Ph.D. Thesis

Anaerobic Digestion of Dairy Manure with Food and Industry Wastes – Enhanced Biogas Production and Digestate Quality

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ABSTRACT

The Ontario biogas industry is relatively young and the overall objective of this research was to help support the growth of the industry with investigating the use of co-substrates and reactor design to enhance biogas production, recommend guidelines on the operation of full scale systems to optimize performance and characterize digestate quality. Laboratory studies evaluated the use of various substrates in the co-digestion with liquid dairy manure. These studies were used to establish ultimate biogas yields, % volatile solids (VS) reduction and minimum hydraulic retention times (HRTs). Box-Wilson Central Composite design models for corn thin stillage and waste grease (as co-substrates with dairy manure) suggest methane yields optimize with increasing proportion of the feed VS from co-substrates (constant total VS in all assays) and increasing temperatures; however, temperature had a great effect. Bench scale studies were conducted to determine a change in digester design to optimize biogas yields and increase digestate stability. A two-phase digestion system was implemented for co-digestion systems using thin stillage and waste grease with dairy manure, and methane yields showed to increase by over 22% when compared with single-phase systems. Based on current FIT contracts of 18 to 20¢/kWhe, the increased electricity and heat production could make the two-phase system economically attractive for producers. Organic loading rates (OLRs) over 4.4 g VS/L led to digester upset and OLRs of over 4.2 g VS/L·day are not recommended. On-farm anaerobic digester systems were studied for digester performance and digestate quality. Residual biogas potential (RBP) yields were effective at evaluating the stability of digestate and the U.K. PAS 110:2014 limit of 0.45 L biogas/g VS (28 days incubation) was assessed too lenient for the Ontario systems studied. A limit of 0.25 L biogas/g VS after 28 days of incubation or 0.45 L biogas/g VS after 60 days of incubation are recommended. VS reductions ranged from 56 to 76% and easily achieved the O. Reg. 267/03 regulated 50% VS reduction. *E.coli* and *Salmonella* were typically 1 to 3 logs CFU/100 mL lower than raw manure and increased HRT did not demonstrate a significant impact on the bacterial log reductions. Intermediate alkalinity (IA)/partial alkalinity (PA) proved to be a valuable tool in determining potential digester upset and has been recommended as a standard performance parameter for on-farm systems.

Table of Contents

List of Figures

List of Tables

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List of Abbreviations and Nomenclature

AAFC	Agriculture and Agri-food Canada
ABC	American Biogas Council
ABR	Anaerobic baffled reactor
ASBR	Anaerobic sequencing batch reactor
ASM	Agricultural source material
AD	Anaerobic digestion
ANOVA	Analysis of variance
BMP	Biochemical methane potential (L CH_4/g VS; L biogas/g VS)
BOD	Biological oxygen demand (mg/L; g/L)
COD	Chemical oxygen demand (mg/L; g/L)
CSTR	Continuously stirred tank reactor
DAF	Dissolved air flotation sludge
DDG	Distillers dried grains
DDGS	Distillers dried grains with solubles
DM	Dairy manure
E(Y)	Expected value of the response variable
ECA	Environmental compliance approval
F-statistic	Evaluation of lack of fit
FOD	Flame ionization detector
FIT	Feed-in-Tariff
F/M	Food/microorganism ratio
FOG	Fats, oils and greases
GC	Gas chromatograph
GHG	Greenhouse gas
GTW	Grease trap waste
HRT	Hydraulic retention times
IA	Intermediate alkalinity (mg $CaCO_3$/L) – represents VFA concentration
LCFA	Long chain fatty acid

k	Number of factors being studied in experimental design
MOECC	Ontario Ministry of Environment and Climate Change
MS_{LF}	Mean squares due to lack of fit
MS_{PE}	Mean squares due to pure error
MSreg	Mean squares due to regression
MSres	Mean squares due to residuals
MSW	Municipal solid waste
N	Total number of design points
n	Total number of samples
n_0	Number of centre points
NH_4^+ -N	Ammonium nitrogen (mg/L; g/L)
NMA	Nutrient Management Act
OLR	Organic loading rate (g VS/L·day)
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
OPA	Ontario Power Authority
p	Number of parameters in model (excluding β_0)
p	Statistical probability
PA	Partial alkalinity (mg $CaCO_3$/L) – represents buffering alkalinity
pCOD	Particulate chemical oxygen demand (mg/L; g/L)
PO_4^{3-} -P	ortho-Phosphate phosphorus (mg/L; g/L)
Q	Daily flow rate (L/day; m^3/day)
r	Pearson correlation coefficient
R^2	Coefficient of determination
RBP	Residual biogas potential (L biogas/g VS)
RSS_{cfm}	Sum of squares due to the regression (corrected for mean)
ResSS	Residual sum of squares
s^2_{PE}	Pure error variance
SCFA	Short chain fatty acid
sCOD	Soluble chemical oxygen demand (mg/L; g/L)
SMA	Specific methane activity
SRT	Solids retention time

SSLF	Lack of fit sum of squares
SSPE	Pure error sum of squares (using response variables at the centre)
SS	Sum of squares
STP	Standard temperature and pressure (0 °C, 1 atm)
TA	Total alkalinity (mg $CaCO_3$/L)
TCD	Thermal conductivity detector
tCOD	Total chemical oxygen demand (mg/L; g/L)
TKN	Total Kjeldahl nitrogen (mg/L; g/L)
TN	Total nitrogen (mg/L; g/L)
TP	Total phosphorus (mg/L; g/L)
TPAD	Temperature phased anaerobic digestion
TS	Total solids (mg/L; g/L)
TSS_{cfm}	Total sum of squares (corrected for mean)
UASB	Upflow anaerobic sludge blanket
VFAs	Volatile fatty acids
V	Volume of tank or digester (L; m^3)
VFA	Volatile fatty acid (mg/L; g/L)
VS	Volatile solids (mg/L; g/L)
X_i and X_j	Coded factors in design model
x_i and x_j	Natural values in design model
Y_i	Experimental value of the response variable

Greek Letters

α	Distance of star points from design center
$\beta_0, \beta_i, \beta_j$	Model parameters of surface response design model

Chapter 1 - Introduction and Research Hypothesis

1.1 **Background on the Agricultural Biogas Industry – Domestic and International**

Over the past several years there has been significant interest in adopting on-farm manure anaerobic digesters in Ontario. The potential of digesting agricultural biomass for energy production, while reducing GHG emissions, mitigating pathogens and odours, and increasing ionized nutrients in the digestate, has made AD an attractive option for manure management. The biogas produced from the anaerobic digester can be used to fuel an electric co-generator, where the electricity produced is sold to the grid and waste heat recovered to be used on the farm. The co-digestion of manure with organic rich substrates can offer significantly increased biogas yields, thereby increasing energy revenues. The digestate offers environmental benefits, where methane gas (CH_4) and nitrous oxide gas (N_2O) emissions are reduced from storage reservoirs and land application. Reduced volatile fatty acids and pathogens in the digestate result in decreased odours and pathogen migration to surface and ground waters; thereby making the land application of digestate more acceptable to rural communities than conventional, untreated manure. The removal of carbon through the digestion of organic compounds results in the ionization of nutrients (e.g. Org-N → NH_4^+; Org-P → o-PO_4^{3-}), making these nutrients more readily available for crop uptake and ultimately increasing crop yields.

The adoption of manure digesters is much more advanced in Europe than in North America, and especially more so than in Canada. Europe faced energy reductions during and after World War II and is still more dependent on imports of oil and natural gas than Canada or the U.S. (Lazarus, 2008). To secure energy independence, Europe has moved aggressively to develop renewable energy technologies, including manure digesters. This long-term European experience indicates that manure digesters do operate well and are of economic value in cooler climates, similar to that of Canada. Of the European countries, Germany, Denmark, the United Kingdom and Sweden have the best-known biogas industries. Bo Holm-Nielson et al. (1997) reported Germany to be primarily interested in fostering the development of small scale farm biogas plants for the agriculture industry, while Denmark was moving more towards centralized units where manure slurries are co-digested with agro-industrial wastes. The leader in agriculture based biogas plants in Europe is Germany with 7850 systems as of 2013, where this number was only 2700 in 2005 and represented 0.8% of Germany's electricity needs (Effenberger, 2006; EBA, 2014). A distant second is Italy with 1121 agriculture based biogas plants (EBA, 2014). Canada and the U.S. are beginning to grow their numbers of manure based digester systems. As of 2011 a total of 167 farm digesters were operating in the U.S., with an industry growth rate of 20 new digester systems annually (Sullivan, 2011). In Canada, by 2013 there were just under 45 operating farm digesters, with 36 systems in Ontario alone (Kelleher Robins, 2013).

Following the European example, both federal and provincial governments have implemented several programs to stimulate the biogas industry in Canada. Agriculture and Agri-food Canada (AAFC), the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the Ontario Power Authority (OPA) have been committed to fostering the adoption of farm based anaerobic digesters. In 2001, AAFC launched the Energy Cogeneration for Agricultural and Municipal Wastes (ECoAMu) program and in 2004 the Environmental Technology Assessment for Agriculture (ETAA) program, where both programs were designed to establish anaerobic digestion and energy co-generation plants as demonstrations of AD technologies for producing renewable energy from agriculture. In 2007 OMAFRA launched the Ontario Biogas Systems Financial Assistance (OBSFA) Program to aid farmers and rural businesses in implementing new on-farm digesters systems, cover both feasibility studies and digester capital costs. On September 30, 2009, the OPA launched the first Feed-in-Tariff (FIT) Program offering guaranteed pricing for renewable electricity production (biogas, wind and solar) (OPA, 2009). Since its launch, there have been numerous applications to the FIT program. The OBSFA and FIT programs have been instrumental in the growth of the biogas industry in Ontario.

Even with the current FIT pricing the business plan for a manure only digester may not net enough profit to justify a large movement of AD systems onto Ontario farms. However, the co-digestion of off-farm materials or energy crops with manure can enhance biogas production by as much as 2 to 4 times (Scott and Ma, 2004a; Banks and Heaven, 2013; Rico et al., 2015; Ebner et al., 2016) and increase profits significantly. The economic benefits are not only limited to increased biogas production; tipping fees for the off-farm materials can also be collected by farmers as a revenue source. Organic rich substrates suitable for on-farm co-digestion with manure include: energy crops (e.g. corn silage, red clover), food wastes generated from food processing, food service and food retail, and plant residues (e.g. from greenhouses, nurseries, residences). Anaerobic digesters that are designed for processing manure can accept co-substrates which are similar to manure in terms of total solids, volatile solids, chemical oxygen demand and moisture (Burke, 2001; Scott and Ma, 2004a; Banks and Heaven, 2013). Co-digestion can also allow the use of organic substrates that otherwise could be problematic to handle, as many organic rich feedstocks are much easier handled as co-substrates to the more homogeneous main substrate manure resulting in a more stable digestion process (CH2M Hill and NIRAS, 2003; El-Mashad and Zhang, 2010; Das and Mondal, 2016).

A trend in Europe is using more energy crops and less manure in on-farm digesters, which is particularly true for Germany and Northern Ireland (Effenberger, 2006; Murphy et al., 2011; EBA 2014). Some on-farm digesters in Europe operate with over 70% energy crops; potential biogas yield of canola cake is 552 m^3/t and that of corn silage is 202 m^3/t versus 22 m^3/t for liquid dairy manure (Effenberger, 2006; Kelleher Robins, 2013). Lindorfer et al. (2008) demonstrated that an agricultural biogas plant in Austria running on a mass VS ratio of 96% energy crops and 4% liquid dairy manure, with an organic loading rate of 4.25 kg VS/m^3·day, could produce a biogas yield of 0.73 m^3/kg VS. Lehtomäki et al. (2008) demonstrated that the co-digestion of grass silage and cow manure at a VS mass ratio 40% silage to 60% manure produced a methane yield of 0.268 m^3 CH_4/kg VS, while Crolla and Kinsley (2008a) reported a methane yield of 0.171 m^3 CH_4/ kg VS for the Fepro Farms digester (Cobden, ON) operating with liquid dairy manure alone. A comprehensive study from Ahlberg-Eliasson et al. (2017) reported that biogas plants in Germany, Denmark and Sweden co-digesting energy crops demonstrated significantly higher specific methane production when compared with plants digesting a single substrate like manure.

With further travel distances between farms and lower electricity purchase prices, it is not anticipated that Ontario will follow Europe's example of using more energy crops and less manure in on-farm digesters. OMAFRA is also committed to discouraging the conversion of farm lands to produce energy crops rather than food crops. Under the Nutrient Management Regulation (O. Reg. 267/03) a "regulated mixed anaerobic digestion system" must operate with at least 50%, by volume, of manure (Ontario Government, 2009). The growing interest in Ontario is the use of off-farm waste materials as potential manure co-substrates, primarily due to the high biogas yields associated with these materials and potential tipping fees. Another important factor is these wastes will be diverted from landfills and treated through digestion before being introduced to the environment. Schedules 1, 2 and 3, under the Nutrient Management Regulation, list materials that are deemed acceptable for co-digestion with manure. O. Reg. 267/03 stipulates that no more than 50%, by volume, of the total amount of materials in an on-farm digester can be off-farm substrates (Ontario Government, 2009).

To date, grease waste and food wastes have been the most popular co-substrates for the Ontario biogas industry. Food waste and waste grease have high VS/TS ratios, typically 80 to 90%, and are easily digestible. Baking wastes and waste grease have very high potential biogas yields of 580-657 m^3/t and 500-600 m^3/t, respectively (Effenberger, 2006; Köttner et al. 2008). The methane content of biogas from food waste is typically higher: 70% methane (Scott and Ma, 2004a) with food waste compared to 55-65% (Burke, 2001) with liquid dairy manure.

The on-farm manure digesters currently operating in Ontario are single phase systems. In 2008, approximately 90% of the full-scale digester plants currently in use in Europe treating solid wastes and biowastes are also one phase systems (Yilmaz and Demirer, 2008). Although the literature suggests two-phase anaerobic systems to be more stable and efficient for digestion, their adoption at the full-scale has been limited (Yilmaz and Demirer, 2008; Demirer and Chen, 2005; Sung and Santha, 2003). Yilmaz and Demirer (2008) suggests a likely reason for this discrepancy is that single phase systems have simpler designs with potentially less technical failures and have lower capital investment costs. However, the AD industry in Ontario is at its early stage and new AD technologies and designers are emerging, which justifies a more detailed investigation in the use of a two-phase digestion system to treat Ontario sourced manure and organic rich substrates. The potential increased methane yields with a two-phase system, particularly at higher organic loads, could justify the higher initial capital costs of such a system.

1.2 Research Objectives

The Ontario biogas industry is relatively young and the overall objective of this research was to help support the growth of the industry with investigating the use of co-substrates and reactor design to enhance biogas production, recommend guidelines on the operation of full scale systems to optimize performance and characterize digestate quality. The specific objectives of the research included:

i. Evaluate the use of co-substrates in enhancing biogas production from the digestion of dairy manure and determine which substrates are most suitable for the Ontario industry;
ii. Investigate the use of a two-phase digester system to enhance biogas yields and improve digestate quality for agricultural applications;
iii. Evaluate the performance of digester systems and develop operational guidelines for improved operation; and,
iv. Evaluate the use of standard digester performance parameters in assessing the stability of on-farm digesters systems.

1.3 Research Hypothesis

The research thesis was designed to address the following hypotheses:

i. The use of Ontario sourced co-substrates can enhance biogas production from the co-digestion of liquid dairy manure and, the proportions of co-substrates and manure can be optimized.
ii. The use of a two-phase digestion system (where are acidogenesis and methanogenesis are separated in different tanks) will significantly enhance methane production and digestate quality; proving to be an economically feasible technology for the Ontario biogas industry.
iii. The performance of on-farm systems can be improved with changing OLRs, HRTs and percentage of co-substrate used.
iv. Digestate quality can be improved when standard performance parameters are monitored and used as tools to adjust digester operation.

1.4 Organization of Thesis

Chapter 1 is an introduction to the biogas industry and sets the scope of the research conducted under the thesis project.

Chapter 2 is a literature review that addresses the operation of anaerobic digesters, including the parameters that most affect their performance and the quality of the digestate produced. The chapter also summarizes relevant research that has been conducted in the field of agricultural anaerobic digestion.

Chapter 3 describes the BMP assay trials used to investigate the use of co-substrates to enhance biogas production from manure.

Chapter 4 describes the research work conducted on using a two-phase digester system to enhance biogas production and improve digestate quality.

Chapter 5 describes the operation of on-farm manure digesters and the use of standard performance parameters to assess digestate stability in on-farm digesters.

Chapter 6 presents the overall conclusions and recommendations of the research experiments and proposed future research.

Chapter 2 - Literature Review

2.1 Anaerobic Digestion of Liquid Dairy Manure

2.1.1 Characteristics of Dairy Manure – Suitability as a Substrate for Digestion

Liquid dairy manure typically consists of animal excreta, urine, washwaters, bedding (e.g. sand, sawdust, wood shavings, etc.) and spilled feed. Typical TS content of liquid manure ranges from 5 to 20%, where 78-83% of these solids are VS (Krich et al., 2005; Sakar et al., 2009; Crolla et al., 2013). VS in manure is primarily composed of fats, carbohydrates and proteins, where these solids can be digested to VFAs and ultimately methane. Wen et al. (2007) and Burke (2001) suggest that over 52% of the VS in manure are cellulose and hemicellulose. Both cellulose and hemicellulose solids can be converted to methane by anaerobic bacteria. It should be noted however, that typically 11-14% of available volatile solids in dairy manure are composed of lignin, which cannot be biodegraded by the anaerobic digestion process (Sakar et al., 2009; Demirer and Chen, 2005; Burke, 2001).

Biodegradability of solids is the basis of the anaerobic digestion process and can be estimated from BOD and COD analyses (de Lemos Chernicharo, 2007). The theoretical relationship between COD and methane production is 2 mols of COD are removed for every 1 mol of methane produced, this results in a theoretical methane yield of 0.35 m^3 CH_4/ kg COD under STP conditions (Unger, 1994). tCOD is made up of pCOD and sCOD. pCOD is defined as particles > 0.45 µm and sCOD are dissolved particles which are ≤ 0.45 µm in size (Roeleveld and Loosdrecht, 2002; Elmitwalli et al., 2001). Colloidal particles are typically part of the pCOD fraction; however, Roeleveld and Loosdrecht (2002) identified that a portion of colloidal particles can pass through the 0.45 µm filter, thereby slightly overestimating the sCOD fraction. sCOD is composed of a readily biodegradable fraction and an inert fraction, while pCOD is composed of a fraction of particles that are slowly biodegradable and a non-biodegradable fraction. Elmitwalli et al. (2001) reported that the degradability of colloidal particles from domestic wastewater under anaerobic conditions occurs at a lower degree than under aerobic conditions and can represent 60-80% of the effluent tCOD of an anaerobic reactor. However, the authors go on to explain that the removal of colloidal particles is possible under batch recirculation conditions with long retention times.

The literature has shown the tCOD of liquid dairy manure can range from 14.2 g/L (2.1 %TS) to 209 g/L (16.9 %TS) (Yilmaz & Demirer, 2008; Crolla and Kinsley, 2008a; Wen et al., 2007; Güngör-Demirci and Demirer, 2004; Katers and Schultz, 2003). The sCOD fraction of dairy manure can be anywhere from 10 to 28% of the tCOD, with values ranging from 8.9 g/L (7.8 %TS) to 44 g/L (7.6 %TS) (Crolla and Kinsley, 2008a; Güngör-Demirci and Demirer, 2004; Katers and Schultz, 2003).

Anaerobic bacteria also require a sufficient concentration of nutrients to achieve optimum growth. Burke (2001) suggests the C:N ratio of digestion substrates should be less than 43:1 and the C:P ratio should be less than 187:1. Liquid dairy manure typically has a suitable C:N ratio that ranges from 13-18:1 (Burke, 2001; OMAFRA, 2000), thereby respecting these criteria. Ultimately the literature suggests the optimal C:N ratio for digester performance when using manures is 20 to 30:1 (Burke, 2001; US EPA, 2004; Sakar et al., 2009; Das and Mondal, 2016). Organic nitrogen present in the feedstock has two important roles (Burke, 2001; Gerardi, 2003):

(i) synthesis of amino acids, proteins and nucleic acids; and
(ii) conversion to ammonia that can neutralize the volatile fatty acids produced by anaerobic bacteria, and helps maintain neutral pH conditions.

Ammonia in the digester can be in the form of ammonium ions (ionized ammonia – NH_4^+) or dissolved ammonia gas (unionized ammonia – NH_3). The two forms are in equilibrium and the relative concentration of each form is dependent on pH. When pH is 7.2 or lower, NH_4^+ is favoured, whereas when the pH is greater than 7.2, the presence of NH_3 is favoured (Gerardi, 2003; Sakar et al., 2009). Unionized ammonia is toxic to bacteria, particularly methane-forming bacteria. Gerardi (2003) and Fulhage et al. (1993) report that if the nitrogen concentration is too high this excess nitrogen can be transformed directly to ammonia (NH_4^+/NH_3). Sakar et al. (2009) suggests NH_3 concentrations in anaerobic digesters should be maintained below 80 mg/L and NH_4^+ concentrations up to 1500 mg/L can be tolerated. Gerardi (2003) agrees and states that an NH_4^+concentration range of 50 - 200 mg/L is needed for anaerobic digestion and a concentration range of 200-1000 mg/L typically has no negative effect; however, an NH_4^+ concentration between 1500-3000 mg/L can be inhibitory at a pH above 7.2. Sung and Santha (2001) suggest slightly higher ammonia-nitrogen concentrations (~ 4000 mg/L) may not be an issue for thermophilic digesters. When there is not enough nitrogen present and nitrogen concentration are too low, metabolic pathways of anaerobic bacteria can be inhibited.

The pH of manure is also ideal for anaerobic digestion. Methane producing bacteria are rather sensitive and require neutral to slightly alkaline conditions with a pH range of 6.8 to 8.5 (Sakar et al., 2009; Burke, 2001), but optimum performance is achieved at a pH range of 6.8 to 7.2 (van Haandel and van der Lubbe, 2007; Gerardi, 2003). The acid-producing bacteria in the digestion process have an optimum pH range of 5 to 6 (Gerardi, 2003; van Haandel and van der Lubbe, 2007; Sakar et al., 2009). pH of dairy manure typically ranges from 6.3 to 7.6 (Nielsen et al., 2004; Sung and Santha, 2003). During digestion, acid forming bacteria grow much faster than methane forming microorganisms. The pH in an anaerobic digester initially decreases with the production of volatile acids; however, as methane forming bacteria consume the volatile acids and alkalinity is produced, the pH of the digester increases and then stabilizes. If acid-producing bacteria grow too fast, they may produce more acid than the methane forming bacteria can consume. Excess acid builds up in the system, the pH drops, and the system may become unbalanced; inhibiting the activity of methane forming bacteria. Therefore, to maintain a stable pH in the digester, a high alkalinity is required. Sufficient alkalinity in the digester buffers acid accumulation and prevents rapid changes in pH; carbonates contribute hydroxide ions which neutralize the acids. TA concentrations in an anaerobic digester should be maintained between 1500 and 4000 mg $CaCO_3$/L to provide adequate buffering of the digestion process (APHA, 1998; Gerardi, 2003; Franco et al., 2007). A study conducted by Crolla and Kinsley (2008a) found TA concentrations to range between 6500 to 9000 mg $CaCO_3$/L in liquid dairy manure from three farms in Eastern Ontario. TA is equivalent to PA plus intermediate alkalinity IA. IA is equivalent to the total VFA content in the reactor and PA is the alkalinity due to bicarbonates. Ratios of IA/TA or IA/PA are used to measure the stability of the anaerobic digestion process and is an important monitoring parameter. One of the simplest methods of keeping a digester operating well is to maintain an acceptable volatile acid-to-alkalinity ratio. Franco et al. (2003) suggests the IA/PA ratio in a stable anaerobic reactor should not be greater than 0.3. Gerardi

(2003) states an acceptable IA/TA ratio ranges between 0.1 and 0.2 and ratios of 0.07-0.08 is a good working ratio, whereas a ratio >0.5 suggests digester upset and possible failure.

2.1.2 Digestion of Manure – Process, Operation, Reactors & Phased Systems

2.1.2.1 Process of Anaerobic Digestion

Simply stated, anaerobic digestion is a biological process where microorganisms metabolize organic matter, in the absence of air (principally oxygen), and produce biogas. These microbes exist naturally in manure from ruminant animals, thereby making the manure an ideal substrate for digestion. Anaerobic digestion can be separated into four stages that occur simultaneously (Figure 2.1):

i. *Hydrolysis*: A first group of microorganisms secrete enzymes which hydrolyze polymeric compounds to monomers (i.e. glucose and amino acids).
ii. *Acidogenesis*: Acidogenic bacteria convert the products from hydrolysis to primarily VFAs, H_2 and acetic acid.
iii. *Acetogenesis*: Hydrogen-producing acetogenic bacteria convert VFAs to acetic acid, H_2 and CO_2.
iv. *Methanogenesis*: Methanogenic bacteria convert acetate, H_2 and CO_2 to biogas, where biogas is composed of CH_4 and CO_2, with trace concentrations of H_2S.

During hydrolysis, macro-molecules, such as proteins, cellulose, poly-saccharides and lipids, are converted to water soluble molecules (i.e. peptides, saccharides and fatty acids). Hydrolysis is a relatively slow process that is carried out by enzymes excreted by bacteria and it is generally considered the rate limiting stage of the overall anaerobic digestion process (van Haandel and van der Lubbe, 2007).

Acidogenesis (acidification) converts the products from hydrolysis into simple, lower molecular weight molecules, like volatile fatty acids (VFAs), alcohols, aldehydes and gases like CO_2, H_2 and NH_3. Most bacteria in the acidogenesis process are strictly anaerobic. These acid-forming bacteria reproduce rapidly and are typically not sensitive to most environmental conditions. Acidogenic bacteria can metabolise organic matter under pH conditions as low as 4 (van Haandel and van der Lubbe, 2007) but are at an optimum in a pH range of 5 to 6 (Sakar et al., 2009; van Haandel and van der Lubbe, 2007; Gerardi, 2003).

VFAs vary in length and most VFAs produced in an anaerobic digester are short-chain acids with low molecular weights. Gerardi (2003) states typical VFAs produced in an anaerobic digester include formic acid (1 carbon unit), acetic acid (2 carbon units), propionic acid (3 carbon units), butyric acid (4 carbon units) and valeric and isovaleric acids (5 carbon units); where acetic acid is the predominant acid. VFA concentrations in liquid dairy manure can vary from 800 mg/L (1.8 %TS) to 6900 mg/L (7.8 %TS) (Crolla and Kinsley, 2008a; Wen et al., 2007; Katers and Schultz, 2003). Gerardi (2003) reports that 85% of the VFA content in an anaerobic digester is acetic acid. Labatut and Gooch (2012) and Murphy and Thamsiriroj (2013) suggest 65 to 70% of the methane produced during anaerobic digestion is from acetate and the balance is produced via hydrogen. Propionate is an important precursor to the production of acetate and hydrogen, and Labatut and Gooch (2012) state 30% of the electron flow that is directly related to methane production is attributed from propionate. Banks et al. (2013) present the optimum ratio of acetate to propionate to be 2:1.

Accumulating VFAs in a digester without increasing alkalinity is indicative of poor operating conditions. Butyric and propionic acids are important intermediates of methane production; however, an accumulation of these acids can be associated with digester instability or stress, and acceptable VFA concentrations in digester effluents are between 50-500 mg/L (Gerardi, 2003).

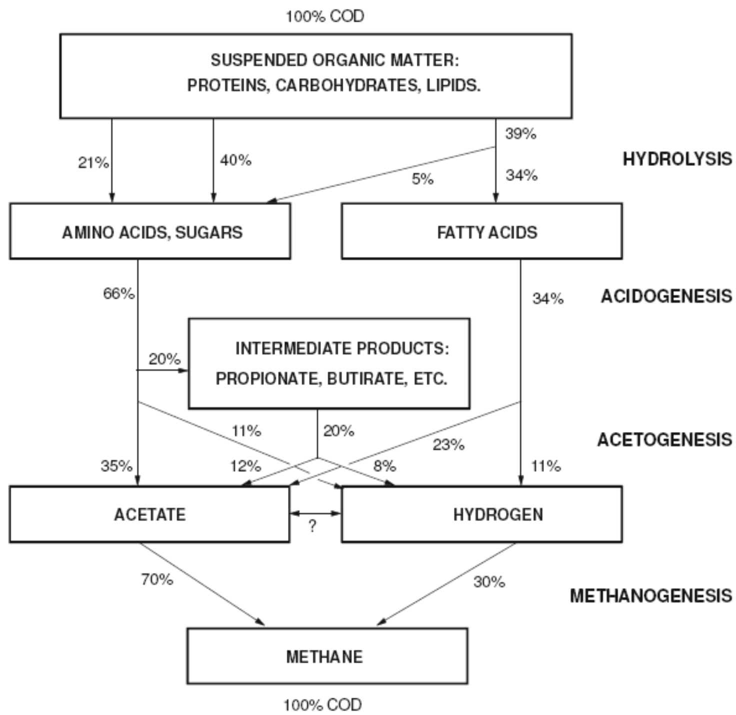

Figure 2.1 Schematic Diagram Demonstrating the Fundamentals of Anaerobic Digestion
(Reproduced from van Haandel and van der Lubbe, 2007)

During the acetogenesis process, the products of acidification are converted into acetic acid, hydrogen and carbon dioxide. The first three stages of the anaerobic digestion process are often grouped together as the acid producing stage. In these first three stages of anaerobic digestion, no organic matter is removed from the liquid phase, only transformed into substrates suitable for methanogenesis (van Haandel and van der Lubbe, 2007).

In the final stage of methanogenesis the products from the acid fermentation (primarily acetic acid) are converted into CH_4 and CO_2 (biogas). Methane-forming bacteria are sensitive to oxygen, temperature, acidity and organic loading. Methanogens require a neutral pH, preferably between 6.8 and 7.2 (van Haandel and van der Lubbe, 2007; Gerardi, 2003). Typical biogas mixtures produced from dairy manure consist of 55-70% CH_4 and 30-45% CO_2 (Krich et al., 2005; Burke, 2001), with traces of inorganic gases that can include CO, H_2, H_2S, NH_3, N_2 and N_2O (Gerardi, 2003).

2.1.2.2 Physical Parameters

Temperature

Anaerobic bacteria function under three temperature ranges: psychrophilic, mesophilic and theromophilic. Psychrophilic temperatures of less than 20^{o}C produce the least amount of bacterial action. Mesophilic digestion occurs between 20 and 40^{o}C and thermophilic digestion occurs between 43 and 71^{o}C (Burke, 2001; Sakar et al., 2009). Gerardi (2003) explains mesophilic digester performance usually does not approach a temperature of 42^{o}C, as this represents the transition from mesophilic to thermophilic organisms. Optimum mesophilic temperature is between 35 and 37^{o}C, while the optimum thermophilic temperature is between 55 and 63^{o}C (USDA, 2003; Burke, 2001; European Commission, 2001). Temperature affects the activity and growth of microorganisms. Methanogenic bacteria are more sensitive to temperature changes than other anaerobic microorganisms, where acid forming bacteria are less sensitive to temperature fluctuations. The rate of VS destruction and methane production is faster under thermophilic conditions (Sakar et al., 2009; Gerardi, 2003; Burke, 2001) and thermophilic digesters can accept higher OLRs than mesophilic reactors (Gerardi, 2003). Figure 2.2 describes the relationship of biogas and methane production as a function of temperature and retention time. Thermophilic digestion can result in higher biogas yields and this optimal biogas production will be achieved at a shorter retention time.

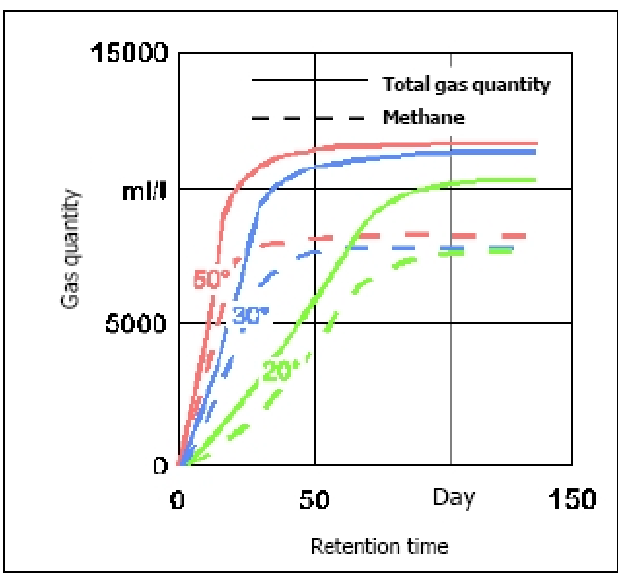

Figure 2.2 Relationship of Biogas Quantity with Temperature
(Köttner et al., 2008)

Gerardi (2003) suggests that although 25 to 50% more activity occurs in thermophilic digesters than in mesophilic digesters, there are several microbial characteristics associated with thermophilic anaerobes that may adversely affect digester performance. The author goes on to suggest the characteristics of concern include: 1) low bacterial growth or yield; 2) high endogenous death rates; and, 3) lack of diversity among anaerobes. As a result, thermophilic anaerobes are responsible for relatively high residual VFAs in digester effluents, >1000 mg/L, leading to possibly more pronounced odours (Gerardi, 2003; Burke, 2001; Han and Dague, 1997). Thermophilic anaerobes can also be sensitive to rapid changes in temperature. Therefore, fluctuations in digester temperature should be as small as possible. Gerardi (2003) suggests for thermophilic digesters to have temperature fluctuations of less than $1^{o}C$ per day and mesophilic digesters to have fluctuations of no greater than $2\text{-}3^{o}C$ per day.

Ontario on-farm manure digesters currently operate under mesophilic conditions. Thermophilic digestion requires a lot of heat energy and thus on-farm systems tend to operate at the lower mesophilic temperatures. In many cases, sufficient heat is not available to operate in the thermophilic range, particularly under Ontario’s cold winter climate. Although there may be some operational challenges associated with operating on-farm manure digesters under thermophilic conditions, current FIT prices have warranted a second look at the potential of having thermophilic digesters operating on farms. The only on-farm manure digester in Canada operating under thermophilic conditions is the Highland Feeders Ltd. digester in Vegreville, Alberta. This digester system utilises manure from 7500 beef cattle and consists of two single-phase mechanically mixed 1,800 m^3 cylindrical concrete digester tanks operating at 55°C and a 14-day retention time (Monreal et al., 2009).

The destruction of pathogens in anaerobic digesters has always been of importance in the treatment of sewage sludge, and is now of greater interest in the digestion of manure as a treatment option to reduce the migration of pathogens to drainage tile lines (potential surface and subsurface water contamination). Pathogens are inactivated during exposure to temperatures above their optimum growth conditions. The European Commission (2001) did extensive work on investigating the relationship between temperature and pathogen destruction in anaerobic digestion systems for sewage sludge. The study concluded that pathogen die-off is most effective under thermophilic conditions, where operating parameters should be more than 7 minutes at 70°C; 30 minutes at 65°C; 2 hours at 60°C; 15 hours at 55°C or 3 days at 50°C. The European Commission (2001) reports that a UK government study in the 1980's concluded that 70°C for 30 minutes or 55°C for 4 hours would produce a digested residue that is virtually pathogen-free or "Class A" (< 1000 fecal coliforms per gram total dry solids).

The European Commission (2001) indicates typical pathogen reductions of 2-3 logs with mesophilic digesters can be expected. Wright et al. (2003) reported a 3-log reduction in faecal coliforms in a mesophilic (37°C) plug flow digester treating dairy manure. Wright et al. (2004) compared the reduction potential of pathogens in five anaerobic digesters (3 plug flow, 1 completed mixed and 1 fixed film) treating dairy cattle manure in New York state, and found an average reduction of 94 to 99% (1-2 log) in faecal coliform counts. Smith et al. (2005) suggest a 2-log reduction for *E.coli* is probably the highest level of bacteria die-off that can be obtained from mesophilic digesters. This observation was corroborated by Crolla et al. (2013) who observed average log reductions of 2.5, 1.3 and 2.3 for *E.coli*, *Salmonella* and Enterococci, respectively, in four on-farm mesophilic digester systems co-digesting liquid dairy manure and waste grease. Manyi-Loh et al. (2014) investigated the inactivation of bacterial pathogens in a mesophilic anaerobic digester treating dairy manure and found 1 log reductions for *E.coli* and *Campylobacter* and up to 2 log reductions for *Salmonella*. Smith et al. (2005) suggest efficient mixing and long retention times are important factors for bacterial reductions in digester systems, while Harikishan and Sung (2003) highlight the importance of operating temperature.

Hydraulic Retention Time (HRT)

HRT is the amount of time the digester liquid remains in the digester tank. HRT is important since it defines the amount of time available for bacteria to grow and convert organic matter to biogas. Optimum HRTs vary with differing technologies, process temperatures and waste composition. Sakar et al. (2009) reported that the digestion of pig manure, with its high fat content, requires lower HRTs than dairy manure, which contains more cellulosic and hemi-cellulosic components. Under mesophilic conditions, Sakar et al. (2009) propose average HRTs for dairy manure of 12 to 25 days, for cattle manure with straw bedding 15 to 35 days and for pig manure 10 to 20 days.

