximum TS removal was observed during period A at 42.3 %TS and the maximum VS removal was observed during period F at 43.1 %VS. The mean digestate pH during the experiment was 7.85, but dropped considerably during period F to a mean pH of 7.53.

Due to the high value of canola meal as a protein source for livestock feed, literature investigating the co-digestion of canola meal was limited. In batch experiments conducted by Amon et al. (2006), the co-digestion of 10 %w/w rapeseed meal with 6%w/w of glycerine and a basic mixture of corn silage (31%w/w), corn grains (15%w/w) and pig manure (54%w/w) resulted in a biogas yield of 0.701 l/gVS (0.432 l CH₄/gVS) compared with the digestion of the basic mixture alone that yielded 0.569 l/gVS (0.335 l CH₄/gVS). The biogas yield of R2 during period F (0.75 l/gVS) exceeded the value reported in Amon et al. (2006). Baserga (1998) reported a volumetric biogas production of 3.5 m³/m³/d at a plant in Switzerland co-digesting pig manure with wheat and canola mill processing residues. The canola meal reactor reached a volumetric biogas production of 2.99 m³/m³/d when set at a OLR of 4.0 gVS/l/d and at a 50 %VS Co-Substrate ratio.

4.2.4 R3 – Cheese Whey Reactor

In Figure 4.18, the volumetric biogas production of cheese whey as the co-substrate is shown from May 22, 2008 through November 28, 2008 with the volumetric biogas production of liquid dairy manure (Figure 4.15) represented by the green dotted line.

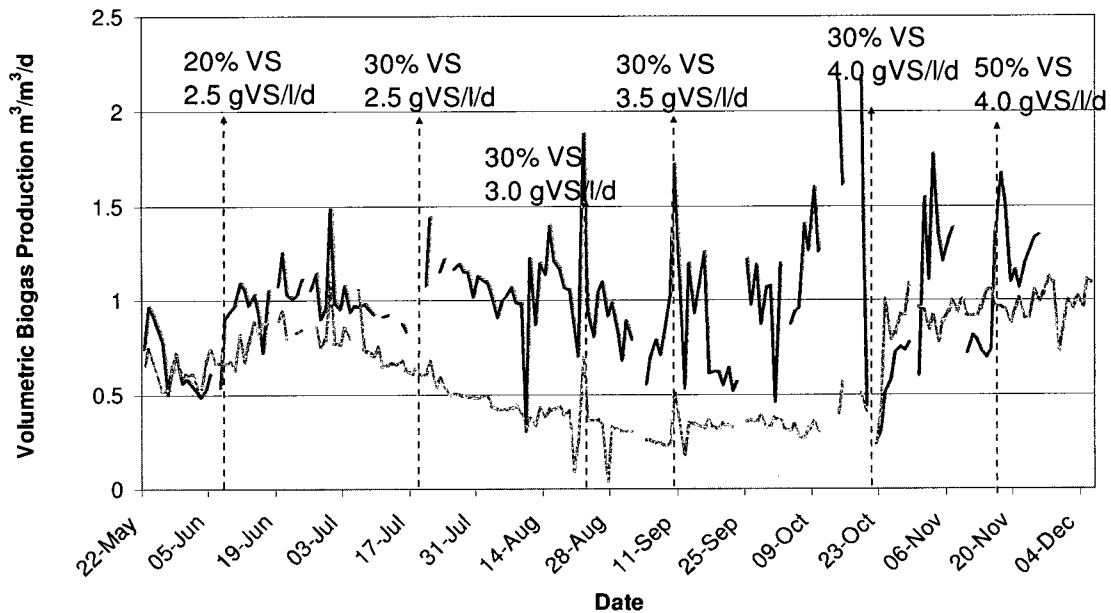


Figure 4.18 Daily Volumetric Biogas Production of R3 Whey Reactor

In comparison to both reactors in this study using solid substrates, reactors R1 and R2, the reactor co-digesting whey was much faster to respond to co-substrate addition on June 7. The nature of rapid degradation of whey into acids explains the more rapid acclimation of the methanogens (Gelegenis et al., 2007; Wildenauer and Winter, 1985). Sudden spikes in the volumetric biogas production during October, November and December due to a tipping bucket malfunction and gas leak in the cover of the reactor renders the situation difficult to draw other observations on the biogas yield because of the high standard deviation of the data. Table 4.17 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the whey reactor.

Table 4.17 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R3 – Whey (Standard Deviation in Parenthesis)

Feeding Regime	Volumetric Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS)	Co-Substrate Biogas Yield (l/gVS)	Methane Content (%Vol.)	Methane Yield (l/gVS)
Control Period - May 7 to June 6 OLR 2.2 gVS/l/d Liquid Dairy Manure	0.66 (0.16)	0.30 (0.07)			
Period A - June 7 to July 16 OLR 2.5 gVS/l/d 20 %VS Co-Substrate	0.99 (0.15)	0.40 (0.06)	0.73		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d 30 %VS Co-Substrate	1.10 (0.13)	0.44 (0.05)	0.98		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d 30 %VS Co-Substrate	0.98 (0.31)	0.33 (0.10)	0.83	50.7 (1.9)	0.17
Period D - September 10 to October 23 OLR 3.5 gVS/l/d 30 %VS Co-Substrate	1.04 (0.52)	0.30 (0.15)	0.71	51.8 (13.7)	0.15
Period E - October 23 to November 14 OLR 4.0 gVS/l/d 30 %VS Co-Substrate	0.95 (0.37)	0.24 (0.09)	0.13	54.1 (5.4)	0.12
Period F - November 14 to November 23 OLR 4.0 gVS/l/d 40 %VS Co-Substrate	1.24 (0.24)	0.31 (0.06)	0.29	51.8 (4.4)	0.16

Table 4.18 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and VS co-substrate ratio for the whey reactor.

Table 4.18 Feedstock and Digestate Properties for R3 – Cheese Whey

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.5	4.0	4.0
HRT (d)	19	18	17	13	11	10	10
% VS Co-Substrate	0	20	30	30	30	30	40
%w/w Co-Substrate	0.0	16.4	26.3	25.1	25.1	25.1	34.8
Duration of HRT (d)	30	41	23	32	44	22	11
Feedstock							
%TS	6.0	5.7	5.3	5.0	5.0	5.0	5.2
%VS	3.92	4.1	4.2	4.1	4.1	4.0	4.2
COD (g/l)	76.1	70.1	74.3	82.1	83.2	75.5	72.2
Digestate							
Mean pH	7.81	7.85	7.64	7.48	7.62	7.47	7.16
Mean PA (g CaCO ₃ /l)	7.6	6.1	5.0	4.5	3.5	3.3	2.9
Mean TA (g CaCO ₃ /l)	-	7.4	6.5	5.6	4.1	4.3	4.2
Mean Ammonia (g NH ₃ -N/l)	1.7	1.1	0.9	0.7	0.5	0.6	0.5
Mean TVFAs (mg/l)	<30	<30	224	130	<30	42	50
Mean %TS	3.22	3.53	3.68	3.17	3.18	3.59	3.30
Mean %VS	2.27	2.59	2.71	2.25	2.17	2.52	2.28
Mean COD (g/l)	37.7	40.1	44.6	42.6	41.9	34.3	34.4
Removal							
Mean %TS	46.4	60.6	50.8	46.1	55.3	56.9	51.6
Mean %VS	42.2	36.3	34.8	45.7	47.7	36.3	45.9
Mean %COD	50.5	42.8	39.9	48.1	49.6	54.5	52.3

For the cheese whey reactor, the missing data from tipping bucket and a reactor leak discovered at the end of the experimentation period explains the high standard variation observed in the data collected during period D, E and F. The mean co-substrate biogas yield of cheese whey ranged from 0.71 to 0.98 l/gVS from period A to D. These results are in agreement with the co-substrate biogas yields reported by Baserga (2000) of 0.800 to 0.900 l/gVS. Due to the lack of reliable data during the last two OLR, the highest biogas yield was observed during period B (OLR of 2.5 gVS/l/d and a 30 %VS Co-Substrate ratio) with a value of 0.43 l/gVS. During period A, the biogas yield obtained was 0.40 l/gVS with a volumetric biogas production of 0.99 m³/m³/d. Compared to the biogas yield of 0.50 l/gVS

obtained in the semi-continuous trials of poultry manure and whey described in Gelegenis et al. (2007), the biogas yields from R3 were slightly lower (0.40 l/gVS for period A and 0.44 l/gVS for period B) when using 15 and 25 %w/w whey in the feed. This difference could be explained by the lower biogas yield of liquid dairy manure obtained in this study (0.12 to 0.33 l/gVS) compared to the theoretical biogas yield of poultry manure (0.88 l/gVS) reported in Gelegenis et al. (2007). Since methane concentrations were only recorded from period C, the methane yield could not be determined for period B. Average methane concentration in the biogas ranged from 50.7 %Vol. during period C to 54.1% during period E. The initial HRT was 18 days when the reactor was loaded at 2.5 gVS/l/d and was successively reduced to 10 days at a loading rate of 4.0 gVS/l/d during period F. The mean PA and TA also dropped along with HRT from 6.1 and 7.4 g CaCO₃/l, respectively, in period A to 2.9 and 4.2 g CaCO₃/l, respectively, in period F. Similarly, the mean ammonia concentration dropped from 1.1 to 0.5 g NH₃-N/l. The mean ammonia levels were stabilised around 0.5 g NH₃-N/l during period D, E and F while the HRT was 10 and 11 days. The solids and COD removal ranged from 46.1 to 60.6 %TS, 34.8 to 47.7 %VS and 39.9 to 54.5 %COD. The highest COD removal was achieved during period E, while the higher removal of TS was during period A while the maximum VS removal was achieved during period D. Because the mean %TS of the first batch of cheese whey collected during period A and most of period B was 6.5 %, the TS of the influent was 5.7%. From period B to F, the cheese whey collected was 5.2 %TS and the average TS of the influent ranged from 5.0 to 5.3 %. Desai et al. (1994) reported a biogas yield of 1.77 l/gVS and production of 1.76 m³/m³/d with a COD removal rate of 67 and 68% when using a 3:1 mixture of cow and poultry manure with cheese whey using a CSTR. The biogas yields observed during this study were significantly lower (0.24 to 0.44 l/gVS) than how Desai et al. (1994) operating their digesters at an OLR of 6 gTS/l/d.

4.2.5 R4 – Glycerine Reactor

In Figure 4.19, the volumetric biogas production of glycerine as the co-substrate is shown from May 22, 2008 through December 4, 2008. In Figure 4.16, the volumetric biogas production of liquid dairy manure (Figure 4.15) is represented by the green dotted line.

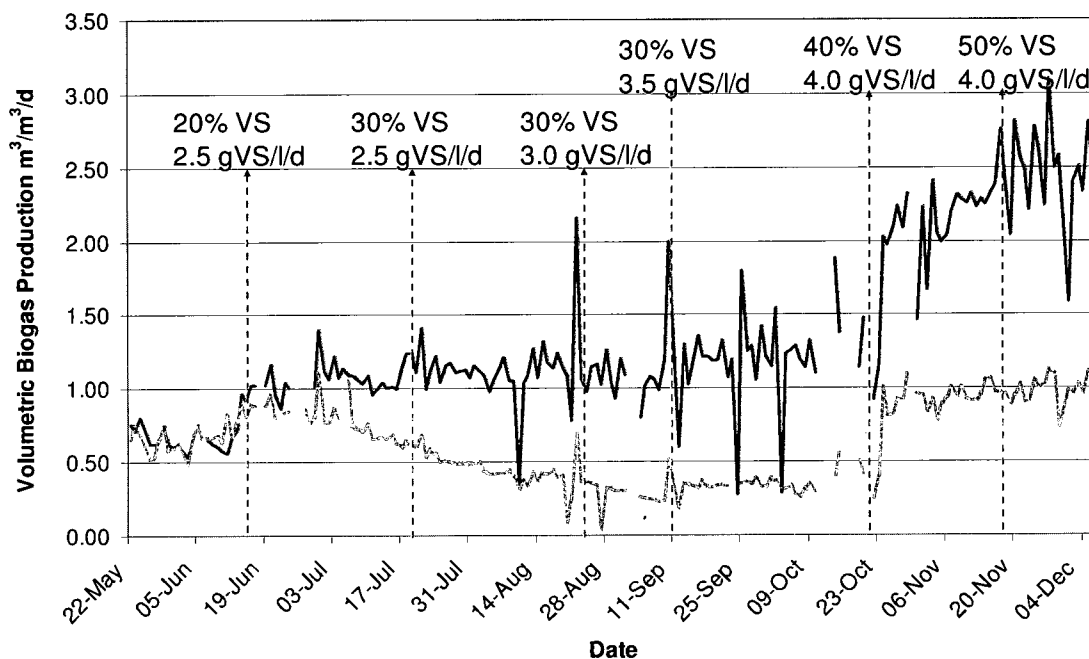


Figure 4.19 Daily Volumetric Biogas Production of R4 Glycerine Reactor

The addition of glycerine on June 13 did not create a sudden increase in volumetric biogas production as opposed to the whey reactor digesting a liquid substrate. This initial reaction to the introduction of glycerine in the feed of R4 could be explained by the time lag seen by Chen et al. (2008) when co-digesting glycerine with dairy manure. During the batch tests, very low biogas production was observed due to a problem related to the digestion. A digestion problem during the batch test could also explain the time lag observed in the semi-continuous reactor. From Table 4.3, glycerine also contained 15% fat from frying residues. As explained in Membrez and Fruteau de Laclos (2004) and Cirne et al. (2007), the presence of LCFA can create inhibition of methanogenesis. Table 4.19 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the glycerine reactor.