The USDA (2003) recommends, under mesophilic conditions, a HRT of greater than 20 days for manure plug flow digesters, greater than 17 days for completely mixed digesters, and 1 to 6 days for fixed film digesters. The US EPA (2004) recommends a HRT of 18-20 days for plug flow digesters and a HRT of 15-20 days for completely mixed digesters. In Germany, the typical HRT is greater than 20 days for mesophilic digestion and in Sweden the typical HRT is greater than 7 days for thermophilic digestion (European Commission, 2001). Under the Ontario Nutrient Management Regulations, the minimum HRT for on-farm manure based digesters is 20 days (Ontario Government, 2009).

Solids Retention Time (SRT)

SRT is an important factor controlling the conversion of solids to biogas and is important for maintaining digester stability. The SRT is defined as:

$$SRT = \frac{(V)(C_d)}{(Q_w)(C_w)} \qquad \text{[2.1] (Burke, 2001)}$$

where V = digester volume, m^3

C_d = solids concentration in digester, kg/m^3

Q_w = volume wasted each day, m^3/day

C_w = solids concentration of waste, kg/m^3

At low SRTs, bacteria cannot grow fast enough to replace any bacteria that has been lost in the effluent. A wash out occurs when the rate of bacteria lost to the effluent exceeds the rate of bacteria growth. The SRT at which wash-out occurs is the “critical SRT” (Burke, 2001). Typically, CSTR and plug flow digesters have an SRT/HRT equal to 1; however, retained biomass reactors (e.g. fixed film digesters) have SRTs greater than digester HRT (van Haandel and van der Lubbe, 2007). The higher SRT/HRT ratio results in much smaller digesters with the same solids conversion to biogas. A direct relationship exists between the SRT and VS converted to biogas and Figure 2.3 illustrates the percentage VS destruction for manure as a function of SRT. Burke (2001) determined that a maximum of 65% of dairy manure’s VS could be converted to biogas at long SRTs, while 65 to 67% of dairy manure's COD could be converted to biogas. The conversion of cellulose to biogas requires long retention times.

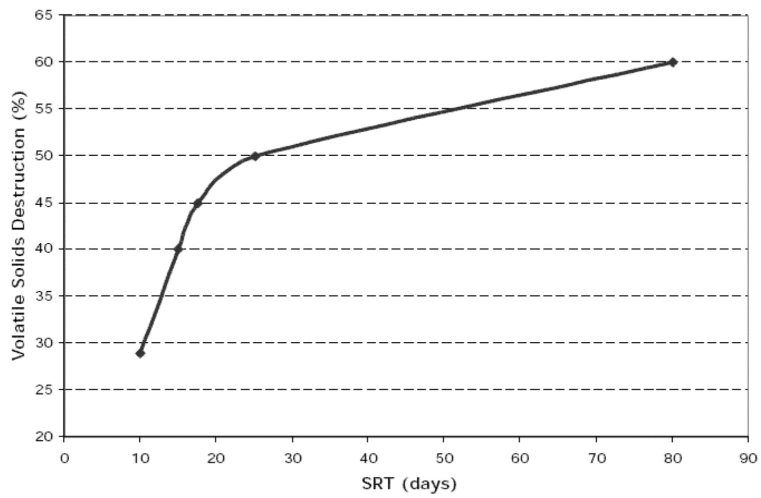

Figure 2.3 Manure Volatile Solids Destruction with Increasing SRT
(reproduced from Burke, 2001)

Organic Loading Rate

OLR is an important parameter for the operation of a digester and maintains an efficient balance between available organic matter and microbial activity during the digestion (Burke, 2001). OLR can be calculated using the digester HRT and influent feedstock VS or COD concentration. The OLR is defined as:

$$OLR = \frac{C_{COD,VSI}}{HRT} \qquad [2.2] \quad \text{(Burke, 2001)}$$

where HRT = volume of tank (V)/daily flow (Q), day

$C_{COD,VSI}$ = total volatile solids concentration of influent waste materials (manure plus co-substrates), kg/m^3

Under mesophilic conditions Sakar et al. (2009) suggest an optimum OLR for dairy manure to be between 2.5 and 3.5 kg VS/m^3/day and for dairy manure with co-substrates to be between 5.0 and 7.0 kg VS/m^3/day. Hilborn (2007) recommends that a mesophilic single phase farm anaerobic digester will maintain its stability if organic loading rates do not exceed 4.5 kg/m^3/day when co-substrates used have VS contents that range from 63 to 98% of TS. Fulhage et al.

(1993) proposes a slightly higher OLR of 5.9 kg VS/m^3/day for dairy and beef manure digesters operating at HRTs between 12 and 17 days.

Food to Microorganism (F/M) Ratio

Burke (2001) describes the F/M ratio as the key factor controlling anaerobic digestion. The F/M parameter expresses the ratio between organic matter available as food and the mass of bacteria available to metabolise the material. F/M ratios are expressed as kg COD/kg VSS/day or kg VS/kg VSS/day and defined as:

$$F/M = \frac{L_D}{VSS_D} \qquad [2.3] \quad \text{(Burke, 2001)}$$

where L_D = COD or VS digester loading rate, kg/m^3/day
VSS_D = volatile suspended solids in digester, kg/m^3

The F/M ratio is the controlling factor in all biological treatment processes. Prashanth et al. (2006) report that too high a F/M ratio can be toxic and too low a ratio can prevent the excretion of enzymes necessary for biodegradation. In a study conducted by Prashanth et al. (2006), optimum F/M ratios were determined to be between 0.57 and 0.68 g COD/g VSS for the digestion of synthetic wastewater (cellulose, sucrose and peptone) in batch mesophilic assays. A study conducted by Ortega et al. (2008) determined that a F/M ratio of 1.15 g VS/g VSS treating food waste under batch thermophilic conditions was suitable; however, a F/M ratio of 4.43 g VS/g VSS pushed methanogenesis towards H_2 production.

2.1.2.3 Anaerobic Digesters Suitable for Processing Dairy Manure

Dairy manure is typically managed as a liquid or semi-solid slurry. Due to its high solids content, anaerobic digesters processing manure must be able to handle the conversion of solids to biogas while allowing for ease of solids movement throughout the system. The digester system will also need to be able to handle bedding material, sand and other foreign materials associated with typical dairy manures. Stratification of solids is another potential concern for dairy manure digesters and must be considered in the design.

There are several types of anaerobic digester designs and they are typically classified as high and low rate biogas systems (Tchobanoglous and Burton, 1991; Burke, 2001; Gerardi, 2003; Bachmann, 2013). High rate digesters are designed to retain solids and keep the methanogenic microbial consortia in the reactor for a longer period (> SRT), thereby achieving process stability with increased conversion of organic matter to biogas. Digester systems considered to be high rate biogas producers include fixed film, sludge blanket (e.g. UASB) and ABR digesters. Burke (2001) suggests effective high rate reactors have a SRT/HRT greater than 3. The design of high rate digesters is best suited for more diluted wastes with high soluble organics and less particulate matter, considering the microbial community has little opportunity to hydrolyze and degrade particulate solids (Gerardi, 2003). Low rate systems, where SRT/HRT equal 1, have been identified as most effective for the digestion of manures and other agricultural residues; sufficient HRT allows for hydrolysis of high solids and particulate matter fraction. Literature identifies low rate digester systems such as covered lagoons, heated plug flow reactors and heated completely mixed reactors as the best suited systems for agricultural substrates (Burke, 2001; US EPA, 2004; Krich et al., 2005; Lazarus, 2008; Bachmann, 2013).

Lazarus (2008), Krich et al. (2005), US EPA (2004) and Burke (2001) suggest the suitable systems for North American farms are ambient temperature covered anaerobic lagoons (low rate), heated plug flow digesters (low rate) and heated completely mixed digesters (mesophilic-low rate; thermophilic-high rate). The US EPA (2004) suggests that a suitable digester should be selected based primarily on climate and characteristics of the existing manure management system. Table 2.1 summarizes digester systems best suited for various manure management systems.

Covered Anaerobic Lagoons

Anaerobic lagoons are essentially covered earthen ponds. Manure enters at one end and the effluent is removed at the other. The inlet and outlet in a lagoon system should be located as far as possible to minimize short circuiting (USDA, 2003). Typically, lagoons operate at psychrophilic (< 20 ^{o}C), or ground temperatures. Since temperatures are quite low, the rate of conversion of solids to biogas is also low. In addition, solids tend to settle to the bottom where digestion continues to occur in the sludge bed. Little contact of bacteria with the bulk liquid occurs.

The concentration of bacterial biomass is low, this results in very low solids conversion to biogas. Periodically covered lagoons must be cleaned out due to solids accumulation. Krich et al. (2005) suggests coarse solids such as hay and silage fibres in dairy manure should be separated in a pre-treatment step and kept from entering the lagoon. If these solids are not removed they will float to the top and form a crust and the crust will thicken which will result in reduced biogas production. However, the authors recognize that a considerable amount of energy potential is lost with the removal of the particulate solids. The advantage of anaerobic lagoons is the low cost; however, the driver of such systems is not energy production but in fact treatment.

Table 2.1 Digester Type for Various Manure Management Systems
(Reproduced from US EPA, 2004)

Climate	Manure Type	Collection System	Estimated Min. Ratio of Water:Manure	% TS	Digester Type
Moderate to Warm (Central to Southern U.S.)	Dairy	Flush	10:1	< 3%	Covered Lagoon Fixed Film
		Scrape & Parlour Washwater	4:1 – 1.1:1	3% - 11%	Complete Mix
		Scrape – Manure Only	N/A	> 11 %	Plug Flow
	Swine	Flush	10:1	< 3%	Covered Lagoon Fixed Film
		Scrape	2:1	3% - 6%	Complete Mix
		Pull Plug	5:1	< 2%	Covered Lagoon
		Managed Pull Plug	3:1	3% - 6%	Complete Mix
Cold (Northern U.S. and Canada)	Dairy	Flush	10:1	< 3%	Limited Possibility for Covered Lagoon
		Scrape & Parlour Washwater	4:1 – 1.1:1	3% - 8%	Complete Mix
		Scrape – Manure Only	N/A	> 11%	Plug Flow
	Swine	Flush	10:1	< 3%	Limited Possibility for Covered Lagoon
		Scrape	2:1	3% - 8%	Complete Mix
		Pull Plug	5:1	< 3%	Limited Possibility for Covered Lagoon
		Managed Pull Plug	3:1	3% - 6%	Complete Mix

A review conducted by Burke (2001) determined that on average lagoon systems accepting screened manure would achieve a 35% conversion of VS to biogas at a loading of 0.04 kg $VS/m^3/day$. The US EPA (2004) recommends a warm climate for lagoon systems to be cost effective unless odour management is the goal. Lagoon systems are best to treat liquid manures with up to 3% TS (US EPA, 2004; USDA, 2003).

Plug Flow Digesters

Plug flow anaerobic digesters have a simple design, are relatively inexpensive and can be either horizontal or vertical in configuration. Horizontal plug flow reactors are most common. Fresh substrates are pumped in, which displaces an equal portion of digestate that gets pushed out from the other end of the digester. The USDA (2003) suggests a length to width ratio of digester flow path to be between 3.5:1 to 5:1. Since bacteria are not conserved in the plug flow digester, a portion of the waste must be converted to new bacteria, which are subsequently wasted with the effluent.

Plug flow systems are subject to stratification, where sand and silt settle to the bottom and organic fibres float to the surface. Stratification can be partially inhibited by maintaining a relatively high solids concentration in the digester (Krich et al., 2005; Burke 2001). Periodically, solids must be removed from the plug flow digester.

The plug flow reactor is a simple and economical system. Applications are probably limited to concentrated liquid dairy manure containing minor amounts of sand and silt. The US EPA (2004) and USDA (2003) suggest using plug flow digesters when TS is between 11 and 14% for ruminant manure and 8 to 14% for other manure sources. Krich et al. (2005) do not recommend a plug flow digester system for manures with less than 10% TS. If stratification occurs because of a dilute waste or excess sand, significant operating costs will be incurred. Generally, a mesophilic plug flow reactor will achieve a 32% conversion of VS to biogas at a loading rate of 2.5 kg $VS/m^3/day$ of dairy and swine manure (Burke, 2001). Typical HRTs of plug flow digesters are between 18 and 20 days (US EPA, 2004; USDA, 2003).

Completely Mixed Digesters

Completely mixed digesters (e.g. CSTR) are perhaps the most common and most flexible in terms of the variety of manures that can be accommodated. Krich et al. (2005), US EPA (2004) and USDA (2003) suggest completely mixed digesters are best suited for manures with TS concentrations from 2.5 to 11%. Completely mixed digesters are usually above ground, heated and insulated round tanks (Krich et al., 2005). Completely mixed reactors can have fixed covers, floating covers, or biogas holding covers. Floating covers are more expensive than fixed covers (Burke, 2001). Removable covers are necessary to allow access to the digester for solids removal. Mixing can be continuous or intermittent, and accomplished by biogas recirculation, mechanical propellers, or circulation of liquid (Burke, 2001). Burke (2001) and Fulhage et al. (1993) explain mixing enhances the digestion process by increasing the distribution of bacteria, substrates and nutrients throughout the digester, as well as balancing temperature dispersion. Gerardi (2003) describes slow gentle mixing as essential for keeping acetate forming bacteria and methane forming bacteria in close contact. Manures with high sand and silt content may require intense mixing to keep manure in suspension; however, intense mixing may inhibit the bacteria consortia (Burke, 2001) and methane forming bacteria are very sensitive to rapid mixing (Gerardi, 2003).

In a completely mixed system, anaerobic bacteria are constantly wasted from the process, and new bacteria must be produced to replace the lost bacteria. Since completely mixed digesters do not retain or concentrate biomass, the SRT = HRT. Completely mixed anaerobic digesters are designed for relatively long HRTs. The USDA (2003) suggests HRTs ≥ 17 days under mesophilic conditions, while the US EPA (2004) suggests a HRT of 15 – 20 days.

The completely mixed digester is a proven technology that achieves reasonable conversion of solids to biogas (Krich et al. 2005). Most of the initial anaerobic digesters installed to treat manure in Canada and the U.S. have been completely mixed mesophilic digesters (Burke, 2001). Many sewage treatment plants and industrial treatment plants use completely mixed reactors to convert solids to biogas (US EPA, 2004). Completely mixed digesters are highly effective for the treatment of slurries like manure. The disadvantages of completely mixed digesters are the large volumes required to provide sufficient HRT, loss of bacteria in the effluent and energy costs associated with mixing (Gerardi, 2003).

Completely mixed thermophilic anaerobic digesters have rapid conversion rates of solids to biogas. It has been shown that the rate of conversion can be three times greater with thermophilic reactors (Burke, 2001). Generally, completely stirred mesophilic digesters have shown to achieve a 42% conversion of volatile solids to biogas at a loading of 5.7 kg VS/m^3/day of dairy and swine manure (Lusk, 1998; Burke, 2001). Thermophilic reactors tend to have better VS conversions, where Burke (2001) suggests as high as 65% VS removal at higher OLRs (> 6.5 kg VS/m^3/day) while mesophilic reactors appear to achieve greater conversions at lower OLRs (< 4.6 kg VS/m^3/day).

Most on-farm dairy manure digesters in Ontario are intermittently mixed systems, either by use of mechanical mixing or recirculation pumps.

2.1.2.4 Two-Phase Digestion of Manure

The two-phase anaerobic digestion process consists of two reactors operating in series, with the initial reactor operated at a much shorter HRT, as little as one tenth of the HRT used in a typical single phase digestion system (Krich et al., 2005; Gerardi, 2003). The second reactor is operated at typical anaerobic digestion HRTs of over 15 days. The overall goal of a two-phase system is to separate the two main microbial processes of anaerobic digestion: acidogenesis and methanogenesis (Figure 2.4). These two processes are carried out by distinct bacterial species and populations. These distinct bacterial consortia differ in terms of their nutritional and pH requirements, but also with respect to their physiology, growth, metabolic kinetics and ability to tolerate environmental stresses (Demirer and Chen, 2005; Krich et al., 2005). In single-phase digestion, the pH and OLR are adjusted to suit the slow growing methanogenic organisms, which may not be optimum conditions for the relatively fast growing acidogens (van Haandel and van der Lubbe, 2007; Demirer and Chen, 2005). The two-phase anaerobic digestion process is based on the concept that each phase will be optimized for the operational characteristics best suited for the respective bacteria: very short HRTs resulting in VFA formation and low pH in the first phase; long HRTs for conversion of acids to methane at neutral pH in the second phase (Demirer and Chen, 2005; Krich et al., 2005; Zhang et al., 2000). The acidogenesis digestion phase takes advantage of the fact that the acid forming bacteria have a much higher growth rate than the methanogens (Burke, 2001).

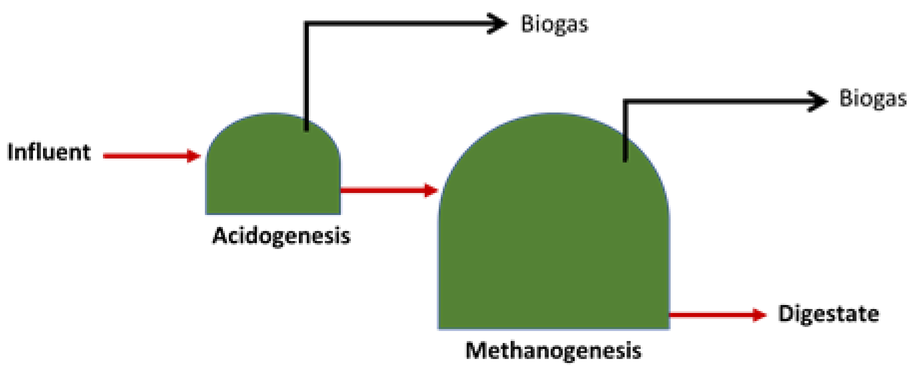

Figure 2.4 Two-Phase Anaerobic Digester System
(adapted from Burke, 2001)

The two-phase digestion configuration has several advantages over conventional single-phase systems. Firstly, it increases the process stability by controlling the acidification phase which prevents overloading and build-up of toxic materials (Yilmaz and Demirer, 2008; Demirer and Chen, 2005; Sung and Santha, 2003). Secondly, the first phase can protect methanogens from being overloaded, thereby preventing pH shock to the methanogenic bacterial population; additionally, a low pH, high OLR and short HRT all favour the establishment of acidogenic bacteria and prevent the growth of methanogens (Demirer and Chen, 2005). Finally, the process can technically be smaller in size and therefore more cost effective (Yilmaz and Demirer, 2008; Demirer and Chen, 2005; Krich et al., 2005; Zhang et al., 2000). Kaparaju et al. (2009) suggest that although biodegradability of recalcitrant materials in manure may be improved in a two-phase system, the syntrophic relationship between bacteria and methanogens is disrupted and can cause product inhibition in the acidogenic reactor.

TPAD is a variation of the two-phase system that optimizes methane production and pathogen destruction. Iowa State University has patented (U.S. Patent No. 5,746,919) the TPAD system (Katers and Schultz, 2003). The TPAD system combines thermophilic and mesophilic processes to increase the efficiency and rates of reaction, thereby reducing reactor size. The first phase digestion operates at thermophilic temperatures ranging from 45^{o}C to 75^{o}C, with typical operating temperatures of approximately 56°C and HRT of up to 10 days. The second phase digestion operates at mesophilic temperatures ranging from 20°C to 45°C, with typical temperatures of approximately 35°C and HRT of up to 20 days (Katers and Schultz, 2003; Pillars, 2002). Sung and Santha (2003; 2001) determined optimal retention times of 4-6 days for the thermophilic phase and retention times of 10-15 days for the mesophilic phase of TPAD systems digesting cattle slurries using completely mixed reactors for each phase.

TPAD has been successfully applied to the stabilization of municipal wastewater sludges and has proven capable of producing "Class A" biosolids (Katers and Schultz, 2003; Sung and Santha, 2003). Initial research using the TPAD system for manure digestion has demonstrated improved odour control, reduced foaming, and increased VS reductions (Pillars, 2002; Sung and Santha, 2001). Also, the thermophilic temperatures during the first phase of digestion increased pathogen kill when compared to mesophilic systems (Katers and Schultz, 2003; Sung and Santha, 2003; Pillars, 2002; Han and Dague, 1997). Biogas production can be increased by 25% using TPAD systems (Katers and Shultz, 2003). A temperature phased sequencing batch reactor when treating dairy manure can achieve a 30 to 41% conversion of volatile solids to biogas, while producing a biogas with 62 to 66 % methane (Burke, 2001). These values are based on a VS loading rate of 2 to 4 kg/m^3/day and a three-day HRT for the first phase thermophilic digester. Pillars (2002) suggests that like one-phase digestion processes, the TPAD system tends to work best with more dilute manures; however, Yilmaz and Demirer (2008) and Demirer and Chen (2004) have reported that a phased digestion process can handle manures with higher solids content (> 10% TS).

Although the TPAD system is promising and has shown to be effective in treating municipal sludges there has been very limited research in applying this system to livestock manures (Yilmaz and Demirer, 2008; Demirer and Chen, 2005; Burke, 2001; Sung and Santha, 2001). Most lab scale applications of TPAD using dairy manure have been limited to screened dairy manure (Demirer and Chen, 2005). On-farm demonstrations are also required to effectively evaluate full scale TPAD systems (Pillars, 2002).

It should be noted that Krich et al. (2005) indicate a two-phase process is not applicable to dairy wastes, stating two-phase systems are only applicable to soluble and readily metabolized sugars and starches, possibly some fats and protein, but not to the more difficult to digest particulate and fibrous materials. The authors indicate the dairy manure in the two-phase system would result in no H_2 production in the first reactor thereby reducing the conversion of CO_2 to CH_4. However, this was the only reference in the review of literature that stated a two-phase system was not appropriate for dairy manure.

2.1.2.5 Research Studies on the Anaerobic Digestion of Dairy Manure

The growing interest in the on-farm anaerobic digestion of manure can be attributed to the following outcomes from the process:

1. Production of biogas, with a methane content of approximately 60% (Krich et al., 2005; Güngör-Demirci and Demirer, 2004; Semmler 2002; Burke, 2001), which can be stored and used on demand to produce electricity and heat.

2. Improved fertilizer value of the digested manure (Yilmaz and Demirer, 2008; Krich et al., 2005; Burke, 2001).

3. Reduction of odours (Yilmaz and Demirer, 2008; Burke, 2001), potentially in the order of 80% (Semmler, 2002).

4. Reduction of pathogens and weed seeds (Yilmaz and Demirer, 2008; Krich et al., 2005; Semmler, 2002).

Several research studies have been conducted to document the feasibility of manure digestion and its advantages. A laboratory study by Lo et al. (1986) was carried out to study the mesophilic (30°C) digestion of screened dairy manure. In this study, 5.5 L reactors were fed with 3.0% VS at a HRT range of 1 to 11 days. The authors determined the maximum biogas (methane) production to be 1.89 L CH_4/L/day at a 1-day HRT, with maximum methane yield of 0.093 L CH_4/g VS at an 11-day HRT. Ten-Hong and Wu-Huann (1996) investigated the performance of four types of anaerobic digesters to treat very dilute dairy manure: continuous stirred tank reactor (CSTR), up-flow anaerobic sludge blanket (UASB) reactor, up-flow anaerobic filter (UAF) reactor and baffled reactor. The laboratory experiments were conducted under mesophilic conditions (35 ± 1°C), HRTs ranging from 2 to 18.8 days with organic loading rates ranging from 0.117 to 1.303 g VS/L/day. The authors reported optimum methane yields of

0.156, 0.222, 0.241 and 0.166 L CH_4/g VS for the CSTR, UASB, UAF and baffled reactors, respectively. The respective COD removals were 66, 71, 74 and 79%. A study conducted by Maranon et al. (2001) investigated the digestion of diluted dairy manure in a UASB reactor. In this study, biogas production ranged between 0.20 and 0.39 m^3 biogas/kg $COD_{removed}$ (up to 64% methane) and the authors reported the highest percentage of COD removed was found to be 75.5% at a HRT of 22.5 days.

BMP tests were conducted by Güngör-Demirci and Demirer (2004) to investigate the effects of initial COD concentration and nutrient addition (micro- and macro- nutrients) on the mesophilic digestion (35 ± 2ºC) of dairy manure. At an initial COD concentration of 12 g/L, biogas yields were reported as 180.3 mL/g COD_{added} (70% methane) and 242.2 mL/g COD_{added} (64% methane) with and without the addition of nutrients, respectively. At a higher initial COD concentration of 53.5 g/L, biogas yields were determined to be much higher at 368.5 mL/g COD_{added} (53% methane) and 359.0 mL/g COD_{added} (49% methane) with and without the addition of nutrients, respectively. The addition of nutrients seemed to have little effect on the biogas and methane production; however, BMP bottles with the lower initial COD concentration produced biogas with higher methane content. In a study conducted by Wen et al. (2007), the digestion of liquid dairy manure from flush barns (< 2.8 %TS) was investigated by comparing completely mixed versus plug flow digesters. The reactors in this study were operated under mesophilic conditions (35ºC) with HRTs ranging from 2 to 15 days and OLRs ranging from 2 to 20 g VS/L/day. The authors reported that the performance of the completely mixed and plug flow reactors was similar. Optimum methane production of 0.88 ± 0.05 L CH_4/L/day (1.21 ± 0.10 L biogas/L/day) was obtained from the completely mixed digester and 0.83 ± 0.03 L CH_4/L/day (1.11 ± 0.07 L biogas/L/day) from the plug flow system. The removal of COD and VS ranged from 11.2 to 37.2% and 9.5 to 35.1%, respectively.

Phased digestion of dairy manure has also been investigated and several studies have been presented in the literature; however, much of the work has been conducted using diluted or screened dairy manure. Dugba and Zhang (1999) present a study investigating the treatment of screened liquid dairy manure using two-phase ASBR systems. One two-phase system consisted of both the acidogenesis and methanogenesis phases operating at 35 ± 1 ºC and the second two-phase system operated as a TPAD system, where the acidogenesis phase operated at 55 ± 1 ^{o}C. Five OLRs (2, 3, 4, 6 and 8 g VS/L/day) were investigated, where each OLR was operated at HRTs of 3 and 6 days. The authors reported that both phased systems were highly dependent on HRT and VS loading rate and each phased system failed at a 3-day HRT when the OLR reached 6 g VS/L/day. The study demonstrated that the TPAD system yielded higher % VS removals and specific methane production rates than the mesophilic-mesophilic phased system, at both 3- and 6-day HRTs. At OLRs of 2, 3 and 4 g VS/L/day, the % VS removal and specific methane production rate of the TPAD system were 43.8 to 44.1% and 0.41 to 0.82 L CH_4/L/day, respectively, for the 3-day HRT and 37.1 to 38.9% and 0.34- to 0.72 L CH_4/L/day, respectively, for the 6-day HRT. The % VS removals and specific methane production rates for the mesophilic-mesophilic system was much lower: 29.3 to 30.2 % and 0.27 to 0.56 L CH_4/L/day for the 3-day HRT, respectively; 26.1 to 29.1 % and 0.27 to 0.48 L CH_4/L/day for the 6-day HRT, respectively. The authors also reported that the TPAD system was better at destroying total coliforms, with non-detectable counts in the effluent.

Sung and Santha (2001) conducted a study investigating the use of a TPAD system treating dairy manure with various TS concentrations (3.5 to 14.5 %TS). The TPAD system was operated using completely mixed reactors with HRTs of 4 days for the thermophilic (58^{o}C) reactor and 10 days for the mesophilic (38^{o}C) reactor, and with OLRs ranging from 1.87 to 7.70 g VS/L/day. Optimum VS removal of 42.6% was achieved at feed influent of 10.35 %TS and OLR of 5.82 g VS/L/day; a drop in system performance was observed when feed influent solids concentrations were higher than 10.35% TS. Methane yields ranged from 0.54 to 0.61 L CH_4/g $VS_{destroyed}$ and pathogen destruction was high with effluent *Salmonella* concentrations < 2 MPN/4g TS. Sung and Santha (2001) also reported operations of the TPAD system under NH_3-N concentrations of 770 ± 90 and 1230 ± 70 mg/L resulted in a significant drop in digester performance.

Demirer and Chen (2004) recognized that much of the phased digestion studies presented in the literature were conducted using screened or diluted dairy manure. The authors therefore investigated the use of a two-phase anaerobic digestion system for the treatment of unscreened dairy manure, with emphasis on the effects of HRTs and OLRs on digestion performance. Twelve daily-fed completely mixed acidogenic anaerobic reactors (160 mL) were operated at

HRTs ranging from 1.25 to 4 days and OLRs ranging from 4 to 30 g VS/L/day under mesophilic conditions (35 ± 1^{o}C). The acidogenic reactor effluents were subsequently fed to methanogenic reactors. The results from this study indicated that the pre-acidification of the dairy manure resulted in improved reduction efficiencies of VS and methane yields. When OLRs ranged from 4 to 10 g VS/L/day, VS reductions ranged from 30.3 to 62.4 % removal and methane yields ranged from 0.066 to 0.243 L CH_4/g VS_{added}. However, at the higher OLRs of 20 to 30 g VS/L/day a relatively lower digestion performance was observed which may be due to the possible wash-out of the acidifiers at the much lower HRTs of 1.25 to 4 days. Demirer and Chen (2004) suggest that the performance at the higher OLRs can be improved by adding a recycle of biomass to the acidification reactor for an increased SRT.

Another study investigating optimum operating conditions for anaerobic acidification of unscreened dairy manure was conducted by Yilmaz and Demirer (2008). Nine completely mixed acidogenic anaerobic reactors (250 mL) were fed daily and operated at HRTs ranging from 1.25 to 4 days and OLRs ranging from 5 to 15 g VS/L/day. The acidogenic reactor effluents were subsequently fed to methanogenic reactors, where both reactors were operated under mesophilic conditions (35 ± 1^{o}C). In this study, the authors focused on determining the relationship of HRT, OLR and pH on the degree of acidification. The degree of acidification was quantified using the percentage of the initial substrate concentration converted to VFAs. The initial substrate concentration was measured as mg COD/L and the quantity of VFAs converted to the theoretical equivalent as mg COD/L (using COD equivalents for each VFA). The authors reported that a HRT of 2 days and an OLR of 15 g VS/L/day yielded maximum acidification, with over 80% degrees of acidification and 600 mg VFA produced/HRT. The average pH value in the acidogenic reactors was within 6.2 and 6.6, which is considered somewhat high for the acidogenesis process. The authors explained that the relatively high pH values were due to the alkalinity generated by the anaerobic biodegradation of nitrogenous organic compounds contained in dairy manure, and consider this the self-buffering capacity of manure that makes it an appropriate substrate for digestion.

Kaparaju et al. (2009) investigated the use of two methanogenic CSTR reactors connected in series to optimize biogas production using diluted dairy manure. Both lab- and pilot-scale studies were conducted under thermophilic conditions (54 ± 1°C), and a one-step CSTR reactor was used as the control. The mean biogas yields from the one-step lab-scale CSTR reactor (HRT = 15 days; OLR = 4 g VS/L/day) ranged from 0.34 to 0.37 L/g VS_{fed} and were found to be 16% lower than the biogas yield of the serial CSTR system (HRT = 15days; OLR = 4 g VS/L/day) that produced a biogas yield of 0.44 L/g VS_{fed}. The VFA concentrations in the digestate of the serial CSTR system were 24 to 43% lower than the one-step CSTR system, which produced effluent VFA concentrations ranging between 0.7 and 2 g/L. The authors reported that optimum results were obtained when serial reactors were operated with 70/30% and 50/50% volume distribution. Pilot-scale experiments also showed that serial digestion with 77/23% volume distribution could improve biogas yields by 1.9 to 6.1% compared to a one-step process, where the biogas yields for the serial digestion ranged from 0.45 to 0.49 L/g VS_{fed}. Kaparaju et al. (2009) concluded from their study that the main reason for improved biogas production in serial digestion was due to a more optimal retention time distribution for particulate matter than the nominal average retention time, thereby minimizing the loss of relatively fresh feed due to short-circuiting which is commonly noticed in CSTRs.

Finally, a study conducted by Nielsen et al. (2004) compared a two-phase thermophilic anaerobic digestion system of cattle manure (5.5 %TS) with a single-phase thermophilic digestion system (55°C; HRT = 15 days). The two-phase system consisted of a digester operating at 68°C with a HRT of 3 days connected to a 55°C reactor with a 12-day HRT. The authors proposed that using a two-phase system with extreme thermophilic pre-treatment of cattle manure at a temperature > 65°C, followed by a traditional moderate thermophilic treatment at 50 to 55°C, would involve a broader spectrum of thermophilic lignocellulose-degrading bacteria, while maintaining an optimal temperature for microbial consortia to convert VFAs to methane. Both the two-phase and conventional single phase systems were operated at a OLR of 3 gVS/L/day and the specific methane yield was determined to be 8% higher with the two-phase system, where the specific methane yield of the two-phase system was 0.26 ± 0.1 L CH_4/g VS (47.1 %VS removal) and that of the single phase system was 0.24 ± 0.1 L CH_4/g VS (43.3 %VS removal). This study was successful in determining that the improved digestion in the two-phase system was caused by increased hydrolysis in the 68°C reactor. Nielsen et al. (2004) concluded their study by conducting a microbial population analysis, which determined that population size and activity of aceticlastic methanogens, syntrophic bacteria and hydrolytic/fermentative bacteria were significantly lower in the 68^{o}C reactor than in the 55^{o}C reactors. The authors also concluded from their study that the bacterial community in the 68^{o}C reactor was stable and was not negatively affected by changes in HRT or feedstock quality.

2.1.2.6 Co-digestion of Manure

The co-digestion of manure with other substrates such as energy crops, industrial wastes, food industry wastes, animal by-products (e.g. slaughterhouse waste), or sewage sludge can result in numerous benefits. The addition of co-substrates to manure can improve the nutrient balance of total organic carbon, nitrogen and phosphorus, which results in a stable and maintainable digestion process and good digestate fertilizer quality (Braun and Wellinger, 2003). Co-digestion also improves the flow qualities of the co-digested substrates (Krich et al., 2005). Perhaps the most important drivers for co-digestion of manure with other substrates are the financial benefits associated with the increased biogas yields due to co-digestion and income generated from tipping fees when wastes are used as co-substrates.

Energy crops have become a popular co-substrate in Europe with the digestion of manure, where some on-farm digesters operate with over 70% energy crops (Effenberger, 2006). Energy crops are especially interesting when the plants are grown on fallow lands. Several crops demonstrate good biogas potentials. Many varieties of grass, clover, cereals and corn, including whole plants, as well as rape or sunflower have shown to be suitable for methane production (Braun et al., 2009). Typically, all C4-plants have very good growth yields and in Germany corn has become the most popular energy crop co-digested with manure, while in Austria grass is most popular (Braun and Wellinger, 2003). Effenberger (2006) reports the potential biogas yields of canola cake and corn silage to be 552 m^3/t and 202 m^3/t, respectively, while that of liquid dairy manure to be only 22 m^3/t. Braun et al. (2003) reported the biogas potential of corn to be 648 m^3/t VS. Recent German experience has shown mean methane yields of 348 m^3 CH_4/t VS for corn (whole plant) and 380 m^3 CH_4/t VS for barley (Braun et al., 2009), whereas methane yields from the digestion of cow manure alone will average a methane yield of 220 m^3CH_4/t VS (Weiland, 2000). Figure 2.5 summarizes the mean potential biogas yields of various substrates suitable for co-digestion with manure. Co-digestion of manure with energy crops has demonstrated to be highly successful in producing high biogas yields. Lindorfer et al. (2008) demonstrated that an agricultural biogas plant in Austria running on a mass VS ratio of 96% energy crops and 4% liquid dairy manure, with an organic loading rate of 4.25 kg VS m^{-3} day^{-1}, produced a biogas yield of 730 m^3/t VS. It should be noted that the biogas plant was operating near digester limits. Lehtomäki et al. (2008) demonstrated that the co-digestion of grass silage and cow manure at a VS mass ratio 40% silage to 60% manure produced a methane yield of 268 m^3 CH_4/t VS. Umetsu et al. (2006) reported that the daily methane yield ranged from 360 to 410 m^3 CH_4/t TS/day when dairy manure was mixed with sugar beets.

Although the use of energy crops in the co-digestion of manure can increase biogas yields by 2 to 4 times (Weiland, 2000), it is anticipated Ontario will not follow the European example of steadily increasing the number of on-farm digesters using energy crops. In Germany for example, the number of digesters using energy crops has increased from 100 in 1990 to nearly 4000 in 2008 (Braun et al., 2009). This discrepancy is primarily due to further travel distances between farms, lower electricity purchase prices and the discouragement from OMAFRA of converting food-based crop lands to produce energy crops.

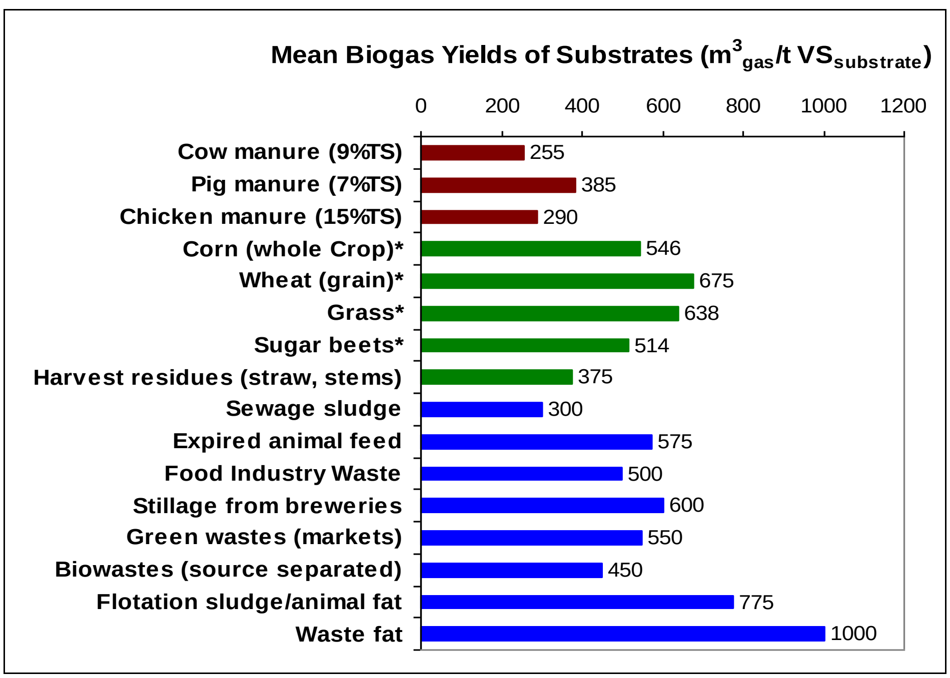

Figure 2.5 Potential Biogas Yields of Various Substrates for Co-digestion
[Compilation of data from Braun et al. (2009), Braun and Wellinger (2003) and Weiland (2000)]
(* Assumes biogas with 60% methane)

In Ontario, there is a growing interest in the use of organic rich wastes as co-substrates in manure co-digestion, primarily due to the high biogas yields associated with these materials and potential tipping fees. There are a number of organic rich substances that are anaerobically feasible to degrade without significant pre-treatment. Among these substrates are leachates, slurries, sludges, oils, fats and whey. Some wastes, such as proteinaceous wastes, can form inhibiting metabolites (e.g. NH_3) during anaerobic digestion which require higher dilutions with substrates like manure. Some other types of substrates (e.g. source separated municipal bio-waste, food leftovers, expired food, market wastes) would require pre-treatment to make the substrates more homogeneous, remove indigestible components or to sterilize/pasteurize the substrates (Braun and Wellinger, 2003). Readily degradable substrates, like fats and proteins, yield the highest percentages of methane (Krich et al., 2005). However, fats and proteins available from industrial wastes such as slaughterhouse operations can inhibit the anaerobic digestion process through the accumulation of VFAs and long chain fatty acids (Salminen et al., 2003). When manure is added to the anaerobic process, it acts as a buffer and provides the necessary nutrients for digestion.

Hilborn (2007) states that in Germany materials that produce biogas yields of 200 to 4,500 m^3/t are ideal for manure anaerobic digesters. Braun et al. (2003) found that a co-substrate addition of 5 to 15% was best suited for a stable digestion process. Figure 2.5 shows that the potential biogas yields of organic wastes can be 2 to 4 times higher than animal manure. Köttner et al. (2008) explain that substrates higher in fats and starch content, which are easy to breakdown, will have higher biogas yields. Baking wastes and food waste grease have very high potential biogas yields of 580-657 m^3/t and 500-600 m^3/t (Köttner et al. 2008; Effenberger, 2006), respectively. Braun et al. (2003) conducted a series of lab studies and determined the biogas yields of corn distillery residues and waste oils to be 400 $m^3/t\ VS_{added}$ and 1104 $m^3/t\ VS_{added}$. The methane content in the biogas depends on the oxidation stage of the substrate and varies according to the fat, protein and carbohydrates present Köttner et al. (2008). Krich et al. (2005) state the theoretical methane content of biogas when using substrates composed of fats, proteins and carbohydrates as 72, 63 and 50% of the total biogas, respectively. For example, the methane content of biogas from the digestion of food waste is typically higher than animal manure: 70% methane (Scott and Ma, 2004) with food waste compared to 55-65% (Burke, 2001) with liquid dairy manure.

2.2 Corn Thin Stillage as Potential Co-substrate for Manure Anaerobic Digestion

2.2.1 Characteristics of Corn Thin Stillage

Stillage is a by-product from the production of ethanol. Ethanol can be produced from sugar crops (sugar beets, sugar cane, molasses, etc.), starch crops (corn, wheat, rice, barley, etc.), dairy products (whey) or cellulosic materials (crop residues, herbaceous energy crops, wood or municipal solid waste). In Canada and the U.S. one of the predominant feedstocks for ethanol production is corn and therefore corn stillage will be considered for this study. In 2006 Canada produced 550 million litres of industrial and beverages ethanol and 200 million litres of fuel ethanol (Krzywonos et al., 2009). Whole stillage (88% H_2O) is the aqueous residue following corn fermentation and the removal of ethanol using distillation and molecular sieves. The whole stillage then undergoes a centrifugation process to remove the denser solids. Distiller's dried grains (DDG) are produced by drying the denser solids. The liquid supernatant from the centrifugation process, known as thin stillage (94% H_2O), is subsequently evaporated to syrup (60% H_2O) and mixed with the DDG to produce distiller's dried grains with solubles (DDGS). DDGS is sold as a livestock feed supplement, where up to 10-20% of animal rations can be made up of DDGS. The market for DDGS is becoming saturated and excess stillage has become a waste problem. Stillage handling is one of the major limitations of the fuel ethanol process, since DDGS drying and stillage evaporation account for approximately 30.3 % and 16.5%, respectively, of the total process energy consumption. Drosg et al. (2008) goes on to explain that almost the same amount of energy is consumed to produce DDGS as is consumed in the ethanol production process, which is already energy intensive due to the distillation process. Wilkie et al. (2000) state that up to 20 litres of stillage can be generated for each litre of ethanol produced; where the thin stillage evaporator condensate is the largest wastewater contributor. The evaporated thin stillage does not add any appreciable value to the DDG as an animal feed product but is a way to dispose of residual organics to achieve "zero discharge" (Schaefer, 2006). Therefore, an alternative disposal option for thin stillage would significantly reduce energy costs for the ethanol plant. Agler et al. (2008) suggests the anaerobic digestion of thin stillage may be advantageous as the energy in the stillage can be recovered in the form of biogas, which could significantly increase the net energy balance.

Wilkie et al. (2000) conducted an extensive review of various types of ethanol stillage and determined the characteristics of stillage to be highly variable and the operations of the ethanol plant had a significant influence on stillage composition. Table 2.2 summarizes the chemical and physical characteristics of corn thin stillage as reported in various sources of literature. COD concentrations of corn whole stillage have been reported to easily exceed 100 g/L (Wilkie et al., 2000; Olguin et al., 1995), with COD values for corn thin stillage ranging from 21.9 to 97.0 g/L (Krzywonos et al., 2009; Schaefer and Sung, 2008; Wilkie et al., 2000). The solids content of corn thin stillage can range from 36.1 to 104.3 g/L for TS and from 31.6 to 93.8 g/L for VS, with VS/TS averaging at 88% (Agler et al., 2008; Schaefer and Sung, 2008). Total nitrogen concentrations have been reported to range from 546-1752 mg/L (Krzywonos et al., 2009; Khanal et al., 2005; Wilkie et al., 2000). Ammonia nitrogen concentrations typically seem to be less than 100 mg/L; Agler et al. (2008) and Khanal et al. (2005) reported average ammonia nitrogen concentrations of 30 mg/L and Krzywonos et al. (2009) reported a concentration as high as 96 mg/L. Depending on plant operations Agler et al. (2008) reported that protein concentrations can be as high as 7,200 mg/L and Khanal et al. (2005) reported an average carbohydrate concentration of 13,600 mg/L (as glucose). Total phosphorus concentrations of corn thin stillage were found to range from 228-1292 mg/L (Khanal et al., 2005; Wilkie et al., 2000). Wilkie et al. (2000) reported that in their review of various stillage sources, corn thin stillage had one of the lowest sulphate concentrations that average below 300 mg/L of total sulphur (as SO_4). Alkalinity of corn thin stillage is negligible as the pH is typically below 4. Because thin stillage has a low pH and no alkalinity, start-up of anaerobic digesters can be a challenge (Schaefer and Sung, 2008) and typically alkalinity is added to the anaerobic process using sodium bicarbonate ($NaHCO_3$) if the pH drops below 7.

Agler et al. (2008) conducted an elemental analysis of corn thin stillage and determined magnesium (Mg) and potassium (K) were present in relatively high concentrations, 370 mg/L and 5560 mg/L, respectively. The authors also determined that cobalt (Co) was not present in detectable concentrations, while manganese (Mn), iron (Fe), nickel (Ni), copper (Cu) and zinc (Zn) were detected at low concentrations, < 6.24 mg/L.

Table 2.2 Chemical and Physical Characteristics of Corn Thin Stillage from Literature

Parameters	Literature References				
	Krzywonos et al. (2009)	Agler et al. (2008)	Schaefer & Sung (2008)	Khanal et al. (2005)	Wilkie et al. (2000)
pH	3.70	3.46 - 4.35	-	4.46	3.3 - 4.0
TS, g/L	-	36.1 - 104.3	65.9 - 90.3	61	-
VS, g/L	-	31.6 - 93.8	59.1 - 83.5	53	-
VS/TS, %	-	88 - 90	90 - 92	87	-
tCOD, g/L	21.9 - 59.4	73.5 - 182.0	90.7 - 121.0	94	59.4 - 64.5
sCOD, g/L	-	39.0 - 71.0	51.0 - 76.0	49	-
VFAs, mg/L	-	-	-	1,310	-
Carbohydrate, mg/L	-	-	-	13,600	-
Protein, mg/L	-	7,200	-	-	-
Total Nitrogen, mg/L	546 - 670	-	-	-	546 - 755
TKN, mg/L	-	-	-	1,720	-
Ammonia, mg/L	96	< 30	-	32.1	-
Total Phosphorus, mg/L	228 - 441	-	-	1,292	228 - 1170

2.2.2 Research Studies Investigating Anaerobic Digestion of Corn Thin Stillage

Wilkie et al. (2000) identifies several studies that considered the application of aerobic treatment for stillage. However, the high COD of stillage will require significant aeration power and almost 50% of the COD will be converted to sludge which would require further disposal (Krzywonos et al., 2008; Wilkie et al., 2000). In an anaerobic digestion process, more than 50% of the COD can be converted to biogas and the process has about 10% of the sludge yield and lower nutrient requirements compared to aerobic treatment (Burke, 2001). The anaerobic digestion of stillage presents an effective and economical treatment option, as the biogas produced can be used as an in-plant fuel.

A considerable amount of research has been conducted on the anaerobic digestion of ethanol stillage from cane molasses; however, limited research has considered corn stillage for anaerobic digestion (Wilkie et al., 2000). Because the anaerobic digestion work in cane molasses stillage is much more advanced, it is important to review some of this work as it give insight to the anaerobic digestion system for corn stillage. Cane molasses stillage has typical COD values above 100 g/L and Tielbaard (1992) found this concentration inhibited stable digestion and the stillage needed to be diluted to a COD concentration of approximately 50 g/L. Deficiencies in the digestion of molasses stillage have also been attributed to high potassium, metals and sulphate levels (Ranade et al., 1999), along with the presence of phenolic compounds. Sulphate in stillage can lead to digester performance problems. In anaerobic treatment sulphate is converted to sulphide and sulphide in the digestate will contribute to odours. Sulphide inhibition is not encountered in anaerobic treatment when the COD/SO_4 ratio is above 10 g/g (Wilkie et al., 2000).

Wilkie et al. (2000) reviewed mesophilic digesters treating cane molasses and identified the average organic loading rate applied was 9-12 g COD/L/day and the average methane yield was 0.25 L CH_4/g COD_{added}. The average BOD_5 and COD treatment efficiencies were greater than 79% and 70%, respectively. The review of thermophilic digesters treating stillage from cane molasses identified that reactors could be dosed with OLRs as high as 23 g COD/L/day and achieve BOD_5 and COD removal efficiencies of 89% and 60%, respectively. The average methane yield from the thermophilic digesters was 0.17 L CH_4/g COD_{added}.

Yeoh (1997) conducted a study treating cane-molasses stillage using a thermophilic two-phase system composed of two bioreactors for each of the acidogenic and methanogenic phases operating in series. The two-phase system was compared with a single-phase process. The two-phase system was operated with HRTs ranging from 32.7 to 5.6 days with OLRs ranging from 4.6 to 20.0 kg COD/m^3/day. The single-phase process was operated with HRTs ranging from 36 to days and OLRs ranging from 3.4 to 14.5 kg COD/m^3/day. The treatment efficiency of the stillage in the two-phase digester system was effective with a BOD_5 removal of 85% and a COD removal of 65%. The author describes the acidogenic phase having had an average rate of 15.6% for the conversion of initial COD to volatile fatty acids (VFAs) and the methanogenic phase was effective at maintaining pH in the range of 7.4 to 7.8. Methane yields were significantly higher in the two-phase system, where the average methane yield for the two-phase system was found to be 0.292 m^3 CH_4/kg VS_{added} and the average methane yield for the single-phase system was 0.082 m^3 CH_4/kg VS_{added}. The methane content of the biogas in the two-phase system was also 17% higher than the single-phase system. It should be noted that the methane yield in the two-phase fermentation process was enhanced only when OLRs were above 2.9 kg COD/m^3/day. The study was successful at demonstrating a two-phase anaerobic digestion system was much more effective at treating cane-molasses stillage than a single-phase fermentation process. In the Yeoh (1997) study the bench-scale experiments were compared with a full-scale two-phase system at an industrial treatment plant for cane-molasses stillage. Despite the variation in characteristics of the raw wastewater the full-scale system demonstrated similar results to the bench-scale reactors, with removal of 84.3% and 63.2% for BOD_5 and COD, respectively.

There is a limited amount of information on the anaerobic digestion of corn stillage. In the Wilkie et al. (2000) review only a small amount of information was presented on the digestion of corn stillage. Stover et al. (1984) conducted research work on the anaerobic digestion of corn thin stillage using mesophilic suspended growth and fixed film processes. Thin stillage was allowed to settle and the supernatant used for the study. The performance of the suspended growth and fixed-film systems were similar and this is probably due to the biomass recycling in the suspended growth process (Schaefer, 2006). The F/M ratios used in this study ranged from 0.32 (SRT=30) to 2.44 (SRT=2). The average methane yield from the suspended growth system was 0.57 m^3 CH_4/kg $COD_{removed}$, while that of the fixed film reactor was 0.63 m^3 CH_4/kg $COD_{removed}$ (Stover et al., 1984).

Wilkinson and Kennedy (2012) successfully digested thin stillage using fixed film mesophilic digesters. The authors found methane yields of 2.05 $LCH_4/L\cdot d$ at an OLR of 7.4 g TCOD/$L\cdot d$ at a 5 d HRT. TCOD and VS removal efficiencies of 89 ± 3 and 85 ± 3%, respectively, were obtained.

Kothari et al. (1986) conducted a study that treated corn thin stillage using a two-phase mesophilic anaerobic system. The wastewater in this study was less concentrated than typical thin stillage and therefore allowed for the use of a fluidized bed with acidogenesis and methanogenesis digesters. The thin stillage in this study had a COD concentration of 9.03 g/L and TSS concentration of 2.2 g/L. The anaerobic system was used as a pre-treatment to an aerobic activated sludge process. Kothari et al. (1986) reported OLRs of up to 25.4 kg COD/m^3/day for the two-phase system with a BOD_5 removal of 83% and a methane yield ranging from 0.21 to 0.31 m^3 CH_4/kg COD. The study showed the two-phase fluidized bed digester worked well in the treatment of thin stillage; however, such a system would not be appropriate for thin stillage sources with higher solids content (Schaefer, 2006).

Lanting and Gross (1985) investigated the use of a mesophilic upflow anaerobic sludge blanket (UASB) reactor to treat corn thin stillage. The anaerobic digestion process was used as a pre-treatment step to the plant's trickling filters. The OLR of the system was 12.3 kg COD/m^3/day and the methane yield averaged at 0.25 m^3/kg COD removed, where 89% of the COD was removed from the effluent (Wilkie et al., 2000). Lanting and Gross (1985) also performed shock loading tests on the UASB reactor and determined that the system could handle loadings of three to four times the average flow (Schaefer, 2006).

Schaefer (2006) suggests the earlier research work conducted in the early to mid-80s on mesophilic digestion of corn stillage was realized with limited success, and the advances made in the past 20 to 25 years offers a new examination of the digestion of corn stillage, particularly with the use of thermophilic systems. Agler et al. (2008) suggest that due to the high COD concentration (~ 100 g/L) of corn thin stillage, substrate digestion requires very high OLRs, and thus increased degradation kinetics, to enable reduced reactor volumes; making thermophilic digestion more advantageous than mesophilic digestion because of higher metabolic rates. Schaefer and Sung (2008) state the additional heat requirements for thermophilic digestion is a common disadvantage for many feedstocks; however ethanol stillage temperatures, typically around 90^{o}C (Wilkie et al., 2000), are above thermophilic levels and thereby eliminating this disadvantage. The advantages of higher loading rates (generally double that of mesophilic systems) are preserved and thermophilic digesters are stable and operable as mesophilic systems (Schaefer and Sung, 2008).

Schaefer and Sung (2008) conducted a study on the treatment of corn thin stillage using two completely mixed digesters operating under thermophilic conditions (55^{o}C). The reactors were fed with a thin stillage feedstock having a COD concentration of 100 mg/L, volatile solids concentration of 60 g/L and a pH of 4.0. Reactor steady state was achieved at 15, 20 and 30 day HRTs; however, the reactors failed at an HRT of 12 days. The removal of VS ranged from 76 to 89% and methane yields ranged from 0.6 to 0.7 m^3 CH_4/kg VS removed. The study also describes the use of an ultrasonic pre-treatment, although no significant improvements were observed.

Finally, a study was conducted by Agler et al. (2008) to investigate the thermophilic digestion of corn thin stillage using semi-batch fed ASBRs. The thin stillage being fed to the reactors had the following characteristics: TS = 36.01-49.93 g/L; VS = 31.55-45.17 g/L; tCOD = 73.5-97.0 g/L; sCOD = 33.5-39.0 g/L. The HRT of the reactors was decreased in a stepwise manner from 40 days (OLR = 2.42 g tCOD/L/day) to 7 days (OLR = 10.71 g tCOD/L/day), after which the HRT was maintained at 8 days (OLR = 9.37 g tCOD/L/day). A solution of trace elements was added weekly to the reactor. The methane potentials and yields for this system ranged from 0.253 to 0.254 L CH_4/g $tCOD_{fed}$ and 0.278 to 0.284 L CH_4/g $tCOD_{removed}$, which are very similar to the theoretical methane yield of 0.350 L CH_4/g $tCOD_{removed}$. The methane concentration in the biogas averaged at 59.7%. The authors from this study determined that an HRT of 10 days was optimum and resulted in a 92.2% removal of tCOD and 95.8% removal of sCOD. Removal efficiencies of TS and VS were 88% and 80.3%, respectively. The results from this study also demonstrated that a 30% increase in ammonia nitrogen was observed in the reactor effects which were attributed to protein degradation.

The research studies presented in this section demonstrate that corn thin stillage is an ideal substrate for anaerobic digestion, under both mesophilic and thermophilic conditions. The high methane yields associated with the biodegradation of thin stillage make it a suitable substrate for the co-digestion of dairy manure for enhanced biogas production. The dairy manure will aid with the digestibility of corn thin stillage as it will be a source of the trace elements (e.g. Co, Cu, Fe, Mn, Ni, and Zn) that are present in low concentrations, or absent all together, in thin stillage. Dairy manure also has an excellent buffering capacity and will be able to provide the alkalinity that is absent in corn thin stillage, thereby eliminating the need for adding $NaHCO_3$ to the digestion process. Research studies describing the co-digestion of thin stillage and manure were not found in the literature reviewed.

2.3 Grease Trap Waste as Potential Co-substrate for Manure Anaerobic Digestion

2.3.1 Characteristics of Grease Trap Waste

Grease traps are a form of wastewater pre-treatment used in the food processing and service industry to separate fats, oils and greases (FOG) from wastewater generated from dishwashers, sinks, floor drains and dumpster pads. Grease traps are designed to slow the incoming wastewater so that the grease coagulates and floats to the top and solids settle to the bottom of the trap. The accumulated grease and solids are pumped out of the trap.

GTW is typically siphoned out of traps by tanker trucks and solids content can range from 5 to 6 % TS. Some haulers, looking to reduce transportation costs, will decant the GTW to increase solids by heating the waste. This causes the GTW to stratify into three layers: sediments at the bottom, water in the middle and grease floating on top. The water layer is discharged to the sanitary sewer and the remaining grease and sediments waste are discarded for disposal. This decanting technique can increase the TS content of GTW to 35 to 40% (Coker, 2006).

GTW or waste grease is primarily composed of grease, water, sediments (mostly food particles) and sanitation chemicals. Grease is essentially composed of fats and oils. Fats and oils (also known as lipids) are made up of triglycerides, which consist of one unit of glycerol ($C_3H_8O_3$) and three units of fatty acids. Typical long chain fatty acids (LCFA) found in GTW include: lauric ($C_{12}H_{24}O_2$), myristic ($C_{14}H_{28}O_2$), palmitic ($C_{16}H_{32}O_2$), stearic ($C_{18}H_{36}O_2$), oleic ($C_{18}H_{34}O_2$), linoleic ($C_{18}H_{32}O_2$) and linolenic ($C_{18}H_{30}O_2$) acids. Fats and oils contain twice the energy of other organic materials, like sugars and starches. Refer to Table 2.3 for chemical and physical characteristics of GTW (or waste grease) from various literature sources. The VS/TS ratio of GTW is typically 97 to 99% and therefore makes it a readily digestible substrate. GTW has low nutrient contents and a low pH with negligible alkalinity.

Much of the waste grease or GTW is disposed of via land application, either directly (if total solids <6%) or after composting. Grease waste can also be disposed of by landfilling and incineration. However, this wet sludge deteriorates landfills and is not favourable to incineration even though the energy content is high with a VS content over 95% of TS (Davidsson et al., 2008). Wastewater treatment plants are increasingly cautious about take in waste grease or GTW due to operational difficulties.

Table 2.3 Chemical and Physical Characteristics of Grease Trap Waste from Literature

Parameters	Literature References			
	Kabouris et al. (2009)	Luostarinen et al. (2009)	Davidsson et al. (2008)	Crolla & Kinsley (2008a)
pH	4.03	5.1	4.38	3.42
TS, g/L	424 ± 8	254	173	149
VS, g/L	409 ± 7	252	170	145
VS/TS, %	96.5	99.2	98.3	97.3
tCOD, g/L	1211 ± 31	-	-	151.4
sCOD, g/L	-	-	-	36.5
VFAs, mg COD/L	3473	-	-	-
Carbohydrate, g/L	60.1	-	-	-
Protein, g/L	29.7	-	-	-
Total Fat, g/L	319.3	-	-	-
TKN, g/L	5.1	-	-	-
Ammonia, mg/L	356 ± 10	-	136	-
Total Phosphorus, mg/L	670	-	-	-

The biological treatment of waste grease and GTW is being increasingly considered over conventional methods since it involves environmental benefits with recovery of nutrients and energy production. The EU policy promotes recycling of biodegradable waste as a priority action and as of 2005 has banned landfilling of organic wastes (Davidsson et al., 2008).

2.3.2 Research Studies Involving Anaerobic Digestion of Waste Grease/GTW

If grease wastes are thickened to a TS > 5%, it can be a suitable substrate for anaerobic digestion. However, the single substrate digestion of grease is usually considered unfavorable since LCFA are known to inhibit anaerobic microorganisms (Hwu and Lettinga, 1997; Rinzema et al., 1994; Angelidaki and Ahring, 1992). Lipid rich materials are known to have high methane production potentials, but the intermediate products, LCFA, may be severely inhibitive to methanogenesis (Luostarinen et al., 2009; Neves et al., 2009; Cirne et al., 2006). There has been much discussion in the literature as to which steps truly limit the conversion of the substrate to biogas when lipids are degraded. Pereira et al. (2003) and Masse et al. (2001) have shown that the hydrolysis of lipids to glycerol and LCFA can be rapid; however, the main problem during digestion is the further degradation of the LCFA. Pereira et al. (2003) and Rinzema et al. (1994) explain that in addition to an acute toxicity of LCFA on the activity of anaerobic microbes, LCFA can be adsorbed onto the biomass, inducing sludge flotation and washout. Cirne et al. (2006) explain the liquefaction of colloids absorbed onto biomass and hydrolysis of suspended solids trapped within the biomass bed were found to be limiting steps in digestion. Salminen et al. (2000) reported that hydrolysis limited the digestion of poultry slaughterhouse wastewater due to high concentrations of propionate, which was the consequence of the presence of LCFA.

The inhibition of LCFA on methanogenesis has been assumed irreversible (Rinzema et al., 1994; Angelidaki and Ahring, 1992), but lately it has been shown to be reversible, where increased consumption of acetate and butyrate were observed after shock loadings of LCFA (Cirne et al., 2006; Pereira et al., 2003). Pereira et al. (2003) demonstrated acetogenic, acetoclastic and hydrogenophilic activities decreased when the amount of adsorbed LCFA increased. This effect was proved to be more severe for methanogenic activity. However, when methanogenic activity measurements indicated a severe inhibition, Pereira et al. (2003) suggested the acetogenic and methanogenic microorganisms could not be killed as was suggested by Rinzema et al. (1994), because methane was produced exclusively from the adsorbed LCFA substrate. Pereira et al. (2003) go on to show that after allowing the depletion of the adsorbed LCFA, the methanogenic activity was significantly enhanced in the presence of acetate, butyrate and H_2/CO_2. The study demonstrated that the toxic effect of adsorbed LCFA is not permanent. It should be noted, when digesting lipid rich materials, a lag phase is usually noticed between LCFA and methane production, and acetogenic bacteria have been shown to grow during the lag phase (Hwu and Lettinga, 1997).

Davidsson et al. (2008) conducted both mesophilic laboratory and pilot scale tests co-digesting grease waste with sewage sludge. The grease waste used in the investigation was thickened to 17.3% TS (VS/TS = 98%) and the sewage sludge was a 50:50 mixture of primary sludge and waste activated sludge. The batch laboratory tests were conducted in 2 L reactors and investigated GTW:VS ratios of 0:100, 10:90, 25:75 and 60:40, based on VS. The results from the batch tests demonstrated an increasing methane potential with increasing amount of grease substrate, where methane potentials ranged from 0.325 to 0.681 L CH_4/g VS_{in} (standardized to

1°C and 1 atm). Davidsson et al. (2008) also found that 90% of the total methane was produced within the first 14 days. When GTW was digested as a single substrate in the batch tests (at various concentrations ranging from 10-60% GTW on VS-basis), the total methane potentials ranged from 0.845 to 0.928 L CH_4/g VS_{in} (standardized to 1°C and 1 atm), approaching the theoretical methane potential for fat of 1.01 L CH_4/g VS_{in} (STP) (Davidsson et al., 2008). No methanogenic inhibition was observed with the single substrate digestion of GTW in batch tests. Davidsson et al. (2008) conducted continuous mesophilic pilot digestion trials in 35 L digesters and investigated GTW:VS ratios of 0:100, 10:90 and 30:70, based on VS, at an organic loading rate of 2.5 kg VS/m^3/day and SRT of 13 days. All combinations of grease trap waste and sewage sludge showed higher methane yields (0.295 to 0.344 m^3/kg VS_{in}) than the control digestion with only sewage sludge (0.271 m^3/kg VS_{in}), thereby resulting in 9-27% higher yields when GTW is added to SS. The continuous single substrate digestion of GTW failed to reach stable operation. After 10 days of slow start up with an organic loading rate of 1.7 kg VS/m^3/day, the pH dropped and the methane production decreased. Davidsson et al. (2008) describe that in spite of the repeated additions of sodium bicarbonate ($NaHCO_3$), the process could not be stabilised. This result contradicts the methane yields obtained from the batch digestion trials where no methanogenic inhibition was observed. Davidsson et al. (2008) suggests increasing the retention time to 30-46 days to reduce the inhibition of methane production for the single substrate digestion of GTW.

Luostarinen et al. (2009) investigated the co-digestion of sewage sludge and grease trap waste from a meat processing plant in Mikkeli, Finland. Mesophilic digestion trials were conducted using a more concentrated grease trap waste, with a TS content of 25.4% (VS/TS = 99%), than that used in the study conducted by Davidsson et al. (2008). In the Luostarinen et al. (2009) study, serum bottle batch experiments determined the methane potential of sewage sludge to be 0.263 m^3CH_4/kg VS_{in} and that of grease trap waste to be 0.918 m^3CH_4/kg VS_{in},; however, the single substrate digestion of grease trap waste did experience a 4-day lag period. When sewage sludge and grease trap waste were co-digested in mixtures ranging from 0.5% to 50% wet wt. of grease, no lag phase was observed and methane potentials increased with increasing grease content, ranged from 0.435 to 0.788 m^3CH_4/kg VS_{in}. A mixture using 70% wet wt. of grease, however, resulted in a lower methane potential of 0.679 m^3CH_4/kg VS_{in}, demonstrating the start of LCFA inhibition. In semi-continuous mesophilic digestion trials conducted in 5 L reactors, Luostarinen et al. (2009) found that the co-digestion of grease trap waste and sewage sludge was feasible up to a grease trap waste addition of 46% of feed VS with a HRT of 16 days and OLR of up to 3.46 $kgVS/m^3/day$. This resulted in significantly increased specific methane production in the co-digestion of grease trap waste and sewage sludge reactor experiments, with a maximum yield of 0.463 $m^3CH_4/kg\ VS_{in}$, compared with the digestion of sewage sludge alone (0.278 $m^3CH_4/kg\ VS_{in}$). However, grease trap waste additions of 55 and 71% of feed VS resulted in decreased methane production and VS removal, indicating overloading and LCFA inhibition. Luostarinen et al. (2009) recommend that the introduction of grease trap waste to an anaerobic digester should be done with stepwise increases to the desired feed ratio in order to acclimatise the bacteria and to prevent overloading.

Kabouris et al. (2009) conducted a study to investigate the acid-methane phased digestion of municipal sludge and FOG, using semi-continuous laboratory scale digesters. A mixture of primary sludge (2.5% TS), thickened waste activated sludge (TWAS) (6.7% TS) and FOG (42.4% TS), at respective proportions of 21, 31, 48% of feed VS, was fed to a 1 L acid digester operated at 35^oC, 1 day HRT and loading rate of 52.5 g VS/L/day. The acid digester effluent was fed to two parallel methane phase digesters operated at 35^oC and 52^oC, respectively, and HRT of 12 days. After an initial period of 20 days with near zero biogas production in the acid digester, biogas production increased and stabilized to 2 mL CH_4 (STP)/g VS_{in}, with a volatile solids destruction of 0.4%. The acid phase digester decreased unsaturated fat by 43% and increased soluble COD, volatile fatty acids and ammonia by 16, 26 and 20%, respectively, indicating acidification had taken place. The methane yields for the methane phase digesters were 473 and 551 mL CH_4 (STP)/g VS_{in}, with 45 and 51% VS and 50.2 and 57.9% COD destructions, at 35^oC and 52^oC, respectively. The thermophilic methane yield was 16% higher than that of the mesophilic digestion. The methane yields were 3 times higher with the addition of FOG when compared with the digestion of primary sludge and TWAS at 35^oC and 52^oC, with methane yields of 159 and 197 mL CH_4 (STP)/g VS_{in}, respectively. Kabouris et al. (2009) concludes that the acid-methane-phased digestion has not only the potential to reduce lipid toxicity but also the potential to significantly accelerate reaction rates and increase VS reduction and process stability by providing the optimum growth environment for acid and methane producing bacteria.

In the Kabouris et al. (2009) study, it was demonstrated that 91% of unsaturated fat in the feed was removed in the acid-methane phased digestion process, regardless of the temperature of the methane producing phase. However, 75% of the saturated fat in the feed was removed when the methanogenic digester was operated at 52^{o}C, while 58% of the saturated fat was removed when methane production was conducted at 35^{o}C. However, there was no preference observed in the degradation of the saturated fat components, where palmitic (16:0), stearic (18:0) and myristic (14:0) acids were observed in the effluent of both the mesophilic and thermophilic methane reactors.

Neves et al. (2009) investigated the co-digestion of liquid dairy (2.8% TS) manure and food waste (23.8% TS), with increasing concentrations of intermittent pulses of residual-oil (97.1% TS) from a canned fish processing company. The overall objective of this study was to determine the optimal lipids concentration that can be added to enhance methane production without inhibiting the process. The pulses of oil were added to 5 L continuously stirred tank reactors operating at 37^{o}C, HRT of 15 days and organic loading rate of 4.6 gCOD/$L_{reactor}$/day. The increased oil concentrations rose to 9, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$ after the pulse feeding in the reactors. The authors reported that all pulse feedings had a positive effect on methane production except for the feeding of 18 $gCOD_{oil}/L_{reactor}$, promoting a persistent inhibition of the process. The inhibition of the digestion process was observed when pH dropped to 6.8 and VFA concentrations in the digester effluent increased significantly to values between 8 and 17 g COD/L. The intermittent pulse feedings of 9, 12 and 15 $gCOD_{oil}/L_{reactor}$ increased methane production by 42, 82 and 80 %, respectively, compared to the control digester which did not receive the fish oil addition. Methane production was increased from 3.78 L CH_4/day (control) to 6.88 L CH_4/day (pulse feeding of 12 $gCOD_{oil}/L_{reactor}$). The TS removal ranged from 43.7 to 46.7 % and VS removal ranged from 49.1 to 53.5 %. In this study, SMA tests were conducted on digester effluents and it was determined that the optimum oil loading was 12 $gCOD_{oil}/L_{reactor}$ which resulted in the highest SMA in acetate value of 110 g COD CH_4/kg VS/day.

A study investigating the effect of oleate on the anaerobic digestion of cattle and pig manure was conducted by Nielsen and Ahring (2006). Oleate was chosen for this study because during the digestion process, lipids are initially hydrolyzed to LCFA (e.g. oleate, stearate). The authors reported that two thermophilic (55^{o}C) CSTRs were fed with 1:1 mixtures of cattle and pig manure. One reactor operated at an OLR of 2.5 – 2.6 g VS/L/day and the other at an OLR of 3.1 – 3.3 g VS/L/day and a 17-day HRT for both. The reactors were dosed with increasing pulses of sodium oleate at loads of 0.5, 1.0 and 2.0 g oleate/$L_{reactor}$. The pulses of 0.5 and 1.0 g oleate/$L_{reactor}$ resulted in increased methane production and low VFA concentrations in the digester effluent, thereby indicating oleate had a positive effect on the digestion process. Methane yields increased from 253-268 mL CH_4/g VS (control) to 314-329 mL CH_4/g VS after oleate addition. However, the addition of 2.0 g oleate/$L_{reactor}$ severely inhibited the process and resulted in significant increases in VFAs in the digester effluent and immediate drop in methane production. The authors of the study concluded that improved acetogenic and methanogenic activity in the reactors was confirmed in batch activity tests. Toxicity tests revealed that the oleate pulses induced an increase in the tolerance level of acetotrophic methanogens towards oleate.

Finally, a study conducted by Crolla and Kinsley (2008a) investigated the addition of GTW to a full scale on-farm digester. The objective of the study was to evaluate the impact of the addition of GTW (24% TS) on biogas production, and reductions in VFA and pathogen concentrations. The Fepro Farm (Cobden, ON) digester was 500m^3 in size and operated at a HRT of 28 days under mesophilic conditions. GTW was added to the digester at an incremental rate of 0.5% (by total volume of digester) per day until reaching a feed load of 3 m^3/day GTW GTW (17% by vol.) and liquid dairy manure of 15 m^3/day (83% by vol.). The GTW was pasteurized at 70^{o}C for 1 hour prior to being fed to the digester. The average daily biogas production at the Fepro Farm digester prior to receiving GTW was 391 ± 77 m^3/day (0.299 ± 0.1 m^3 biogas/kg VS) and after the addition of GTW biogas production increased to 1133 ± 478 m^3/day (0.732 ± 0.2 m^3 biogas/kg VS), thereby increasing productivity by 2.9 folds. The average methane concentration in the biogas ranged from 60 to 65% and peaked to 70% after the addition of the GTW. The authors also reported that VFA reductions ranged between 95 and 98%. The digester was also effective at reducing pathogen concentrations. *E.coli* had the greatest reduction with a 3.29 log reduction from the digestion process; while *Salmonella* was reduced by 0.92 log, *C.perfringens* was reduced by 0.75 log and Enterococci was reduced by 1.05 logs. The addition of GTW to the digester did not significantly ($p>0.05$) affect the pathogen reductions in the digester.

The literature demonstrates that lipid containing wastes can be readily digested and have a high methane potential; however, their potential toxicity to the anaerobic digestion process suggest they would be better suited as substrates in co-digestion with municipal sludge and dairy manure (Palatsi et al., 2009). Grease based wastes typically have low nutrients content and low alkalinity, thereby not ideal for single substrate anaerobic digestion. By co-digesting manure with grease, the manure will be able to stabilize the digestion process by supplying a high buffering capacity and a wide variety of nutrients necessary for optimal bacterial growth.