Table 4.19 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R4 – Glycerine (Standard Deviation in Parenthesis)

Feeding Regime	Volumetric Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS)	Co-Substrate Biogas Yield (l/gVS)	Methane Content (%Vol.)	Methane Yield (l/gVS)
Control Period – May 7 to June 12 OLR 2.2 gVS/l/d Liquid Dairy Manure	0.67 (0.08)	0.31 (0.04)			
Period A – June 13 to July 16 OLR 2.5 gVS/l/d 20 %VS Co-Substrate	0.96 (0.20)	0.39 (0.08)	0.673		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d 30 %VS Co-Substrate	1.13 (0.09)	0.45 (0.04)	1.02		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d 30 %VS Co-Substrate	1.12 (0.31)	0.37 (0.10)	0.99	51.9 (1.6)	0.19
Period D - September 10 to October 23 OLR 3.5 gVS/l/d 30 %VS Co-Substrate	1.23 (0.35)	0.35 (0.10)	0.89	54.2 (2.8)	0.19
Period E - October 23 to November 14 OLR 4.0 gVS/l/d 40 %VS Co-Substrate	2.13 (0.23)	0.53 (0.06)	0.86	56.0 (6.9)	0.30
Period F - November 14 to December 4 OLR 4.0 gVS/l/d 50 %VS Co-Substrate	2.47 (0.31)	0.62 (0.08)	0.90	59.2 (2.0)	0.37

Table 4.20 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio for the glycerine reactor.

Table 4.20 Feedstock and Digestate Properties for R4 – Glycerine

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.5	4.0	4.0
HRT (d)	19	22	23	19	15	15	19
% VS Co-Substrate	0	20	30	30	30	40	50
%w/w. Co-Substrate	0.0	1.3	2.0	2.0	1.9	2.9	4.4
Duration of HRT (d)	36	35	23	32	44	22	20
Feedstock							
%TS	6.0	6.5	6.6	6.6	6.2	7.1	8.4
%VS	3.92	5.0	5.5	5.5	5.4	6.2	7.4
COD (g/l)	76.1	80.7	92.0	102.2	102.7	101.2	109.6
Digestate							
Mean pH	7.80	7.85	7.81	7.65	7.86	7.65	7.45
Mean PA (g CaCO ₃ /l)	7.2	6.2	5.8	5.4	4.9	4.4	3.9
Mean TA (g CaCO ₃ /l)	0.0	7.7	7.6	6.8	5.9	5.4	5.1
Mean Ammonia (g NH ₃ -N/l)	1.6	1.1	0.9	0.8	0.6	0.6	0.6
Mean TVFAs (mg/l)	193	<30	74	96	59	94	83
Mean %TS	3.09	3.57	3.74	3.56	4.25	5.27	5.50
Mean %VS	2.23	2.64	2.85	2.52	3.15	3.94	3.97
Mean COD (g/l)	38.3	56.8	52.3	52.4	44.6	59.9	59.9
Removal							
Mean %TS	48.4	45.4	43.7	45.8	32.0	25.5	34.4
Mean %VS	43.1	46.7	48.4	54.2	41.9	36.9	46.6
Mean %COD	49.6	29.6	43.2	48.7	56.5	40.8	45.3

The volumetric biogas production from co-digestion of glycerine was initially increased from 0.67 m³/m³/d to 0.96 m³/m³/d with 1.3 %w/w and then doubled to 2.47 m³/m³/d using a 50% VS ratio of glycerine (4.4 %/w) with liquid dairy manure at a OLR of 4.0 gVS/l/d and solids influent concentration of 8.4 %TS. According to Holm-Nielsen et al. (2008), the recommended amount of pure glycerol addition to the influent is situated between 3 and 5g/l. Amon et al. (2006) also recommended a maximum of 6% glycerine to be digested with a manure and corn silage mixture, which resulted in a significant increase in methane yield from 0.569 to 0.679 l/gVS. A similar laboratory trial was conducted by Chen et al. (2008) where the OLR ranged from 1 to 6 gVS/l/d. At the OLR of 4 gVS/l/d and 45% VS glycerine

mixture, the reactor achieved a stable biogas yield of approximately 0.55 l/gVS with a volumetric biogas production rate of 2.21 m³/m³/d. These observations are right in the middle of the results observed during period E (40% VS glycerine) and F (50% VS glycerine) of this study where the specific biogas yield was 0.53 and 0.62 l/gVS and volumetric biogas production rate was 2.13 and 2.47 m³/m³/d. Methane content observed in this trial ranged from 51.9 to 59.2 %Vol., as opposed to 62.6 to 69.7%/Vol. as seen by Chen et al. (2008). The co-substrate biogas yield was significantly higher during period B, C, D and F because of the lag in response in the biogas production during period A, also observed in the batch test study of Chen et al. (2008). The co-substrate biogas yield of glycerine was similar for all periods, except period A (0.67 l/gVS), with values ranging from 0.86 to 1.02 l/gVS which highly exceeded values obtained in the batch test. In comparison with the results of the batch test of crude glycerine by Chen et al. (2008), the biogas yield of 0.67 l/gVS for 14 days of digestion of glycerine is also significantly lower than the co-substrate biogas yield observed during this semi-continuous co-digestion trial (0.86 to 1.02 l/gVS). This could be explained by the 15% fat content of our raw glycerine. Fat has a higher methane potential (1.014 l CH₄/gVS) compared to carbohydrates (0.415 l CH₄/gVS) as shown in Table 2.1 by Angelidaki and Sanders (2004). However, the fat content of the glycerine Chen et al. (2008) used in their trial was not verified. The mean digestate pH ranged from 7.45 in period F to 7.86 in period D when the highest COD removal of 56.5 % was recorded. During the co-digestion experiment, the reactor ammonia concentration dropped from the initial levels recorded in period A of 1.1 g NH₃-N /l and did not contribute significantly to TA and PA levels. Similarly with the corn silage reactor R1, TA and PA values considerably dropped during the co-digestion, initially from 6.2 and 7.7 g CaCO₃/l, respectively, to 3.9 and 5.1 g CaCO₃/l, respectively. The lowest solids removal was also observed at period E, as observed from the corn silage reactor at the lowest HRT of 15 days. Mean TS and VS removal were 25.5 %TS, 36.9 %VS and 40.8% COD, respectively. The highest solids removal was observed in period C with values of 45.8 %TS and 54.2 %VS which is significantly lower than the solids removal observed by Chen et al. (2008) ranging from 51.8 to 59.7 %TS and 57.6 to 66.6 %VS removal.

4.2.6 R5 – DAF Sludge Reactor

In Figure 4.20, the volumetric biogas production of DAF sludge as the co-substrate is shown from May 22, 2008 through December 4, 2008 with the volumetric biogas production of liquid dairy manure (Figure 4.15) represented by the green dotted line.

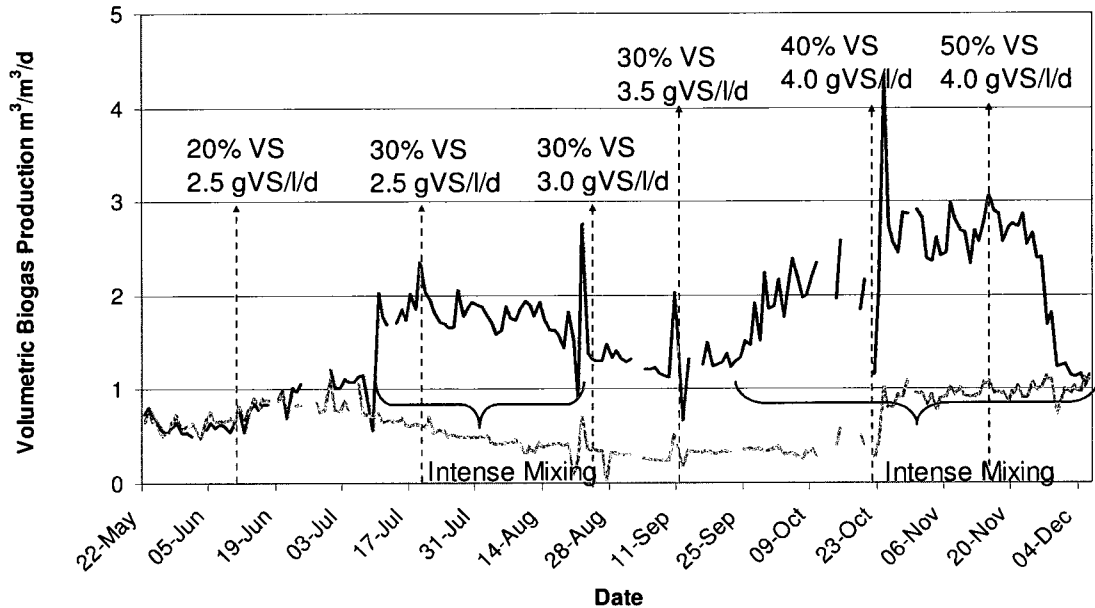


Figure 4.20 Daily Volumetric Biogas Production of R5 DAF Sludge Reactor

When the DAF sludge was added to reactor R5, the volumetric biogas production was similar to that of the R6 manure control reactor up until the last week of June, after which the biogas production in R5 increased considerably. Cirne et al. (2007) demonstrated that due to the LCFA present in the grease, an acclimation period and lag phase is expected. However, half of the inoculum used in the reactors was taken from Terryland Farm digester, which is already acclimated to the DAF sludge waste. From visual observations taken during the first loading rate, DAF sludge floated to the crust layer and started to accumulate. For this reason, on July 9, intense mixing was induced until the crust was disturbed and the DAF sludge layer was mixed in with the reactor contents after each daily feeding until August 23. The intense mixing took at least 30 seconds to mix in the contents of the crust with the DAF sludge. During the last two weeks of the OLR 2.5 gVS/l/d at a 20% co-substrate VS ratio, the

volumetric biogas production almost doubled because of the mixing. This also adds to the fact that the design chosen for this study was not suitable to digest waste containing high concentrations of fats. The DAF sludge contained 49% Crude Fat (Table 4.3). For one month, the intense mixing was stopped to see the effects on the volumetric biogas production. Volumetric biogas production dropped slightly but still considerably higher than that of the liquid dairy manure. On September 23, intense mixing resumed and volumetric biogas production increased to over $2.0 \text{ m}^3/\text{m}^3/\text{d}$ after only a day. Figure 4.21 illustrates a photo taken of the R5 reactor before feeding on November 19 where the grease layer accumulated under the effluent sampling port. When the contents of the reactor were mixed, the color of the digestate turned light brown and the grease/manure mixture inside the reactor started to flocculate, due to emulsion and saponification of fats.



Figure 4.21 Photograph of R5 DAF Sludge Reactor Before Reactor Failure

Reactor failure due to accumulation of LCFA and intense mixing occurred on November 27. Looking back at the batch test of 60%VS DAF sludge, this BMP assay digested successfully since there was no accumulation of grease layer and the contact with the bacteria and the grease layer was controlled.

Table 4.21 shows the different levels of fatty acids on a wet basis present in two digestate samples of R5 reactor. The sample identified as “A” was a composite of five digestate samples taken from the R5 reactor on the following dates:

- September 30
- October 4
- October 10
- October 20
- October 28

Sample “B” was a composite of five digestate samples taken on the following dates:

- November 8
- November 13
- November 20
- November 24
- December 3

Table 4.21 Fatty Acid Levels in Two Digestate Samples for R5- DAF Sludge

Nutritional Parameters	Units	R5 Effluent - A	R5 Effluent - B
C16 Palmitic	g/100g	0.071	0.228
C18 Stearic	g/100g	0.020	0.051
C18:1 Oleic	g/100g	0.013	0.024
C18:2 Linoleic	g/100g	0.004	0.004
Fat (GC/FID)	g/100g	0.129	0.370
Saturated Fatty Acids	g/100g	0.097	0.288

From the composite sample “B”, it is clear that the levels of LCFA is in the limit of the LCFA inhibition range of 0.3 g/100g to 1.5 g/100g identified by Membrez and Fruteau de Laclos (2002). The intense mixing necessary to mix the crust of grease was beyond recommendations of Vavilin and Angelidaki (2005) who stated that at vigorous mixing, methanogenic bacteria cannot establish themselves properly and were not protected from rapid acidification. On the other hand, vigorous mixing was used to increase the contact of the grease with the bacteria present in the reactor. Negative and positive effects of the mixing were not rigorously assessed. Future design of mixing systems should be optimized to mix the grease layer with the reactor contents with as little disturbance of the methanogens as possible, as shown in Membrez and Fruteau de Laclos (2002) with the pilot anaerobic reactor

from VA Tech Webag SA. Table 4.22 presents biogas yield, methane content and methane yield for each OLR and %VS co-substrate ratio of the DAF sludge reactor.