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Chapter 3 - Co-digestion of Liquid Dairy Manure with Cellulosic substrates, Grease-based wastes, Food wastes and Industry wastes

Abstract

Various substrates were evaluated for enhanced biogas production and volatile solids (VS) reduction in the co-digestion with liquid dairy manure. Cellulosic substrates (corn silage, switchgrass, wheat straw and woodchips), grease based substrates (waste grease and dissolved air flotation (DAF) sludge) and industry and food processing wastes (grocery food waste, fish offal, corn thin stillage and spent barley) were evaluated. Energy crops like corn silage, switchgrass and wheat straw were effective at increasing biogas yields and biogas was optimum when 50% of the feed VS came from the energy crop. Woodchips proved to be non-biodegradable. Co-digestion of dairy manure with DAF sludge and waste grease produced the highest biogas yields of 0.59 ± 0.03 and 0.67 ± 0.02 L biogas/g VS (at 50% of feed VS), respectively. Grocery food waste, fish offal and corn thin stillage all increased biogas yields when co-digested with manure. Biogas production for these substrates was slow but steady. Box-Wilson Central Composite Circumscribed Models were established for both co-digestion of dairy manure with corn thin stillage and co-digestion with grease waste. Both models suggest methane yields optimize with increasing proportion of the feed VS from co-substrates (constant total VS in all assays) and increasing temperatures. Although the models suggest the proportion of feed VS from co-substrate to be positively correlated to methane production, temperature was shown to have a great influence on yields. The models also suggest there is minimal interaction between temperature and proportion of feed VS from co-substrates on methane yields.

Keywords: BMP, cellulosic substrates, energy crops, grease, DAF, food processing wastes, biogas yield, central composite design

3.1 Introduction

The co-digestion of manure with other substrates such as energy crops, industrial wastes, food industry wastes, animal by-products, or sewage sludge can result in increased biological activity of digester systems, leading to significant biogas production. The addition of co-substrates to manure can improve the nutrient balance of total organic carbon, nitrogen and phosphorus, which results in a stable and maintainable digestion process and good digestate fertilizer quality (Braun and Wellinger, 2003). Das and Mondal (2016) suggest that the increased load of mixed nutrients during co-digestion bio-stimulates microbial activity and accelerates the breakdown of macro-molecules of the feedstock mixture. Perhaps the most important drivers for co-digestion of manure with other substrates are the financial benefits associated with the increased biogas yields and the fertilizer value of the digested material.

Three macro-nutrients that can significantly affect microbial activity are: lipids, carbohydrates and proteins. Lubatut et al. (2011) and Das and Mondal (2016) explain lipids are easily degradable (main from the SCFA) but can have long HRTs due to retarded (lagged) biodegradability and can be prone to inhibition due to the accumulation of LCFA. Whereas carbohydrates and proteins respond quickly to biogas conversion but have lower biogas yields. Substrates rich in cellulose and hemi-cellulose can also significantly increase biogas production; biodegradability varies widely among substrates.

Energy crops have become a popular co-substrate in Europe with the digestion of manure, where some on-farm digesters operate with over 70% energy crops (Effenberger, 2006). Energy crops are especially interesting when the plants are grown on fallow lands. Many varieties of grass, clover, cereals and corn, including whole plants, as well as rape or sunflower have shown to be suitable for methane production (Braun et al., 2009). Effenberger (2006) reports the potential biogas yields of canola cake and corn silage to be 552 m^3/t and 202 m^3/t, respectively, while that of liquid dairy manure to be only 22 m^3/t. Braun et al. (2003) reported the biogas potential of corn to be 648 m^3/t VS. German experience has shown mean methane yields of 348 m^3 CH_4/t VS for corn (whole plant) and 380 m^3 CH_4/t VS for barley (Braun et al., 2009), whereas methane yields from the digestion of cow manure alone will average a methane yield of 220 m^3CH_4/t VS

(Weiland, 2000). Das and Mondal (2016) reported specific methane yields of 268, 229 and 213 L CH_4/kg VS for grass silage, sugar-beet tops and oat straw, respectively.

In Ontario, the trend has been to favour the use of food or industry wastes as co-substrates. El-Mashad and Zhang (2010), Labatut et al. (2011) and Das and Mondal (2016) explain the use of organic rich food wastes to be highly effective at increasing biogas during co-digestion and maintain reactor stability. Köttner et al. (2008) explain that food wastes higher in fats and starch, which are easy to breakdown, will significantly increase biogas yields. Baking wastes and food waste grease demonstrated very high potential biogas yields of 580-657 m^3/t and 500-600 m^3/t (Köttner et al. 2008; Effenberger, 2006), respectively. Baking wastes and food waste grease have very high potential biogas yields of 580-657 m^3/t and 500-600 m^3/t (Köttner et al. 2008; Effenberger, 2006), respectively. Braun et al. (2003) conducted a series of lab studies and determined the biogas yields of corn distillery residues and waste oils to be 400 m^3/t VS_{added} and 1104 m^3/t VS_{added}. The methane content in the biogas depends on the oxidation stage of the substrate and varies according to the fat, protein and carbohydrates present Köttner et al. (2008). El-Mashad and Zhang (2010) reported the co-digestion of unscreened manure and food waste, at mixtures of 68/32% and 52/48%, resulted in methane yields of 282 and 311 L CH_4/kg VS after 30 days of digestion.

The overall objective of this study was to evaluate the use of various substrates in co-digestion with liquid dairy manure. Although the literature already reports potential biogas and methane yields from various substrates, the aim of the study was to establish a baseline of biogas data specific for Ontario based substrates. Over the past few years, the trend for on-farm digesters in Ontario has been to favour the use of grease based substrates as a co-substrate for manure digesters; however, as the industry grows and becomes more competitive these sources of grease waste will become more and more depleted thereby increasing the need to source other types of substrates.

BMP assays were conducted in this study to determine the effects of co-digesting various quantities of co-substrates with liquid manure in enhancing biogas and methane production. The BMP assays were conducted under controlled mesophilic conditions (37 ± 2 °C) and trials conducted until biogas production came to completion. A second-order central composite experimental design was also implemented to investigate the effects of co-substrate quantity (corn thin stillage and waste grease) and digestion temperature on methane yields.   Central composite designs are appropriate for calibrating the full quadratic modes of surface response models and the accuracy of the circumscribed design estimates are good over the entire design space.

3.2 Materials and Methods

3.2.1 Biogas and Methane Production from the Co-digestion of Various Substrates with Dairy Manure

3.2.1.1 Description of Biogas Assays

Experiments were conducted to determine the effects on biogas and methane production from the co-digesting of organic rich substrates with liquid dairy manure. To determine the biodegradability of the substrates, Biochemical Methane Potential (BMP) assays were conducted. The basic approach of a BMP is to incubate a small amount of the waste with an anaerobic inoculum and measure the methane generation by simultaneous measurements of biogas volume and biogas composition. The BMP assays conducted in this study follow standard methods described by Owen et al. (1979) and Hansen et al. (2004), with some modifications. In addition to incubating a substrate with an anaerobic inoculum, the Owen et al. (1979) method describes also adding a defined media to the assay supplying nutrients and vitamins for the anaerobic culture. Due to its sufficient nutrient content, the liquid dairy manure used as co-substrate replaced the defined media described in the Owen et al. (1979) method.

The BMP assays in this study were conducted using 500mL Wheaton serum bottles (300 mL liquid volume and 295 mL void volume) and agitated at 100 rpm and incubated under mesophilic conditions (37 ± 2ºC) using a New Brunswick Controlled Environment incubator shaker. For thermophilic assay trials (50 to 56 ± 2°C) a Lab Line incubator shaker was used. A defined volume of organic rich co-substrate was added to the serum bottle (run in triplicates) with a known volume of liquid dairy manure and anaerobic inoculum added. Section 3.2.1.2 describes the quantity of substrates and inoculum used for the various BMP assays. Sufficient alkalinity of

4000-8000 mg/L (as $CaCO_3$) using $NaHCO_3$ and $KHCO_3$ was also added. Hansen et al. (2004) recommends 2 gVS/100 mL in assay bottles is suitable to avoid acidifying the process; however, their method does not include the addition of bicarbonate for added alkalinity. After all substrates were added to the Wheaton bottles, the assay bottles were flushed for 2 minutes using pure N_2 gas or a gas mixture of 80% N_2 + 20% CO_2, and then sealed shut with 43 mm butyl rubber lyophilisation stoppers and Teflon® faced silicone lined caps. Refer to Figure 3.1 for photos of the BMP setup. The assay bottles were then equilibrated for 2 hours at the incubation temperature and biogas volumes zeroed to ambient pressure with a manometer. The bottles were returned to the incubator and the digestion process commenced. Seed-blanks (no manure or co-substrate), manure blanks (no co-substrate) and co-substrate blanks (no manure) were also prepared.

All assays were run until cumulative biogas production stabilized, which was typically 64 days (1536 hours) for most substrates while some substrates had an incubation period of up to 104 days (2496 hours). Biogas measurements were taken daily for the first 14 days and subsequently three times per week. A water manometer and B-D 21-gauge, 4 cm needle were used to measure biogas volume by recording the displacement of water in the manometer column as the assay bottle and atmospheric pressures equilibrated. All biogas volumes were adjusted to STP (0 °C, 1 atm). A B-D 3 mL syringe equipped with a 21-gauge needle was used to take a biogas sample from the Wheaton bottle headspace to be analyzed for CH_4 and CO_2 composition. A liquid sample was taken of each assay mixture at the start and end of the digestion trial. The liquid samples of the assay mixtures were analyzed for pH, alkalinity, TS and VS. Individual substrates were analysed for pH, alkalinity, TS, VS, tCOD, sCOD, pCOD, C:N, TKN, total ammonia (NH_3-N + NH_4^+-N) and TP. Refer to Section 3.2.3 for descriptions of the analytical methods used. All samples were refrigerated at 4 ± 1 °C after being collected and before analysis. The BMP assays were used to evaluate mixtures of manure and co-substrates that enhanced biogas production and those that inhibited or acidified the anaerobic digestion process.

a) Prepared substrates for BMP assays in Wheaton Bottles

b) Water displacement manometer

c) Controlled environment incubator shakers

Figure 3.1 Prepared BMP assays and equipment used for assay incubation and biogas measurements

3.2.1.2 Preparation of Assay Bottles and Characteristics of Substrates and Inoculum

To determine a baseline of biogas and methane production from manure, four different manure types were investigated: liquid dairy manure, horse manure, poultry manure and swine manure. The manure substrates were obtained from farms in the Eastern Ontario region: Fepro Farms (Cobden, ON) for the liquid dairy manure, Lepage Farm (Vankleek Hill, ON) for the horse manure, Ferme Drouin (Plantagenet, ON) for the poultry manure and Henrard Farm-Petrocorn Inc. (Pendleton, ON) for the swine manure. The composite manure samples collected from each of the farms were composed of 15 subsamples. All manure substrates collected were refrigerated at 4 ± 1 ^{o}C prior to be being used for the BMP assays. Table 3.1 summarizes the characteristics of the various manure types used for the study.

Table 3.1 Characteristics of Various Manure Types

Parameter (wet basis)	Liquid Dairy Manure	Horse Manure	Poultry Manure	Swine Manure
	n=10[1]	n=5	n=5	n=5
pH	7.35 (0.17)[2]	8.33 (0.24)	8.89 (0.31)	7.95 (0.22)
Alkalinity (mg $CaCO_3$/kg)	9564 (423)	5489 (187)	16350 (607)	4898 (201)
TS (g/kg)	78 (20)	408 (111)	231 (78)	48 (18)
VS (g/kg)	57 (19)	263 (39)	146 (47)	33 (11)
VS/TS (%)	73	65	63	69
tCOD (g/kg)	74.6 (14.1)	---	---	55.4 (11.3)
sCOD (g/kg)	21.9 (9.5)	---	65.1 (5.5)	29.1 (5.6)
pCOD (g/kg)	52.6 (12.3)	---	---	26.3 (9.2)
C:N	21:1 (3)	32:1 (4)	9:1 (3)	23:1 (4)
TKN (g/kg)	3.9 (0.9)	3.3 (0.5)	14.2 (5.1)	4.2 (0.7)
Ammonium - NH_4^+-N (g/kg)	2.1 (0.7)	3.6 (0.5)	7.8 (2.3)	3.3 (0.6)
TP (g/kg)	0.6 (0.1)	0.6 (0.3)	0.3 (0.1)	0.7 (0.1)

1 Number of samples used to prepare substrate mixture for digestion
2 Values reported in brackets are calculated standard deviations on average values

The BMP assays were prepared by adding enough substrate to each bottle to deliver 8 g VS/bottle along with the addition of 100 g of acclimated inoculum. Hansen et al. (2004) and Angelidaki et al. (2009) explain that adding enough inoculum is crucial to the success of BMP assays. The inoculum used in this study was taken from the effluent line of the Terryland Farms (St. Eugene, ON) mesophilic (39 ± 1°C) dairy manure digester which co-digested waste grease. The inoculum was acclimated in a 5 L lab reactor with semi-batch feeding of corn silage and waste grease (HRT 30 days) under mesophilic conditions (36 ± 2°C). Refer to Table 3.2 for the characteristics of the acclimated inoculum. The liquid volume in each BMP assay was brought to a final 300 mL volume using distilled water. Due to the volume of swine manure required to obtain 8g VS/bottle, 1 L Wheaton bottles were used for the assays with a final working volume of 500 mL. As described in Section 3.2.1.1, an equal mixture of $NaHCO_3$ and $KHCO_3$ was added, when necessary, to provide sufficient alkalinity of 4000-8000 mg/L (as $CaCO_3$), with a target value of 5000 mg/L, and the headspace purged with N_2 gas or a N_2+CO_2 gas mixture prior to bottles being sealed for incubation.

Table 3.2 Characteristics of Acclimated Mesophilic Inoculum

Parameter (wet basis)	**Acclimated Inoculum** n=25[1]
pH	7.97 (0.30)[2]
Alkalinity (mg $CaCO_3$/kg)	11782 (174)
TS (g/kg)	41 (5)
VS (g/kg)	28 (4)
VS/TS (%)	68
tCOD (g/kg)	29.3 (18.2)
sCOD (g/kg)	5.4 (2.5)
pCOD (g/kg)	23.9 (12.3)
C:N	11:1 (3)
TKN (g/kg)	3.7 (0.7)
Ammonium - NH_4^+-N (g/kg)	2.4 (0.3)
TP (g/kg)	0.4 (0.2)

1 Number of samples used for average values
2 Values reported in brackets are calculated standard deviations on average values

The substrates used for the co-digestion with liquid dairy manure were divided into three categories: energy crops (cellulosic substrates), grease-based wastes and, food and industry wastes. The energy crops used for the study were corn silage, switchgrass, wheat straw and woodchips. The corn silage was grown and collected at the University of Guelph – Alfred Campus research farm, while the switchgrass and wheat straw substrates were obtained from the University of Guelph – Kemptville Campus research farm. The woodchips were obtained from the horse stables at Woodbine Racetrack (Toronto, ON). Table 3.3 describes the characteristics of the four cellulosic substrates used in this study. The grease-based substrates used were DAF, which was obtained from Olymel (Cornwall, ON), that is a pork and poultry processing plant, and grease trap waste from Organic Resources Management Inc.'s Ottawa transfer depot. Table 3.4 describes the characteristics of the two grease-based substrates. The food and industry wastes used for the study were grocery food waste, fish offal, corn thin stillage and spent barley. The food waste was composed of organic solid waste from deli, fruits and vegetables, and fish grocery departments. The substrate mixture was obtained from Organic Resources Management Inc.'s Ottawa transfer depot and when received was already ground to a thick pulp-like consistency. The fish offal was a mixture of fish offal, bones and skin of trout and was obtained from Meeker's Aquaculture (Manitoulin Island, ON). The fish offal was pulsed in a Braun® food processor for 1 minute to make a thick paste before being used for the assay trials. The corn thin stillage was obtained from the GreenField corn ethanol plant in Johnstown, ON. Spent barley was obtained from Molson Canada (Etobicoke, ON) and is the leftover mash after the barley steeping process has extracted the sugars for the brewing fermentation process. Table 3.5 describes the characteristics of the food and industry wastes used for the study.

Table 3.3 Characteristics of Cellulosic Substrates used for Co-digestion with Dairy Manure

Parameter (as received)	**Corn Silage**	**Switchgrass**	**Straw**	**Woodchips**
	n=8[1]	n=8	n=5	n=5
pH	7.22 (0.11)[2]	6.99 (0.23)	7.22 (0.19)	7.73 (0.17)
Alkalinity (mg $CaCO_3$/kg)	111 (22)	130 (19)	178 (46)	120 (20)
TS (g/kg)	600 (144)	622 (137)	684 (121)	470 (132)
VS (g/kg)	551 (173)	569 (181)	641 (149)	450 (101)
VS/TS (%)	92	91	94	96
C:N	37:1 (4)	34:1 (5)	42:1 (3)	53:1 (6)
TN (g/kg)	11.9 (7.1)	9.8 (5.5)	6.7 (3.6)	7.1 (2.3)
TP (g/kg)	1.7 (0.5)	1.2 (0.5)	1.9 (0.7)	0.4 (0.3)

1 Number of samples used to prepare substrate mixture for co-digestion
2 Values reported in brackets are calculated standard deviations on average values

All cellulosic substrates were chopped to approximately 1 cm by 1 cm pieces prior to being used for the BMP assays. The co-digestion BMP assays were prepared by adding enough substrate to each bottle to deliver a total of 10 g VS/bottle. Two sets of BMP assay trials were conducted, where one trial evaluated co-digestion with 25% of the total VS from cellulosic substrates and the remaining 75% of the VS from liquid dairy manure. A second trial evaluated co-digestion with 50% of the total VS from cellulosic substrates and the remaining 50% of the VS from liquid dairy manure. 100 g of acclimated inoculum (as characterized in Table 3.2) was added to each assay and when necessary the liquid volume was brought to a final 300 mL using distilled water. An equal mixture of $NaHCO_3$ and $KHCO_3$ was added to provide sufficient alkalinity of approximately 5000 mg/L (as $CaCO_3$) and the headspace purged with N_2 gas or a N_2+CO_2 gas mixture prior to bottles being sealed for incubation.

Table 3.4 Characteristics of Grease-Based Substrates used for Co-digestion with Dairy Manure

Parameter (wet basis)	**DAF Sludge**	**Waste Grease**
	n=8[1]	n=8
pH	5.87 (0.45)[2]	3.99 (0.22)
Alkalinity (mg $CaCO_3$/kg)	7073 (301)	0
TS (g/kg)	172 (57)	278 (79)
VS (g/kg)	151 (37)	267 (41)
VS/TS (%)	88	96
tCOD (g/kg)	202 (34)	407 (29)
sCOD (g/kg)	179 (31)	375 (23)
pCOD (g/kg)	23 (12)	32 (9)
C:N	37:1 (7)	34:1 (4)
TKN (g/kg)	2.7 (0.7)	5.1 (0.3)
Ammonium - NH_4^+-N (g/kg)	0.8 (0.2)	0.9 (0.2)
TP (g/kg)	0.3 (0.2)	0.4 (0.3)

1 Number of samples used to prepare substrate mixture for co-digestion
2 Values reported in brackets are calculated standard deviations on average values

The BMP assays using grease-based wastes were prepared by adding enough substrate to each bottle to deliver a total of 8 g VS/bottle. Three sets of BMP assay trials were conducted:

i. one trial evaluated co-digestion with 25% of the total VS from grease-based wastes and the remaining 75% of the VS from liquid dairy manure;
ii. a second trial evaluated co-digestion with 50% of the total VS from grease-based wastes and the remaining 50% of the VS from liquid dairy manure; and,
iii. a third trial evaluated co-digestion with 75% of the total VS from grease-based wastes and the remaining 25% of the VS from liquid dairy manure.

Each assay received 100 g of acclimated inoculum (as characterized in Table 3.2) and when necessary the liquid volume was brought to a final 300 mL using distilled water. An equal mixture of $NaHCO_3$ and $KHCO_3$ was added, when necessary, to provide sufficient alkalinity of 5000 mg/L (as $CaCO_3$) and the headspace purged with N_2 gas or a N_2+CO_2 gas mixture prior to bottles being sealed for incubation. With assays receiving 50% of the VS, and greater, from the grease-based waste, additional alkalinity was added to the bottles (using an equal mixture of $NaHCO_3$ and $KHCO_3$) during the first 5 days of incubation.

The BMP assays using food and industry wastes were prepared by adding enough substrate to each bottle to deliver a total of 8 g VS/bottle. Three sets of BMP assay trials were conducted:

iv. one trial evaluated co-digestion with 25% of the total VS from food and industry wastes and the remaining 75% of the VS from liquid dairy manure;

v. a second trial evaluated co-digestion with 50% of the total VS from food and industry wastes and the remaining 50% of the VS from liquid dairy manure; and,

vi. a third trial evaluated co-digestion with 75% of the total VS from food and industry wastes and the remaining 25% of the VS from liquid dairy manure.

Each assay received 100 g of acclimated inoculum (as characterized in Table 3.2) and when necessary the liquid volume was brought to a final 300 mL using distilled water. An equal mixture of $NaHCO_3$ and $KHCO_3$ was added, when necessary, to provide sufficient alkalinity of 5000 mg/L (as $CaCO_3$) and the headspace purged with N_2 gas or a N_2+CO_2 gas mixture prior to bottles being sealed for incubation.

Table 3.5 Characteristics of Food and Industry Wastes used for Co-digestion with Dairy Manure

Parameter (wet basis)	**Grocery Food Waste**	**Fish Offal**	**Corn Thin Stillage**	**Spent Barley**
	n=8[1]	n=5	n=8	n=7
pH	5.69 (0.22)[2]	6.86 (0.12)	4.64 (0.20)	5.49 (0.13)
Alkalinity (mg $CaCO_3$/kg)	5942 (232)	7620 (311)	0	681 (200)
TS (g/kg)	197 (31)	336 (29)	85 (11)	240 (53)
VS (g/kg)	183 (27)	289 (33)	67 (11)	211 (41)
VS/TS (%)	93	86	79	88
tCOD (g/kg)	205 (33)	369 (45)	141 (18)	289 (33)
sCOD (g/kg)	87 (15)	97 (23)	64 (21)	115 (30)
pCOD (g/kg)	118 (21)	273 (33)	77 (12)	175 (24)
C:N	36:1	19:1	39:1	31:1
TKN (g/kg)	2.6 (0.6)	12.1 (2.1)	2.1 (0.9)	3.3 (0.5)
Ammonium - NH_4^+-N (g/kg)	1.2 (0.4)	5.9 (1.3)	0.9 (0.4)	1.6 (0.9)
TP (g/kg)	0.4 (0.2)	1.0 (0.3)	1.1 (0.2)	0.9 (0.6)

1 Number of samples used to prepare substrate mixture for co-digestion
2 Values reported in brackets are calculated standard deviations on average values

3.2.2 Box-Wilson Central Composite Circumscribed Models

A second order central composite design was applied using BMP assays with two design factors: co-substrate (thin stillage or grease trap waste) content in feed (X_1) and temperature (X_2). Two models were developed to describe the factor interactions for the co-digestion of corn thin stillage with liquid dairy manure and the co-digestion of waste grease with liquid dairy manure. The Box-Wilson central composite circumscribed design was attributed in 1951 and has shown to be efficient in determining main effects, two-factor interaction effects and the quadratic effects (Burns, 2010). The selected optimization parameters (model responses) were methane yield (L CH_4/kg VS_{in}) (Y_1) and biogas yield (L biogas/kg VS_{in}) (Y_2).

A two-factor (2^k) central composite design is described as:

$$E(Y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j \geq 1}^{k} \beta_{ij} X_i Y_j \quad [3.1]$$

where E(Y) is the expected value of the response variable
B_0, β_i, β_j are the model parameters
X_i and X_j are the coded factors being studied
k is the number of factors being studied

The design consists of a 2^k factorial or fractional factorial design augmented by 2k axial (star) points at (±α, 0, 0,…, 0), (0, 0, ±α,…, 0),…, (0, 0, 0,…, ±α) and n_0 centre points at (0, 0,…, 0), where α is the distance of the star point from the centre. The choice of α establishes the rotatability of a central composite design. For a two factor circumscribed design, α should be set as 1.41, as discussed in Burns (2010). The design matrix is presented in Table 3.6.

The coded levels and the natural values of the factors set in the statistical designs for thin stillage and waste grease models are presented in Tables 3.7 and 3.8, respectively.

Each substrate model consisted of 13 BMP assay bottles, which were prepared as described in Section 3.2.1.1. The quantity of co-substrate added to each bottle is described in Tables 3.7 and 3.8 for each of the respective models. The balance in total VS (6g VS for thin stillage model and

7 g for grease waste model) for each bottle was added as liquid dairy manure. 100 g of acclimated inoculum (as described in Table 3.2) was added to each assay bottle, except for assays incubated at the 50 and 56°C temperatures which received an acclimated thermophilic inoculum. An equal mixture of $NaHCO_3$ and $KHCO_3$ was added, when necessary, to provide sufficient alkalinity of 5000 mg/L (as $CaCO_3$) and the headspace purged with N_2 gas or a N_2+CO_2 gas mixture prior to bottles being sealed for incubation.

Table 3.6 Box-Wilson Central Composite Circumscribed Design for Two Factors

Bottle No.	Co-substrate Content (g VS) (X_1)	Temperature (X_2)
1	-1.00	-1.00
2	1.00	-1.00
3	-1.00	1.00
4	1.00	1.00
5	-1.41	0.00
6	1.41	0.00
7	0.00	-1.41
8	0.00	1.41
9	0.00	0.00
10	0.00	0.00
11	0.00	0.00
12	0.00	0.00
13	0.00	0.00

Table 3.7 Coded and Natural Levels of the Design Factors for the Box-Wilson Central Composite Circumscribed Model - Co-digestion of Corn Thin Stillage and Manure

Parameter	-1.41	-1	0	+1	+1.41
x_1: VS from Corn Thin Stillage (g)[1] [% VS from thin stillage]	0.7 [11]	1.1 [18]	2.1 [35]	3.1 [52]	3.5 [59]
x_2: Temperature (°C)	14	20	35	50	56

1 Total VS = 6g per bottle

Table 3.8 Coded and Natural Levels of the Design Factors for the Box-Wilson Central Composite Circumscribed Model - Co-digestion of Waste Grease and Manure

Parameter	-1.41	-1	0	+1	+1.41
x_1: VS from Grease (g)[1] [% VS from waste grease]	0.7 [10]	1.2 [17]	2.4 [34]	3.6 [51]	4.1 [58]
x_2: Temperature (°C)	14	20	35	50	56

1 Total VS = 7 g per bottle

The inoculum used in this study was taken from the effluent line of the Fepro Farms (Cobden, ON) mesophilic dairy manure digester which co-digested grease trap waste. The inoculum was acclimated in a 5 L lab reactor with semi-batch feeding of waste grease (HRT 28 days) under thermophilic conditions (51 ± 2°C). To achieve a well acclimatized thermophilic inoculum, the semi-batch reactor was operated for 4 to 5 months before it could be used. Refer to Table 3.9 for the characteristics of the acclimated thermophilic inoculum. The responses to the central composite design were biogas and methane yields.

Table 3.9 Characteristics of Acclimated Thermophilic Inoculum

Parameter (wet basis)	Acclimated Inoculum n=17[1]
pH	7.97 (0.30)[2]
Alkalinity (mg $CaCO_3$/kg)	11782 (174)
TS (g/kg)	45 (11)
VS (g/kg)	31 (9)
VS/TS (%)	69
tCOD (g/kg)	32.1 (11.1)
sCOD (g/kg)	8.8 (2.3)
pCOD (g/kg)	23.3 (12.3)
TKN (g/kg)	3.4 (0.9)
Ammonium - NH_4^+-N (g/kg)	2.1 (0.6)

1 Number of samples used for average values
2 Values reported in brackets are calculated standard deviations on average values

3.2.3 Analytical Methods

Table 3.10 describes the analytical methods used in the study. All substrate samples and inoculum used for the BMP assays were analysed for pH, Alkalinity, TS, VS, tCOD, sCOD, pCOD, C:N, TKN, NH_4^+ -N and TP. During incubation, assays were measured for biogas production and CH_4 + CO_2 composition of the biogas. Digested substrates were analysed for pH, TS and VS.

Table 3.10 Description of Analytical Methods Used for Sample Analysis

Lab Analyses	Description
pH	• Adapted from Standard Method 4500-H^+ B. (APHA, 2005)
Alkalinity	• Adapted from Standard Method 2320 B. Titration Method (APHA, 2005)
TS	• Adapted from Standard Method 2540 B. Total Solids dried at 103-105°C (APHA, 2005)
VS	• Adapted from Standard Method 2540 E. Fixed and Volatile Solids ignited at 550°C (APHA, 2005)
tCOD	• Adapted from Standard Method 5220 D. Closed Reflux, Colorimetric (APHA, 2005)
sCOD	• Adapted from Standard Method 5220 D. Closed Reflux, Colorimetric (APHA, 2005) • Sample preparation: centrifugation of 15 mins. at 6230 relative centrifugal force (RCF) using a Thermo Scientific Sorvall bentop centrifuge followed by filtration with a Millipore 0.45 µm disc filter
pCOD	• Difference between tCOD and sCOD
C:N	• Total and organic carbon were measured at an external lab (University of Guelph Lab Services in Guelph, ON) • Nitrogen was measured as TKN
TKN	• Adapted from Standard Method 4500-N_{org} B. Macro-Kjeldahl Method (APHA, 2005)
Ammonium (NH_4^+ -N)	• Adapted from Standard Method 4500-NH_3 D. Ammonia – Gas Selective Electrode Method (APHA, 2005)
TP	• Adapted from Standard Method 4500-P Phosphorus (Persulfate Digestion Method) and 4500-P E. Ascorbic Acid Method (APHA, 2005)
Biogas	• Volume displacement method using manometer • Corrected to STP
CH_4 & CO_2 (Biogas)	• Adapted from method as outlined in Zhou et al. (2003) • Varian CP-3800 GC with metal packed column and TCD detection • H_2 carrier gas and He balance gas

- Heater: 260°C; Column oven temperature: 50°C
- TCD temperature: 120°C
- HayeSep® N 80/100 mesh, 0.5 m x 1/8 in.
- SST Porapak® QS 80/100 mesh, 2 m x 1/8 in.
- SST HayeSep® D, 80/100 mesh, 2 m x 1/8 in. SST
- Column Flow (H_2): 4.8 mL/min
- He reference flow: 25 mL/min
- Injection volume: 1µL

3.2.4 Analysis of Experimental Data

3.2.4.1 Analysis of Data from BMP Assay Trials for the Co-digestion of Liquid Dairy Manure with Various Organic Rich Substrates

To normalize the biogas and methane data from the BMP assay trials, biogas yields (L biogas/g VS) and methane yields (L CH_4/g VS) were calculated. Cumulative biogas and methane production at the end of each trial was divided by the total mass of VS of the initial assay, respectively, thereby expressing biogas and methane production in terms of available organic matter. The percent VS reduction of the BMP assays was calculated as the percent difference of the VS concentration at the start and end of the digestion period. Since the BMP assays were conducted in triplicate, biogas and methane yields are reported as an average of the triplicate assays and the standard deviation presented.

Standard statistical procedures have been implemented: in addition to standard deviation and mean averaging, single factor ANOVA, with one-sided t-test, have been used to determine if significant differences exist between BMP assay trials. Statistical significance was established at the $p< 0.05$.

3.2.4.2 Analysis of Data from BMP Assay Trials for Box-Wilson Central Composite Circumscribed Models

Methane yields were calculated for each of the BMP assays used for the central composite design models. MS Excel 2016 was used to model the design using least squares analysis. An analysis of the residuals from the model was performed to determine the adequacy of the least squares fit. An ANOVA analysis was also performed. For the ANOVA analysis, the variation was separated into regression and residual variance, as shown in Table 3.11.

Table 3.11 ANOVA Table for Multiple Regression Models
(adapted from Burns, 2010)

Source Variance	Degrees of Freedom	Sum of Squares	Mean Squares	F
Regression	p	RSS_{cfm}	$MSreg=RSS_{cfm}/p$	MSreg/MSres
Residual	N-p-1	ResSS	MSres=ResSS/N-p-1	
(lack of fit)	N-p-1-(n_0-1)	SSLF	MS_{LF}=SSLF/N-p-1-(n_0-1)	MS_{LF}/MS_{PE}
(pure error)	n_0-1	SSPE	MS_{PE}=SSPE/(n_0-1)	
Total	N-1	TSS_{cfm}		

Note: p = Number of parameters in model (excluding β_0)
N = Total number of design points
n_0 = Number of centre points

The F ratio (MSreg/MSres) was used to determine the significance of the models and the L ratio (MS_{LF}/MS_{PE}) was used to determine whether there were model inadequacies (lack of fit).

3.3 Results and Discussion

3.3.1 Co-digestion of Dairy Manure with Various Substrates

3.3.1.1 Digestion of Various Manure Types

BMP assays were conducted on four different manure types: liquid dairy, horse, poultry (layers) and swine. For the liquid dairy manure assays, the substrate was composed of a composite from 10 different subsamples to capture variability in sampling. The horse, poultry and swine manure trials were composed of a composite of 5 different subsamples, respectively. Average biogas yields during 64 days (1536 hours) of digestion are presented in Figure 3.2.

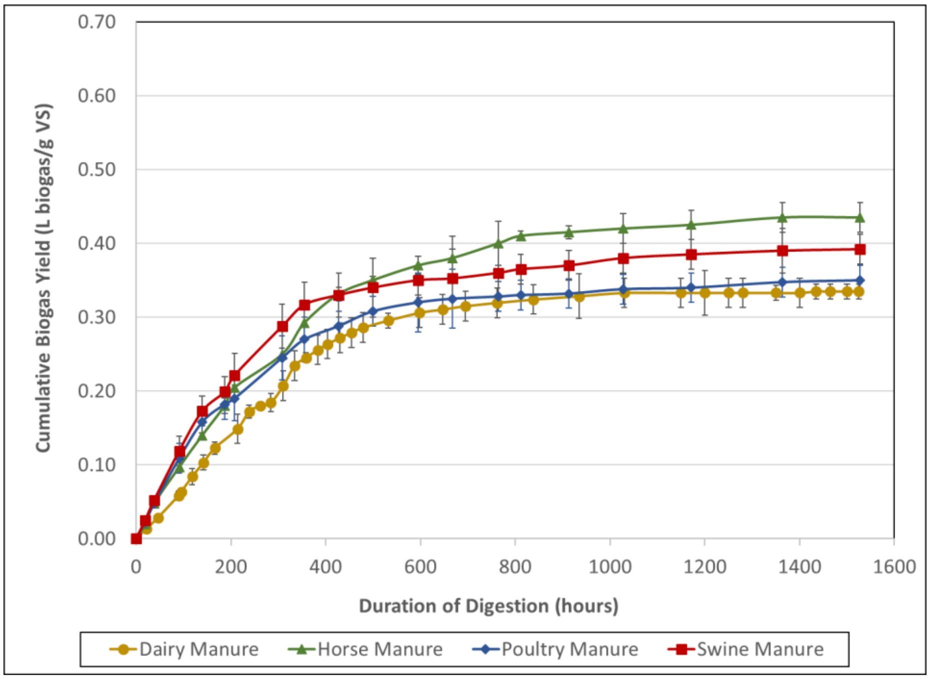


Figure 3.2 Cumulative Biogas Production from Various Manure Types
(Each point is the average cumulative biogas yield from triplicate BMP assays and error bars represent the standard deviation on the average)

The lowest biogas yields were observed with the liquid dairy and poultry manure BMP trials, with yields of 0.34 ± 0.02 and 0.35 ± 0.02 L biogas/g VS, respectively. There was no significant difference (p>0.05) between these yields. The average % CH_4 in the biogas was 56 and 57 % (Table 3.12) for dairy and poultry manure trials, respectively, which is consistent with the range of 55 to 70 % CH_4 reported in literature (Burke, 2001; Gerardi, 2003; Krich et al., 2005; Das and Mondal, 2016). Luna-delRisco et al. (2011) showed BMP biogas yields for cattle slurry to be slightly higher at 0.397 L biogas/g VS (238 ± 42 L CH_4/g VS). El-Mashad and Zhang (2010) reported a similar biogas yield to our study with 0.366 L biogas/gVS for unscreened dairy manure; however, biogas production was significantly higher for the coarse fraction of dairy manure with a biogas yield of 0.404 L/g VS. The authors suggested the higher fraction of solids in the coarse dairy manure contributed to the higher biogas yield. However, in our study the higher solids poultry manure did not demonstrate a higher biogas yield, in fact, the biogas yield was not significantly different from that of dairy manure despite having almost three times the solids content. The poultry manure's low C:N ratio (9:1) and high TKN concentration of 14.2 ± 5.1 g/kg may been the key factors for the relatively low biogas yield due to accumulating ammonium levels during digestion (Bujoczek et al., 2000; Gerardi, 2003; Kukkonen, 2014).

Table 3.12 Digestion of Various Manure Types (64 days of digestion)

Substrate Mixtures	Biogas Yield (L biogas/g VS)	Rate of Biogas Production [1] (L biogas/kg VS·day)	Average CH_4 Content in Biogas [1] (%)	VS Reduction (%)	pH of Digestate
Dairy Manure (8% TS)	0.34 (0.02) [2]	12.3 (0.8)	57 (2)	45.8 (1.1)	8.11 (0.16)
Horse Manure (39% TS)	0.44 (0.02)	12.4 (0.6)	59 (4)	51.2 (3.5)	8.30 (0.11)
Poultry Manure (28% TS)	0.35 (0.02)	12.7 (0.8)	56 (7)	47.7 (2.4)	8.63 (0.27)
Swine Manure (5% TS)	0.39 (0.02)	14.2 (0.8)	61 (3)	40.2 (2.0)	7.80 (0.13)

1 Calculated during exponential phase of biogas production (90% of total biogas produced)
2 Values reported in brackets are calculated standard deviations on average values

The highest biogas yield of 0.44 ± 0.02 L biogas/g VS was observed with horse manure, followed by swine manure with a yield of 0.39 ± 0.02 L biogas/g VS; % CH_4 in the biogas was consistent with literature at 59 and 61 %, respectively (Table 3.12). Biogas production from both horse and swine manure trials were significantly higher than liquid dairy manure ($p<0.05$). Although swine manure was lower in solids content than liquid dairy manure, it had a higher sCOD/tCOD ratio of 0.53 compared to 0.29 for dairy manure which may explain the higher biogas yield.