Table 4.22 Measured Parameters of Volumetric Biogas Production, Biogas Yield and Methane Yield for R5 – DAF Sludge (Standard Deviation in Parenthesis)

Feeding Regime	Volumetric Biogas Production (m ³ /m ³ /d)	Biogas Yield (l/gVS)	Co-Substrate Biogas Yield (l/gVS)	Methane Content (%Vol.)	Methane Yield (l/gVS)
Control Period - May 7 to June 12 OLR 2.2 gVS/l/d Liquid Dairy Manure	0.61 (0.09)	0.28 (0.04)			
Period A - June 13 to July 16 OLR 2.5 gVS/l/d 20 %VS Co-Substrate	1.05 (0.41)	0.42 (0.16)	0.85		
Period B - July 16 to August 9 OLR 2.5 gVS/l/d 30 %VS Co-Substrate	1.83 (0.17)	0.73 (0.07)	1.96		
Period C - August 9 to September 10 OLR 3.0 gVS/l/d 30 %VS Co-Substrate	1.52 (0.36)	0.51 (0.12)	1.43	60.5 (0.8)	0.31
Period D - September 10 to October 23 OLR 3.5 gVS/l/d 30 %VS Co-Substrate	1.76 (0.45)	0.50 (0.13)	1.40	57.2 (3.4)	0.29
Period E - October 23 to November 14 OLR 4.0 gVS/l/d 40 %VS Co-Substrate	2.72 (0.42)	0.68 (0.11)	1.24	60.4 (0.7)	0.41
Period F - November 14 to November 26 OLR 4.0 gVS/l/d 50 %VS Co-Substrate	2.76 (0.75)	0.69 (0.19)	1.05	58.6 (3.6)	0.40

Table 4.23 presents pH, PA, TA, Ammonia, TVFAs, solids and COD if applicable, along with COD and solids removal for each OLR and %VS co-substrate ratio for the DAF sludge reactor.

Table 4.23 Feedstock and Digestate Properties for R5 – DAF Sludge

Feeding Regime	Control Period	Period A	Period B	Period C	Period D	Period E	Period F
OLR (gVS/l/d)	2.2	2.5	2.5	3.0	3.5	4.0	4.0
HRT (d)	19	22	22	18	15	15	19
% VS Co-Substrate	0	20	30	30	30	40	50
%w/w. Co-Substrate	0.0	1.6	2.9	1.6	1.6	2.5	3.8
Duration of HRT (d)	36	35	23	32	44	22	20
Feedstock							
%TS	6.0	6.5	6.6	6.2	6.2	7.0	8.3
%VS	3.92	5.5	5.6	5.4	5.4	6.3	7.5
COD (g/l)	76.1	93.1	116.4	108.4	109.8	112.2	126.3
Digestate							
Mean pH	7.86	7.86	7.81	7.60	7.94	7.29	6.85
Mean PA (g CaCO ₃ /l)	6.8	6.1	5.4	4.8	3.9	2.7	1.7
Mean TA (g CaCO ₃ /l)	-	6.6	6.3	6.0	4.7	3.3	2.8
Mean Ammonia (g NH ₃ -N/l)	1.6	1.1	0.9	0.8	0.6	0.6	0.9
Mean TVFAs (mg/l)	<30	<30	154	<30	44	104	940
Mean %TS	3.44	3.47	4.03	3.58	3.24	5.64	6.86
Mean %VS	2.50	2.67	3.07	2.76	2.41	4.28	5.42
Mean COD (g/l)	40.6	58.7	51.9	57.6	72.1	84.3	-
Removal							
Mean %TS	42.7	47.0	39.2	42.4	48.1	19.9	17.7
Mean %VS	36.3	51.1	44.7	49.2	55.7	31.7	27.4
Mean %COD	46.6	36.9	55.4	46.8	34.3	24.9	-

DAF sludge had the highest co-substrate biogas yield of all the co-substrate tested during the lab digestion trials. During period A, the co-substrate biogas yield was 0.85 l/gVS and considerably lower than period B (1.96 l/gVS) when the reactor was intensively mixed daily after feeding. For period C, D, E and F the co-substrate biogas yield dropped from 1.43 to 1.05 l/gVS when the reactor reached its highest volumetric biogas production of 2.76 m³/m³/d with a biogas yield of 0.69 l/gVS. Baserga (2000) reported similar biogas yields of 1.20 to 1.30 l/gVS for DAF sludge. The methane concentration in the biogas ranged from 57.2 to 60.5 %Vol. throughout the co-digestion study. The highest methane yield (0.41 l/gVS) was reached during period E with an OLR of 4.0 gVS/l/d and a 40 %VS DAF sludge

ratio in the influent. Membrez and Fruteau de Laclos (2002) also reached an optimum methane yield of 0.31 l/gVS (calculated) at an OLR of 3.5 to 3.8 gVS/l/d (calculated) during their third loading period with a total fat content of the influent at 41 %TS. The biogas yield was 0.55 l/gVS with an average of 56.6 % methane content in the biogas, reaching high levels of acetic acid (5 g/l) in the reactor. The mean TVFAs concentration of R5 during period E was 140 mg/l but reached 67 mg/l of propionic acid on November 26. When the %VS Co-Substrate ratio was increased to 50%VS DAF Sludge on November 26, the biogas yield, daily volumetric biogas production and methane yield did not changed significantly. After almost one week, the reactor eventually failed at this organic loading rate on December 3. From four VFA samples taken on December 4, two samples showed levels of butyric acid exceeding 1000 mg/l. The PA and TA alkalinity reached threshold values during this feeding period, with values of 1.7 and 2.8 g CaCO₃/l, respectively (Membrez, 1993; Bjornsson et al., 2000). The pH also dropped considerably, with a mean pH of 6.85, reaching threshold levels laid-out in Bjornsson et al. (2000). Solids removal was also low with only 17.7 %TS and 27.4 %VS. These solids removal rates were much lower compared to the range of values observed during period A, B, C and D of 39.2 to 48.1 %TS, 44.7 to 54.7 %VS and 34.3 to 54.6 %COD removal. Because of the high concentration of VS in the DAF sludge, the %TS influent reached 8.3 %TS with a 3.8 %w/w. concentration of DAF sludge in the influent during the last feeding period.

The optimal VS Co-Substrate: VS Liquid Dairy Manure for each reactor compared to liquid dairy manure with a biogas yield of 0.32 l/gVS at an OLR of 3.0 gVS/l/d is listed below:

- 40 %VS Corn Silage co-digested with 60 %VS LDM at an OLR of 4.0 gVS/l/d with a biogas yield of 0.55 l/gVS
- 50 %VS Canola Meal co-digested with 50 %VS LDM at an OLR of 4.0 gVS/l/d with a biogas yield of 0.75 l/gVS
- 30 %VS Whey co-digested with 70 %VS LDM at an OLR of 2.5 gVS/l/d with a biogas yield of 0.44 l/gVS
- 50 %VS Raw Glycerine co-digested with 50 %VS LDM at an OLR of 4.0 gVS/l/d with a biogas yield of 0.62 l/gVS
- 40 %VS DAF Sludge co-digested with 60 %VS LDM at an OLR of 4.0 gVS/l/d with a biogas yield of 0.68 l/gVS

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The anaerobic digestion of regionally available on farm and off farm co-substrates substantially benefits the agricultural community of Eastern Ontario. Not only does it fit with the new directive of Ontario's government for the commitment of production of renewable energy, but it also reduces treatment costs and environmental impacts associated with the disposal of organic wastes in landfills. This study on co-digestion of on-farm and off-farm materials clearly shows the advantages of processing these substrates with liquid dairy manure both during batch and semi-continuous laboratory trials. The use of liquid dairy manure proved to be beneficial to the AD process by supplementing necessary buffering capacity, nitrogen and micro and macro elements for the co-digestion of high carbon content organic materials.

Both solid and liquid co-substrates were processed with liquid dairy manure without difficulty. It was found that the most interesting co-substrate from the farmer's perspective is raw glycerine. Raw glycerine is a very concentrated source of carbon that does not require expensive solid input technology and can be added to the liquid feeding system already installed for pumping liquid dairy manure to the digester. On the other hand, canola meal represented the most interesting co-substrate for further study because of its protein content which supplied enough nitrogen for bacteria growth as opposed to carbon-only co-substrates. Additionally, the semi-continuous pilot digester that co-digested canola meal achieved the highest biogas yield of the trials at 0.75 l/gVS as opposed to liquid dairy manure at 0.32 l/gVS. The other interesting property of canola meal was its rate limiting step in anaerobic degradation. Hydrolysis limited the ability of the acid producing bacteria to degrade canola meal rapidly and acidify the semi-continuous pilot digester during high organic loading rates.

During the initial BMP assays to evaluate the inhibitory limits of co-substrate in various volatile solid mixes, the AD of co-substrates with liquid dairy manure increased the biogas yield from 5 to 50% in the first batch and from 50 to 250% in the second batch. When digestion of these co-substrates was evaluated in semi-continuous pilot digester using liquid

dairy manure that had volumetric biogas production of $0.9 \text{ m}^3/\text{m}^3/\text{d}$ at 13 days of HRT, biogas productivity increased in relation to the co-substrate being co-digested:

- two-fold increase with 7 %w/w. (40 %VS co-substrate) corn silage for dairy livestock and a volumetric biogas production of $2.2 \text{ m}^3/\text{m}^3/\text{d}$ at 15 days of HRT
- three-fold increase with 4 %w/w. (50 %VS co-substrate) cold-pressed canola meal and a volumetric biogas production of $3.0 \text{ m}^3/\text{m}^3/\text{d}$ at 19 days of HRT
- two-fold increase with 26 %w/w. (30 %VS co-substrate) cheese whey and a volumetric biogas production of $1.1 \text{ m}^3/\text{m}^3/\text{d}$ at 17 day of HRT (during period B with LDM pilot digester running at $0.5 \text{ m}^3/\text{m}^3/\text{d}$)
- two and half-fold increase with 4 %w/w. (50 %VS co-substrate) raw glycerine from biodiesel production of yellow grease and a volumetric biogas production of $2.5 \text{ m}^3/\text{m}^3/\text{d}$ at 19 days of HRT
- almost three-fold increase with 4 %w/w. (40 %VS co-substrate) DAF sludge from meat processing and a volumetric biogas production of $2.7 \text{ m}^3/\text{m}^3/\text{d}$ at 19 days of HRT

In general, AD of the co-substrates selected was shown to be effective in CSTRs with short HRTs without digestate recirculation, however this was at the expense of lower %VS and %TS removal efficiencies. Digestion of high carbon co-substrates in volatile solid mixes of 40 to 50% VS was feasible and compares with long term co-digestion trials at the pilot scale level reported in the literature.

5.2 Recommendations for Further Research

These recommendations should be investigated to fine-tune various elements not elaborated and studied in this initial co-digestion experiment:

- to determine the increase in biogas production and solids removal by increasing the solid retention time while keeping a high OLR with process water recirculation (digestate recirculation)

- to determine the effects of pre-treatment of agricultural residues, agri-food and organic fraction of municipal solid waste to optimize biogas yields and solids removal
- to determine possible co-digestion synergistic effect and interaction such as increase in biogas yield between mixtures of various substrates
- to establish appropriate operational conditions of AD digesters to limit the post-methane potential of the digestate and consequently reduce the possible risk of releasing methane from poor digestate management such as uncovered earthen and concrete digestate storage
- to determine the economic and environmental feasibility of replacing cropland in cultivation to feed livestock and to bring back in production fallow and marginal land in Ontario for cultivation of energy crops to produce renewable energy via AD

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Appendix A:

RAW EXPERIMENTAL DATA

This appendix contains all raw data obtained experimentally, except for the daily biogas measurements for the BMP and pilot scale tests since these sets of data would have taken a considerable amount of paper. The data for the daily biogas measurements have been stored at the Alfred Campus of University of Guelph and are available under request.

A.1 Ammonia

Table A.1 Ammonia Calibration Curves

May 30 2008		$y = -25.158 \ln(x) + 128.92$	R2 = 0.9998
Ammonia (mg/l)		Reading (mV)	Recuperation (mV)
	100	13	
	200	-4	
	300	-15	-14
	800	-39	
	1000	-45	-44
Jun 20 2008		$y = -24.723 \ln(x) + 128.92$	R2=0.9998
Ammonia (mg/l)		Reading (mV)	Recoup (mV)
	1000	-42	
	800	-36	-36
	500	-25	
	200	-2	-2
July 18 2008		$y = -22.209 \ln(x) + 116.34$	R2 = 0.997
Ammonia (mg/l)		Reading (mV)	Recoup (mV)
	1000	-38	
	800	-32	-31
	600	-25	
	400	-16	-16
	200	2	
August 1 2008		$y = -26.169 \ln(x) + 143.11$	R2 = 0.9992
Ammonia (mg/l)		Reading (mV)	Recoup (mV)
	1000	-38	-38
	800	-32	-32
	600	-24	
	400	-13	-13
	200	2	

Table A.1 (Continued) Ammonia Calibration Curves

Sep 19 2008	$y = -24.416 \ln(x) + 134.74$	R2=0.9990
Ammonia (mg/l)	Reading (mV)	Recoup (mV)
1000	-34	-36
800	-29	-30
600	-21	
400	-11	-12
200	5	
Nov 12 2008	$y = -24.58 \ln(x) + 156.97$	R2=0.9992
Ammonia (mg/l)	Reading (mV)	Recoup (mV)
1000	-13	
800	-7	-6
600	0	-1
400	9	
200	27	
Dec 15 2008	$y = -24.238 \ln(x) + 121.63$	R2=0.9990
Ammonia (mg/l)	Reading (mV)	Recoup (mV)
1000	-46	
800	-40	-39
600	-34	
400	-23	-23
200	-7	

Table A.2 Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
22-May			
R1-Corn Silage	0.5	-31	1818
R2-Canola Meal	0.5	-30	1767
R3-Cheese Whey	0.5	-27	1616
R4-Raw Glycerine	0.5	-29	1717
R5-DAF Sludge	0.5	-28	1667
R6-Manure (Control)	0.5	-27	1616
29-May			
R1-Corn Silage	0.5	-33	1918
R2-Canola Meal	0.5	-32	1868
R3-Cheese Whey	0.5	-32	1868
R4-Raw Glycerine	0.5	-30	1767
R5-DAF Sludge	0.5	-29	1717
R6-Manure (Control)	0.5	-27	1616
04-Jun			
R1-Corn Silage	0.5	-25	1494
R2-Canola Meal	0.5	-25	1494
R3-Cheese Whey	0.5	-25	1494
R4-Raw Glycerine	0.5	-21	1296
R5-DAF Sludge	0.5	-23	1395
R6-Manure (Control)	0.5	-23	1395
13-Jun			
R1-Corn Silage	0.5	-25	1494
R2-Canola Meal	0.5	-25	1494
R3-Cheese Whey	0.5	-23	1395
R4-Raw Glycerine	0.5	-23	1395
R5-DAF Sludge	0.5	-24	1445
R6-Manure (Control)	0.5	-23	1395
21-Jun			
R1-Corn Silage	0.5	-19	1197
R2-Canola Meal	0.5	-23	1395
R3-Cheese Whey	0.5	-17	1098
R4-Raw Glycerine	0.5	-18	1148
R5-DAF Sludge	0.5	-18	1148
R6-Manure (Control)	0.5	-19	1197

Table A.2 (Continued) Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
01-Jul			
R1-Corn Silage	0.5	-17	988
R2-Canola Meal	0.5	-24	1299
R3-Cheese Whey	0.5	-17	988
R4-Raw Glycerine	0.5	-15	899
R5-DAF Sludge	0.5	-15	899
R6-Manure (Control)	0.5	-17	988
08-Jul			
R1-Corn Silage	0.5	-19	1077
R2-Canola Meal	0.5	-22	1210
R3-Cheese Whey	0.5	-14	855
R4-Raw Glycerine	0.5	-16	943
R5-DAF Sludge	0.5	-15	899
R6-Manure (Control)	0.5	-16	943
17-Jul			
R1-Corn Silage	0.5	-16	943
R2-Canola Meal	0.5	-24	1299
R3-Cheese Whey	0.5	-15	899
R4-Raw Glycerine	0.5	-14	855
R5-DAF Sludge	0.5	-16	943
R6-Manure (Control)	0.5	-16	943
23-Jul			
R1-Corn Silage	0.5	-14	1019
R2-Canola Meal	0.5	-24	1542
R3-Cheese Whey	0.5	-12	914
R4-Raw Glycerine	0.5	-13	967
R5-DAF Sludge	0.5	-12	914
R6-Manure (Control)	0.5	-15	1071
31-Jul			
R1-Corn Silage	0.5	-15	1071
R2-Canola Meal	0.5	-26	1647
R3-Cheese Whey	0.5	-12	914
R4-Raw Glycerine	0.5	-14	1019
R5-DAF Sludge	0.5	-13	967
R6-Manure (Control)	0.5	-16	1124

Table A.2 (Continued) Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
06-Aug			
R1-Corn Silage	0.5	-15	1002
R2-Canola Meal	0.5	-27	1588
R3-Cheese Whey	0.5	-11	807
R4-Raw Glycerine	0.5	-13	904
R5-DAF Sludge	0.5	-12	855
R6-Manure (Control)	0.5	-14	953
11-Aug			
R1-Corn Silage	0.5	-12	855
R2-Canola Meal	0.5	-27	1588
R3-Cheese Whey	0.5	-10	758
R4-Raw Glycerine	0.5	-13	904
R5-DAF Sludge	0.5	-11	807
R6-Manure (Control)	0.5	-13	904
22-Aug			
R1-Corn Silage	0.5	-12	855
R2-Canola Meal	0.5	-29	1686
R3-Cheese Whey	0.5	-8	660
R4-Raw Glycerine	0.5	-11	807
R5-DAF Sludge	0.5	-11	807
R6-Manure (Control)	0.5	-14	953
29-Aug			
R1-Corn Silage	0.5	-11	807
R2-Canola Meal	0.5	-27	1588
R3-Cheese Whey	0.5	-9	709
R4-Raw Glycerine	0.5	-10	758
R5-DAF Sludge	0.5	-10	758
R6-Manure (Control)	0.5	-17	1100
07-Sep			
R1-Corn Silage	0.5	-11	807
R2-Canola Meal	0.5	-28	1637
R3-Cheese Whey	0.5	-8	660
R4-Raw Glycerine	0.5	-10	758
R5-DAF Sludge	0.5	-12	855
R6-Manure (Control)	0.5	-14	953

Table A.2 (Continued) Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
12-Sep			
R1-Corn Silage	0.5	10	611
R2-Canola Meal	0.5	-6	1854
R3-Cheese Whey	0.5	14	561
R4-Raw Glycerine	0.5	13	572
R5-DAF Sludge	0.5	13	572
R6-Manure (Control)	0.5	9	627
20-Sep			
R1-Corn Silage	0.5	12	584
R2-Canola Meal	0.5	-5	1704
R3-Cheese Whey	0.5	28	457
R4-Raw Glycerine	0.5	14	561
R5-DAF Sludge	0.5	14	561
R6-Manure (Control)	0.5	9	627
30-Sep			
R1-Corn Silage	0.5	10	611
R2-Canola Meal	0.5	-6	1854
R3-Cheese Whey	0.5	16	541
R4-Raw Glycerine	0.5	14	561
R5-DAF Sludge	0.5	12	584
R6-Manure (Control)	0.5	8	644
07-Oct			
R1-Corn Silage	0.5	9	627
R2-Canola Meal	0.5	-7	2003
R3-Cheese Whey	0.5	14	561
R4-Raw Glycerine	0.5	12	584
R5-DAF Sludge	0.5	12	584
R6-Manure (Control)	0.5	6	688
20-Oct			
R1-Corn Silage	0.5	13	572
R2-Canola Meal	0.5	-3	1405
R3-Cheese Whey	0.5	19	515
R4-Raw Glycerine	0.5	15	550
R5-DAF Sludge	0.5	14	561
R6-Manure (Control)	0.5	10	611

Table A.2 (Continued) Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
28-Oct			
R1-Corn Silage	0.5	12	584
R2-Canola Meal	0.5	-8	2153
R3-Cheese Whey	0.5	17	532
R4-Raw Glycerine	0.5	15	550
R5-DAF Sludge	0.5	14	561
R6-Manure (Control)	0.5	10	611
08-Nov			
R1-Corn Silage	0.5	15	550
R2-Canola Meal	0.5	-8	2153
R3-Cheese Whey	0.5	22	493
R4-Raw Glycerine	0.5	21	500
R5-DAF Sludge	0.5	20	507
R6-Manure (Control)	0.5	12	584
13-Nov			
R1-Corn Silage	0.5	-13	873
R2-Canola Meal	0.5	-37	2037
R3-Cheese Whey	0.5	-11	776
R4-Raw Glycerine	0.5	-8	631
R5-DAF Sludge	0.5	-8	631
R6-Manure (Control)	0.5	-16	1019
20-Nov			
R1-Corn Silage	0.5	-13	873
R2-Canola Meal	0.5	-41	2231
R3-Cheese Whey	0.5	-7	583
R4-Raw Glycerine	0.5	-8	631
R5-DAF Sludge	0.5	-9	680
R6-Manure (Control)	0.5	-17	1067
24-Nov			
R1-Corn Silage	0.5	-14	922
R2-Canola Meal	0.5	-43	2328
R3-Cheese Whey	0.5	-4	437
R4-Raw Glycerine	0.5	-7	583
R5-DAF Sludge	0.5	-12	825
R6-Manure (Control)	0.5	-20	1213

Table A.2 (Continued) Ammonia Readings for Digestate and Fresh Manure Samples

Date/Label	Dilution Factor	Reading (mV)	Ammonia (mg/l)
03-Dec			
R1-Corn Silage	0.5	-16	1019
R2-Canola Meal	0.5	-45	2425
R3-Cheese Whey	-	-	-
R4-Raw Glycerine	0.5	-8	631
R5-DAF Sludge	0.5	-21	1261
R6-Manure (Control)	0.5	-23	1358
Fresh Manure May 28	0.5	-19	1214
Fresh Manure May 30	0.5	-20	1264
Fresh Manure June 15	0.5	-21	1296
Fresh Manure July 8	0.5	-17	988
Fresh Manure July 13	0.5	-16	943
Fresh Manure July 25	0.5	-15	899
Fresh Manure Sept 7	0.5	-10	758
Fresh Manure Sept 12	0.5	-10	758
Fresh Manure Aug 17	0.5	-13	904
Fresh Manure Oct 31	0.5	20	507
Fresh Manure Oct 21	0.5	22	493
Fresh Manure Nov 8	0.5	16	541
Fresh Manure Sept 19	0.5	15	550
Fresh Manure Nov 15	0.5	-19	1164
Fresh Manure Nov 21	0.5	-22	1310
Fresh Manure Nov 28	0.5	-24	1407

Table A.3 Partial Alkalinity and Total Alkalinity of Digestate, Inoculum, Cheese Whey and Fresh Manure Samples

Date/Label	Sample size (ml)	Titrant volume for PA (ml)	Rounded PA (mg/l CaCO ₃)	Titrant volume for TA (ml)	Rounded TA (mg/l CaCO ₃)
29-May					
R1-Corn Silage	50	0	0	-	-
R2-Canola Meal	50	59.5	5950	-	-
R3-Cheese Whey	50	75.5	7550	-	-
R4-Raw Glycerine	50	71.5	7150	-	-
R5-DAF Sludge	50	67.5	6750	-	-
R6-Manure	50	62.5	6250	-	-
08-Jun					
R1-Corn Silage	50	74.5	7450	93.6	9360
R2-Canola Meal	25	35.4	7080	45.5	9100
R3-Cheese Whey	25	34.5	6900	41.5	8300
R4-Raw Glycerine	25	34.7	6940	40.5	8100
Fresh Manure	25	14.8	2960	32.5	6500
Cheese Whey	50	0	0	3	300
Cheese Whey	50	0	0	2.9	290
R5-DAF Sludge	25	30.6	6120	35.1	7020
R6-Manure	25	30	6000	37	7400
R6-Manure	25	29	5800	35.4	7080
Fresh Manure	25	14.2	2840	33.1	6620
09-Jun					
R1-Corn Silage	25	34	6800	41.4	8280
R2-Canola Meal	25	35	7000	43	8600
R3-Cheese Whey	25	33.5	6700	40.5	8100
R4-Raw Glycerine	25	31.5	6300	38	7600
R5-DAF Sludge	25	29	5800	35	7000
R6-Manure	25	27.5	5500	33.5	6700
20-Jun					
Inoculum Reactor	25	31.3	6260	38	7600
R1-Corn Silage	25	32	6400	40	8000
R2-Canola Meal	25	34	6800	42.5	8500
R3-Cheese Whey	25	30.2	6040	37.4	7480
R4-Raw Glycerine	25	30.5	6100	37	7400
R5-DAF Sludge	25	29.5	5900	35	7000
R6-Manure	25	29.5	5900	36	7200
Fresh Manure	25	12.5	2500	33	6600

Table A.3 (Continued) Partial Alkalinity and Total Alkalinity of Digestate, Inoculum, Cheese Whey and Fresh Manure Samples

Date/Label	Sample size (ml)	Titration volume for PA (ml)	Rounded PA (mg/l CaCO ₃)	Titration volume for TA (ml)	Rounded TA (mg/l CaCO ₃)
30-Jun					
R1-Corn Silage	25	29	5800	37	7400
R2-Canola Meal	25	34	6800	42.3	8460
R3-Cheese Whey	25	27.7	5540	34.5	6900
R4-Raw Glycerine	25	31	6200	41	8200
R5-DAF Sludge	25	25.5	5100	31	6200
R6-Manure	25	27.8	5560	34	6800
Fresh Manure	25	14.9	2980	41.5	8300
10-Jul					
R1-Corn Silage	25	28.9	5780	36	7200
R2-Canola Meal	25	34.3	6860	42.4	8480
R3-Cheese Whey	25	26	5200	32	6400
R4-Raw Glycerine	25	28	5600	35	7000
R5-DAF Sludge	25	23.8	4760	29.5	5900
R6-Manure	25	29.8	5960	37.5	7500
Fresh Manure	25	23	4600	35	7000
Fresh Manure	25	25	5000	37	7400
25-Jul					
Inoculum	25	58.9	11780		
R1-Corn Silage	25	29.4	5880	37.4	7480
R2-Canola Meal	25	35.5	7100	45	9000
R3-Cheese Whey	25	25.5	5100	32.5	6500
R4-Raw Glycerine	25	28.3	5660	35.4	7080
R5-DAF Sludge	25	24	4800	30	6000
R6-Manure	25	29.5	5900	36.4	7280
01-Jul					
R1-Corn Silage (unmixed)	25	23.6	4720	30	6000
R1-Corn Silage (mixed)	25	27.4	5480	35	7000
R2-Canola Meal	25	35.6	7120	44	8800
R3-Cheese Whey	25	24.5	4900	32	6400
R4-Raw Glycerine	25	29.2	5840	40.5	8100
R5-DAF Sludge	25	29.6	5920	33	6600
R6-Manure	25	26.5	5300	32.9	6580
Fresh Manure	25	25.6	5120	37.3	7460

Table A.3 (Continued) Partial Alkalinity and Total Alkalinity of Digestate, Inoculum, Cheese Whey and Fresh Manure Samples