When considering the exponential phase of the BMP curves, we can assume that its slope is a good indicator of the rate of biogas production while the microbial consortia is most active during the digestion process (under the batch mesophilic conditions of the trial). Table 3.12 lists the rate of biogas production for the four manure types (at 90% of total biogas production). The rate of biogas production was relatively the same for dairy, horse and poultry manure, while that of swine manure was slightly higher at 14.2 L biogas/kg VS·day. The length of the exponential phase suggests the number of days of residence time required to achieve the biochemical biogas potential of the substrate (Labatut et al., 2011), ranged between 26 and 33 days for the four manure types. This data could be further extrapolated to suggest the residence time required to achieve the biochemical biogas potential may be a suitable HRT for a batch or semi-batch reactor to main optimum microbial activity (El-Mashad and Zhang, 2010). VS reduction in the manure assay trials ranged from 40.2 to 51.2 %.

3.3.1.2 Digestion of Dairy Manure with Cellulosic Substrates

BMP assays were conducted to investigate biogas production when co-digesting cellulosic substrates with dairy manure. Four different co-substrates were selected: corn silage, switchgrass, wheat straw and woodchips. Figures 3.3, 3.4 and 3.5 illustrate the biogas yields from co-digestion of dairy manure with corn silage, switchgrass and wheat straw, respectively, during 104 days (2496 hours) of digestion.

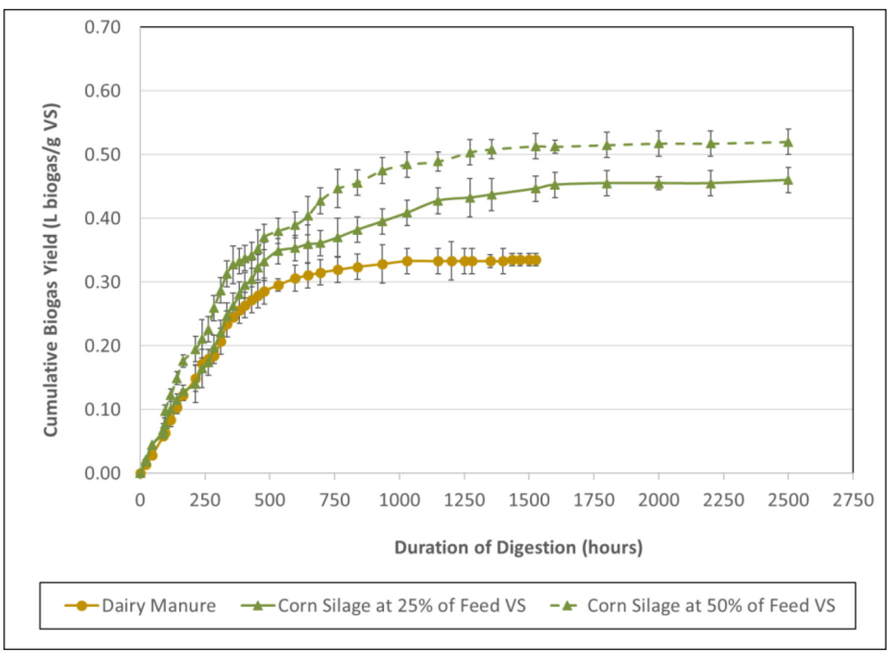


Figure 3.3 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Corn Silage **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

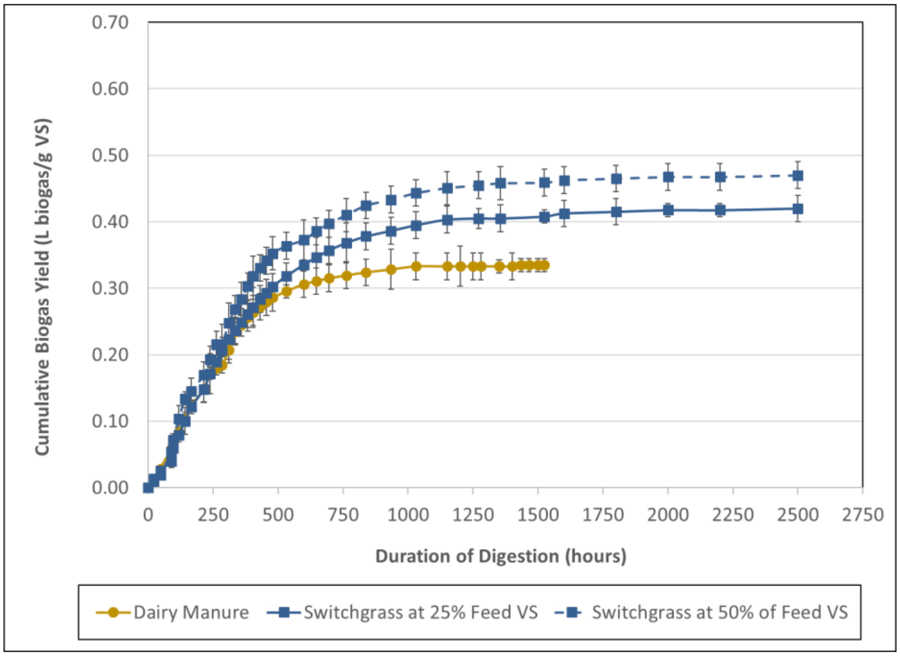


Figure 3.4 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Switchgrass **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

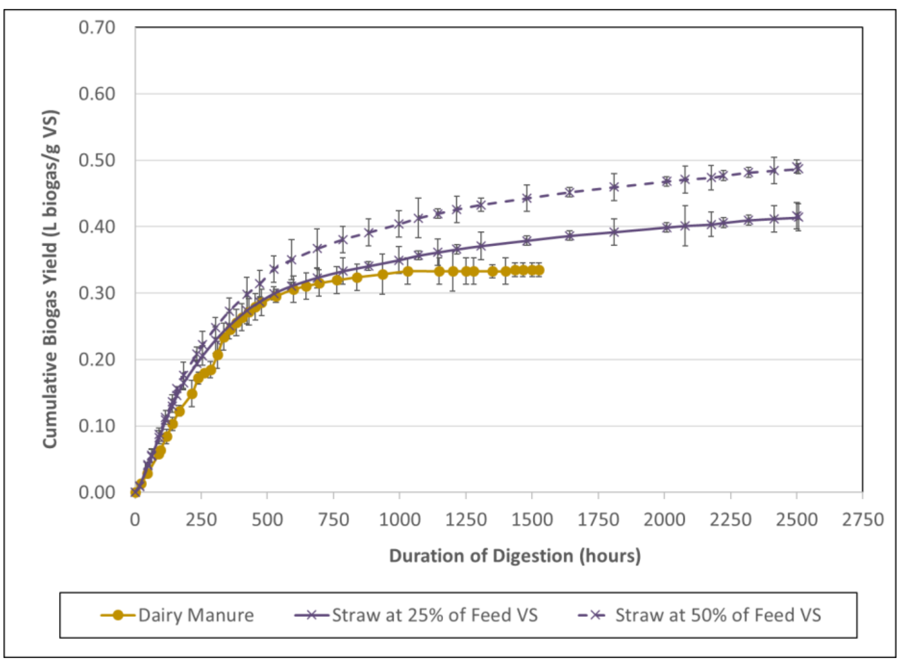


Figure 3.5 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Wheat Straw **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

Except for woodchips, biogas yields were significantly (*p*<0.05) higher with co-digestion when compared to the single substrate digestion of liquid dairy manure. Optimum yields were achieved when 50% of the feed VS was comprised of cellulosic substrate, with yields of 0.51 ± 0.02, 0.46 ± 0.02 and 0.38 ± 0.02 L biogas/g VS for corn silage, switchgrass and wheat straw, respectively. % CH_4 in the biogas ranged from 54 to 62 % (Table 3.13). Methane yields observed in this study were consistent with yields reported in literature for co-digestion of cellulosic substrates, which ranged from 0.286 to 0.370 L CH_4/g VS for substrates like corn silage, grass silage and hay (Pobeheim et al., 2010; Labatut et al., 2011; Luna-delRisco et al., 2011). It is of no surprise that co-digestion of corn silage produced the higher biogas/methane yields; due in part to its higher sugar content from corn kernels. % VS reductions were also higher with co-digestion and ranged from 46.3 to 55.7 %.

Table 3.13 Co-digestion of Cellulosic Substrates with Dairy Manure (104 days of Digestion)

Substrate Mixtures	Biogas Yield (L biogas/g VS)	Rate of Biogas Production [1] (L biogas/kg VS·day)	Average CH_4 Content in Biogas [1] (%)	VS Reduction (%)	pH of Digestate
Corn Silage (25% of VS Feed)	0.46 (0.02)[2]	9.6 (0.5)	59 (3)	48.9 (2.9)	7.63 (0.12)
Corn Silage (50% of VS Feed)	0.51 (0.02)	13.4 (0.6)	62 (2)	55.7 (3.1)	7.81 (0.21)
Switchgrass (25% of VS Feed)	0.42 (0.02)	10.8 (0.6)	55 (4)	44.9 (1.9)	7.54 (0.18)
Switchgrass (50% of VS Feed)	0.46 (0.02)	12.1 (0.6)	54 (3)	46.3 (2.7)	7.56 (0.10)
Straw (25% of VS Feed)	0.42 (0.02)	6.1 (0.2)	58 (4)	47.9 (2.9)	7.33 (0.21)
Straw (50% of VS Feed)	0.38 (0.02)	7.2 (0.2)	57 (2)	48.7 (2.1)	7.67 (0.27)
Woodchips (25% of VS Feed)	0.29 (0.03)	5.2 (0.2)	49 (4)	28.9 (3.6)	7.23 (0.21)
Woodchips (50% of VS Feed)	0.21 (0.02)	3.6 (0.2)	49 (3)	19.7 (4.1)	7.09 (0.11)

1 Calculated during exponential phase of biogas production (90% of total biogas produced)
2 Values reported in brackets are calculated standard deviations on average values

When observing the exponential phase of the BMP curves, the slope is similar for both co-digestion and single substrate digestion, which can be explained by the proportion of bedding in the liquid dairy manure. The slope of the exponential phase of the assay plots indicate biogas production rates (at 90% of total biogas produced) to be highest with corn silage and switchgrass (50% of VS feed) at 13.4 and 12.1 L biogas/kg VS·day, followed by wheat straw at 7.2 L biogas/kg VS·day. The length of the exponential phase of the plots suggests residence times required to achieve the biochemical biogas potential for cellulosic substrates to be higher than manure, at closer to 41 days. This is not unexpected given the cellulosic and lignocellulosic content of the co-digestion mixture.

Co-digestion of woodchips with liquid dairy manure produced biogas yields that were lower than the single substrate digestion of liquid dairy manure, suggesting inhibition or non-biodegradability. Figure 3.6 illustrates the biogas yields from the woodchip co-digestion trials and shows these yields to be up to 38% lower than when digesting dairy manure alone. Toscano et al. (2013) and Ahring et al. (2015) suggest that without substrate pre-treatment the anaerobic digestion of wood fibers is difficult due to the slowly degradable lignocellulosic composition of the substrate and the non-degradable lignin. Considering the difficulty in digesting wood based substrates without pre-treatment, farms with anaerobic digesters should avoid (or limit) the use of woodchips or shavings as animal bedding, as the wood substrate will add solids load to the system but little biological benefit for the digestion process.

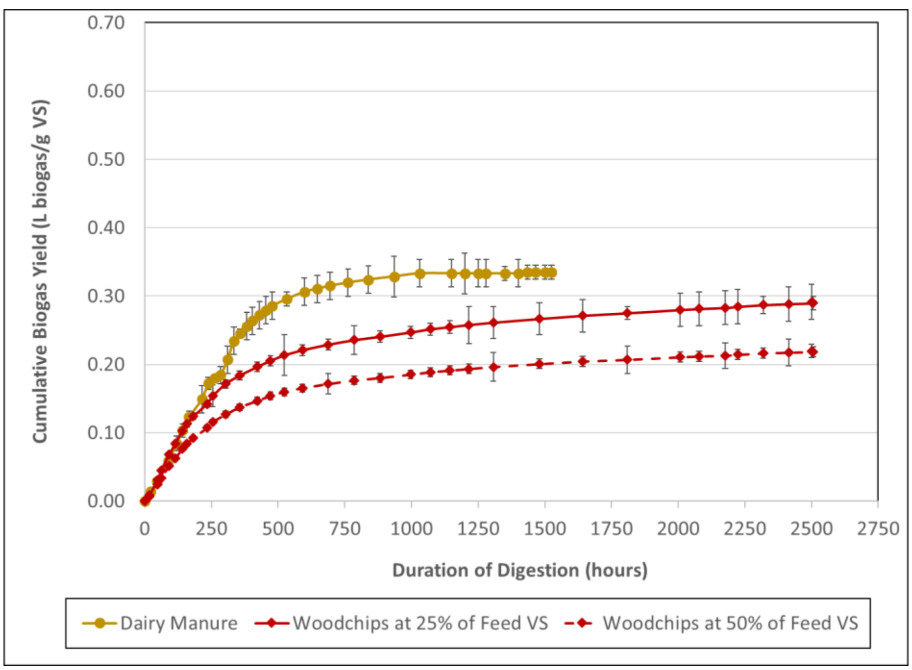


Figure 3.6 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Woodchips
(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)

3.3.1.3 Digestion of Dairy Manure with Grease Based Wastes

A series of BMP assays were conducted to investigate biogas production when co-digesting liquid dairy manure with grease based substrates. Figures 3.7 and 3.8 illustrate the biogas yields from co-digestion of dairy manure with DAF and waste grease (primarily composed of grease trap waste), respectively, during 64 days (1536 hours) of digestion. Co-digestion trials were conducted at 25, 50 and 75% of the feed VS from grease based wastes.

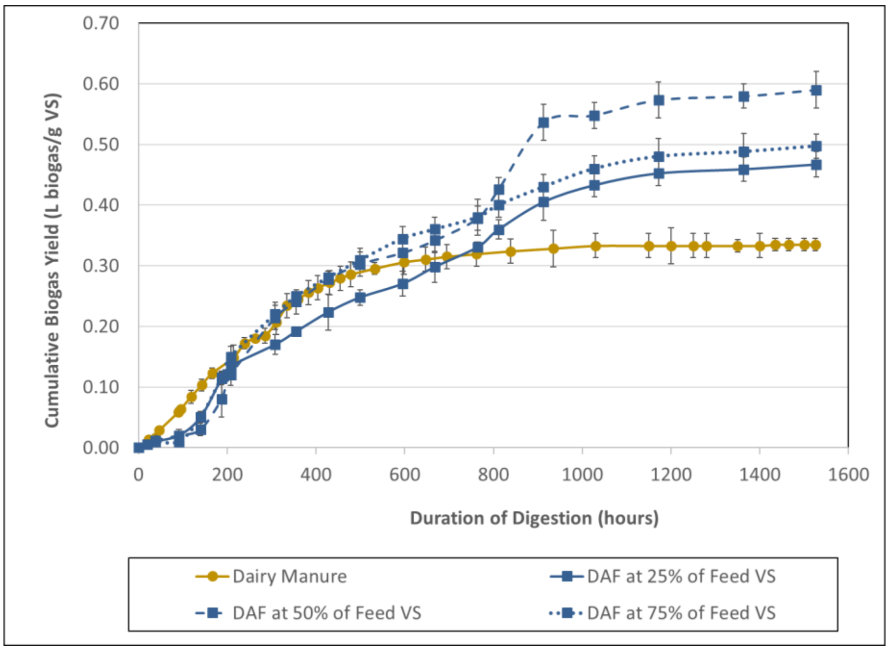


Figure 3.7 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with DAF
(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)

In all cases, biogas yields were significantly (p<0.05) higher when co-digesting lipid rich substrates compared to single substrate digestion of liquid dairy manure. Optimum biogas yields were achieved when 50% of the feed VS was comprised of the grease based waste, with yields of 0.59 ± 0.03 and 0.69 ± 0.02 L biogas/g VS for DAF and waste grease, respectively. % CH_4 in the biogas ranged from 60 to 67 % (Table 3.14). Labatut et al. (2011) and Das and Mondal (2016) explain that lipids are easily degradable under anaerobic conditions but experience longer HRTs due to potential inhibition from the accumulation of LCFA. This could explain the slow start in biogas production for the DAF trials (Figure 3.7) followed by a sharp increase in biogas production after 33 days (800 hours) of incubation. For the co-digestion trials using waste grease, long retention times were experienced before sharp increases in biogas production. The potential inhibition due to accumulating LCFA could also explain why lower biogas yields were observed in trials receiving 75% of the feed VS from the grease based substrates.

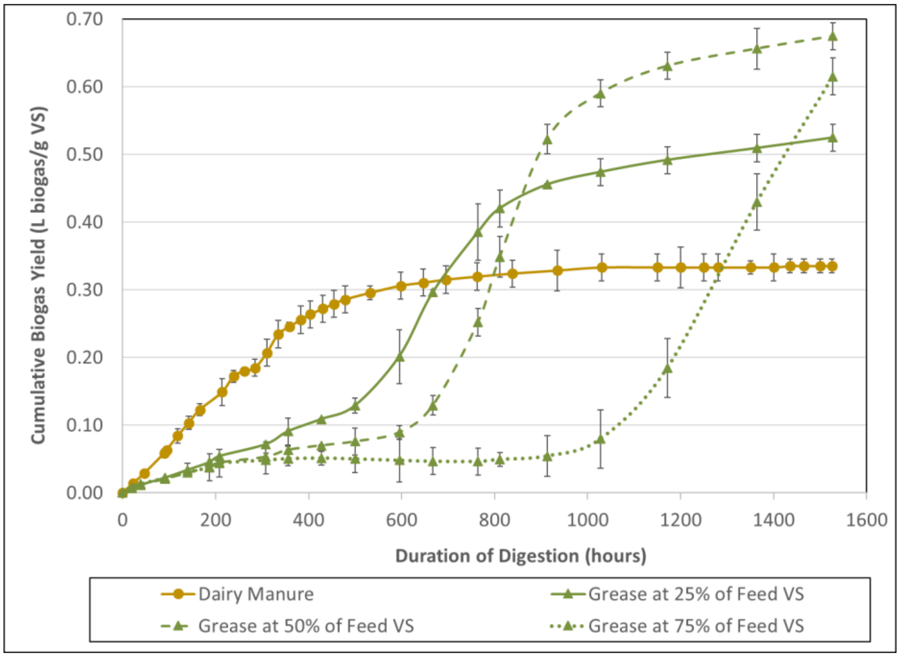


Figure 3.8 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Waste Grease **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

Table 3.14 Co-digestion of Grease-based Substrates with Dairy Manure (64 days of Digestion)

Substrate Mixtures	Biogas Yield (L biogas/g VS)	Rate of Biogas Production [1] (L biogas/kg VS·day)	Average CH_4 Content in Biogas [1] (%)	VS Reduction (%)	pH of Digestate
DAF Sludge (25% of Feed VS)	0.47 (0.02)[2]	12.4 (0.6)	63 (5)	53.7 (2.9)	7.57 (0.15)
DAF Sludge (50% of Feed VS)	0.59 (0.03)	17.1 (1.0)	67 (3)	69.9 (1.8)	7.80 (0.14)
DAF Sludge (75% of Feed VS)	0.50 (0.02)	13.3 (0.8)	60 (5)	61.7 (2.2)	7.41 (0.22)
Waste Grease (25% of Feed VS)	0.52 (0.02)	13.6 (0.6)	67 (4)	57.9 (3.5)	8.07 (0.11)
Waste Grease (50% of Feed VS)	0.67 (0.02)	27.5 (0.9)	65 (3)	69.4 (2.8)	8.12 (0.19)
Waste Grease (75% of Feed VS)	0.62 (0.03)	26.8 (1.1)	66 (4)	71.3 (2.1)	7.58 (0.17)

1 Calculated during exponential phase of biogas production (90% of total biogas produced)
2 Values reported in brackets are calculated standard deviations on average values

Methane yields observed in this study were on the low to middle end of the wide range of yields (0.250 to 0.508 L CH_4/g VS) reported in literature for co-digestion of substrates like grease, vegetable oil, DAF and animal fat (Labatut et al., 2011; Das and Mondal, 2016; Ebner et al., 2016).

Table 3.14 lists the rate of biogas production for various BMP assays using DAF and waste grease. The highest biogas production rates (at 90% of total biogas production) were observed when dairy manure was co-digested with waste grease at 50% of feed VS and 75% of feed VS, with rates of 27.5 ± 0.9 and 26.8 ± 1.1 L biogas/kg VS·day, respectively. Waste grease biogas production rates did not include the lag phase period. The lower biogas production rates of the DAF assays reflect the slow but steady increase in biogas production observed over the entire digestion period. The length of the exponential phase suggests 35 and 25 days are required to achieve the biochemical biogas potential of DAF and waste grease, respectively. VS reduction in the grease waste co-digestion trials ranged from 53.7 to 71.3 %.

3.3.1.4 Digestion of Dairy Manure with Food and Industry Wastes

BMP assays were conducted to determine biogas yields when liquid dairy manure is co-digested with food or industry wastes. Figures 3.9, 3.10, 3.11 and 3.12 illustrate the biogas yields from co-digestion trials using grocery waste, fish offal, corn thin stillage and spent barley, respectively. Co-digestion trials were conducted at 25, 50 and 75% of the feed VS from food or industry wastes, with a digestion period of 64 days (1536 hours) for all trials except those with spent barley which had a digestion period of 108 days (2592 hours).

Biogas yields were significantly (p<0.05) higher for most food or industry waste co-digestion trials when compared with single substrate digestion of liquid dairy manure. Optimum biogas yields were achieved when 50% of the feed VS was comprised of either grocery food waste, fish offal and corn thin stillage, resulting in biogas yields of 0.57 ± 0.02, 0.51 ± 0.02 and 0.54 ± 0.02 L biogas/g VS, respectively. While for spent barley, optimum biogas yields were achieved when the substrate composed only 25% of the feed VS resulting in a biogas yield of 0.45 ± 0.02 L biogas/g VS. % CH_4 in the biogas ranged from 58 to 66 % (Table 3.15).

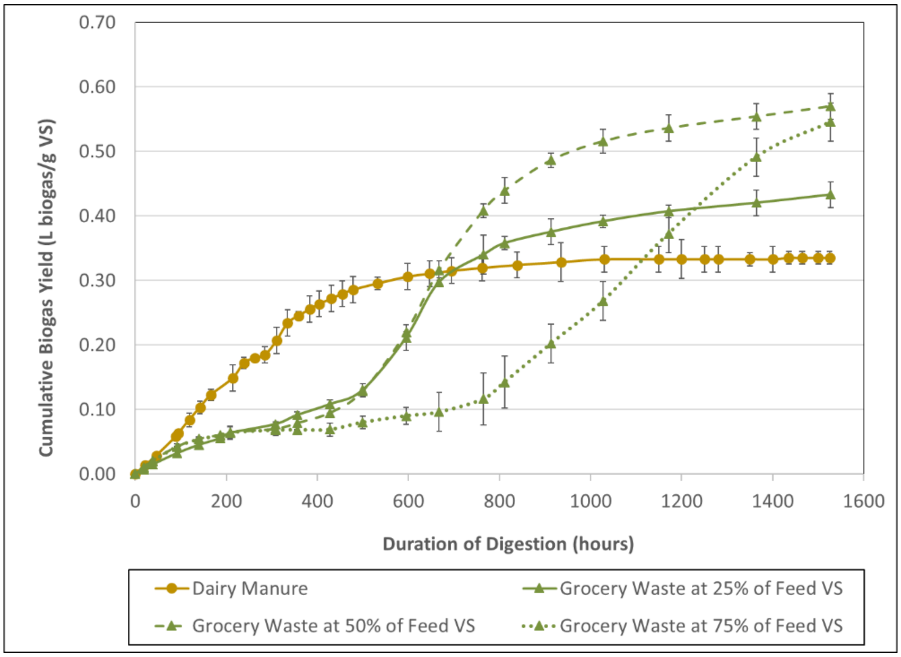


Figure 3.9 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Grocery Food Waste **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

Like the waste grease trials, co-digestion with grocery waste and fish offal experienced long lag phases (16 days and longer) prior to a sharp increase in biogas production. Both the grocery waste and fish offal were rich in animal fat and the lag phase could be explained by the presence LCFA and potential inhibition at the start of the digestion process.

Co-digestion trials involving corn thin stillage and spent barley demonstrated slow and steady increases in biogas production. Further increasing the proportion of thin stillage and spent barley in the feed to 75% of the feed VS decreased biogas yields and demonstrated no significant (p>0.05) difference to yields from single substrate dairy manure digestion.

Table 3.15 Co-digestion of Food and Industry Waste with Dairy Manure (64 days of Digestion)

Substrate Mixtures	Biogas Yield (L biogas/g VS)	Rate of Biogas Production [1] (L biogas/kg VS·day)	Average CH_4 Content in Biogas [1] (%)	VS Reduction (%)	pH of Digestate
Grocery Food Waste (25% of Feed VS)	0.43 (0.02)[2]	11.1 (0.6)	63 (4)	50.9 (2.6)	8.03 (0.22)
Grocery Food Waste (50% of Feed VS)	0.57 (0.02)	17.0 (0.7)	66 (3)	61.3 (4.1)	8.14 (0.19)
Grocery Food Waste (75% of Feed VS)	0.55 (0.03)	16.9 (1.0)	64 (5)	65.7 (5.1)	8.17 (0.10)
Fish Offal Waste (25% of Feed VS)	0.38 (0.02)	11.0 (0.6)	57 (2)	52.3 (1.3)	7.99 (0.18)
Fish Offal Waste (50% of Feed VS)	0.51 (0.02)	16.4 (0.7)	62 (4)	59.9 (3.7)	7.87 (0.21)
Fish Offal Waste (75% of Feed VS)	0.40 (0.02)	8.1 (0.4)	64 (3)	55.5 (3.0)	7.85 (0.13)
Corn Thin Stillage (25% of Feed VS)	0.41 (0.01)	12.6 (0.3)	58 (3)	52.3 (3.0)	7.91 (0.17)
Corn Thin Stillage (50% of Feed VS)	0.54 (0.02)	13.6 (0.6)	61 (1)	59.1 (2.5)	8.08 (0.11)
Corn Thin Stillage (75% of Feed VS)	0.31 (0.02)	9.9 (0.7)	57 (1)	45.6 (2.9)	7.21 (0.20)
Spent Barley (25% of Feed VS) [3]	0.45 (0.01)	7.0 (0.2)	58 (3)	48.6 (2.6)	7.50 (0.14)
Spent Barley (50% of Feed VS) [3]	0.39 (0.02)	4.8 (0.3)	53 (4)	43.7 (1.9)	7.47 (0.10)
Spent Barley (75% of Feed VS) [3]	0.23 (0.03)	2.2 (0.3)	49 (3)	37.8 (3.0)	7.03 (0.18)

1 Calculated during exponential phase of biogas production (90% of total biogas produced)
2 Values reported in brackets are calculated standard deviations on average values
3 Incubation period for Spent Barley trials was 108 days

The highest biogas production rates (at 90% of total biogas production) for the various substrates were observed as 17.0 ± 0.7 L biogas/kg VS·day for grocery waste (50% VS of feed), 16.4 ± 0.7 L biogas/kg VS·day for fish offal (50% VS of feed), 13.6 ± 0.6 L biogas/kg VS·day for corn thin stillage (50% VS of feed) and 7.0 ± 0.2 L biogas/kg VS·day for spent barley (25 % VS of feed). The length of the exponential phase of the digestions profiles of the substrates suggests 25 to 35 days are required to achieve biochemical biogas potential. VS reduction between the various substrate co-digestion trials ranged from 48.6 to 65.7 %.

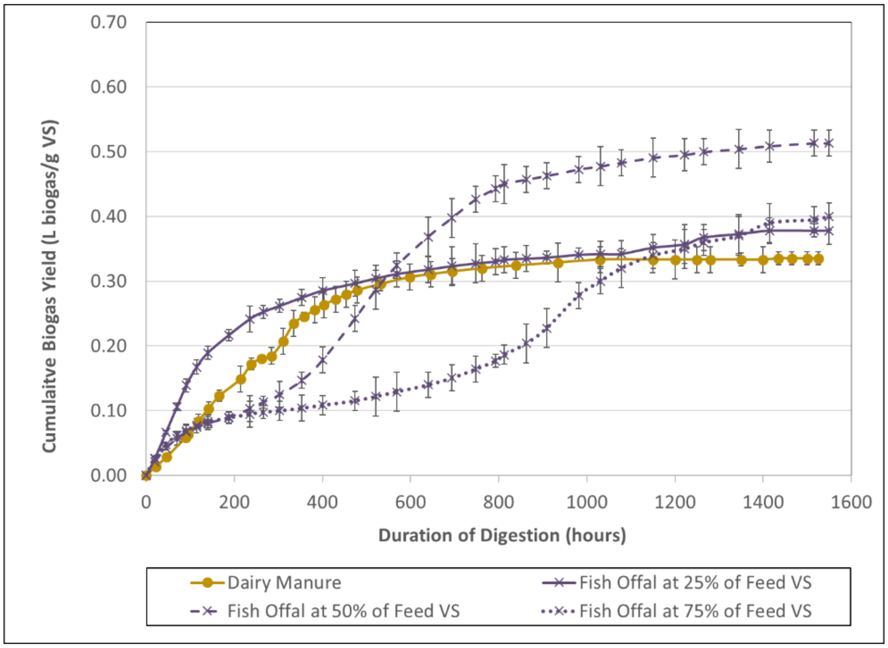


Figure 3.10 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Fish Offal (Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)

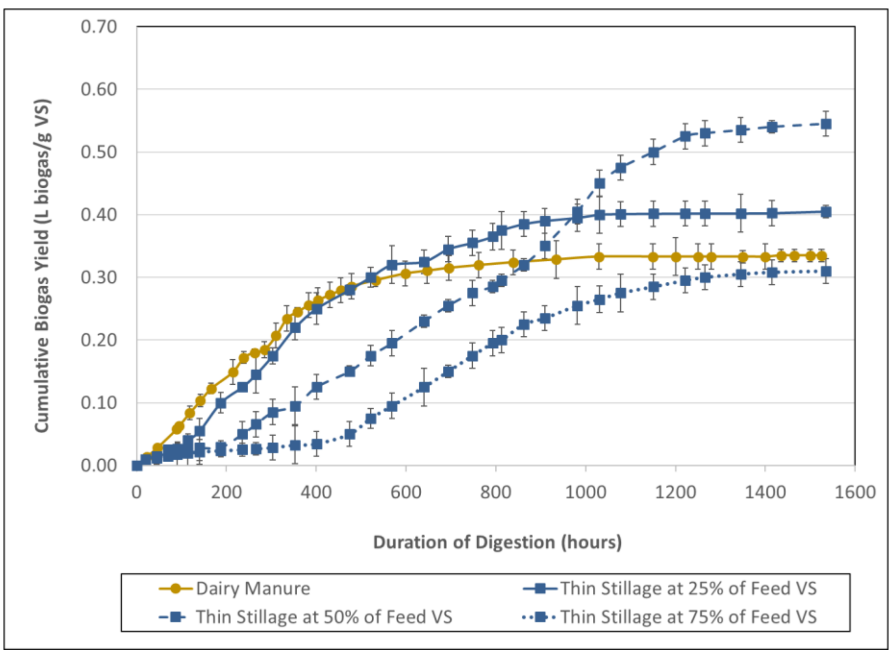


Figure 3.11 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Corn Thin Stillage **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

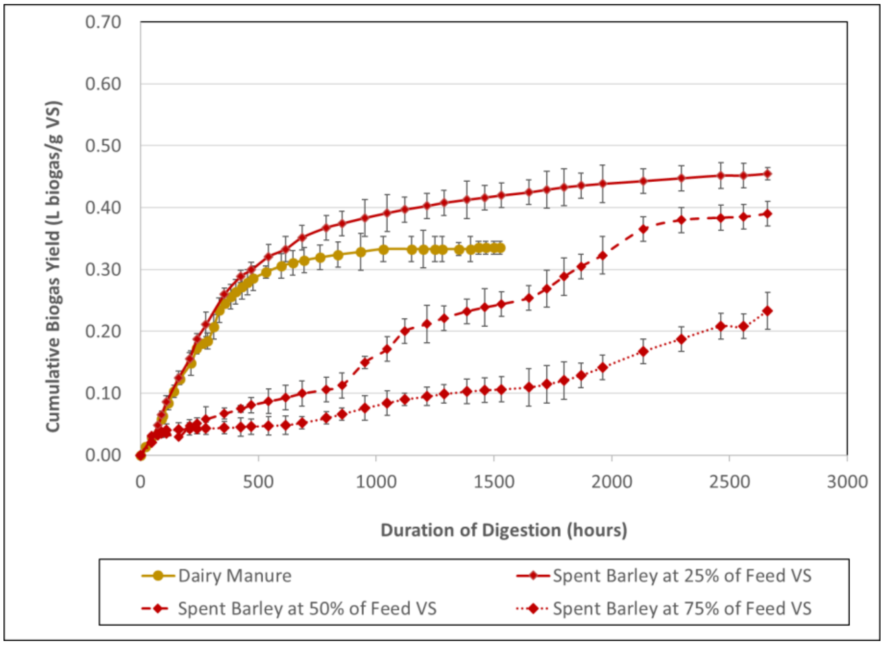


Figure 3.12 Cumulative Biogas Production from Co-digestion Liquid Dairy Manure with Spent Barley **(Each point is the average cumulative biogas yield from the triplicate BMP assays and the error bars represent the standard deviation on the average)**

Figure 3.13 summarizes the optimum biogas yields, in ascending order, for each of the substrates tested in this study, after 30, 60 and 90 days of digestion (typical retention times for full scale digester systems). Dairy manure demonstrates the lowest biogas yield of 0.34 L biogas/g VS at 60 days of digestion, while the highest biogas yield of 0.67 L biogas/g VS was observed with the co-digestion of waste grease (at 50% VS of feed) and liquid dairy manure at 60 days of digestion.

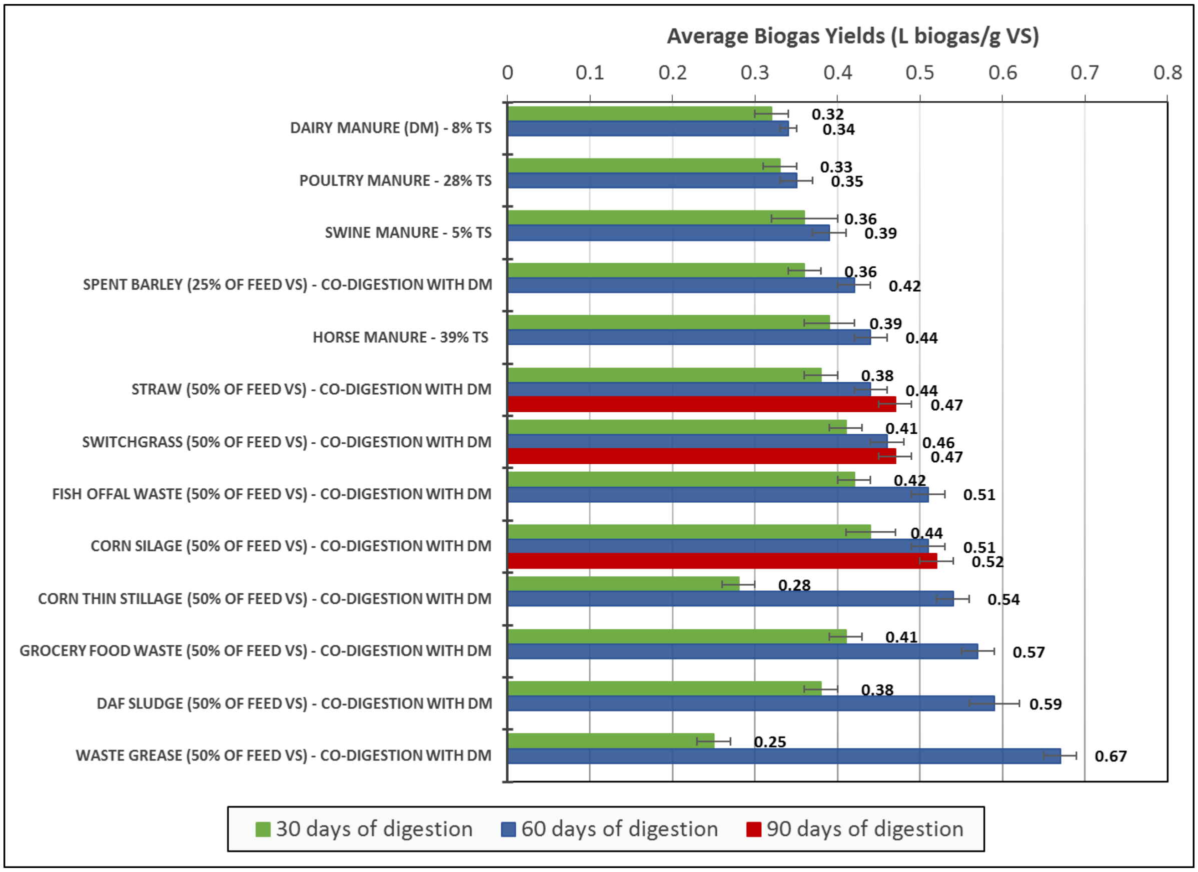


Figure 3.13 Average Biogas Yields for 30, 60 and 90 days of digestion for Optimum Manure and Co-substrate Ratios **(error bars represent the standard deviation on average values)**

3.3.2 Central Composite Design

Central composite design experiments using BMP assays were conducted over a 65-day period to determine the effects of temperature and % VS of the feed from co-substrate (corn thin stillage or waste grease). The digestion process was allowed to progress until an increase in cumulative methane production was no longer observed. The plateau in cumulative methane production was defined as less than a 5% increase in methane production over a 10-day period. Cumulative methane production and initial VS concentration were used to calculate the methane yield (L CH_4/kg VS_{in}) for each BMP assay. Tables 3.16 and 3.17 summarize the operating parameters and respective methane yields for both the corn thin stillage and waste grease models.