Date/Label	Sample size (ml)	Titrant volume for PA (ml)	Rounded PA (mg/l CaCO ₃)	Titrant volume for TA (ml)	Rounded TA (mg/l CaCO ₃)
11-Aug					
R1-Corn Silage	25	28	5600	34	6800
R2-Canola Meal	25	37.2	7440	49	9800
R3-Cheese Whey	25	22.4	4480	28	5600
R4-Raw Glycerine	25	26.5	5300	33.5	6700
R5-DAF Sludge	25	23.5	4700	29.5	5900
R6-Manure	25	24.5	4900	30	6000
22-Aug					
R1-Corn Silage	25	27.2	5440	35	7000
R2-Canola Meal	25	37.5	7500	47	9400
R3-Cheese Whey	25	24.2	4840	30.5	6100
R4-Raw Glycerine	25	26.5	5300	34.5	6900
R5-DAF Sludge	25	26.7	5340	33	6600
R6-Manure	25	28.4	5680	35	7000
10-Sep					
R1-Corn Silage	25	24.3	4860	29.8	5960
R2-Canola Meal	25	39	7800	47.3	9460
R3-Cheese Whey	25	21	4200	26	5200
R4-Raw Glycerine	25	27.5	5500	34.3	6860
R5-DAF Sludge	25	22.4	4480	27	5400
R6-Manure	25	25.6	5120	31	6200
20-Sep					
R1-Corn Silage	10	10	5000	11.6	5800
R2-Canola Meal	15	17.4	5800	21	7000
R3-Cheese Whey	15	7	2333	8.7	2900
R4-Raw Glycerine	15	15.2	5067	18	6000
R5-DAF Sludge	15	12	4000	14.6	4867
R6-Manure	15	14.7	4900	17.7	5900
04-Oct					
R1-Corn Silage	15	15	5000	18.2	6067
R2-Canola Meal	15	20.2	6733	24.3	8100
R3-Cheese Whey	15	12.5	4167	15	5000
R4-Raw Glycerine	15	14.6	4867	19.2	6400
R5-DAF Sludge	15	11.5	3833	14.5	4833
R6-Manure	15	15	5000	18.5	6167

Table A.3 (Continued) Partial Alkalinity and Total Alkalinity of Digestate, Inoculum, Cheese Whey and Fresh Manure Samples

Date/Label	Sample size (ml)	Titration volume for PA (ml)	Rounded PA (mg/l CaCO ₃)	Titration volume for TA (ml)	Rounded TA (mg/l CaCO ₃)
20-Oct					
R1-Corn Silage	15	15.5	5167	18.5	6167
R2-Canola Meal	15	22.2	7400	25.8	8600
R3-Cheese Whey	15	11.7	3900	13.5	4500
R4-Raw Glycerine	15	14.7	4900	16.2	5400
R5-DAF Sludge	15	12	4000	13.5	4500
R6-Manure	15	15.5	5167	18.1	6033
08-Nov					
R1-Corn Silage	15	12.9	4300	15.3	5100
R2-Canola Meal	15	18.7	6233	22.2	7400
R3-Cheese Whey	15	10	3333	11.5	3833
R4-Raw Glycerine	15	13.5	4500	15.3	5100
R5-DAF Sludge	15	8.5	2833	10.5	3500
R6-Manure	15	13.5	4500	16	5333
Fresh Manure	15	8.3	2767	13.5	4500
Fresh Manure	15	5.1	1700	12.8	4267
Fresh Manure	15	9.1	3033	18.2	6067
13-Nov					
R1-Corn Silage	15	14	4667	15.6	5200
R1-Corn Silage	15	13.5	4500	17	5667
R2-Canola Meal	15	20.7	6900	26.4	8800
R3-Cheese Whey	15	9.9	3300	14	4667
R4-Raw Glycerine	15	12.6	4200	17.2	5733
R5-DAF Sludge	15	7.4	2467	9.3	3100
R6-Manure	15	12.2	4067	-	-
20-Nov					
R1-Corn Silage	15	11	3667	14.9	4967
R2-Canola Meal	15	20	6667	26.6	8867
R3-Cheese Whey	15	9.1	3033	14	4667
R4-Raw Glycerine	15	11.5	3833	15.4	5133
R5-DAF Sludge	15	6.3	2100	9	3000
R6-Manure	15	11.5	3833	15	5000

Table A.3 (Continued) Partial Alkalinity and Total Alkalinity of Digestate, Inoculum, Cheese Whey and Fresh Manure Samples

Date/Label	Sample size (ml)	Titration volume for PA (ml)	Rounded PA (mg/l CaCO ₃)	Titration volume for TA (ml)	Rounded TA (mg/l CaCO ₃)
24-Nov					
R1-Corn Silage	15	11.7	3900	-	-
R2-Canola Meal	15	21.5	7167	27.6	9200
R3-Cheese Whey	15	8.3	2767	11	3667
R4-Raw Glycerine	15	11.9	3967	15.6	5200
R5-DAF Sludge	15	6.3	2100	9.3	3100
R6-Manure	15	11.7	3900	14.7	4900
03-Dec					
R1-Corn Silage	15	12.4	4133	16.7	5567
R2-Canola Meal	15	22.3	7433	28.4	9467
R4-Raw Glycerine	15	11.6	3867	15.3	5100
R5-DAF Sludge	15	2.4	800	6.7	2233
R6-Manure	15	13.8	4600	16.6	5533

Table A.4 COD Calibration Curves

COD (mg/l)	Absorbance	COD (mg/l)	Absorbance
0	0.012	0	0.010
100	0.052	100	0.058
200	0.086	200	0.093
300	0.140	300	0.132
400	0.168	400	0.171
500	0.208	500	0.200
600	0.254	600	0.250
700	0.292	700	0.262
800	0.343	800	0.308
900	0.374	900	0.360
1000	0.380	1000	0.395
y = 2661.7x - 41.787 R2 = 0.9962		y = 2456.2x - 23.798 R2 = 0.9984	

Table A.5 COD for Manure Samples

Date/Label	Absorbance	Dilution Factor	Actual COD (mg/l)
Aug 12 Man	0.295	100	70078
Aug 12 Man	0.337	100	80394
Aug 16 Manure	0.371	100	88745
Sept 8 Manure	0.343	100	81868
Sept 15 Manure	0.342	100	81622
Oct 21 Manure	0.384	100	91938
Oct 31 Manure	0.318	100	75727
Nov 8 Manure	0.324	100	77201
Nov 15 Manure	0.256	100	60499
Nov 21 Manure	0.35	100	83587
Nov 28 Manure	0.217	100	50920
May 6 Manure	0.323	100	76955
May 12 Manure	0.343	100	81868
June 1 Manure	0.292	100	69341
June 16 Manure	0.23	100	54113
June 22 Manure	0.342	100	81622
July 26 Manure	0.312	100	74254
July 1 Manure	0.307	100	73026
July 16 Manure	0.288	100	68359

Table A.6 Soluble COD for Manure Samples

Date/Label	Absorbance	Dilution Factor	Actual sCOD (mg/l)
June 1 Manure	0.078	50	8389
June 16 Manure	0.053	50	5319
Nov 28 Manure	0.079	50	8512
Nov 15 Manure	0.058	50	5933
Nov 8 Manure	0.076	50	8144
Sept 8 Manure	0.035	50	3108
Oct 31 Manure	0.082	50	8881
June 1 Manure	0.087	50	9495
Nov 21 Manure	0.065	50	6793
Sept 15 Manure	0.039	50	3600
Aug 12 Manure	0.034	50	2986
July 1 Manure	0.083	50	9003
May 12 Manure	0.118	50	13302
Oct 21 Manure	0.088	50	9617
June 22 Manure	0.106	50	11828
July 16 Manure	0.048	50	4705
May 6 Manure	0.104	50	11582
July 26 Manure	0.045	50	4337
Sept 8 Manure	0.038	50	3477
Aug 16 Manure	0.043	50	4091
Aug 12 Manure	0.029	50	2372

Table A.7 COD and sCOD of Liquid Feedstock and Checks

Sample	Absorbance	Dilution Factor	Actual COD (mg/l)
Whey COD	0.307	100	73026
Whey COD	0.303	100	72043
Whey sCOD	0.301	100	71552
Whey sCOD	0.296	100	70324
Glycerine	0.376	1000	899733
Glycerine	0.365	1000	933893
Glycerine	0.492	500	592326
Glycerine	0.378	1000	968725
500 mg/l (Check)	0.203	-	475
100 mg/l (Check)	0.053	-	106
300 mg/l (Check)	0.138	-	326
700 mg/l (Check)	0.291	-	736

Table A.8 COD for Effluent Samples

Date/Label	Absorbance	Dilution Factor	Actual COD (mg/l)
22-May			
R1-Corn Silage	0.173	100	41945
R2-Canola Meal	0.214	100	52930
R2-Canola Meal	0.182	100	44356
R3-Cheese Whey	0.168	100	40605
R4-Raw Glycerine	0.143	100	33907
R5-DAF Sludge	0.181	100	44088
R6-Manure (Ctrl)	0.196	100	48107
31-May			
R1-Corn Silage	0.135	100	31763
R2-Canola Meal	0.166	100	40069
R3-Cheese Whey	0.146	100	34710
R4-Raw Glycerine	0.176	100	42749
R5-DAF Sludge	0.155	100	37122
R6-Manure (Ctrl)	0.177	100	43017
22-Jun			
R1-Corn Silage	0.173	100	41945
R2-Canola Meal	0.203	100	49983
R3-Cheese Whey	0.166	100	40069
R4-Raw Glycerine	0.252	100	63112
R5-DAF Sludge	0.287	100	72490
R6-Manure (Ctrl)	0.307	100	77849
R6-Manure (Ctrl)	0.211	100	52127
18-Jul			
R1-Corn Silage	0.078	100	16491
R2-Canola Meal	0.213	100	52662
R3-Cheese Whey	0.183	100	44624
R4-Raw Glycerine	0.205	100	50519
R5-DAF Sludge	0.184	100	44892
R6-Manure (Ctrl)	0.215	100	53198
11-Aug			
R1-Corn Silage	0.276	100	69543
R2-Canola Meal	0.238	100	59361
R3-Cheese Whey	0.191	100	46768
R4-Raw Glycerine	0.218	100	54002
R5-DAF Sludge	0.236	100	58825
R6-Manure (Ctrl)	0.229	100	56949

Table A.8 (Continued) COD for Effluent Samples

Date/Label	Absorbance	Dilution Factor	Actual COD (mg/l)
29-Aug			
R1-Corn Silage	0.246	100	61504
R2-Canola Meal	0.259	100	64988
R3-Cheese Whey	0.16	100	38462
R4-Raw Glycerine	0.206	100	50787
R5-DAF Sludge	0.227	100	56414
R6-Manure (Ctrl)	0.205	100	50519
30-Sep			
R1-Corn Silage	0.375	100	96069
R2-Canola Meal	0.281	100	70882
R3-Cheese Whey	0.173	100	41945
R4-Raw Glycerine	0.293	100	40337
R5-DAF Sludge	0.167	100	74098
R6-Manure (Ctrl)	0.359	100	91782
10-Oct			
R1-Corn Silage	0.327	100	83208
R2-Canola Meal	0.211	100	52127
R3-Cheese Whey	0.173	100	41945
R4-Raw Glycerine	0.199	100	48911
R5-DAF Sludge	0.278	100	70079
R6-Manure (Ctrl)	0.202	100	49715
13-Nov			
R1-Corn Silage	0.255	100	63916
R2-Canola Meal	0.289	100	73026
R3-Cheese Whey	0.138	100	32567
R4-Raw Glycerine	0.258	100	64720
R5-DAF Sludge	0.331	100	84279
R6-Manure (Ctrl)	0.257	100	64452
20-Nov			
R1-Corn Silage	0.237	100	59093
R2-Canola Meal	0.252	100	63112
R3-Cheese Whey	0.151	100	36050
R4-Raw Glycerine	0.243	100	60701
R5-DAF Sludge	0.363	100	92853
R6-Manure (Ctrl)	0.244	100	60969

Table A.8 (Continued) COD for Effluent Samples

Date/Label	Absorbance	Dilution Factor	Actual COD (mg/l)
24-Nov			
R1-Corn Silage	0.247	100	61772
R2-Canola Meal	0.265	100	66595
R3-Cheese Whey	0.145	100	34443
R4-Raw Glycerine	0.237	100	59093
R5-DAF Sludge	0.167	100	40337
R5-DAF Sludge	0.151	100	36050
R6-Manure (Ctrl)	0.247	100	61772
03-Dec			
R1-Corn Silage	0.203	100	49983
R2-Canola Meal	0.217	100	53734
R4-Raw Glycerine	0.321	100	81600
R5-DAF Sludge	0.193	100	47304
R5-DAF Sludge	0.195	100	47840
R6-Manure (Ctrl)	0.196	100	48107

Table A.9 Volatile Fatty Acids Concentration in Effluent and Manure (ND = Non Detectable)

Date/Label	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)
16-May			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	34	31	127
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	58	ND	ND
Fresh Manure	3540	823	523
04-Jun			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
23-Jul			
R1 Corn Silage Effluent	577	ND	ND
R2 Canola Effluent	74	ND	ND
R3 Whey Effluent	418	ND	ND
R4 Glycerine Effluent	ND	54	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
07-Sep			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	108	ND	ND
R4 Glycerine Effluent	76	20	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
16-Sep			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	167	ND	ND
R3 Whey Effluent	10	ND	ND
R4 Glycerine Effluent	24	ND	ND
R5 DAF Effluent	53	12	ND
R6 Manure Effluent	ND	ND	ND

Table A.9 (Continued) Volatile Fatty Acids Concentration in Effluent and Manure (ND = Non Detectable)

Date/Label	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)
20-Oct			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	63	16	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
27-Oct			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	663	69	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	167	53	ND
R6 Manure Effluent	23	26	ND
26-Nov			
R1 Corn Silage Effluent	ND	95	ND
R2 Canola Effluent	332	44	ND
R3 Whey Effluent	ND	14	ND
R4 Glycerine Effluent	37	ND	ND
R5 DAF Effluent	57	67	ND
R6 Manure Effluent	ND	ND	ND
04-Dec			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	452	340	60
R5 DAF Effluent	503	185	49
R5 DAF Effluent	602	429	1086
R5 DAF Effluent	615	432	1058
R6 Manure Effluent	26	20	ND