Table 3.16 Experimental Methane Yields from the Box-Wilson Central Composite Circumscribed Model Design for Corn Thin Stillage BMP Assays

BMP Assay No.	Coded Variables		Natural Variables		Y_1: Methane Yield (L CH_4/kgVS_{in})
	X_1	X_2	x_1: VS from Thin Stillage (g)	x_2: Temperature (°C)	
1	-1.00	-1.00	1.1	20	108
2	1.00	-1.00	3.1	20	132
3	-1.00	1.00	1.1	50	241
4	1.00	1.00	3.1	50	325
5	-1.41	0.00	0.7	35	99
6	1.41	0.00	3.5	35	275
7	0.00	-1.41	2.1	14	87
8	0.00	1.41	2.1	56	316
9	0.00	0.00	2.1	35	239
10	0.00	0.00	2.1	35	288
11	0.00	0.00	2.1	35	254
12	0.00	0.00	2.1	35	280
13	0.00	0.00	2.1	35	229

The model described by Equation [3.1] was fitted to the data obtained from the 13 BMP assay trials and the following fitted model was obtained for the co-digestion of liquid dairy manure and corn thin stillage:

$$\boxed{E(Y_1) = 258.1 + 44.7X_1 + 81.4X_2 + 15.0X_1X_2 - 33.8X_1^{\,2} - 26.5X_2^{\,2}} \qquad [3\text{-}2]$$

where $$X_1 = \frac{x_1 - 2.1}{1} \qquad [3\text{-}3]$$

$$X_2 = \frac{x_2 - 35}{15} \qquad [3\text{-}4]$$

The fitted model for the co-digestion of liquid dairy manure and waste grease is:

$$\boxed{E(Y_1) = 298.7 + 47.6X_1 + 78.7X_2 - 3.8X_1X_2 - 34.8X_1^2 - 21.0X_2^2} \quad [3\text{-}5]$$

where $$X_1 = \frac{x_1 - 2.4}{1.2} \quad [3\text{-}6]$$

$$X_2 = \frac{x_2 - 35}{15} \quad [3\text{-}7]$$

Tables 13.18 and 13.19 summarize the model parameter values and their respective upper and lower limits at the 95% confidence interval for the thin stillage and waste grease models respectively.

Table 3.17 Experimental Methane Yields from the Box-Wilson Central Composite Circumscribed Model Design for Waste Grease BMP Assays

BMP Assay No.	Coded Variables		Natural Variables		Y_1: Methane Yield (L CH_4/kgVS_{in})
	X_1	X_2	x_1: VS from Grease (g)	x_2: Temperature (^oC)	
1	-1.00	-1.00	1.2	20	156
2	1.00	-1.00	3.6	20	220
3	-1.00	1.00	1.2	50	311
4	1.00	1.00	3.6	50	360
5	-1.41	0.00	0.7	35	116
6	1.41	0.00	4.1	35	305
7	0.00	-1.41	2.4	14	120
8	0.00	1.41	2.4	56	356
9	0.00	0.00	2.4	35	295
10	0.00	0.00	2.4	35	275
11	0.00	0.00	2.4	35	323
12	0.00	0.00	2.4	35	290
13	0.00	0.00	2.4	35	311

Table 3.18 Model Parameter Values with 95% Confidence Intervals for the Box-Wilson Central Composite Circumscribed Model Design for Corn Thin Stillage BMP Assays

Model Parameters		Standard Error	t-statistic	Lower 95%	Upper 95%
β_0	258.1	11.37	22.7	226.5	289.7
β_1	44.7	9.00	5.0	19.7	69.7
β_2	81.4	9.00	9.0	56.4	106.4
β_{12}	15.0	12.71	1.2	-20.3	50.3
β_{11}	-33.8	9.68	3.5	-60.7	-6.9
β_{22}	-26.5	9.68	2.7	-53.4	0.4

Table 3.19 Model Parameter Values with 95% Confidence Intervals for the Box-Wilson Central Composite Circumscribed Model Design for Waste Grease BMP Assays

Model Parameters		Standard Error	t-statistic	Lower 95%	Upper 95%
β_0	298.7	8.34	35.8	275.5	321.9
β_1	47.6	6.61	7.2	29.3	65.9
β_2	78.7	6.61	11.9	60.4	97.0
β_{12}	-3.8	9.33	0.4	-29.7	22.1
β_{11}	-34.8	7.10	4.9	-54.5	-15.1
β_{22}	-21.0	7.10	3.0	-40.7	-1.3

The significance of the fitted models is evaluated by determining whether the amount of variation explained by the fitted model (RSS) is significant compared to the amount it leaves unexplained (ResSS). An ANOVA describing the variation between experimental data and the thin stillage fitted model is summarized in Table 3.20. The F ratio (MSreg/MSres) for the corn thin stillage fitted model is 21.8, which is greater than the $F_{5,7,0.05}$ value of 3.97 (Burns, 2010); indicating the fitted model explains a significant amount of variation in the data at the 5% significance level.

Table 3.20 ANOVA Table for the Box-Wilson Central Composite Circumscribed Model Design for the Corn Thin Stillage Assays

Source Variance	Degrees of Freedom	Sum of Squares	Mean Squares	F
Regression	5	80943.2	16188.6	MSreg/MSres = 21.8
Residual	7	5201.0	743.0	
(lack of fit)	3	2616.4	872.1	MS_{LF}/MS_{PE} = 1.3
(pure error)	4	2584.6	646.2	
Total	12	86144.2		

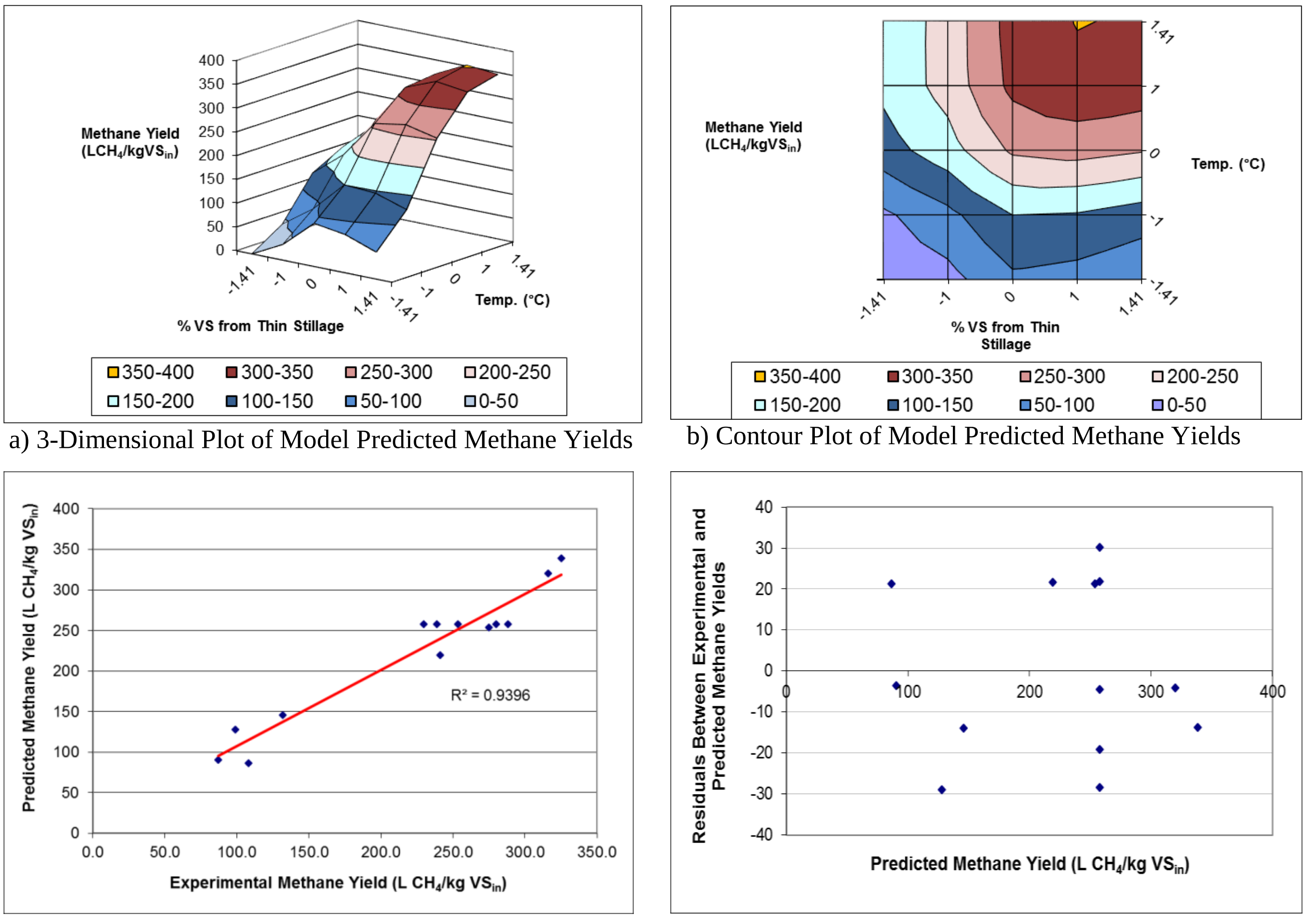
The F ratio (MSreg/MSres) for the waste grease fitted model is 14.6 (Table 3.21), which is greater than the $F_{5,7,0.05}$ value of 3.97 (Burns, 2010); there is a significant amount of variation in the data at the 5% significance level. An ANOVA describing the variation between experimental data and the waste grease fitted model is summarized in Table 3.21.

Table 3.21 ANOVA Table for the Box-Wilson Central Composite Circumscribed Model Design for Waste Grease BMP Assays

Source Variance	Degrees of Freedom	Sum of Squares	Mean Squares	F-value
Regression	5	77802.0	15560.4	MSreg/MSres = 14.6
Residual	7	7449.8	1064.3	
(lack of fit)	3	6057.1	2019.0	MS_{LF}/MS_{PE} = 5.8
(pure error)	4	1392.7	348.2	
Total	12	85251.8		

Figures 3.14 and 3.15 present the 3-dimensional and contour plots of the model predicted methane yields for thin stillage and waste grease, respectively. Graphs illustrating the variation in the experimental and model predicted methane yields, along with residual scatter plots, are also presented in the figures. The R^2 statistics (RSS_{cfm}/TSS_{cfm}) of 94.0% and 91.2%, for the thin stillage and waste grease models respectively, describe a significant fit of the predicted models to the experimental data, suggesting that most of the total variation about the mean is explained by the regression. Figures 13.14 c) and 13.15 c) clearly illustrate a good correlation of predicted methane yield to the experimental data.

The residuals from the experimental and predicted methane yields are illustrated in Figures 13.14 d) and 13.15 d) for thin stillage and waste grease, respectively. These two residuals plots illustrate there is a random scatter of residuals about the zero-line for much of the data, suggesting the fitted models are significant at predicting the experimental data at the centre (0), upper and lower factorial (+1, -1) and upper and lower axial (+α, -α) levels.



a) 3-Dimensional Plot of Model Predicted Methane Yields

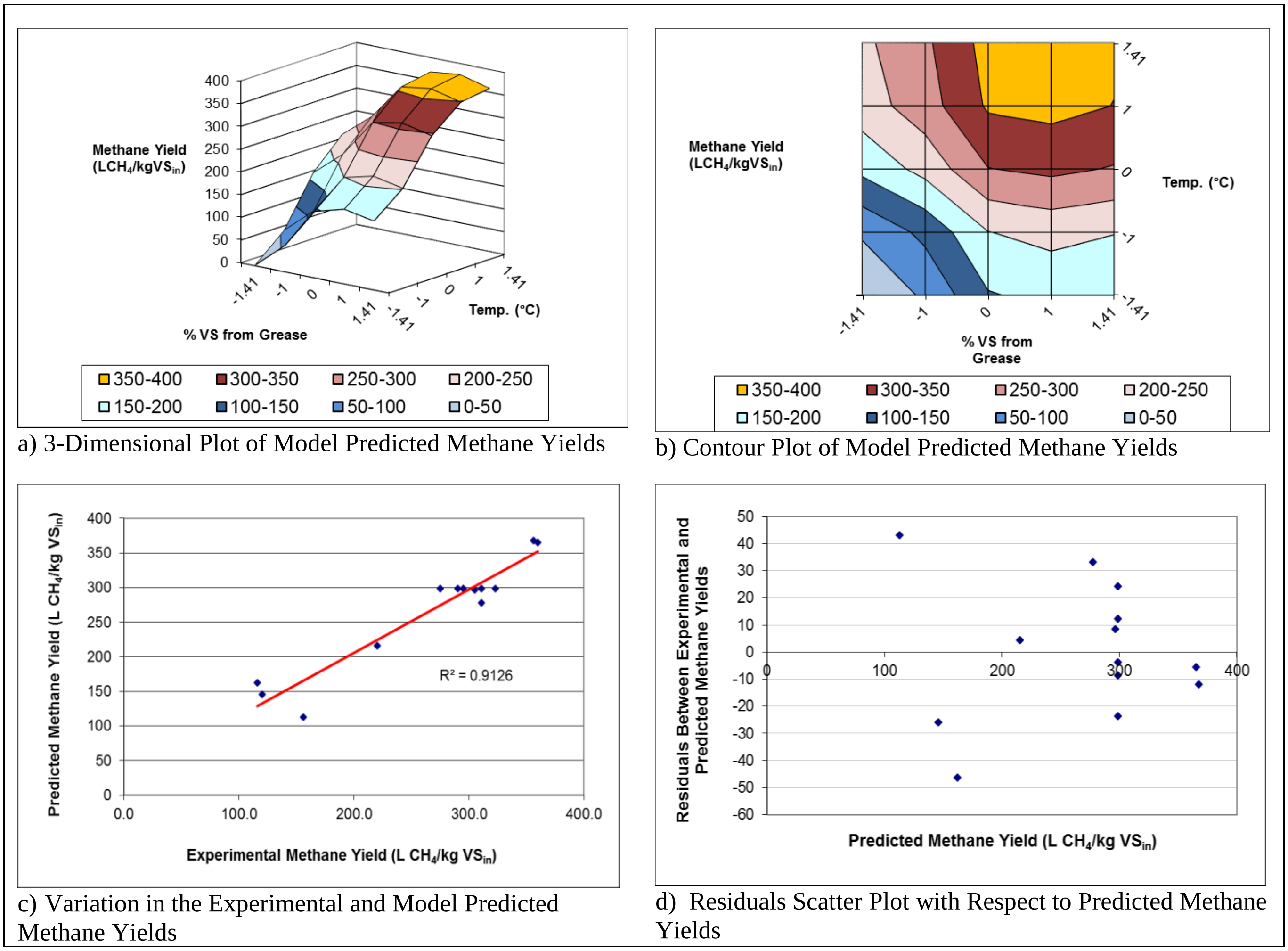
b) Contour Plot of Model Predicted Methane Yields

c) Variation in the Experimental and Model Predicted Methane Yields

d) Residuals Scatter Plot with Respect to Predicted Methane Yields

Figure 3.14 3-Dimensional and Contour Plots for Model Predicted Methane Yields (L CH4/kg VSin) with Changing Temperature and g VS from Corn Thin Stillage in Feed

Although the fitted model describes a significant fit, the adequacy of the model must also be determined. The L ratio (MS_{LF}/MS_{PE}) of 1.3 for the thin stillage fitted model (Table 3.20) is less than the $F_{3,4,0.05}$ value of 6.59 (Burns, 2010), suggesting model adequacy (no lack of fit). A similar observation was made for the waste grease model, where the L ratio of 5.8 is less than the $F_{3,4,0.05}$ value of 6.59 and the model is considered adequate (no lack of fit).



a) 3-Dimensional Plot of Model Predicted Methane Yields

b) Contour Plot of Model Predicted Methane Yields

c) Variation in the Experimental and Model Predicted Methane Yields

d) Residuals Scatter Plot with Respect to Predicted Methane Yields

Figure 3.15 3-Dimensional and Contour Plots for Model Predicted Methane Yields (L CH4/kg VSin) with Changing Temperature and g VS from Waste Grease in Feed

The 3-dimensional and contour plots illustrate similar responses for the thin stillage and waste grease models. Optimum methane yields are observed at temperature design levels ranging from 0 (35°C) to +1.41 (56°C) and % VS from co-substrate in feed from co-substrate design levels ranging from 0 (35 % of feed VS) to +1.41 (59 % of feed VS). Overall methane yields are predicted to be higher with the waste grease model. The model also predicts grease co-digestion to be more responsive at lower temperatures than co-digestion using thin stillage.

The two fitted model equations, Eq. 3.2 and 3.5, have higher β_2 model parameters than β_1, suggesting more weight is placed on the X_2 variable (temperature) in contributing to the model response (methane yield). Also, the relatively low β_{12} model parameter suggests there is minimal interaction between the X_1 and X_2 variables in determining methane yields, and these variables are more independent on their influence on yield. It should be noted that the central composite design models established for corn thin stillage co-digestion and waste grease co-digestion may be more useful in determining trends in methane yields and less important are the absolute values of the yields.

3.4 Conclusions

The main objective of this study was to evaluate the use of various substrates in co-digestion with liquid dairy manure. Grease based wastes have been the co-substrates of choice for Ontario on-farm digesters; however, as the industry grows other types of substrates need to be considered. The major conclusions from this study include:

1. Energy crops like corn silage, switchgrass and wheat straw were effective at significantly increasing biogas yields in co-digestion of dairy manure when compared to digesting manure alone. Woodchips decreased biogas production which suggest inhibition or non-biodegradability. Design HRTs of greater than 40 days were determined to be essential when co-digesting cellulosic substrates. At optimum biogas productions, VS reductions were just under 50%, suggesting O. Reg. 267/03 requirement of 50% VS reductions in on-farm digesters may not be suitable for cellulosic substrates.
2. Co-digestion of dairy manure with DAF sludge and waste grease produced the highest biogas yields. Co-digestion was most effective when these substrates represented 50% of the feed VS but when the fraction of grease based substrate increased to 75% biogas yields decreased. Design HRTs of at least 25 days were determined necessary for grease based substrates and VS reductions of 50% were achievable.
3. Grocery food waste, fish offal and corn thin stillage all increased biogas yields when co-digested with manure. Biogas production for these substrates was slow but steady. Design HRTs of 25 to 30 days were determined suitable for industry waste substrates.
4. Box-Wilson Central Composite Circumscribed Models were established for both co-digestion of dairy manure with corn thin stillage and co-digestion with grease waste. Both models suggest methane yields optimize with increasing proportion of the feed VS from co-substrates (constant total VS in all assays) and increasing temperatures.

Although the models suggest the proportion of feed VS from co-substrate to be positively correlated to methane production, temperature was shown to have a great influence on yields. The models also suggest there is minimal interaction between temperature and proportion of feed VS from co-substrates on methane yields.

Acknowledgements

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Chapter 4 - Enhanced Biogas Production Using Single and Two-Phase Anaerobic Digestion Systems: Co-digestion of Corn Thin Stillage with Liquid Dairy Manure and Waste Grease with Liquid Dairy Manure

Abstract

The co-digestion of corn thin stillage with liquid dairy manure and waste grease with dairy manure demonstrated to enhance biogas production. The implementation of a two-phase digestion system enhanced biogas production even further. Methane yields were increased by over 22% using two-phase digestion systems compared with single-phase, for both corn thin stillage and waste grease co-digestion. Based on current Feed-in-Tariff (FIT) contracts of 18 to 20¢/kWhe, the increased electricity and heat production could make the two-phase system economically attractive for producers. Organic loading rates (OLRs) over 4.4 g volatile solids (VS)/L led to digester upset and OLRs of over 4.2 g VS/L·day are not recommended. The sudden peak and continued rise of intermediate alkalinity (IA)/partial alkalinity (PA) was an effective indicator of impending digester upset. Significant VS reductions of 51 to 81% were obtained in the co-digestion of corn thin stillage and waste grease with liquid dairy manure. Final digestate VS concentrations were over 50% lower than raw dairy manure. 1 to 3 log CFU/100 mL reductions in pathogen indicators (*E.coli* and *Salmonella*) were observed in digestate when compared with raw dairy manure. The unfavorable accumulated acids and low pH in the acid reactor (two-phase system) helped achieve the higher log reductions in *E.coli* but did not appear to have as significant an impact on *Salmonella* die-off. NH_4^+-N/TKN ratios ranged from 0.6 to 0.8 in digestate from single-phase digestion (3 to 4 times higher than raw manure); however, the two-phase system made little impact on increasing this ratio of nutrient solubility.

Keywords: single-phase digestion, two-phase, organic loading rates, residual biogas potential, VS reductions, pathogen die-off, nutrients

4.1 Introduction

There are several types of anaerobic digester designs and they are typically classified as high and low rate biogas systems (Tchobanoglous and Burton, 1991; Burke, 2001; Gerardi, 2003; Bachmann, 2013). High rate digesters are designed to retain solids and keep the methanogenic microbial consortia in the reactor for a longer period (> SRT), thereby achieving process stability with increased conversion of organic matter to biogas. Digester systems considered to be high rate biogas producers include fixed film, sludge blanket (e.g. UASB) and ABR digesters. Burke (2001) suggests effective high rate reactors have a SRT/HRT greater than 3. The design of high rate digesters is best suited for more diluted wastes with high soluble organics and less particulate matter, considering the microbial community has little opportunity to hydrolyze and degrade particulate solids (Gerardi, 2003). Low rate systems, where SRT/HRT equal 1, have been identified as most effective for the digestion of manures and other agricultural residues; sufficient HRT allows for hydrolysis of high solids and particulate matter fraction. Literature identifies low rate digester systems such as covered lagoons, heated plug flow reactors and heated completed mixed reactors as the best suited systems for agricultural substrates (Burke, 2001; US EPA, 2004; Krich et al., 2005; Lazarus, 2008; Bachmann, 2013).

Phased digester systems have been implemented to enhance biogas and methane production. A two-phase system essentially consists of two anaerobic digesters operating in series, where the objective is to separate the two main microbial processes of anaerobic digestion: acidogenesis and methanogenesis. The first reactor operates at a much shorter HRT, which can be as short as one tenth the HRT of single-phase systems, and the second digester at higher HRTs of over 15 days (Gerardi, 2003; Krich et al., 2005). Two-phase systems are also referred to as two-stage systems in the literature. In a single-phase digester, the pH and OLR are optimized to suit the requirements of the more sensitive and slow growing methanogenic organisms, while these conditions may not be ideal for the faster growing acidogens (Demirer and Chen, 2005; van Haandel and van der Lubbe, 2007). In a two-phase digestion process, operating parameters are optimized to favour the respective bacterial growth stage: very short HRTs for VFA production and low pH in the first phase, and long HRTs with conversion of VFAs to methane at neutral pH in the second phase (Zhang et al., 2000; Demirer and Chen, 2005; Krich et al., 2005). The overall objective of the research work presented in this thesis chapter was to investigate the use of a two-phase digestion system to enhance co-digestion of liquid dairy manure with corn thin stillage and co-digestion of liquid dairy manure with waste grease, and determine the effects on biogas and methane yields, process stability and digestate quality.

In a two-phase system, the digestion process becomes more stable since the acidification stage is controlled, while overloading and build-up of toxic materials such as VFAs that can occur in single-phase systems are prevented (Sung and Santha, 2003; Demirer and Chen, 2005; Yilmaz and Demirer, 2008). Demirer and Chen (2005) go on to explain the first phase can protect methanogens from overloads and possible pH shock; and, the low pH, high OLR and short HRT of the first phase favour acidogenic bacteria and prevent the growth of methanogens. Literature has shown that depending on the waste type the process can be smaller in size and ultimately more cost effective (Zhang et al., 2000; Demirer and Chen, 2005; Krich et al., 2005; Yilmaz and Demirer, 2008). TPAD is a variation of the two-phase system, where the first phase acidogenesis process is conducted under thermophilic temperatures and the second methanogenic phase is conducted under mesophilic conditions. The TPAD system was designed to optimize methane recovery and pathogen destruction. Iowa State University has patented (U.S. Patent No. 5,746,919) the TPAD system for treatment of municipal sludges; first stage digestion operates at 56^{o}C with a HRT of up to 10 days and the second stage digestion operates at 35^{o}C with a HRT of up to 20 days (Pillars, 2002; Katers and Schultz, 2003). Biogas production can be increased by 25% using TPAD systems (Katers and Shultz, 2003). Sung and Santha (2003; 2001) determined optimal HRTs of 4-6 days for the thermophilic stage and 10-15 days for the mesophilic stage of TPAD systems digesting cattle slurries using completely mixed reactors for each stage.

Dugba and Zhang (1999) investigated the treatment of screened liquid dairy manure using two sets of two-phase ASBR systems. One two-phase system operated both reactors at 35 ± 1 ^{o}C and the second two-phase system operated as a TPAD system. Five OLRs (2, 3, 4, 6 and 8 g VS/L/day) were investigated, where each OLR was operated at HRTs of 3 and 6 days. The authors reported that both phased systems were highly dependent on HRT and VS loading rate and each system failed at a 3-day HRT when the OLR reached 6 g VS/L/day. The study demonstrated that the TPAD system yielded higher percent VS removals and specific methane production rates than the mesophilic-mesophilic phased system. At OLRs of 2, 3 and 4 g VS/L/day, the % VS removal and specific methane production rate of the TPAD system were 43.8 to 44.1% and 0.41 to 0.82 L CH_4/L/day, respectively, for the 3-day HRT and 37.1 to 38.9% and 0.34- to 0.72 L CH_4/L/day, respectively, for the 6-day HRT. The authors also reported that the TPAD system was better at increasing die-off of total coliforms, with non-detectable counts in the effluent.

Sung and Santha (2001) conducted a study investigating the use of a TPAD system treating dairy manure with various TS concentrations (3.5 to 14.5 %TS). The TPAD system was operated using completely mixed reactors with HRTs of 4 days for the thermophilic (58^{o}C) reactor and 10 days for the mesophilic (38^{o}C) reactor, and with OLRs ranging from 1.87 to 7.70 g VS/L/day. Optimum VS removal of 42.6% was achieved at feed influent of 10.35 %TS and OLR of 5.82 g VS/L/day. Methane yields ranged from 0.54 to 0.61 L CH_4/g $VS_{destroyed}$ and pathogen destruction was high with effluent *Salmonella* concentrations < 2 MPN/4g TS.

Demirer and Chen (2004) recognized that much of the phased digestion studies presented in the literature were conducted using screened or diluted dairy manure. The authors therefore investigated the use of a two-phase anaerobic digestion system for the treatment of unscreened dairy manure, with emphasis on the effects of HRTs and OLRs on digestion performance; HRTs ranged from 1.25 to 4 days and OLRs ranged from 4 to 30 g VS/L/day under mesophilic conditions (35 ± 1^{o}C). When OLRs ranged from 4 to 10 g VS/L/day, VS reductions ranged from 30.3 to 62.4 % removal and methane yields ranged from 0.066 to 0.243 L CH_4/g VS_{added}. However, at the higher OLRs of 20 to 30 g VS/L/day a relatively lower digestion performance was observed which may be due to the possible wash-out of the acidifiers at the much lower HRTs of 1.25 to 4 days. The authors suggest that the performance at the higher OLRs can be improved by adding a recycle of biomass to the acidification reactor for an increased SRT.

Optimum operating conditions for anaerobic acidification of unscreened dairy manure were conducted by Yilmaz and Demirer (2008). HRTs ranging from 1.25 to 4 days and OLRs ranging from 5 to 15 g VS/L/day were investigated. The acidogenic reactor effluents were subsequently fed to methanogenic reactors, where both reactors were operated under mesophilic conditions

($35 \pm 1^{o}C$). The degree of acidification was quantified using the percentage of the initial substrate concentration converted to VFAs. The authors reported that a HRT of 2 days and an OLR of 15 g VS/L/day yielded maximum acidification. The average pH value in the acidogenic reactors was within 6.2 and 6.6, which is considered somewhat high for the acidogenesis process.

Nielsen et al. (2004) compared a two-phase thermophilic anaerobic digestion system of cattle manure (5.5 %TS) with a single-phase thermophilic digestion system ($55^{o}C$; HRT = 15 days). The two-phase system consisted of a digester operating at $68^{o}C$ with a HRT of 3 days connected to a $55^{o}C$ reactor with a 12-day HRT. The authors proposed that using a two-phase system with a relative high thermophilic temperature would involve a broader spectrum of thermophilic lignocellulose-degrading bacteria. Both the two-phase and single stage systems were operated at an OLR of 3 gVS/L/day and the specific methane yield was determined to be 8% higher with the two-phase system; specific methane yield of the two-phase system was 0.26 ± 0.1 L CH_4/g VS (47.1 %VS removal) and that of the single stage system was 0.24 ± 0.1 L CH_4/g VS (43.3 %VS removal). The authors concluded from their study that the bacterial community in the $68^{o}C$ reactor was stable and was not negatively affected by changes in HRT or feedstock quality. Kaparaju et al. (2009) concluded from their study that the main reason for improved biogas production in two- phase digestion was due to a more optimal retention time distribution for particulate matter than the nominal average retention time, thereby minimizing the loss of relatively fresh feed due to short-circuiting which they suggest is commonly noticed in CSTRs.

Although some research work has been conducted to investigate the use of two-phase systems in enhancing the digestion of manure, more work is required in fully understanding the applicability of these systems for agriculture based digesters. Pillars (2002) suggests the TPAD system tends to work best with more dilute manures, while Yilmaz and Demirer (2008) and Demirer and Chen (2004) reported that a phased digestion process can handle manures with higher solids content (> 10% TS). However, Krich et al. (2005) go even further to suggest a two-phase process is not applicable to dairy wastes at all, stating two-phase systems are only applicable to soluble and readily metabolized sugars and starches, possibly some fats and protein, but not to the more difficult to digest particulate and fibrous materials. The authors indicate the dairy manure in the two-phase system would result in no H_2 production in the first reactor thereby reducing the conversion of CO_2 to CH_4. However, this was the only reference in the review of literature that stated a two-phase system was not appropriate for dairy manure. Although there has been some work conducted on using a two-phase digestion system for manure, there has been very little work conducted on investigating the use of a two-phase system in co-digestion manure with organic rich substrates. The objective of this study was to help address this gap of knowledge by determining the effectiveness of two-phase systems in co-digesting manure with co-substrates high in soluble organics.

The research work presented in this chapter investigates the use of corn thin stillage and waste grease as co-substrates to enhance biogas production in manure digesters. The study also investigates the use of a two-phase mesophilic digestion process to enhance the co-digestion of liquid dairy manure with co-substrates (corn thin stillage and waste grease). The use of anaerobic digestion as a treatment for manure and off-farm wastes was an important component of this study and digestate quality was investigated (as compared to raw manure for land application). The applicability of such systems and possible recommendations for the Ontario agricultural biogas industry was also considered.

4.2 Materials and Methods

4.2.1 Description of Bench Scale Digesters

Bench-scale digestion trials operated under semi-continuous mesophilic conditions were conducted to optimize the production of biogas and methane, along with degradation of organic matter, from the co-digestion of dairy manure with thin stillage and waste grease. The bench-scale digesters were operated under single-phase and two-phase digestion.

Four 30 L digesters (24 L liquid operating volume) were operated under simultaneous trials throughout the study. These digester vessels were used as the single-phase digesters and the methanogenic phase digesters of the two-phase systems. A smaller 10 L reactor (up to 8 L operating volume) was used for the acidogenesis reactor of the two-phase system. The operating volume of the acid reactor was allowed to change to accommodate a 4-day HRT for the various trials. The bench-scale digesters were made of machined clear Plexi-Lexan (0.95 cm thickness), with dimensions of 30.5 cm (12 inches) internal diameter and 40.6 cm (16 inches) in height. The reactor contents were intermittently mixed using a three-paddle stainless steel impeller for mixing. Impeller rotation was controlled by a Cole-Parmer (Model No. 7553-70) motor and the intermittent mixing (RPM of 45 to 55) had a daily cycle of 3 hours on and then off for 1 hour. All biogas was collected from the reactors and volume measured using a calibrated wet tip meter. Refer to Figure 4.1 for a schematic of the lab digesters. The reactors were housed in a 2.4 m (8 ft) x 1.8 m (6 ft) insulated heat room, where base board heaters and an air blower were used to maintain an ambient room temperature of 36 ± 2°C for mesophilic operation. The heat room and reactors are pictured in Figure 4.2. For health and safety purposes, the heat room was equipped with gas monitors and alarms for CH_4, O_2 and H_2S.

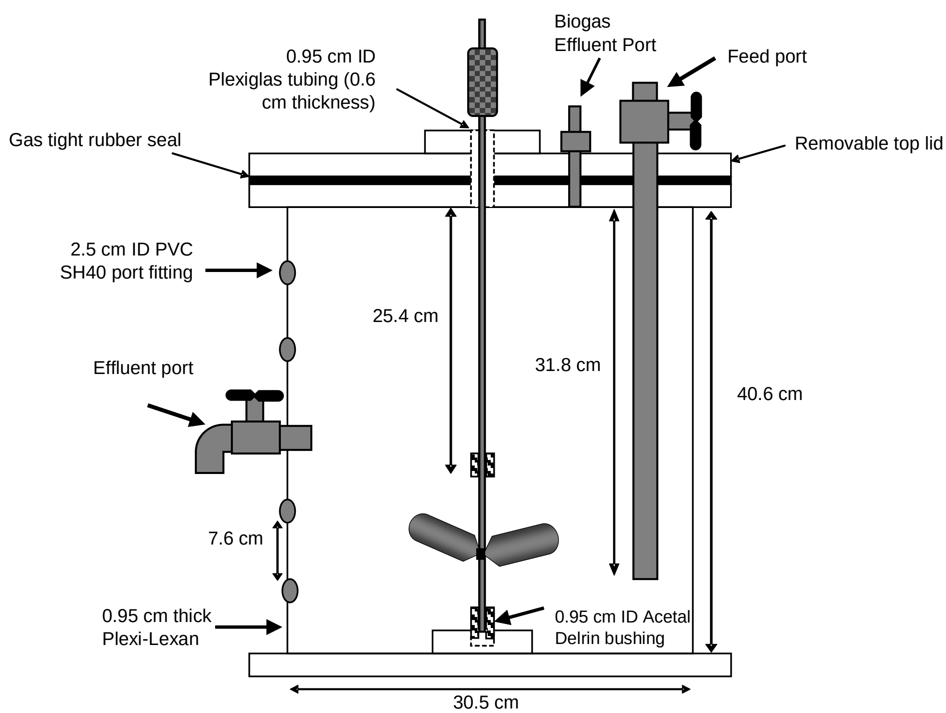


Figure 4.1 Schematic of 30 L Bench-Scale Anaerobic Digester

Biogas samples were collected for CH_4 and CO_2 composition analysis by attaching a Tedlar® bag to the gas outlet of the reactor and the bag allowed filling. A Hamilton Gas-tight, Leak-free syringe was used to collect a sample of the biogas for injection into the GC for CH_4 and CO_2 percent composition analysis. Refer to Section 4.2.4 for a description of the CH_4 and CO_2 gas composition analysis.

a) Reactor Feeding Tube

b) Bench-scale Reactors

c) Heat Room for Mesophilic Incubation (36±2°C)

Figure 4.2 Photos of Bench-scale Reactors and Heat Room

4.2.2 Characteristics of Liquid Dairy Manure, Inoculums and Co-substrates (Thin Stillage and Waste Grease)

The liquid dairy manure used for this study was collected from the University of Guelph - Campus d'Alfred (Alfred, ON) organic dairy farm. At the time of this study, the Alfred dairy farm had a total herd size of 60 Holstein cows (45 cows in lactation and 15 cows in gestation). The feed ratio for the cows was 40% grain and 60% hay/grass, a typical feed for organic dairy cows. The bedding used in the barn was composed primarily of straw, with small quantities of fine wood shavings. The manure collection system at the barn consisted of grated floors that allowed faeces and urine to be collected in a concrete pit beneath the barn. Washwaters from barn floors and the milking parlour were also added to the in-barn manure pit, thereby making the Alfred dairy barn a liquid manure operation. Liquid manure used to feed the reactors was collected every two weeks from the in-barn pit and large pieces of straw were screened from the manure using a 1.5 x 1.0 cm (mesh opening) stainless steel sieve. The manure was stored refrigerated (4 ± 1°C) until needed for digester feeding. Any feed manure that lasted longer than two weeks was discarded. It should be noted the liquid dairy manure from the Alfred in-barn pit was slightly more diluted than typical liquid dairy manure from Ontario farms, which can typically range between 5 and 12 %TS (OMAFRA, 2009). However, for the purposes of this study the use of liquid manure with slightly lower solids was ideal in alleviating operational concerns of potential clogging of valves and incomplete mixing.

Each new batch of liquid manure collected from the Alfred dairy barn was analyzed for pH, alkalinity, TS, VS, tCOD, sCOD, pCOD, TKN, NH_4^+-N, Organic N, TP, VFAs and pathogen indicators (*E.coli* and *Salmonella*). Section 4.2.4 describes the laboratory methods used for the analyses listed. Monthly samples were taken of the liquid manure from the in-barn pit between January and June 2009, and the average TS, VS and tCOD concentrations were 42 g/L (4.2 %TS), 29 g/L (2.9 %VS) and 32 g/L, respectively. The average TS concentration in liquid dairy manure from Ontario farms is 84 g/L (8.4 %TS) and typically ranges from 6 to 12 %TS. Table 4.1 summarizes average concentrations of physical and chemical parameters characterizing the liquid dairy manure used in this study.

Table 4.1 Average Concentrations of Physical and Chemical Parameters Characterizing

Parameter	Liquid Dairy Manure	Mesophilic Inoculum	Thin Stillage	Waste Grease
n	95	22	88	64
pH	7.21 (0.48)[1]	7.88 (0.23)	4.22 (0.35)	5.01 (0.12)
Alkalinity (g $CaCO_3$/L)	5.8 (0.4)	9.7 (0.6)	0	0.8 (0.1)
TS (g/L)	54 (15)	46 (11)	104 (25)	203 (43)
VS (g/L)	41 (10)	33 (14)	82 (19)	181 (45)
VS/TS (%)	76	72	79	89
tCOD (g/L)	65.2 (15.4)	34.9 (18.2)	127.6 (44.1)	236.8 (43.5)
sCOD (g/L)	18.3 (4.3)	5.4 (2.5)	54.4 (17.5)	67.3 (19.5)
pCOD (g/L)	52.9 (12.7)	29.5 (8.7)	73.2 (30.6)	101.6 (36.7)
TKN (g/L)	1.8 (0.6)	3.7 (0.7)	2.6 (0.9)	3.1 (0.4)
Organic-N (g/L)	1.1 (0.4)	1.3 (0.5)	2.5 (0.6)	2.3 (0.4)
NH_4^+-N (g/L)	0.7 (0.2)	2.4 (0.3)	0.1 (0.06)	0.8 (0.2)
TP (g/L)	0.5 (0.1)	0.4 (0.2)	1.3 (0.3)	0.3 (0.2)
TK (g/L)	1.9 (0.4)	2.0 (0.2)	2.2 (0.4)	---
Total VFAs (g/L)	2.73 (0.48)	0.49 (0.23)	1.30 (0.52)	3.81 (0.62)
Acetic Acid (g/L)	1.64 (0.66)	0.31 (0.12)	0.82 (0.68)	1.33 (0.35)
Propionic Acid (g/L)	0.68 (0.34)	0.18 (0.16)	0.40 (0.28)	1.86 (0.58)
Butyric Acid (g/L)	0.38 (0.21)	<0.05[2]	0.07 (0.03)	0.62 (0.09)
Fat[3] (g/kg)	---	---	---	117 (27)
Protein[3] (mg/kg)	---	---	16 (4)	18 (5)
Carbohydrates[3] as glucose (mg/kg)	---	---	23 (3)	27 (11)
Ash[3] (mg/kg)	---	---	---	6 (1)
Arsenic (As)[3] (mg/kg)	---	---	---	<0.7
Cadmium (Cd)[3] (mg/kg)	---	---	<0.005	<0.9
Chromium (Cr)[3] (mg/kg)	---	---	0.021	17 (2)
Cobalt (Co)[3] (mg/kg)	---	---	<0.005	<1
Copper (Cu)[3] (mg/kg)	---	---	0.165	1870 (58)
Lead (Pb)[3] (mg/kg)	---	---	<0.01	<10
Mercury (Hg)[3] (mg/kg)	---	---	---	<0.05
Molybdenum (Mo)[3] (mg/kg)	---	---	0.017	<2
Nickel (Ni)[3] (mg/kg)	---	---	0.013	10 (2)
Selenium (Se)[3] (mg/kg)	---	---	---	<0.5
Zinc (Zn)[3] (mg/kg)	---	---	1.77	665 (21)

1 Standard deviations are presented in parentheses
2 Less than detection limit of 0.05 g/L
3 Analyses conducted periodically; n=6

The source of mesophilic inoculum used for this study was the effluent digestate from the Terryland Farms (St. Eugene, Ontario) mesophilic on-farm digester. The Terryland digester is a single tank system ($1000 m^3$) and operates under mesophilic conditions of 39 ± 1 °C. The digester was fed with liquid dairy manure and grease (75% waste grease from bacon rendering and 25% grease trap waste) at a daily feeding rate of 16 m^3 of manure and 4m^3 of grease, resulting in an OLR of 1.94 kg VS/m^3·day. To maintain the viability of the inoculum prior to being used in laboratory trials, the digestate was placed in 10 L carboys (4 L headspace), incubated at 36 ± 2^{o}C and agitated at 120 rpm using a Cole Parmer orbital shaker. The inoculum was fed with fresh dairy manure and waste grease (75:25 v/v) at an OLR of 1.8 kg VS/m^3·day. The inoculum was only used to seed the bench scale anaerobic reactors at the start of the study, where each reactor received 12 L of inoculum and 12 L of fresh dairy manure and allowed to incubate for 3 months period (with bi-weekly feeding of fresh dairy manure). Once the reactors established an active anaerobic microbial consortium the reactors were ready for bench-scale trials. Refer to Table 4.1 for a characterization of the mesophilic inoculum.

Corn thin stillage was obtained from GreenField Ethanol corn ethanol plant in Johnstown, Ontario. The Johnstown plant operates with an annual feedstock of 20 million bushels of corn and produces 254 million litres of fuel ethanol per year. WDG is produced at the plant and 154,000 tonnes is used on farms annually as animal feed. Thin stillage was collected from the plant and delivered back to the lab within 4 to 6 hours. Stillage was collected in ten 20 L pail batches every 4 to 5 months. Upon arrival at the lab, the pails of thin stillage were immediately frozen at -15 ± 2^{o}C for later use. When thin stillage was needed for laboratory experiments the required number of pails were removed from the freezer and allowed to thaw in the refrigerator at a temperature of 4 ± 1^{o}C. Because of the sugar content in stillage, pails of raw material were never allowed to stand at room temperature for long periods of time as mold could grow on the liquid surface as quickly as overnight. Each new batch of thin stillage collected from the GreenField plant was analyzed for pH, alkalinity, TS, VS, tCOD, sCOD, pCOD, TKN, NH_4^+-N, Organic N, TP, VFAs and pathogen indicators (*E.coli* and *Salmonella*). The characterization of corn thin stillage is summarized in Table 4.1.

The waste grease for this study was supplied by Organic Resources Management Inc. (ORMI). ORMI is one of the largest grease collection companies in Ontario and services over 6000 customers. Waste grease was collected by ORMI, using vacuum trucks, from grease interceptors at industrial and commercial facilities, including: food processing, bakeries, food services, grocery stores, restaurants and hotels. The collected grease was then transported to its Ottawa location to be dewatered and uniformly blended. The dewatered grease was transported to the lab in 20 L pails. Each new batch of waste grease received from ORMI was analyzed for pH, alkalinity, TS, VS, tCOD, sCOD, pCOD, TKN, NH_4^+-N, Organic N, TP, VFAs and pathogen indicators (*E.coli* and *Salmonella*). Refer to Table 4.1 for a characterization of the ORMI waste grease.

4.2.3 Bench-scale Co-digestion Trials using Liquid Dairy Manure with Corn Thin Stillage and Liquid Dairy Manure with Waste Grease – Single and Two-phase Digestion

The digestion of co-substrate (either corn thin stillage or waste grease) and liquid dairy manure was investigated using both a single-phase digestion process and a two-phase digestion process. In the single-phase operation, the digestion process proceeded with both the acidogenesis and methanogenesis stages taking place in the same reactor under mesophilic conditions (36 ± 2°C). Refer to Figure 4.3 for a schematic of the laboratory setup for the single-phase digestion setup. Co-substrate and manure were manually fed to the digester, as a pump would not have been suitable due to clogging potentials. The reactor codes were R1 and R3 for the single-phase co-digestion of corn thin stillage and waste grease with liquid dairy manure, respectively.

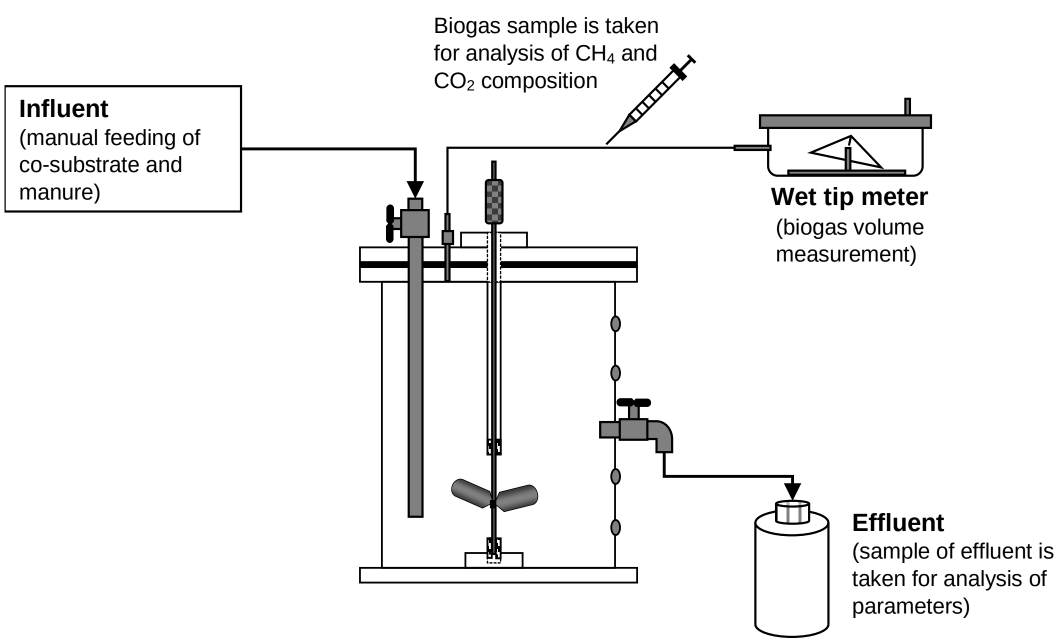


Figure 4.3 Singe Phase Digestion of Liquid Dairy Manure and Co-substrate

For the two-phase digestion process, the acidogenesis and methanogenesis stages were separated into two distinct digesters, where the effluent of the acidogenesis reactor was fed to the methanogenesis reactor and both reactors operated under mesophilic conditions (36 ± 2^{o}C). Raw substrates and acidogenesis effluent were manually fed to the acidogenesis and methanogenesis reactors, respectively. The reactor codes were R2 and R4 for the two-phase co-digestion of corn thin stillage and waste grease with liquid dairy manure, respectively. Refer to Figure 4.4 for a schematic of the two-phase system.

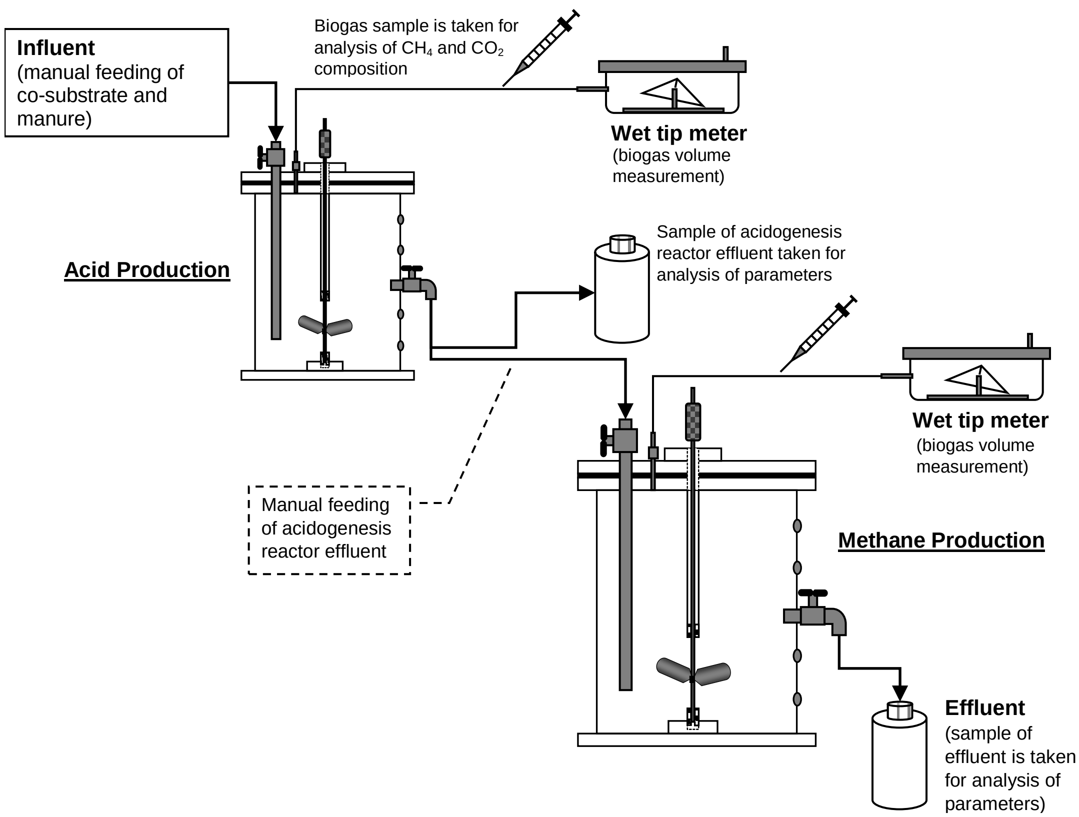


Figure 4.4 Two-phase Digestion of Liquid Dairy Manure and Co-substrate

Table 4.2 summarizes the OLRs and HRTs for the trials using thin stillage and liquid dairy manure. The OLRs ranged between 1.67 and 4.40 gVS/L·day, with total HRTs ranging from 30 down to 15 days. Proportions of thin stillage and liquid dairy manure in the feedstock were varied between volumetric ratios of 25:75 and 50:50 (proportion limits governed by Ontario regulations O. Reg. 267/03). The volumetric proportions of thin stillage and dairy manure were adjusted so HRTs were never lowered beyond 15 days.

Table 4.3 summarizes the OLRs and HRTs for trials using waste grease and liquid dairy manure. The OLRs ranged between 1.83 and 4.60 g VS/L·day and HRTs ranged from 45 to 25 days. As previously mentioned for the thin stillage trials, proportions of waste grease and liquid dairy manure in the feedstock were varied between volumetric ratios of 25:75 and 50:50 (proportion limits governed by Ontario regulations O. Reg. 267/03). Proportions of waste grease and dairy manure were adjusted so HRTs never decreased beyond 25 days.

As described in Section 4.2.2, all digesters were seeded with inoculum and operated under mesophilic conditions (36 ± 2°C) for 3 months (while being fed with fresh dairy manure) to establish a baseline microbial consortium. At the start of the experiment, all four digesters were operated as single-phase mesophilic systems and fed semi-continuously with their respective feedstock mixtures of dairy manure and thin stillage, or dairy manure and waste grease. Once the digesters reached steady state conditions, R2 and R4 began operations as two-phase systems (Trials III). For all trials, reactors were operated until reaching steady-state conditions and then maintained an additional 1 to 2 HRTs at steady-state. Steady state was defined as less than 5% variation in daily biogas production over a one to two week period (Dugba and Zhang, 1999). A steady state of 2 HRTs was desirable; however, 1 HRT was used in some cases due to time constraints.

Prior to commencing digestion trials acidification experiments were conducted to determine the optimum HRT for acidogenesis reactors. The acidogenesis reactors were operated at various OLRs (ranging from 10 to 20 g VS/L·day) and VFA concentrations (acetic, propionic and butyric acids) measured. Optimum HRTs were determined as a function of OLRs and VFA production, and were found to be 4 and 5 days, respectively, for thin stillage and waste grease acid reactors. pH in acid reactors were maintained at between 5.24 and 6.23.

Table 4.2 Single-Phase and Two-Phase Operating Conditions for R1 (Thin Stillage - Single-Phase) and R2 (Thin Stillage - Two-Phase)

Trial	System OLR[1] (g VS/L·day)	% Stillage (by volume)	% Manure (by volume)	HRT in Acidogenic Phase (days)	HRT in Methanogenic Phase (days)	Total HRT (days)
Trials for R1:						
I	1.67	25	75	0	30	30
II	2.08	25	75	0	24	24
III	2.38	25	75	0	21	21
IV	2.38	25	75	0	21	21
V	3.00	50	50	0	20	20
VI	3.30	50	50	0	18	18
VII	4.00	50	50	0	15	15
VIII	4.13	55	45	0	15	15
IX	4.40	60	40	0	15	15
Trials for R2:						
I	1.67	25	75	4	26	30
II	2.08	25	75	4	20	24
III [2]	2.38	25	75	4	17	21
IV	2.38	25	75	4	17	21
V	3.00	50	50	4	16	20
VI	3.30	50	50	4	14	18
VII	4.00	50	50	4	11	15
VIII	4.13	55	45	4	11	15
IX	4.40	60	40	4	11	15

1 OLR delivered to entire digestion system
2 Trial where reactor transitions from single-phase to two-phase

Daily biogas volumes were recorded from the wet tip meters of the respective digesters, while biogas composition (CH_4, CO_2) was measured periodically. When digesters transitioned to a new organic loading rate, effluent samples were collected every two days and analyzed. During steady state periods of digestion, effluent samples were collected every two days and weekly composite samples prepared and analyzed. All substrate and digester effluent samples were analysed for pH, alkalinity, tCOD, pCOD, sCOD, TS, VS, VFAs, TKN, total ammonium (NH_4^+-

N + NH_3-N), TP, o-PO_4^{3-} and bacteria indicators (*E.coli* and *Salmonella*). Residual Biogas Potential (RBP) tests were also conducted during steady state digestion of the four reactors. Refer to Section 4.2.4 for a description of the analytical methods used in this study.

Table 4.3 Single-Phase and Two-Phase Operating Conditions for R3 (Waste Grease - Single-Phase) and R4 (Waste Grease - Two-Phase)

Trial	System OLR[1] (g VS/L·day)	% Grease (by volume)	% Manure (by volume)	HRT in Acidogenic Phase (days)	HRT in Methanogenic Phase (days)	Total HRT (days)
Trials for R3 (Single-Phase):						
I	1.83	25	75	0	45	45
II	2.12	25	75	0	39	39
III	2.36	25	75	0	35	35
IV	2.36	25	75	0	35	35
V	2.75	25	75	0	30	30
VI	3.44	25	75	0	24	24
VII	3.93	25	75	0	21	21
VIII	4.11	50	50	0	28	28
IX	4.60	50	50	0	25	25
Trials for R4 (Two-Phase):						
I	1.83	25	75	5	40	45
II	2.12	25	75	5	34	39
III [2]	2.36	25	75	5	30	35
IV	2.36	25	75	5	30	35
V	2.75	25	75	5	25	30
VI	3.44	25	75	5	19	24
VII	3.93	25	75	5	16	21
VIII	4.11	50	50	5	23	28
IX	4.60	50	50	5	20	25

1 OLR delivered to entire digestion system

2 Trial where reactor transitions from single-phase to two-phase

4.2.4 Analytical Methods

Table 4.4 describes the analytical methods used in the study. All substrate and digestate samples were analysed for pH, alkalinity, TS, VS, tCOD, sCOD, pCOD, TKN, total ammonium (NH_4^+-N + NH_3-N), TP, o-PO_4^{3-}, VFAs and pathogen indicators. Digestate samples collected during steady state conditions were also analysed for RBP. Biogas volumes and composition were also measured.

Table 4.4 Description of Analytical Methods Used for Sample Analysis

Lab Analyses	Description
pH	• Adapted from Standard Method 4500-H^+ B. (APHA, 2005)
Alkalinity	• Adapted from Standard Method 2320 B. Titration Method (APHA, 2005)
IA/PA	• Adapted from Standard Method 2320 B. Titration Method (APHA, 2005) • PA is determined for a 0.1 N H_2SO_4 titration to an endpoint of 5.75 • TA is determined for a 0.1 N H_2SO_4 titration to an endpoint of 4.50 • IA = TA - PA
TS	• Adapted from Standard Method 2540 B. Total Solids dried at 103-105°C (APHA, 2005)
VS	• Adapted from Standard Method 2540 E. Fixed and Volatile Solids ignited at 550°C (APHA, 2005)
tCOD	• Adapted from Standard Method 5220 D. Closed Reflux, Colorimetric (APHA, 2005)
sCOD	• Adapted from Standard Method 5220 D. Closed Reflux, Colorimetric (APHA, 2005) • Sample preparation: centrifugation of 15 mins. at 6230 relative centrifugal force (RCF) using a Thermo Scientific Sorvall bentop centrifuge followed by filtration with a Millipore 0.45 µm disc filter
pCOD	• Difference between tCOD and sCOD
C:N	• Total and organic carbon were measured at an external lab (University of Guelph Lab Services in Guelph, ON) • Nitrogen was measured as TKN
TKN	• Adapted from Standard Method 4500-N_{org} B. Macro-Kjeldahl Method (APHA, 2005)
Ammonium (NH_4^+ -N)	• Adapted from Standard Method 4500-NH_3 D. Ammonia – Gas Selective Electrode Method (APHA, 2005)

Organic N	• Difference between TKN and Ammonium
TP	• Adapted from Standard Method 4500-P Phosphorus (Persulfate Digestion Method) and 4500-P E. Ascorbic Acid Method (APHA, 2005)
E.coli	• Adapted from Standard Method 9222 Membrane Filter Technique for Members of The Coliform Group (APHA, 2005) • Difco m-FC Basal Medium with BCIG • Agar plate and filter incubation at 44.5 ± 0.2°C for 24 ± 2 hours
Salmonella	• Adapted from Standard Method 9222 Membrane Filter Technique for Members of The Coliform Group (APHA, 2005) • Difco SS Agar • Agar plate and filter incubation at 35 ± 2°C for up to 48 ± 2 hours
VFAs	• Adapted from method as outlined in Adorno et al. (2014) • Varian GC 3800 with FID for VFA detection • Varian Capillary Column CP-FFAP CB (25m, 0.32mm, 0.3 µm) for detection of acetic, propionic, butyric and iso-butyric acids • H_2 gas and Air used for sample combustion, while He was used as a carrier gas and N_2 gas as make-up • FID Temperature: 300°C; make-up N_2 at 20 mL/min • H_2 gas flow: 40 mL/min • Air flow: 250 mL/min • Column flow: 1.0 mL/min • Injector volume: 1 µL • Injector temperature: 260°C; split 20 • Oven temperature: 80°C (1 min) 20°C/min; 120°C (1 min) 20°C/min; 205°C (2 min); 10.25 min of chromatographic time
CH_4 & CO_2 (Biogas)	• Adapted from method as outlined in Zhou et al. (2003) • Varian CP-3800 GC with metal packed column and TCD detection • H_2 carrier gas and He balance gas • Heater: 260°C • Column oven temperature: 50°C • TCD temperature: 120°C • HayeSep® N 80/100 mesh, 0.5 m x 1/8 in. • SST Porapak® QS 80/100 mesh, 2 m x 1/8 in. • SST HayeSep® D, 80/100 mesh, 2 m x 1/8 in. SST • Column Flow (H_2): 4.8 mL/min • He reference flow: 25 mL/min • Injection volume: 1µL
RBP	• Adapted from Walker et al. (2010) and Banks et al. (2013) • Replicated assays were conducted using 500 mL Wheaton serum bottles (150 mL digestate + 75 mL inoculum; 2:1 ratio), agitated at 100 rpm and incubated under mesophilic conditions (37 ± 2 °C) for 60 days

- Inoculum source was digestate from Farm B that had undergone lab incubation and high rate feeding for 2 weeks prior to use
- Biogas measurements were taken regularly using a water displacement manometer to measure biogas accumulation in the serum bottle headspace
- Biogas composition measurements (%CH_4 and %CO_2) were taken only periodically to confirm methane production during incubation. A GC was used to measure biogas composition.

4.2.5 Analysis of Experimental Data

To normalize the biogas and methane data from the semi-continuous digestion trials, biogas yields (L biogas/g VS) and methane yields (L CH_4/g VS) were calculated. Daily biogas and methane production were divided by the VS being fed to digesters, thereby expressing biogas and methane production in terms of available organic matter. Percent VS reduction was calculated as the percent difference of the VS concentration at the start and end of the digestion period, under steady state conditions.

Standard statistical procedures were implemented. In addition to standard deviation and mean averaging, single factor ANOVA, with one-sided t-test were used to determine if significant differences exist between digestion trials. Statistical significance was established at the $p < 0.05$.

Pearson r regression analysis was used to determine correlations between two variables. Essentially the Pearson coefficient is the covariance of the two variables divided by the product of their standard deviations.

Box and Whisker plots were used for the pathogen indicator data. The plots show the degree of dispersion of the data and depicts the skewness of the data sets. The Box and Whisker plots identify the mean and median of the log cts/100mL concentrations of the bacteria data as an x and horizontal band in the middle of the box, respectively.

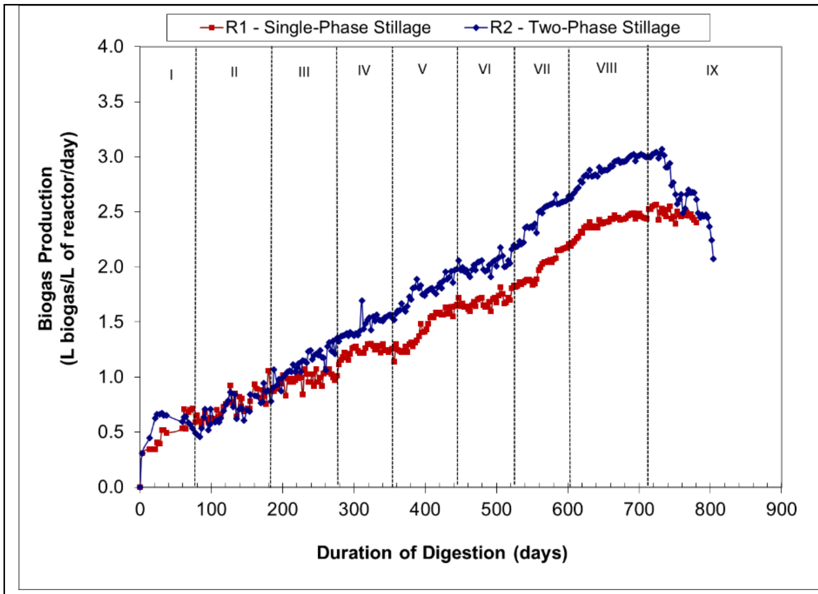
4.3 Results and Discussion

4.3.1 Biogas and Methane Production from Single and Two-Phase Co-digestion of Liquid Dairy Manure with Corn Thin Stillage and Liquid Dairy Manure with Waste Grease

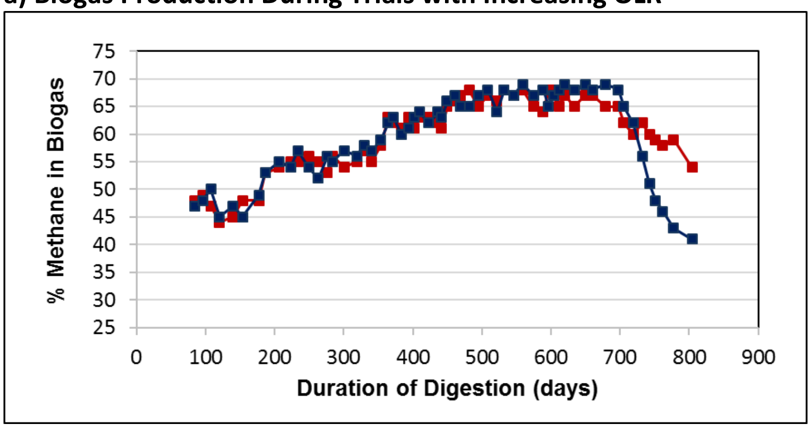
Bench-scale single and two-phase co-digestion trials were conducted for over 2 years. Conducting trials for this extended period allowed for the collection of large data sets of biogas performance and digestate quality; accounting for variability of data during steady state periods. Transition between increasing OLRs were conducted slowly so as not to stress digester performance. Digester performance parameters, including biogas production, IA/PA, pH and VFA concentrations, were monitored closely during periods of increasing OLRs.

The co-digestion of liquid dairy manure with corn thin stillage was conducted under mesophilic conditions (36 ± 2°C) using single and two-phase systems. Figure 4.5 a) illustrates biogas production with increasing OLR. When operating the single-phase system, OLRs were increased from 1.67 to 4.40 g VS/L·day and biogas production increased from 0.48 to 2.52 L biogas/L·day (HRT ranged from 30 to 15 days) under these conditions. Optimum biogas production of 2.52 ± 0.06 L biogas/L·day was reached at an OLR of 4.13 g VS/L·day and HRT of 15 days. At stable biogas production (digestion day 360 to 715), the percentage methane in biogas ranged from 62 to 68 % CH_4 (Figure 4.5 b)) and pH levels remained neutral in the reactor (Figure 4.5 c)). When organic loading further increased to 4.40 g VS/L·day (HRT of 15 days), biogas production began to plateau and the %CH_4 in biogas decreased to 49%. The pH in the reactor decreased steadily along with an increase in IA/PA (Figure 4.5 c)), indicating potential digester upset.

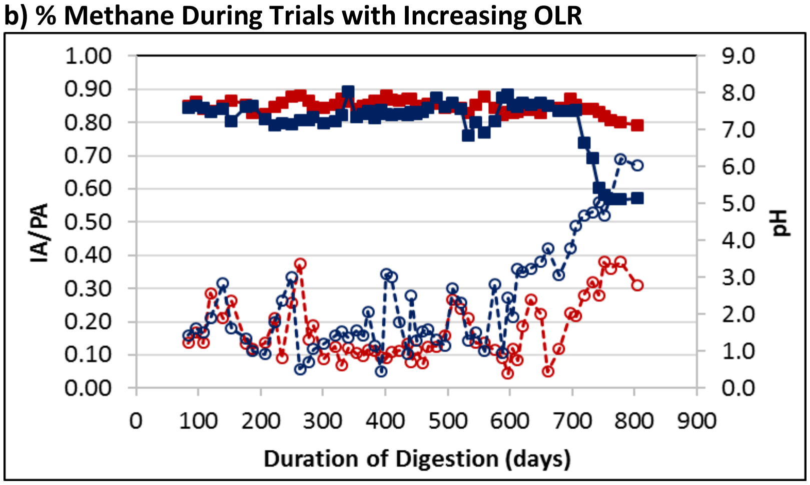
When the co-digestion of liquid dairy manure and corn thin stillage was conducted in a two-phase system significant increases (p<0.05) in biogas production were observed. When OLR were increased from 1.67 to 4.40 g VS/L·day (OLR to entire two-phase system), biogas production ranged from 0.65 to 3.02 L biogas/L·day (Figure 4.5 a)). Optimum biogas production of 3.02 ± 0.07 L biogas/L·day was achieved at an OLR of 4.13 g VS/L·day (total HRT of 15 days) with a CH_4 composition of 67% in the biogas, resulting in a 20% increase in biogas production compared with the single-phase system.



a) Biogas Production During Trials with Increasing OLR



b) % Methane During Trials with Increasing OLR



c) IA/PA and pH During Trials with Increasing OLR

Figure 4.5 : Biogas Production, % Methane and Changing IA/PA (○) and pH (■) with Increasing OLR under Single and Two-Phase Co-digestion of Corn Thin Stillage and Liquid Dairy Manure

Although significant biogas production was achieved with the two-phase system, digester upset and possible inhibition was observed at an OLR of 4.40 g VS/L·day (HRT of 15 days), as was experienced with the single-phase digestion system. However, digester upset was much more pronounced in the two-phase system with a drastic drop in biogas production to 2.30 ± 0.04 L biogas/L·day and pH to 5.13. IA/PA also increased to over 0.6. IA/PA ratios should be kept below the range of 0.3 to 0.4 for stable digester performance and an IA/PA over 0.5 typically means an impending digester upset (Gerardi, 2003; Martin-Gonzalez et al., 2013; Murphy and Thamsiriroj, 2013). The sharp peaks and sudden drops in IA/PA throughout the digestion trials (Figure 4.5 c)) correspond to transition periods to higher OLRs.

Figure 4.6 illustrates the correlation of specific methane yield with OLR for both the single and two-phase systems. This graph excludes the methane yields of 0.29 and 0.23 L CH_4/g VS·day for single and two-phase co-digestion, respectively, at OLR of 4.40 g VS/L·day. The single-phase digestion shows a strong correlation of specific methane yield with OLR, with an R^2 of 0.81 and Pearson r of 0.90. Digestion under two-phase digestion demonstrates a slightly stronger positive correlation of specific methane yield with OLR; R^2 of 0.87 and Pearson r of 0.94.

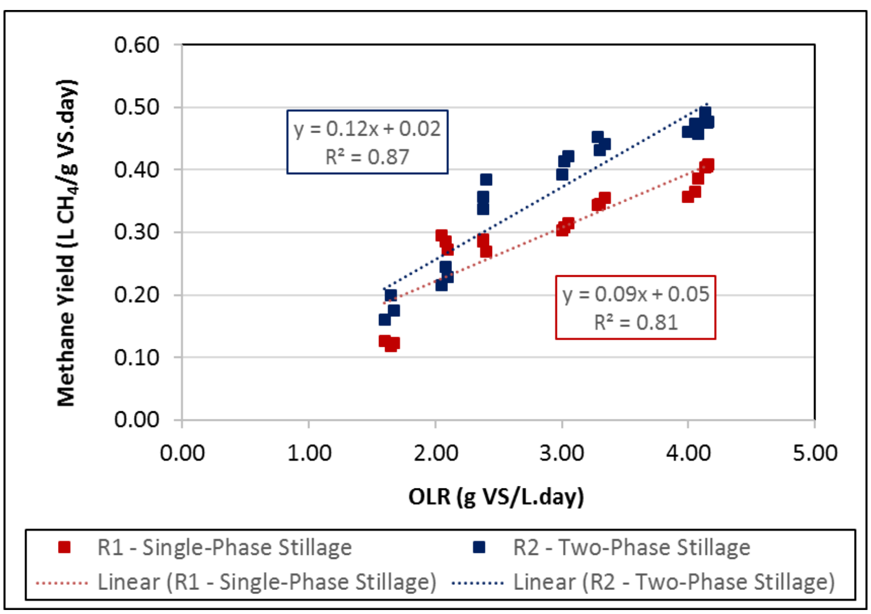
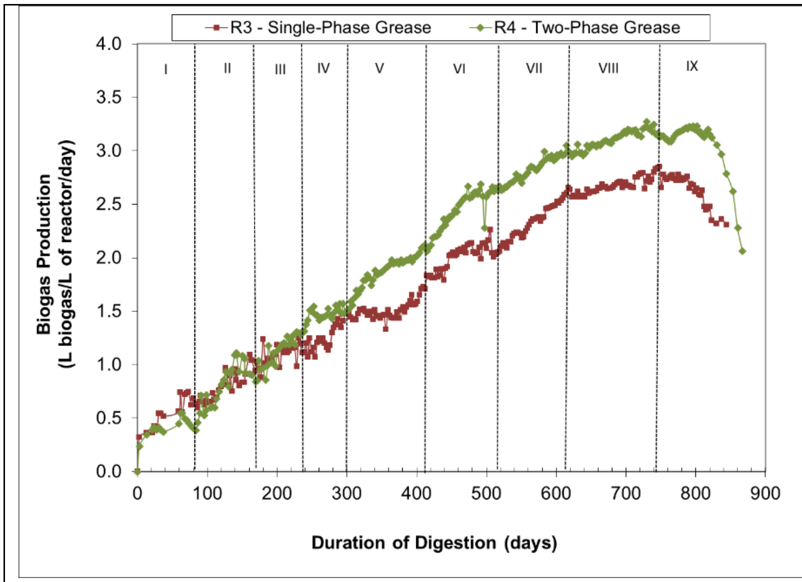


Figure 4.6 Correlation of Specific Methane Yields with OLR under both Single and Two-Phase Co-digestion of Corn Thin Stillage and Liquid Dairy Manure
(Note: Excludes methane yields of 0.29 and 0.23 L CH_4/g VS·day for single and two-phase co-digestion, respectively, at OLR of 4.40 g VS/L·day)

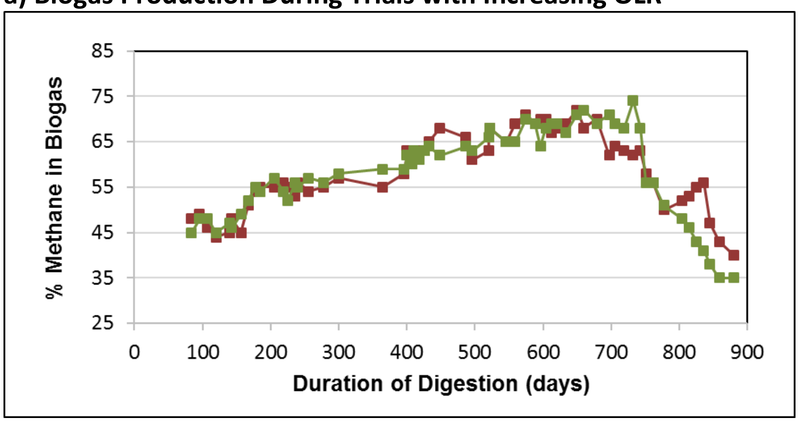
Optimum methane production was achieved at the OLR of 4.13 g VS/L·day and specific methane yields averaged 0.40 ± 0.03 L CH_4/gVS·day (0.61 ± 0.05 L biogas/gVS·day) and 0.49 ± 0.04 L CH_4/gVS·day (0.73 ± 0.06 L biogas/gVS·day) for the single and two-phase digestion systems, respectively. Although optimum methane yields were obtained at an OLR of 4.13 g VS/L·day, the increasing IA/PA values during the digestion period (Figure 4.5 c)) indicate there is some digester instability due to acid build up in the reactor. Therefore, it can be concluded that OLR 4.13 g VS/L·day is the threshold where digester upset can quickly occur without operator intervention. An important objective of this study was to evaluate biogas production and digestate quality at digester upset, therefore no digester intervention was implemented to mitigate potential digester upsets.