Table A.9 (Continued) Volatile Fatty Acids Concentration in Effluent and Manure (ND = Non Detectable)

Date/Label	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)
09-Jun			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	36	ND	ND
17-Jun			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	21	13	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
31-Jul			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	ND	ND	ND
R5 DAF Effluent	34	110	ND
R6 Manure Effluent	50	ND	ND
Aug-11			
R2 Canola Effluent	ND	ND	ND
Aug-22			
R2 Canola Effluent	62	ND	ND
R6 Manure Effluent	ND	ND	ND

Table A.9 (Continued) Volatile Fatty Acids Concentration in Effluent and Manure (ND = Non Detectable)

Date/Label	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)
04-Oct			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	50	ND	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	15	79	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	ND	ND
10-Oct			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	187	103	ND
28-Oct			
R1 Corn Silage Effluent	26	ND	ND
R2 Canola Effluent	ND	13	ND
R3 Whey Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	ND	ND	ND
R6 Manure Effluent	ND	12	ND
03-Nov			
R1 Corn Silage Effluent	56	28	ND
R2 Canola Effluent	269	95	ND
R3 Whey Effluent	46	ND	ND
R4 Glycerine Effluent	136	77	ND
R5 DAF Effluent	33	ND	ND
R6 Manure Effluent	ND	97	ND
03-Dec			
R1 Corn Silage Effluent	ND	ND	ND
R2 Canola Effluent	ND	ND	ND
R4 Glycerine Effluent	ND	ND	ND
R5 DAF Effluent	273	299	ND
R6 Manure Effluent	ND	ND	ND

Table A.10 Total and Volatile Solids Results for the Substrates

Substrate	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
Canola Meal	46.52	68.99	66.73	47.87	89.9%	83.9%
Canola Meal	53.76	72.16	70.45	54.86	90.7%	84.7%
Corn Silage	46.76	58.09	50.93	46.97	36.8%	35.0%
Corn Silage	50.93	64.31	55.53	51.17	34.4%	32.6%
Inoculum	38.94	93.45	41.69	39.74	5.0%	3.6%
Inoculum	40.95	95.87	43.76	41.72	5.1%	3.7%
Whey	37.97	95.23	41.28	38.56	5.8%	4.8%
Whey	46.07	100.67	49.3	46.67	5.9%	4.8%
Corn Syrup	40.07	79.29	50.36	42.22	26.2%	20.8%
Corn Syrup	36.4	72.25	46.17	37.33	27.3%	24.7%
DAF	45.45	90	49.15	45.93	8.3%	7.2%
DAF	50.59	95.64	54.3	51.08	8.2%	7.1%
FOG	38.36	84.73	45.5	38.62	15.4%	14.8%
FOG	46.73	92.94	53.7	46.91	15.1%	15.1%
Manure	46.95	87.87	48.46	47.2	3.7%	3.1%
Manure	39.09	75.6	40.75	39.38	4.5%	3.8%
Whey	50.56	90.86	53.21	51.03	6.6%	5.4%
Canola	50.86	60.79	59.71	51.42	89.1%	83.5%
Corn Silage	39.82	52.69	44.21	40.03	34.1%	32.5%
Grease Trap	37.74	64.43	41.56	37.83	14.3%	14.0%
Inoculum	36.85	65.94	38.21	37.23	4.7%	3.4%
Manure	52.33	96.1	54.06	52.69	4.0%	3.1%
Manure	56.47	100.09	58.32	56.84	4.2%	3.4%
DAF	52.22	92.37	79.42	53.64	67.7%	64.2%
Grease Trap	50	96.08	56.78	50.45	14.7%	13.7%
Whey	56.58	89.91	58.87	57.02	6.9%	5.6%
Whey	52.35	90.3	55.01	52.85	7.0%	5.7%
Canola Meal	52.2	61.64	60.9	50.65	92.2%	108.6%
Canola Meal	54.69	58.12	57.3	54.72	76.1%	75.2%
Corn Silage	54.29	63.97	58.03	54.87	38.6%	32.6%
Corn silage	50.18	68.16	57.61	50.48	41.3%	39.7%
Manure May 6	36.91	82.14	39.89	37.49	6.6%	5.3%
Manure May 9	38	80.97	40.72	38.55	6.3%	5.1%
Manure June 6	50.2	105.49	52.88	50.75	4.8%	3.9%
Manure June 9	39.18	92.56	42.18	39.82	5.6%	4.4%
Manure June 10	45.5	101.79	48.83	46.12	5.9%	4.8%
Manure June 18	46.14	107.09	49.63	46.86	5.7%	4.5%
Manure June 18	38	92.05	40.99	38.61	5.5%	4.4%

Table A.10 (Continued) Total and Volatile Solids Results for the Substrates

Substrate	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
Canola	38.99	56.39	54.67	40.28	90.1%	82.7%
Corn Silage	47.03	65.75	53.26	47.35	33.3%	31.6%
Whey	37.12	78.2	39.62	37.67	6.1%	4.7%
DAF	41.47	77.08	56.84	-	43.2%	-
Glycerine	46.6	101.31	94.44	-	87.4%	-
Glycerine	46.83	116.7	108.09	-	87.7%	-
DAF B Jul 18	36.66	50.71	44.87	37.01	58.4%	55.9%
DAF A Jul 18	54.62	68.79	63.21	55.03	60.6%	57.7%
Corn A Sept	47.03	54.51	49.57	47.12	34.0%	32.8%
Corn B Sept	38	44.33	40.15	38.08	34.0%	32.7%
Whey A Sept	50.99	81.5	52.59	51.36	5.2%	4.0%
Whey B Sept	56.63	97.55	58.8	57.13	5.3%	4.1%
DAF Sept	41.94	50.17	50.02	42	98.2%	97.4%
DAF Sept	47.38	59.84	59.6	47.48	98.1%	97.3%
Canola A Sept	49.97	53.76	53.35	50.21	89.2%	82.8%
Canola B Sept	54.66	60	59.41	54.99	89.0%	82.8%
Glycerine Sept	37.29	44.61	43.76	37.47	88.4%	85.9%
Glycerine Sept	51.14	66.84	65.15	51.53	89.2%	86.8%
Manure Aug 15	46.03	95.23	47.67	46.44	3.3%	2.5%
Manure Sept 8	47.18	103.66	49.43	47.69	4.0%	3.1%
Manure Sept 12	48.35	106.4	51.07	48.94	4.7%	3.7%
Manure July 16	36.44	66.45	38.05	36.76	5.4%	4.3%
Manure July 26	50.64	101.34	53.71	51.21	6.1%	4.9%
Glycerine	36.43	59.97	56.9	37.03	87.0%	84.4%
Manure Sept 8	37.99	78.41	39.46	38.34	3.6%	2.8%
Manure Sept 19	47.02	88.67	48.99	47.46	4.7%	3.7%
Manure Oct 21	50.3	84.59	53.37	50.78	9.0%	7.6%
Manure Nov 15	39.17	77.61	40.94	39.64	4.6%	3.4%
Manure Nov 28	36.91	80.45	38.77	37.35	4.3%	3.3%
Manure Aug 16	50.94	101.31	53.08	51.4	4.2%	3.3%
Manure Oct 21	48.02	93.14	50.17	48.42	4.8%	3.9%

Table A.11 Total and Volatile Solids for First Batch of BMP Assays

Label of BMP	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
M7S3.1	36.9	82.03	38.1	37.29	2.7%	1.8%
M7S3.2	41.78	79.7	42.73	42.1	2.5%	1.7%
M5S5.1	50.98	94.76	52.03	51.31	2.4%	1.6%
M5S5.2	39.16	80.46	40.17	39.5	2.4%	1.6%
M7G3.1	46.57	81.55	47.42	46.85	2.4%	1.6%
M7G3.2	46.8	78.42	47.57	47.03	2.4%	1.7%
C1.1	50.63	94.02	51.54	50.97	2.1%	1.3%
C1.2	50.93	105.57	52.06	51.36	2.1%	1.3%
M8C2.1	46.11	81.83	47.29	46.49	3.3%	2.2%
M8C2.2	38.96	69.98	39.92	39.26	3.1%	2.1%
M6C4.1	37.79	66.17	38.55	38.05	2.7%	1.8%
M6C4.2	40.98	79.69	42.05	41.33	2.8%	1.9%
M6W1S3.1	38.38	59.93	38.88	38.56	2.3%	1.5%
M6W1S3.2	49.51	87.1	50.43	49.85	2.4%	1.5%
M4W1S5.1	39.89	65.8	40.58	40.14	2.7%	1.7%
M4W1S5.2	45.47	85.07	46.36	45.81	2.2%	1.4%
M5G2S2.1	37.99	73.97	38.79	38.26	2.2%	1.5%
M5G2S2.2	36.43	70.94	37.22	36.69	2.3%	1.5%
M7W3.2	47.93	94.64	49.28	48.41	2.9%	1.9%
G1.1	47.02	91.28	48.47	47.35	3.3%	2.5%
G1.2	37.67	85.15	39.12	38	3.1%	2.4%
M1.1	45.5	84.48	46.35	45.79	2.2%	1.4%
M1.2	39.9	85.15	40.83	40.22	2.1%	1.3%
I1.1	39.18	60.42	39.58	39.35	1.9%	1.1%
I1.2	50.95	73.71	51.42	51.16	2.1%	1.1%
I1.3	36.45	53.66	37.02	36.68	3.3%	2.0%
I1.4	40.99	65.74	41.75	41.3	3.1%	1.8%
M4G6.1	50.64	86.37	51.52	50.97	2.5%	1.5%
M4G6.2	46.83	84.2	47.82	47.16	2.6%	1.8%
M3W7.1	50.67	92.15	51.6	51.11	2.2%	1.2%
M3W7.2	51.02	90.97	51.8	51.42	2.0%	1.0%
W1.1	37.13	79.35	38.08	37.64	2.3%	1.0%
W1.2	38.02	75.06	38.97	38.48	2.6%	1.3%
S1.1	39.92	76.01	40.69	40.22	2.1%	1.3%
S1.2	39	74.09	39.83	39.31	2.4%	1.5%

Table A.12 Total and Volatile Solids for Second Batch of BMP Assays

Label of BMP	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
d6.a	47.91	68.87	48.65	48.11	3.5%	2.6%
d4.b	54.67	84	55.74	54.95	3.6%	2.7%
is.a	47.5	67.37	48.19	47.71	3.5%	2.4%
d6.b	38	57.63	38.72	38.2	3.7%	2.6%
ms.b	38.97	71.46	40.25	39.31	3.9%	2.9%
g4.a	56.63	81.33	57.57	56.88	3.8%	2.8%
g6.a	37.11	51.26	37.63	37.25	3.7%	2.7%
ms.a	47.03	73.1	48.03	47.31	3.8%	2.8%
gly4.b	47.18	67.47	47.92	47.41	3.6%	2.5%
g6.b	47.05	73.84	48.06	47.32	3.8%	2.8%
is.b	58.69	85.83	59.82	59.01	4.2%	3.0%
gly20	53.83	73.54	54.78	54.09	4.8%	3.5%
daf20	48.36	65.56	50.05	48.54	9.8%	8.8%
cm2.a	46.04	68.86	46.98	46.31	4.1%	2.9%
cm1.a	49.97	71.63	50.8	50.22	3.8%	2.7%
cm2.b	36.43	63.03	37.48	36.73	3.9%	2.8%
cm1.b	49.07	71.09	49.87	49.25	3.6%	2.8%
cm4.b	50.23	68.38	51.02	50.44	4.4%	3.2%
cm3.b	38.4	57.61	39.21	38.62	4.2%	3.1%
cm5.a	46.78	73.62	48.37	47.19	5.9%	4.4%
cm3.a	46.82	72.01	47.92	47.11	4.4%	3.2%
c2.b	49.02	67.71	49.82	49.26	4.3%	3.0%
c3.a	49.54	69.05	50.41	49.78	4.5%	3.2%
c4.b	49.92	73.21	51	50.25	4.6%	3.2%
c3.b	50.63	72.14	51.52	50.89	4.1%	2.9%
c5.a	40	62.27	41.63	40.32	7.3%	5.9%
c1.a	50.15	75.8	51.09	50.44	3.7%	2.5%
c1.b	40.1	58.5	40.79	40.31	3.7%	2.6%
c2.a	47.57	66.7	48.35	47.8	4.1%	2.9%
d4.a	38.08	60.52	38.9	38.3	3.7%	2.7%
mb.a	46.58	98.67	48.92	47.19	4.5%	3.3%
ib.b	56.63	93.72	57.92	57.09	3.5%	2.2%
ib.a	41.74	89.37	43.55	42.3	3.8%	2.6%
c5.b	47.06	78.7	49.43	47.53	7.5%	6.0%
c4.b	54.26	90.97	55.95	54.75	4.6%	3.3%
blank	54.66	54.66	54.66	54.67	0.0%	0.0%
cm4.a	54.97	97.56	56.76	55.46	4.2%	3.1%
cm5.b	39.91	79.83	42.01	40.44	5.3%	3.9%

Table A.13 Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
13-May						
R1-Corn Silage	39.89	78.17	41.33	40.3	3.8%	2.7%
R2-Canola Meal	41.77	75.21	43.21	42.12	4.3%	3.3%
R3-Cheese Whey	47.93	93.24	49.58	48.4	3.6%	2.6%
R4-Raw Glycerine	38.97	84.71	40.69	39.41	3.8%	2.8%
R5-DAF Sludge	40.97	91.9	42.84	41.48	3.7%	2.7%
R6-Manure (Ctrl)	50.63	114.83	53.08	51.25	3.8%	2.9%
06-May						
R1-Corn Silage	49.52	99.12	51.48	50.05	4.0%	2.9%
R2-Canola Meal	36.67	84.94	38.66	37.2	4.1%	3.0%
R3-Cheese Whey	50.99	101.67	52.91	51.51	3.8%	2.8%
R4-Raw Glycerine	37.11	84.07	38.78	37.55	3.6%	2.6%
R5-DAF Sludge	47.06	92.66	48.72	47.5	3.6%	2.7%
R6-Manure (Ctrl)	38.08	87.4	39.85	38.54	3.6%	2.7%
22-May						
R1-Corn Silage	49.94	93	51.3	50.34	3.2%	2.2%
R2-Canola Meal	50.34	94.54	51.84	50.75	3.4%	2.5%
R3-Cheese Whey	52.67	99.85	54.25	53.1	3.3%	2.4%
R4-Raw Glycerine	56.67	101.25	57.73	56.99	2.4%	1.7%
R5-DAF Sludge	50.18	92	51.56	50.52	3.3%	2.5%
R6-Manure (Ctrl)	50.24	89.88	51.45	50.56	3.1%	2.2%
29-May						
R1-Corn Silage	50	84.84	51.24	50.3	3.6%	2.7%
R2-Canola Meal	49.81	88.3	51.13	50.14	3.4%	2.6%
R3-Cheese Whey	50.22	87.41	51.44	50.57	3.3%	2.3%
R4-Raw Glycerine	58.73	93.47	59.91	59.05	3.4%	2.5%
R5-DAF Sludge	54.53	96.67	55.85	54.94	3.1%	2.2%
R6-Manure (Ctrl)	54.98	97.34	56.09	55.34	2.6%	1.8%
04-Jun						
R1-Corn Silage	38.1	75.79	38.96	38.43	2.3%	1.4%
R2-Canola Meal	37.62	79.63	38.52	37.97	2.1%	1.3%
R3-Cheese Whey	40.1	79.67	40.9	40.43	2.0%	1.2%
R4-Raw Glycerine	50.64	108.06	52.51	51.17	3.3%	2.3%
R5-DAF Sludge	50.32	89.26	50.96	50.59	1.6%	1.0%
R6-Manure (Ctrl)	41.78	75.81	42.75	42.07	2.9%	2.0%

Table A.13 (Continued) Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
13-Jun						
R1-Corn Silage	58.7	89.01	59.37	58.95	2.2%	1.4%
R2-Canola Meal	52.65	98.6	53.88	53.05	2.7%	1.8%
R3-Cheese Whey	38.4	85.47	39.85	38.82	3.1%	2.2%
R4-Raw Glycerine	40.99	73.89	41.82	41.25	2.5%	1.7%
R5-DAF Sludge	47.07	85.58	47.7	47.31	1.6%	1.0%
R6-Manure (Ctrl)	50.95	91.24	51.81	51.23	2.1%	1.4%
19-Jun						
R1-Corn Silage	47.54	81.98	-	47.86	-	-
R2-Canola Meal	52.24	85.35	-	52.54	-	-
R3-Cheese Whey	53.88	91.26	55.09	54.23	3.2%	2.3%
R4-Raw Glycerine	56.67	83.05	57.53	56.92	3.3%	2.3%
R5-DAF Sludge	38.43	69.72	39.63	38.7	3.8%	3.0%
R6-Manure (Ctrl)	51.03	84.87	52.13	51.31	3.3%	2.4%
27-Jun						
R1-Corn Silage	47.61	68.45	48.38	47.8	3.7%	2.8%
R2-Canola Meal	41.81	68.02	42.77	42.05	3.7%	2.7%
R3-Cheese Whey	48.06	79.63	49.39	48.39	4.2%	3.2%
R4-Raw Glycerine	41.01	71.08	42.21	41.32	4.0%	3.0%
R5-DAF Sludge	52.36	83.14	53.33	52.61	3.2%	2.3%
R6-Manure (Ctrl)	52.69	86.03	53.81	52.97	3.4%	2.5%
01-Jul						
R1-Corn Silage	38.11	67.66	39.29	38.39	4.0%	3.0%
R2-Canola Meal	36.95	67.59	38.28	37.26	4.3%	3.3%
R3-Cheese Whey	58.73	92.93	60.11	59.08	4.0%	3.0%
R4-Raw Glycerine	50.69	82.36	51.83	50.98	3.6%	2.7%
R5-DAF Sludge	37.64	67.45	38.61	37.86	3.3%	2.5%
R6-Manure (Ctrl)	50.68	85.99	51.97	50.95	3.7%	2.9%
08-Jul						
R1-Corn Silage	50.25	85.38	51.64	50.59	4.0%	3.0%
R2-Canola Meal	36.47	67.02	37.65	36.75	3.9%	2.9%
R3-Cheese Whey	54.99	77.7	55.8	55.19	3.6%	2.7%
R4-Raw Glycerine	50.23	78.67	51.21	50.47	3.4%	2.6%
R5-DAF Sludge	39	70.22	40.08	39.24	3.5%	2.7%
R6-Manure (Ctrl)	37.14	73.67	38.39	37.45	3.4%	2.6%

Table A.13 (Continued) Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
15-Jul						
R1-Corn Silage	45.53	78.42	46.97	45.84	4.4%	3.4%
R2-Canola Meal	38.03	66.84	39.33	38.31	4.5%	3.5%
R3-Cheese Whey	39.2	69.84	40.14	39.47	3.1%	2.2%
R4-Raw Glycerine	46.16	78.02	46.86	46.39	2.2%	1.5%
R5-DAF Sludge	47.98	94.35	49.68	48.37	3.7%	2.8%
R6-Manure (Ctrl)	47.06	86.6	48.31	47.39	3.2%	2.3%
26-Jul						
R1-Corn Silage	38.4	75.93	39.93	38.73	4.1%	3.2%
R2-Canola Meal	47.95	99.53	50.18	48.42	4.3%	3.4%
R3-Cheese Whey	50.2	96.57	51.93	50.64	3.7%	2.8%
R4-Raw Glycerine	40.97	95.53	42.94	41.44	3.6%	2.7%
R5-DAF Sludge	47.51	87.64	49.37	47.97	4.6%	3.5%
R6-Manure (Ctrl)	38.98	87.78	41.12	39.43	4.4%	3.5%
31-Jul						
R1-Corn Silage	40.11	75.47	41.46	40.42	3.8%	2.9%
R2-Canola Meal	50.95	91.35	52.55	51.33	4.0%	3.0%
R3-Cheese Whey	48.03	99.2	49.96	48.54	3.8%	2.8%
R4-Raw Glycerine	47.91	95.57	49.61	48.34	3.6%	2.7%
R5-DAF Sludge	52.2	99.59	54	52.63	3.8%	2.9%
R6-Manure (Ctrl)	52.31	98.63	54.2	52.72	4.1%	3.2%
06-Aug						
R1-Corn Silage	58.69	107.74	60.8	59.15	4.3%	3.4%
R2-Canola Meal	52.32	104.25	54.7	52.84	4.6%	3.6%
R3-Cheese Whey	37.12	82.71	38.73	37.56	3.5%	2.6%
R4-Raw Glycerine	50.31	106.89	52.59	50.82	4.0%	3.1%
R5-DAF Sludge	53.84	111.68	60.38	54.36	11.3%	10.4%
R6-Manure (Ctrl)	38.08	81.35	39.84	38.46	4.1%	3.2%
09-Aug						
R1-Corn Silage	47.9	94.94	49.95	48.34	4.4%	3.4%
R2-Canola Meal	50.95	103.07	53.36	51.47	4.6%	3.6%
R3-Cheese Whey	47.54	91.8	49.04	47.95	3.4%	2.5%
R4-Raw Glycerine	45.46	92.44	47.37	45.89	4.1%	3.2%
R5-DAF Sludge	49.94	98.26	51.77	50.35	3.8%	2.9%
R6-Manure (Ctrl)	56.59	115.74	59.15	57.11	4.3%	3.4%

Table A.13 (Continued) Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
19-Aug						
R1-Corn Silage	41.43	94.08	43.37	41.9	3.7%	2.8%
R2-Canola Meal	52.26	102.97	54.37	52.76	4.2%	3.2%
R3-Cheese Whey	56.39	104.33	57.93	56.84	3.2%	2.3%
R4-Raw Glycerine	40.07	87.35	41.73	40.84	3.5%	1.9%
R5-DAF Sludge	46.55	108.68	48.87	47.09	3.7%	2.9%
R6-Manure (Ctrl)	39.14	92.06	41.05	39.62	3.6%	2.7%
29-Aug						
R1-Corn Silage	37.08	83.27	38.85	37.5	3.8%	2.9%
R2-Canola Meal	47.47	107.39	49.91	48.07	4.1%	3.1%
R3-Cheese Whey	41.72	88.44	43.19	42.16	3.1%	2.2%
R4-Raw Glycerine	45.98	98.63	47.73	46.41	3.3%	2.5%
R5-DAF Sludge	40.94	94.51	42.87	41.38	3.6%	2.8%
R6-Manure (Ctrl)	50.6	113.2	52.82	51.14	3.5%	2.7%
07-Sep						
R1-Corn Silage	50.91	107.55	52.92	51.36	3.5%	2.8%
R2-Canola Meal	47.03	98.47	48.92	47.47	3.7%	2.8%
R3-Cheese Whey	47.99	90.02	49.23	48.37	3.0%	2.0%
R4-Raw Glycerine	46.1	97.89	47.83	46.52	3.3%	2.5%
R5-DAF Sludge	36.65	77.53	37.96	36.95	3.2%	2.5%
R6-Manure (Ctrl)	46.01	87.5	47.32	46.33	3.2%	2.4%
12-Sep						
R1-Corn Silage	47.89	98.18	49.62	48.28	3.4%	2.7%
R2-Canola Meal	50.16	98.35	51.9	50.57	3.6%	2.8%
R3-Cheese Whey	48.33	106.05	49.99	48.84	2.9%	2.0%
R4-Raw Glycerine	47	108.41	49.08	47.5	3.4%	2.6%
R5-DAF Sludge	39.87	86.3	41.38	40.22	3.3%	2.5%
R6-Manure (Ctrl)	36.41	80.96	37.93	36.77	3.4%	2.6%
20-Sep						
R1-Corn Silage	48.99	99.87	50.85	49.39	3.7%	2.9%
R2-Canola Meal	50.27	106.96	52.46	50.74	3.9%	3.0%
R3-Cheese Whey	38.95	87.93	40.53	39.39	3.2%	2.3%
R4-Raw Glycerine	54.91	106.15	56.79	55.32	3.7%	2.9%
R5-DAF Sludge	49.5	114.99	51.5	49.96	3.1%	2.4%
R6-Manure (Ctrl)	46.78	109.5	49.68	47.38	4.6%	3.7%

Table A.13 (Continued) Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
30-Sep						
R1-Corn Silage	36.63	93.96	38.44	37.2	3.2%	2.2%
R2-Canola Meal	54.61	122.55	58.87	55.74	6.3%	4.6%
R3-Cheese Whey	50.25	92.56	51.47	50.66	2.9%	1.9%
R4-Raw Glycerine	41.71	89.94	43.95	42.35	4.6%	3.3%
R5-DAF Sludge	45.98	94.52	47.22	46.34	2.6%	1.8%
R6-Manure (Ctrl)	36.39	82.67	38.31	36.86	4.1%	3.1%
04-Oct						
R1-Corn Silage	38.35	80.09	40.76	38.97	5.8%	4.3%
R2-Canola Meal	37.96	83.46	40.62	38.65	5.8%	4.3%
R3-Cheese Whey	50.57	98.88	52	51.03	3.0%	2.0%
R4-Raw Glycerine	47.13	103.76	50.58	48	6.1%	4.6%
R5-DAF Sludge	53.77	109.31	55.37	54.18	2.9%	2.1%
R6-Manure (Ctrl)	47.51	104.66	49.44	48.04	3.4%	2.4%
10-Oct						
R1-Corn Silage	45.3	93.53	47.38	45.84	4.3%	3.2%
R2-Canola Meal	50.93	114.82	53.88	51.67	4.6%	3.5%
R3-Cheese Whey	38.03	88.46	39.73	38.59	3.4%	2.3%
R4-Raw Glycerine	36.88	88.43	38.82	37.41	3.8%	2.7%
R5-DAF Sludge	37.54	91.57	39.93	38.17	4.4%	3.3%
R6-Manure (Ctrl)	52.24	97.47	52.99	52.56	1.7%	1.0%
20-Oct						
R1-Corn Silage	49.73	90.98	51.63	50.21	4.6%	3.4%
R2-Canola Meal	49.92	97.88	51.96	50.46	4.3%	3.1%
R3-Cheese Whey	41.41	87.49	43.14	41.99	3.8%	2.5%
R4-Raw Glycerine	45.95	97.24	47.96	46.49	3.9%	2.9%
R5-DAF Sludge	46.52	94.74	48.09	46.93	3.3%	2.4%
R6-Manure (Ctrl)	47	92.26	48.59	47.43	3.5%	2.6%
28-Oct						
R1-Corn Silage	47.84	101.48	49.91	48.37	3.9%	2.9%
R2-Canola Meal	47.89	105.19	50.63	48.54	4.8%	3.6%
R3-Cheese Whey	47.44	99.66	49.18	47.93	3.3%	2.4%
R4-Raw Glycerine	50.16	97.98	51.75	50.57	3.3%	2.5%
R5-DAF Sludge	48.97	97.73	50.69	49.39	3.5%	2.7%
R6-Manure (Ctrl)	50.58	102.13	52.25	51.01	3.2%	2.4%

Table A.13 (Continued) Total and Volatile Solids for Reactor Effluent

Date/Label	Empty (g)	Filled (g)	Dry (g)	Ash (g)	TS%	VS%
08-Nov						
R1-Corn Silage	45.45	101.72	48.55	46.19	5.5%	4.2%
R2-Canola Meal	56.37	111.61	59.52	57.13	5.7%	4.3%
R3-Cheese Whey	48.3	91.58	49.9	48.74	3.7%	2.7%
R4-Raw Glycerine	40.05	83.6	42.73	40.68	6.2%	4.7%
R5-DAF Sludge	56.57	113.11	58.73	57.02	3.8%	3.0%
R6-Manure (Ctrl)	46.98	105.01	50.95	48.03	6.8%	5.0%
13-Nov						
R1-Corn Silage	50.28	101.76	53.14	51.02	5.6%	4.1%
R2-Canola Meal	54.25	103.23	57.16	55.05	5.9%	4.3%
R3-Cheese Whey	39.16	84.83	40.54	39.63	3.0%	2.0%
R4-Raw Glycerine	45.48	93.52	48.52	46.29	6.3%	4.6%
R5-DAF Sludge	37.56	78.01	41.43	38.54	9.6%	7.1%
R6-Manure (Ctrl)	50.62	109.71	54.25	51.83	6.1%	4.1%
20-Nov						
R1-Corn Silage	56.42	101.41	58.69	56.99	5.0%	3.8%
R2-Canola Meal	50.92	111.73	54.33	51.81	5.6%	4.1%
R3-Cheese Whey	56.54	121.33	58.68	57.2	3.3%	2.3%
R4-Raw Glycerine	47.04	97.33	49.83	47.81	5.5%	4.0%
R5-DAF Sludge	46.11	103.12	50.02	46.93	6.9%	5.4%
R6-Manure (Ctrl)	49.77	99.98	52.47	50.56	5.4%	3.8%
24-Nov						
R1-Corn Silage	52.28	105.03	55.19	53.01	5.5%	4.1%
R2-Canola Meal	41.73	91.6	44.76	42.52	6.1%	4.5%
R4-Raw Glycerine	41.44	97.5	44.47	42.27	5.4%	3.9%
R5-DAF Sludge	39.88	87.98	41.19	40.16	2.7%	2.1%
R6-Manure (Ctrl)	47.87	93.28	50.38	48.61	5.5%	3.9%
03-Dec						
R1-Corn Silage	45.34	100.57	48.05	46.05	4.9%	3.6%
R2-Canola Meal	48.01	101.66	50.92	48.76	5.4%	4.0%
R4-Raw Glycerine	46.57	106.99	49.92	47.53	5.5%	4.0%
R5-DAF Sludge	47.92	85.91	49.26	48.17	3.5%	2.9%
R6-Manure (Ctrl)	36.9	82.37	39.46	37.69	5.6%	3.9%

Table A.14 Cumulative Biogas Production of BMP Assays (With Inoculum Biogas)

First Batch of BMP				Second Batch of BMP			
Label/Duration	43 days	61 days	75 days	Label/Duration	41 days	Label/Duration	41 days
M1.1	2712	3004	3179	C1.A	815	GLY4.A	1359
M1.2	2675	2960	3122	C1.B	829	GLY4.B	1656
S1.1	2004	2853	4388	C2.A	1421	GLY6.A	1791
S1.2	1451	1995	4211	C2.B	1412	GLY6.B	1753
G1.1	758	790		C3.A	2038	GLY20	972
G1.2	747	777		C3.B	2002	MB.A	2122
C1.1	4800	4919		C4.A	2581	MB.B	2133
C1.2	4748	4871		C4.B	2561	IB.A	446
W1.1	2015	2236	4422	C5.A	1013	IB.B	445
W1.2	1920	1945	1968	C5.B	1029		
M7S3.1	3397	3682		CM1.A	1029		
M7S3.2	3375	3635		CM1.B	1100		
M5S5.1	3700	4089		CM2.A	1458		
M5S5.2	3821	4187		CM2.B	1484		
M7G3.1	3794	4141		CM3.A	1965		
M7G3.2	3821	4160		CM3.B	1992		
M4G6.1	2769	4281	4642	CM4.A	2379		
M4G6.2	3010	4277	4526	CM4.B	2454		
M8C2.1	3153	3505		CM5.A	4019		
M8C2.2	3095	3413		CM5.B	3953		
M6C4.1	3433	3772		MS.A	1282		
M6C4.2	3456	3815		MS.B	1177		
M7W3.2	3325	3665	3665	IS.A	231		
M3W7.1	2763	4124	4364	IS.B	286		
M3W7.2	2852	3841	3990	DAF4.A	2650		
I1.1	571	701	731	DAF4.B	2601		
I1.2	593	721	755	DAF6.A	3410		
I1.3	570	707	754	DAF6.B	3481		
I1.4	507	623	666	DAF20	949		

Table A.15 Biogas Composition

Date/Label	N ² %	CH ₄ %	CO ₂ %
28-Aug-08			
R1 Corn Silage Effluent	2.8	43.6	53.7
R1 Corn Silage Effluent	2.2	43.6	54.2
R2 Canola Effluent	3.5	50.5	46.1
R2 Canola Effluent	3.6	50.8	45.6
R3 Whey Effluent	2.2	49.3	48.5
R4 Glycerine Effluent	1.9	50.8	47.3
R5 DAF Effluent	1	59.9	38.9
R6 Manure Effluent	6.3	49.2	44.6
09-Sep			
R1 Corn Silage Effluent	3	49	48
R2 Canola Effluent	5	56	39
R3 Whey Effluent	1	52	47
R4 Glycerine Effluent	6	53	41
R5 DAF Effluent	6	61	33
R6 Manure Effluent	10	41	49
30-Sep			
R1 Corn Silage Effluent	6.9	45	48.1
R2 Canola Effluent	7.4	52	40.6
R3 Whey Effluent	7.4	42.1	45.5
R4 Glycerine Effluent	3.1	52.2	44.7
R5 DAF Effluent	8.2	54.8	37
R6 Manure Effluent	18	42.4	39.6
14-Oct			
R1 Corn Silage Effluent	2.5	45.3	52.2
R2 Canola Effluent	0	55	45
R3 Whey Effluent	0	61.5	38.5
R4 Glycerine Effluent	2.2	56.2	41.6
R5 DAF Effluent	4.8	59.6	35.6
R6 Manure Effluent	0	51	49
28-Oct			
D6.B	4.6	62.5	32.9
CM3.A	4.8	66.5	28.7
MS.A	9.7	45.8	44.5
CM4.B	0	57.4	42.6
C2.B	6.8	52.5	40.7
G1.B	8.6	44.3	47.2
G2.A	8.8	44.5	37.9

Table A.15 (Continued) Biogas Composition

Date/Label	N ₂ %	CH ₄ %	CO ₂ %
27-Oct			
R1 Corn Silage Effluent	13.2	45.5	41.3
R2 Canola Effluent	5.2	53.7	41.1
R3 Whey Effluent	3.4	57.9	38.7
R4 Glycerine Effluent	6.4	60.8	32.8
R5 DAF Effluent	6.7	60.9	32.4
R6 Manure Effluent	4.3	55.1	40.6
11-Nov			
R1 Corn Silage Effluent	1.1	48.4	50.5
R2 Canola Effluent	0	56	44
R3 Whey Effluent	2.4	50.2	47.4
R4 Glycerine Effluent	8.6	51.1	40.3
R5 DAF Effluent	0	59.9	43.1
R6 Manure Effluent	1.1	53.8	45.1
24-Nov			
R1 Corn Silage Effluent	2.8	51.5	45.7
R2 Canola Effluent	0	56.8	43.2
R3 Whey Effluent	0	54.9	45.1
R4 Glycerine Effluent	3.3	57.8	38.9
R5 DAF Effluent	0	58.1	41.9
R5 DAF Effluent -rep	0	59.3	40.7
R6 Manure Effluent	0	53.9	46.1
02-Dec			
R1 Corn Silage Effluent	5.33	48.33	46.35
R2 Canola Effluent	0	58.47	41.53
R3 Whey Effluent	5.14	48.67	46.18
R4 Glycerine Effluent	0	60.63	39.37
R5 DAF Effluent	2.46	54.18	43.36
R6 Manure Effluent	0	53.68	46.32
05-Dec			
R1 Corn Silage Effluent	6.95	47.89	45.16
R1 Corn Silage Effluent	6.79	48.05	45.16
R2 Canola Effluent	5.44	57	37.56
R2 Canola Effluent	5.72	56.75	37.53
R5 DAF Effluent	4.22	62.85	32.94
R6 Manure Effluent	6.28	60.46	33.26

Appendix B:

OLR MS Excel Spreadsheet

This appendix shows two MS Excel spreadsheets used for the first design of BMP assays and throughout the pilot scale experiments. It consists of one input area where the desired OLR and volume of reactor is entered. The second section contains the characteristics of the feedstocks. Finally, each reactor has its own section, showing the amount of fresh mass to add each day, HRT of the reactor, TS of the influent, %Volume of co-substrate and the %Mass of co-substrate in the feed using the different VS co-substrate / VS Manure ratios.

OLR [gVs added / Vr / dl] =

Volume of reactor, Vr (l) –

Volatile Solids Added per day, gVS/d – OLR * Vr –

4.0 (Input Cell)

20.0 (Input Cell, here fixed at 20 l)

80.0

Feedstocks		g TS / g Liq	g VS / g TS	g VS / g Liq	g liq / ml
Slurries	Manure	0.055	0.800	0.044	1.01
	Inoculum	0.050	0.720	0.036	1.01
	Whey	0.055	0.800	0.044	1.01
	Heinzle DAF	0.900	0.970	0.873	0.9
	Glycerine	0.870	0.970	0.844	1.23
Solids	Corn Silage	0.340	0.950	0.323	0.85
	Canola Cake	0.900	0.920	0.828	1.2

Reactor #6 Inoculum-Manure						
Feedstock	VS Co-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.
M	0	1818	0	11	0%	0%
Reactor #4 Manure - Glycerine						
Feedstock	VS Co-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.
M	0.9	1636	0.06	12	0%	1%
GI	0.1	9				
M	0.8	1455	0.07	14	1%	1%
GI	0.2	19				
M	0.7	1273	0.07	16	2%	2%
GI	0.3	28				
M	0.6	1091	0.08	18	3%	3%
GI	0.4	38				
M	0.5	909	0.10	21	4%	5%
GI	0.5	47				
M	0.4	727	0.11	26	6%	7%
GI	0.6	57				
M	0.3	545	0.14	34	9%	11%
GI	0.7	66				
Reactor #5 Manure - DAF Heinzle						
Feedstock	VS Co-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.
M	0.9	1636	0.06	12	1%	1%
DAF	0.1	9				
M	0.8	1455	0.07	14	1%	1%
DAF	0.2	18				
M	0.7	1273	0.07	15	2%	2%
DAF	0.3	27				
M	0.6	1091	0.08	18	4%	3%
DAF	0.4	37				
M	0.5	909	0.10	21	5%	5%
DAF	0.5	46				
M	0.4	727	0.11	26	8%	7%
DAF	0.6	55				

Figure A.1 Snapshot of the First Page of the OLR MS Excel Spreadsheet

Reactor #1 Manure - Corn Silage							
Feedstock	VS Cc-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.	
M	0.9	1636	0.06	12	2%	1%	
CS	0.1	25					
M	0.8	1455	0.06	13	4%	3%	
CS	0.2	50					
M	0.7	1273	0.07	15	6%	6%	
CS	0.3	74					
M	0.6	1091	0.08	17	10%	8%	
CS	0.4	99					
M	0.5	909	0.09	19	14%	12%	
CS	0.5	124					
M	0.4	727	0.10	22	20%	17%	
CS	0.6	149					
M	0.3	545	0.12	27	27%	24%	
CS	0.7	173					
Reactor #2 Manure - Canola							
Feedstock	VS Cc-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.	
M	0.9	1636	0.06	12	0%	1%	
C	0.1	10					
M	0.8	1455	0.07	14	1%	1%	
C	0.2	19					
M	0.7	1273	0.07	16	2%	2%	
C	0.3	29					
M	0.6	1091	0.08	18	3%	3%	
C	0.4	39					
M	0.5	909	0.10	21	4%	5%	
C	0.5	48					
M	0.4	727	0.12	26	6%	7%	
C	0.6	58					
M	0.3	545	0.15	34	9%	11%	
C	0.7	68					
Reactor #3 Manure - Whey							
Feedstock	VS Cc-S / VS LDM	g Fresh Mass added per day	TS of Influent	HRT (d)	% Volume of Co-Sub.	% Mass of Co-Sub.	
M	0.9	1636	0.06	11	10%	10%	
W	0.1	182					
M	0.8	1455	0.06	11	20%	20%	
W	0.2	364					
M	0.7	1273	0.06	11	30%	30%	
W	0.3	545					
M	0.6	1091	0.06	11	40%	40%	
W	0.4	727					
M	0.5	909	0.06	11	50%	50%	
W	0.5	909					
M	0.4	727	0.06	11	60%	60%	
W	0.6	1091					
M	0.3	545	0.06	11	70%	70%	
W	0.7	1273					

Figure A.1 Snapshot of the Second Page of the OLR MS Excel Spreadsheet