The co-digestion of liquid dairy manure with waste grease under mesophilic conditions (36 ± 2°C) resulted in very similar observations. Figure 4.7 a) illustrates biogas production with increasing OLR, where OLRs increased from 1.83 to 4.60 g VS/L·day with biogas production increasing from 0.43 to 2.81 L biogas/L·day (total HRT ranged from 45 to 28 days) for the single-phase system co-digesting liquid dairy manure with waste grease. Optimum biogas production of 2.78 ± 0.04 L biogas/L·day was reached at an OLR of 4.11 g VS/L·day and total HRT of 28 days (5 days in acid reactor and 23 days in methane producing reactor). At stable biogas production (digestion day 385 to 685), the percentage methane in biogas ranged from 65 to 69 % CH_4 (Figure 4.7 b)) and pH levels remained neutral (Figure 4.7 c)). When organic loading further increased to 4.60 g VS/L·day (total HRT of 25 days), biogas production dropped dramatically to 2.11 ± 0.03 L biogas/L·day and the % CH_4 in biogas decreased to 43%. The pH in the reactor decreased steadily, with an increase in IA/PA, indicating potential digester upset.

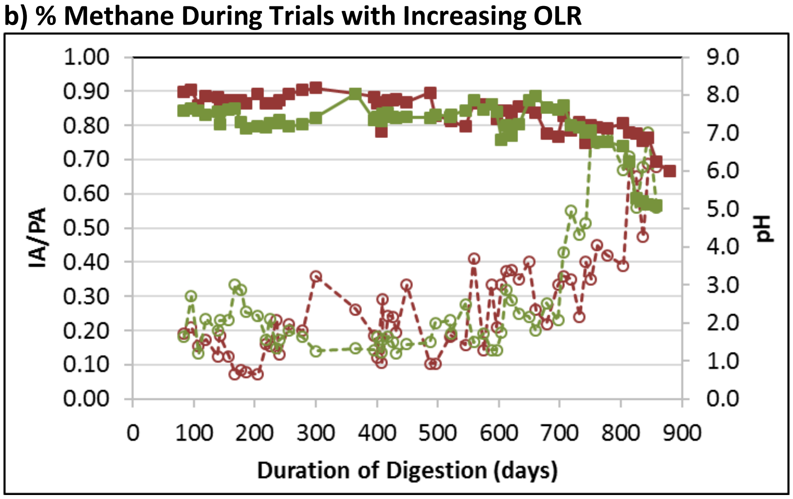
The co-digestion of liquid dairy manure with waste grease in a two-phase system resulted in significant increases ($p<0.05$) in biogas production. When OLR were increased from 1.83 to 4.11 g VS/L·day (OLR to entire two-phase system), biogas production ranged from 0.67 to 3.20 L biogas/L·day (Figure 4.7 a)). Optimum biogas production of 3.20 ± 0.05 L biogas/L·day was achieved at an OLR of 4.11 g VS/L·day (total HRT of 28 days) with a CH_4 composition of 66% in the biogas, resulting in a 15% increase in biogas production compared with the single-phase system.



a) Biogas Production During Trials with Increasing OLR



b) % Methane During Trials with Increasing OLR



c) IA/PA and pH During Trials with Increasing OLR

Figure 4.7 Biogas Production, % Methane and Changing IA/PA (○) and pH (■) with Increasing OLR under Single and Two-Phase Co-digestion of Waste Grease and Liquid Dairy Manure

Digester upset and possible inhibition was observed at an OLR of 4.60 g VS/L·day (HRT of 25 days), as was experienced with the single-phase digestion system. However, digester upset was much more pronounced in the two-phase system with a dramatic drop in biogas production to 2.22 ± 0.05 L biogas/L·day, a %CH_4 drop to 37% and pH to 5.09. IA/PA also increased to over 0.62; well above the recommended IA/PA ratio range of 0.3 to 0.4 for stable digester performance (Gerardi, 2003; Martin-Gonzalez et al., 2013; Murphy and Thamsiriroj, 2013). As observed with the corn stillage trials, the sharp peaks and sudden drops in IA/PA throughout the digestion trials (Figure 4.7 c)) correspond to transition periods to higher OLRs.

Figure 4.8 illustrates the correlation of specific methane yield with OLR for both the single and two-phase systems. This graph excludes the methane yields of 0.23 and 0.19 L CH_4/g VS·day for single and two-phase co-digestion, respectively, at OLRs of 4.60 g VS/L·day. The single-phase digestion shows a good correlation of specific methane yield with OLR, with an R^2 of 0.77 and Pearson r of 0.88. Digestion under a two-phase system demonstrates a much stronger positive correlation of specific methane yield with OLR; R^2 of 0.93 and Pearson r of 0.97.

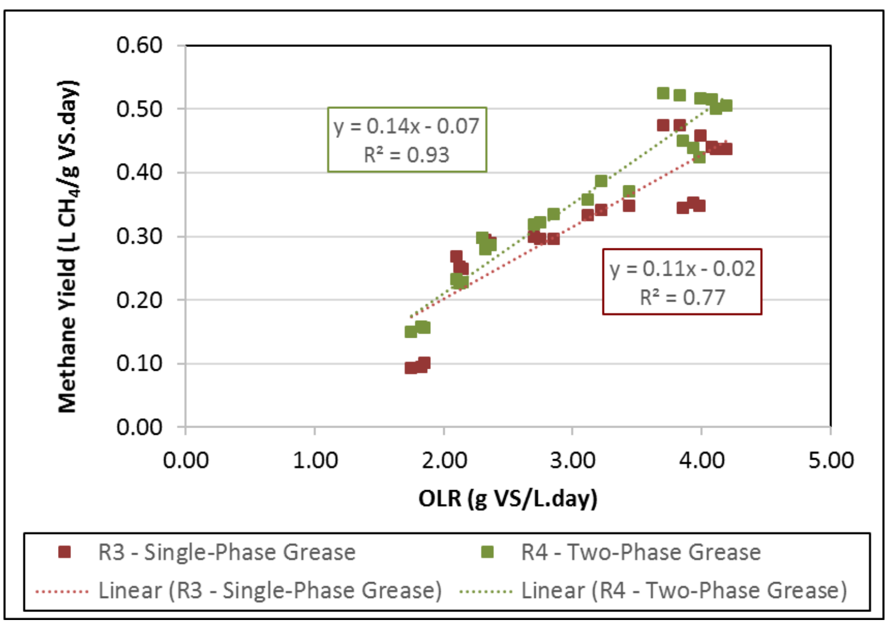


Figure 4.8 Correlation of Specific Methane Yields with OLR under both Single and Two-Phase Co-digestion of Waste Grease and Liquid Dairy Manure

(Note: Excludes methane yields of 0.23 and 0.19 L CH_4/g VS·day for single and two-phase co-digestion, respectively, at OLR of 4.60 g VS/L·day)

Optimum methane production was achieved at OLRs of 3.93 and 4.11 g VS/L·day and specific methane yields averaged 0.43 ± 0.04 L CH_4/gVS·day (0.67 ± 0.06 L biogas/gVS·day) and 0.53 ± 0.03 L CH_4/gVS·day (0.79 ± 0.05 L biogas/gVS·day) for the single and two-phase digestion systems, respectively. Although optimum methane yields were obtained at an OLR of 4.11 g VS/L·day, the increasing IA/PA values to greater than 0.5 during the digestion period (Figure 4.7 c)) indicate there is digester instability due to acid build up in the reactor. Therefore, it can be concluded that the OLR of 4.11 g VS/L·day is the threshold where digester upset can quickly occur without operator intervention.

The co-digestion trials of this study demonstrated that either the addition of corn thin stillage or waste grease to liquid dairy manure (at 50:50 v/v) significantly increased biogas production. During single-phase digestion, biogas yields for the co-digestion of liquid dairy manure with corn thin stillage and waste grease were found to be 0.61 ± 0.05 L biogas/gVS·day and 0.67 ± 0.06 L biogas/gVS·day, respectively. These yields are significantly ($p<0.05$) higher than the ultimate biogas yield of 0.34 L biogas/g VS obtained from the batch BMP trials presented in Chapter 3 and yields reported in literature that ranged between 0.366 and 0.404 L biogas/g VS for cattle manure (El-Mashad and Zhang, 2010; Labatut et al., 2011; Luna-delRisco et al., 2011). Biogas yields were increased even further with the two-phase digestion system, with increases of up to 20% in biogas yields when compared to the single-phase system. This increased biogas yield is higher than yields reported by Dugba and Zhang (1999), Nielsen et al. (2004) and Kaparaju et al. (2009). In fact, Dugba and Zhang (1999), Pillars (2002) and Krich et al. (2005) suggest two-phase anaerobic digestion systems are not suited to dairy manure or are only applicable to diluted manure. It should be noted these studies only considered two-phase digestion systems with manure alone and did not investigate the potential of adding co-substrates to increase the soluble organics fraction of the digestion mixture.

Table 4.5 presents estimated energy yields that can be expected from increased biogas production using co-digestion and two-phase systems (calculated based on optimum biogas yields obtained from BMP and bench-scale trials). Expressed per tonne of wet material, electricity and heat yields double when co-substrates are added to manure (50:50 v/v) compared to yields obtained when manure is digested alone. Two-phase digestion increases electrical and heat yields by up to 20%. OMAFRA (2010) suggests for the Ontario biogas industry, that during winter months as much as 50% of the heat produced from the co-generation system will be used to heat a one tank digester system; however, heat requirements become significantly depleted during warmer months. Although a two-phase system will require heating two tanks to mesophilic temperatures (given each digester tank will be smaller in size than a one-tank system), the 20% increase in heat yield may be sufficient energy to heat both tanks and only supplemental heat required during cold winter months. The increased potential electricity and heat at the farm may make a two-phase system economically attractive, given a Feed-In-Tariff (FIT) contract averaging of over 18 to 20¢/kWh$_e$. The potential increased electrical and heat yields calculated in Table 4.5 are based on biogas yields from lab studies and these values may be somewhat different when based on full-scale biogas yields.

Table 4.5 Calculated Potential Electrical and Heat Yields from Co-digestion of Liquid Dairy Manure with Corn Thin Stillage and Waste Grease (with Single and Two-Phase Systems)

Material	Biogas Yield (L/g VS)	Biogas per Wet Material (m^3/t)	BTU from Biogas[1] (BTU/t)	Electrical Yield (kWh$_e$/t)[2]	Heat Yield (kWh$_h$/t)[3]
Single-Phase – Manure only	0.34	21	437833	45	58
Single-Phase – Co-digestion with Stillage	0.61	37	775188	79	102
Two-Phase – Co-digestion with Stillage	0.73	44	927684	95	122
Single-Phase – Co-digestion with Waste Grease	0.67	50	1064295	109	140
Two-Phase – Co-digestion with Waste Grease	0.79	59	1254915	129	165

1 At 60% CH_4: 1 m^3 biogas = 21180 BTU (OMAFRA, 2010)
2 1 million BTU = 293 kWh$_e$; assume 35% efficiency for conversion to electricity (OMAFRA, 2010)
3 Assume 45% efficiency for conversion to heat (OMAFRA, 2010)

4.3.2 Digestate Quality from Single and Two-Phase Co-digestion of Liquid Dairy Manure with Corn Thin Stillage and Liquid Dairy Manure with Waste Grease

In addition to the advantage of producing biogas on-farm, the treatment potential of using anaerobic digesters to treat manure and off-farm wastes is of interest to both the Ontario biogas industry and OMAFRA. Under the Nutrient Management Act and O. Reg. 267/03, digestate from a Regulated Mixed Anaerobic Digestion Facility on Ontario farms is land applied on agricultural fields as an agricultural source material (ASM). Digestate quality was evaluated as it compared with the characteristics of the raw substrates. Tables 4.6 and 4.7 summarise the physical and chemical characteristics of digestate under steady state single-phase and two-phase co-digestion, respectively, of liquid dairy manure with corn thin stillage operated under various OLRs. Tables 4.8 and 4.9 summarise the physical and chemical characteristics of digestate under steady state single-phase and two-phase co-digestion, respectively, of liquid dairy manure with waste grease operated under various OLRs.

4.3.2.1 Volatile Solids Reduction

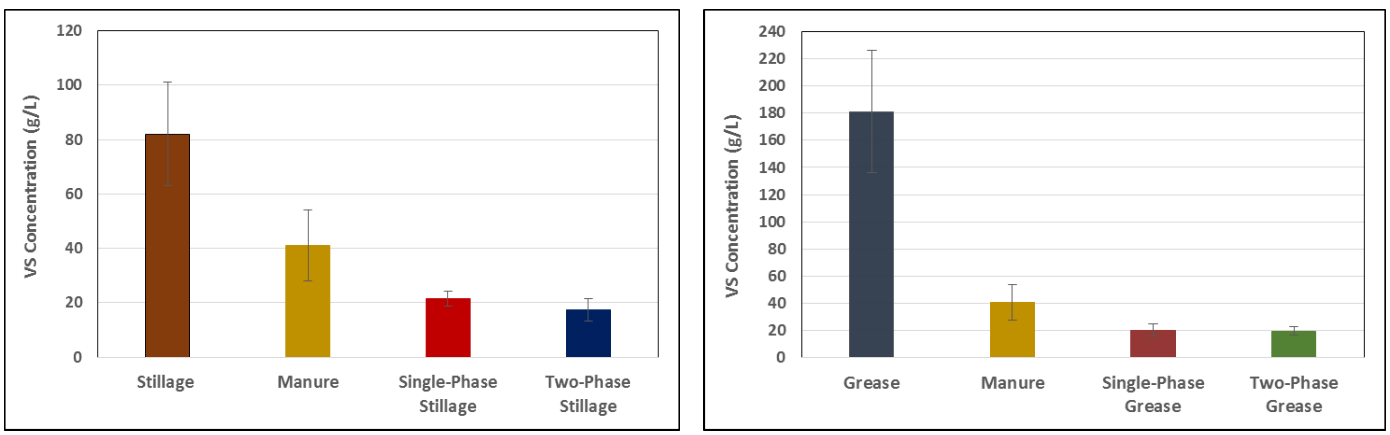
When co-digesting corn thin stillage with liquid dairy manure in a single-phase system, significant (p<0.05) VS reductions were observed when compared to the raw substrate VS concentrations. At the various OLRs the percent VS reductions ranged from 51 to 71%, with an optimum reduction of 71% at an OLR of 4.0 ± 0.1 g VS/L·day (Table 4.6). VS reductions in Trials V to VII, with OLRs ranging from 3.0 ± 0.1 to 4.0 ± 0.1 g VS/L·day, were significantly (p<0.05) higher than the other trials. VS reductions in the two-phase thin stillage digestion system also observed significant (p<0.05) VS reductions when compared with the raw substrates. VS reductions ranged from 55 to 80 % for the various OLRs studied, and the optimum VS reduction of 80% was achieved at an OLR of 3.0 ± 0.1 g VS/L·day (Table 4.7). VS reductions in Trials V and VI were considered significantly (p<0.05) higher than those obtained in the single-phase digestions using thin stillage.

Similar VS reductions were observed with waste grease co-digestion trials. The single-phase co-digestion of waste grease with liquid dairy manure resulted in significant VS reductions (p<0.05) when compared with the raw substrates. At the various OLRs the percent VS reductions ranged from 57 to 80%, with optimum reductions of 79 and 80% at OLRs of 3.4 ± 0.1 and 3.9 ± 0.1 g VS/L·day, respectively (Table 4.7). VS reductions in Trials V to VII, with OLRs ranging from 2.8 ± 0.1 to 3.9 ± 0.1 g VS/L·day, were significantly (p<0.05) higher than the other trials. VS reductions in the two-phase thin stillage digestion system also observed significant (p<0.05) VS reductions when compared with the raw substrates. VS reductions ranged from 67 to 79 % for the various OLRs studied, and the optimum VS reduction of 79% was achieved at an OLR of 2.8

± 0.1 g VS/L·day (Table 4.8). VS reductions among the various trials of changing OLR were consistent and significant differences between VS reductions were not observed (p>0.05).

Figure 4.10 illustrates average final VS concentrations in digestate compared with those of the raw substrates and demonstrates that both the single and two-phase co-digestion trials produced digestate with significantly lower VS concentrations. Lower VS concentrations applied to agricultural land will help reduce soil biological activity that contributes to odours and GHG emissions, thereby reducing environmental impacts (Crolla et al., 2013).

The % VS reductions in the co-digestion trials of this study ranged from 51 to 81%, which are slightly higher VS reductions than reported in literature for mesophilic digesters (Burke, 2001; Al Seadi et al., 2013; Banks et al., 2013; Crolla et al., 2013).



a) Thin Stillage Co-Digestion Trials b) Waste Grease Co-Digestion Trials

Figure 4.9 Digestate VS Concentrations as Compared to Raw Substrates for both Single and Two-Phase Digestion for Corn Thin Stillage and Waste Grease Co-Digestion Trials

(Note: Excludes trials VIII and IX where digesters experienced upset)

Table 4.6 Corn Thin Stillage Single-phase Digestion (R1) – Average Physical and Chemical Characteristics of Digestate

Parameter	Raw Influent		Digestate Effluent							
	Stillage	Manure	I [1]	II	III & IV [2]	V	VI	VII	VIII	IX
n [3]	88	95	35	18	28	18	18	18	18	12
ORL (g VS/L·day)	---	---	1.7 (0.1) [4]	2.1 (0.1)	2.4 (0.0)	3.0 (0.1)	3.3 (0.0)	4.0 (0.1)	4.2 (0.0)	4.4 (0.1)
HRT (days)	---	---	30	24	21	20	18	15	15	15
% vol. Manure	---	---	75	75	75	50	50	50	45	40
% vol. Stillage	---	---	25	25	25	50	50	50	55	60
TS (g/L)	104 (25)	54 (15)	32 (3)	31 (4)	27 (3)	28 (2)	24 (2)	26 (3)	27 (6)	45 (6)
VS (g/L)	82 (19)	41 (13)	25 (4)	25 (6)	21 (2)	22 (3)	19 (2)	18 (1)	21 (3)	33 (5)
VS/TS (%)	79	76	78	81	78	79	79	69	78	73
% VS reduction	---	---	51	51	59	64	69	71	67	49
tCOD (g/L)	127.6 (44.1)	65.2 (15.4)	28.9 (2.5)	25.7 (3.1)	20.3 (4.4)	21.2 (2.1)	19.3 (2.1)	18.9 (2.6)	25.2 (3.1)	29.8 (8.9)
sCOD (g/L)	54.4 (17.5)	18.3 (4.3)	3.1 (0.4)	2.8 (0.5)	2.0 (0.2)	2.6 (0.6)	2.8 (0.7)	3.1 (0.5)	2.8 (0.7)	3.4 (0.8)
pCOD (g/L)	73.2 (30.6)	52.9 (12.7)	25.8 (0.9)	22.9 (0.7)	18.3 (0.8)	18.6 (0.9)	16.5 (0.5)	15.8 (0.6)	23.4 (1.0)	26.8 (1.5)
sCOD/tCOD	0.43	0.28	0.11	0.11	0.10	0.12	0.15	0.16	0.11	0.11
pH	4.22 (0.35)	7.21 (0.48)	7.48 (0.11)	7.63 (0.12)	7.71 (0.12)	7.79 (0.09)	7.64 (0.05)	7.55 (0.17)	7.61 (0.12)	7.37 (0.18)
IA/PA	---	---	0.21 (0.03)	0.18 (0.06)	0.15 (0.05)	0.11 (0.02)	0.16 (0.07)	0.12 (0.05)	0.24 (0.08)	0.33 (0.04)
Acetic Acid (g/L)	0.82 (0.68)	1.64 (0.66)	0.24 (0.09)	0.19 (0.11)	0.15 (0.08)	0.09 (0.02)	0.09 (0.03)	0.12 (0.02)	0.23 (0.09)	0.29 (0.16)
Propionic Acid (g/L)	0.40 (0.28)	0.68 (0.34)	0.17 (0.10)	0.17 (0.05)	0.09 (0.07)	0.06 (0.04)	<0.05	0.08 (0.03)	0.22 (0.07)	0.25 (0.13)
Butyric Acid (g/L)	0.07 (0.03)	0.38 (0.21)	0.08 (0.02)	<0.05	0.06 (0.01)	<0.05	<0.05	<0.05	0.12 (0.10)	0.07 (0.05)
TKN (g/L)	2.51 (0.85)	1.82 (0.61)	1.91 (0.33)	2.12 (0.54)	1.98 (0.26)	2.32 (0.12)	1.73 (0.44)	1.71 (0.39)	1.89 (0.56)	2.45 (0.99)
NH_4^+-N (g/L)	0.08 (0.06)	0.37 (0.23)	0.85 (0.12)	0.96 (0.22)	0.82 (0.36)	0.83 (0.20)	0.88 (0.19)	0.95 (0.21)	0.99 (0.41)	1.2 (0.58)
TP (g/L)	1.28 (0.32)	0.51 (0.14)	0.63 (0.10)	0.59 (0.15)	0.46 (0.22)	0.43 (0.21)	0.53 (0.18)	0.47 (0.19)	0.59 (0.23)	0.40 (0.22)
PO_4^{3-} -P (g/L)	0.92 (0.29)	0.38 (0.11)	0.32 (0.08)	0.27 (0.06)	0.21 (0.09)	0.32 (0.09)	0.28 (0.10)	0.29 (0.10)	0.31 (0.09)	0.36 (0.11)
RBP (L biogas/g VS)	---	---	0.22 (0.01)	0.24 (0.02)	0.29 (0.01)	0.33 (0.03)	0.35 (0.03)	0.36 (0.02)	0.39 (0.04)	0.48 (0.10)

1 Data for Trial I are the average values R1 and R2 when operating under the same conditions of the single-phase digestion

2 Trials III & IV were conducted as single-phase digestion for entire trial period (parallels period when R2 transitioned from single to two-phase)

3 n = number of samples (weekly composites) used for average calculations

4 Number within brackets is standard deviation on average value

Table 4.7 Corn Thin Stillage Two-phase Digestion (R2) – Average Physical and Chemical Characteristics of Digestate

Parameter	Raw Influent		Digestate Effluent							
	Stillage	Manure	II	III [1]	IV	V	VI	VII	VIII	IX
n [2]	88	95	18	12	16	18	18	18	18	12
ORL (g VS/L·day)	---	---	2.1 (0.1) [3]	2.4 (0.1)	2.4 (0.1)	3.0 (0.1)	3.3 (0.0)	4.0 (0.1)	4.2 (0.0)	4.4 (0.1)
HRT (days)	---	---	24	21	21	20	18	15	15	15
% vol. Manure	---	---	75	75	75	50	50	50	45	40
% vol. Stillage	---	---	25	25	25	50	50	50	55	60
TS (g/L)	104 (25)	54 (15)	28 (5)	26 (5)	17 (4)	18 (2)	24 (5)	22 (3)	23 (7)	36 (6)
VS (g/L)	82 (19)	41 (13)	23 (3)	22 (4)	14 (2)	12 (3)	17 (3)	16 (3)	18 (6)	28 (7)
VS/TS (%)	79	76	82	85	82	67	71	73	78	78
% VS reduction	---	---	55	57	73	80	72	74	72	57
tCOD (g/L)	127.6 (44.1)	65.2 (15.4)	31.7 (4.3)	28.4 (3.9)	16.3 (3.3)	17.6 (2.3)	18.7 (3.7)	22.5 (2.9)	26.7 (4.5)	36.7 (7.5)
sCOD (g/L)	54.4 (17.5)	18.3 (4.3)	4.0 (1.1)	3.5 (0.9)	2.5 (0.7)	2.7 (0.5)	2.3 (1.1)	2.8 (0.3)	2.7 (0.4)	4.4 (4.6)
pCOD (g/L)	73.2 (30.6)	52.9 (12.7)	27.7 (2.3)	24.8 (2.7)	13.8 (2.0)	14.9 (1.1)	16.4 (2.1)	19.7 (1.9)	24.1 (2.9)	32.2 (5.2)
sCOD/tCOD	0.43	0.28	0.13	0.12	0.15	0.15	0.12	0.12	0.10	0.12
pH	4.22 (0.35)	7.21 (0.48)	7.43 (0.09)	7.55 (0.13)	7.23 (0.08)	7.44 (0.22)	7.64 (0.14)	7.42 (0.42)	7.63 (0.09)	5.56 (0.62)
IA/PA	---	---	0.23 (0.11)	0.18 (0.06)	0.16 (0.07)	0.20 (0.10)	0.20 (0.07)	0.21 (0.10)	0.39 (0.05)	0.58 (0.07)
Acetic Acid (g/L)	0.82 (0.68)	1.64 (0.66)	0.16 (0.05)	0.07 (0.02)	0.09 (0.08)	0.13 (0.04)	0.15 (0.11)	0.13 (0.04)	0.20 (0.88)	0.37 (0.10)
Propionic Acid (g/L)	0.40 (0.28)	0.68 (0.34)	<0.05	0.06 (0.01)	0.04 (0.07)	0.07 (0.01)	0.09 (0.02)	0.05 (0.02)	0.13 (0.13)	0.23 (0.09)
Butyric Acid (g/L)	0.07 (0.03)	0.38 (0.21)	<0.05	<0.05	0.05 (0.01)	0.06 (0.01)	0.06 (0.01)	<0.05	0.14 (0.11)	0.21 (0.08)
TKN (g/L)	2.51 (0.85)	1.82 (0.61)	2.01 (0.24)	1.75 (0.21)	2.09 (0.19)	1.97 (0.21)	1.87 (0.30)	1.69 (0.27)	1.73 (0.53)	2.56 (0.75)
NH_4^+-N (g/L)	0.08 (0.06)	0.37 (0.23)	1.42 (0.53)	1.13 (0.36)	0.93 (0.19)	1.02(0.46)	0.89 (0.22)	0.97 (0.23)	1.11 (0.23)	0.83 (0.35)
TP (g/L)	1.28 (0.32)	0.51 (0.14)	0.54 (0.09)	0.41 (0.11)	0.52 (0.13)	0.49 (0.18)	0.53 (0.16)	0.55 (0.16)	0.57 (0.20)	0.58 (0.23)
PO_4^{3-} -P (g/L)	0.92 (0.29)	0.38 (0.11)	0.21 (0.10)	0.23 (0.13)	0.27 (0.11)	0.22 (0.17)	0.30 (0.10)	0.19 (0.08)	0.31 (0.09)	0.36 (0.09)
RBP (L biogas/g VS)	---	---	0.23 (0.01)	0.27 (0.03)	0.28 (0.02)	0.27 (0.03)	0.30 (0.02)	0.32 (0.04)	0.36 (0.04)	0.51 (0.11)

1 Trial where R2 transitions for single-phase to two-phase operation

2 n = number of samples (weekly composites) used for average calculations

3 Number within brackets is standard deviation on average value

Table 4.8 Waste Grease Single-phase Digestion (R3) – Average Physical and Chemical Characteristics of Digestate

Parameter	Raw Influent		Digestate Effluent							
	Grease	Manure	I [1]	II	III & IV [2]	V	VI	VII	VIII	IX
n [3]	64	95	18	18	28	18	18	18	18	12
ORL (g VS/L·day)	---	---	1.8 (0.1) [4]	2.1 (0.1)	2.4 (0.0)	2.8 (0.1)	3.4 (0.1)	3.9 (0.1)	4.1 (0.0)	4.6 (0.1)
HRT (days)	---	---	45	39	35	30	24	21	28	25
% vol. Manure	---	---	75	75	75	75	75	75	50	50
% vol. Grease	---	---	25	25	25	25	25	25	50	50
TS (g/L)	203 (43)	54 (15)	31 (9)	33 (15)	33 (11)	27 (5)	25 (10)	23 (11)	44 (13)	48 (23)
VS (g/L)	181 (45)	41 (13)	24 (8)	23 (13)	25 (9)	19 (4)	15 (8)	16 (11)	35 (10)	36 (19)
VS/TS (%)	89	76	77	70	76	70	60	70	80	75
% VS reduction	---	---	68	70	67	75	80	79	68	67
tCOD (g/L)	236.8 (43.5)	65.2 (15.4)	26.4 (3.3)	29.9 (2.3)	27.2 (2.0)	19.9 (2.9)	21.6 (2.2)	33.8 (7.3)	45.4 (4.1)	40.7 (9.7)
sCOD (g/L)	67.3 (19.1)	18.3 (4.3)	2.8 (0.8)	2.7 (0.7)	2.5 (0.3)	2.0 (0.5)	1.9 (0.4)	2.8 (1.1)	4.7 (1.9)	2.9 (2.5)
pCOD (g/L)	169.5 (41.3)	52.9 (12.7)	23.6 (1.1)	27.2 (1.6)	24.7 (0.9)	17.9 (1.5)	19.7 (1.8)	31.0 (5.6)	40.7 (3.1)	37.8 (5.6)
sCOD/tCOD	0.28	0.28	0.11	0.09	0.09	0.10	0.09	0.08	0.10	0.07
pH	5.01 (0.12)	7.21 (0.48)	7.78 (0.17)	7.92 (0.14)	7.92 (0.11)	7.69 (0.33)	7.54 (0.23)	7.49 (29)	7.09 (0.29)	6.86 (0.42)
IA/PA	---	---	0.16 (0.07)	0.15 (0.05)	0.18 (0.07)	0.20 (0.08)	0.27 (0.11)	0.32 (0.08)	0.34 (0.06)	0.55 (0.14)
Acetic Acid (g/L)	2.33 (0.35)	1.64 (0.66)	0.10 (0.05)	0.16 (0.03)	0.25 (0.06)	0.16 (0.04)	0.11 (0.04)	0.18 (0.12)	0.38 (0.17)	0.42 (0.21)
Propionic Acid (g/L)	1.86 (0.58)	0.68 (0.34)	<0.05	0.60 (0.06)	0.08 (0.05)	<0.05	<0.05	0.20 (0.11)	0.14 (0.09)	0.29 (0.14)
Butyric Acid (g/L)	0.62 (0.09)	0.38 (0.21)	<0.05	<0.05	0.06 (0.03)	<0.05	<0.05	0.08 (0.01)	0.16 (0.09)	0.11 (0.09)
TKN (g/L)	3.11 (0.40)	1.82 (0.61)	1.73 (0.21)	2.41 (0.32)	2.13 (0.79)	2.29 (0.25)	1.99 (0.61)	2.37 (0.15)	2.09 (0.55)	2.78 (0.73)
NH_4^+-N (g/L)	0.79 (0.21)	0.37 (0.23)	1.11 (0.20)	1.53 (0.31)	1.21 (0.24)	1.57 (0.34)	1.72 (0.25)	1.67 (0.28)	1.83 (0.34)	1.08 (0.43)
TP (g/L)	0.31 (0.17)	0.51 (0.14)	0.41 (0.12)	0.28 (0.15)	0.43 (0.18)	0.45 (0.15)	0.36 (0.11)	0.49 (0.23)	0.52 (0.16)	0.48 (0.21)
PO_4^{3-} -P (g/L)	0.17 (0.10)	0.38 (0.11)	0.25 (0.14)	0.20 (0.11)	0.19 (0.13)	0.23 (0.15)	0.21 (0.13)	0.30 (0.13)	0.29 (0.17)	0.27 (0.19)
RBP (L biogas/g VS)	---	---	0.19(0.01)	0.21 (0.04)	0.25 (0.05)	0.30 (0.02)	0.28 (0.05)	0.31 (0.05)	0.41 (0.05)	0.46 (0.09)

1 Data for Trial I are the average values R1 and R2 when operating under the same conditions of the single-phase digestion
2 Trials III & IV were conducted as single-phase digestion for entire trial period (parallels period when R2 transitioned from single to two-phase)
3 n = number of samples (weekly composites) used for average calculations
4 Number within brackets is standard deviation on average value

Table 4.9 Waste Grease Single-phase Digestion (R4) – Average Physical and Chemical Characteristics of Digestate

Parameter	Raw Influent		Digestate Effluent							
	Grease	Manure	II	III [1]	IV	V	VI	VII	VIII	IX
n [2]	64	95	18	12	16	18	18	18	18	12
ORL (g VS/L·day)	---	---	2.1 (0.1) [3]	2.4 (0.0)	2.4 (0.0)	2.8 (0.1)	3.4 (0.1)	3.9 (0.1)	4.1 (0.0)	4.6 (0.1)
HRT (days)	---	---	39	35	35	30	24	21	28	25
% vol. Manure	---	---	75	75	75	75	75	75	50	50
% vol. Grease	---	---	25	25	25	25	25	25	50	50
TS (g/L)	203 (43)	54 (15)	31 (4)	27(5)	27 (3)	25(3)	26 (5)	25 (10)	41 (7)	49 (11)
VS (g/L)	181 (45)	41 (13)	25(6)	21 (5)	20 (4)	16 (2)	17 (6)	19 (9)	32 (6)	33 (13)
VS/TS (%)	89	76	81	78	74	64	65	76	78	67
% VS reduction	---	---	67	72	74	79	78	75	71	70
tCOD (g/L)	236.8 (43.5)	65.2 (15.4)	25.9 (2.9)	23.5 (3.7)	21.3 (1.9)	17.5 (3.2)	18.7 (1.9)	20.9 (2.2)	38.6 (5.4)	35.2 (7.3)
sCOD (g/L)	67.3 (19.1)	18.3 (4.3)	2.9 (0.9)	2.3 (0.9)	2.9 (0.5)	2.2 (0.3)	2.1 (0.4)	2.5 (0.9)	5.9 (2.6)	5.5 (2.7)
pCOD (g/L)	169.5 (41.3)	52.9 (12.7)	23.0 (1.0)	21.2 (1.5)	18.4 (0.9)	15.3 (1.7)	16.6 (0.8)	18.4 (1.3)	32.7 (3.9)	29.7 (4.7)
sCOD/tCOD	0.28	0.28	0.11	0.10	0.14	0.13	0.11	0.13	0.15	0.16
pH	5.01 (0.12)	7.21 (0.48)	7.55 (0.13)	7.23 (0.08)	7.44 (0.31)	7.44 (0.06)	7.64 (0.14)	7.42 (0.42)	7.63 (0.09)	5.56 (0.62)
IA/PA	---	---	0.18 (0.06)	0.16 (0.10)	0.16 (0.01)	0.20 (0.10)	0.20 (0.07)	0.21 (0.10)	0.39 (0.05)	0.58 (0.07)
Acetic Acid (g/L)	2.33 (0.35)	1.64 (0.66)	0.11 (0.05)	0.08 (0.02)	0.20 (0.08)	0.12 (0.07)	0.17 (0.03)	0.25 (0.14)	1.61 (0.36)	1.20 (0.62)
Propionic Acid (g/L)	1.86 (0.58)	0.68 (0.34)	<0.05	0.10 (0.05)	0.11 (0.06)	0.09 (0.03)	0.07 (0.02)	0.19 (0.13)	0.17 (0.05)	0.56 (0.20)
Butyric Acid (g/L)	0.62 (0.09)	0.38 (0.21)	<0.05	0.07 (0.01)	<0.05	<0.05	0.08 (0.03)	<0.05	0.08 (0.04)	0.27 (0.13)
TKN (g/L)	3.11 (0.40)	1.82 (0.61)	1.86 (0.27)	1.77 (0.21)	2.08 (0.20)	2.44 (0.67)	2.85 (0.47)	2.50 (0.77)	2.76 (0.50)	3.39 (0.87)
NH_4^+-N (g/L)	0.79 (0.21)	0.37 (0.23)	1.23 (0.41)	1.16 (0.46)	1.37 (0.18)	1.29 (0.08)	1.33 (0.12)	1.20 (0.27)	1.48 (0.35)	1.35 (0.29)
TP (g/L)	0.31 (0.17)	0.51 (0.14)	0.22 (0.10)	0.23 (0.07)	0.37 (0.05)	0.41 (0.11)	0.33 (0.09)	0.41 (0.22)	0.43 (0.17)	0.39 (0.18)
PO_4^{3-} -P (g/L)	0.17 (0.10)	0.38 (0.11)	0.17 (0.8)	0.19 (0.05)	0.22 (0.16)	0.29 (0.12)	0.22 (0.08)	0.31(0.12)	0.33 (0.10)	0.21 (0.09)
RBP (L biogas/g VS)	---	---	0.18 (0.04)	0.17 (0.06)	0.19 (0.04)	0.20 (0.03)	0.21 (0.04)	0.23 (0.06)	0.38 (0.09)	0.55 (0.14)

1 Trial where R2 transitions for single-phase to two-phase operation
2 n = number of samples (weekly composites) used for average calculations
3 Number within brackets is standard deviation on average value

4.3.2.2 VFA Concentrations

VFAs are important intermediates in the anaerobic digestion process, where essentially organic matter, through several stages, gets metabolically converted to short chain VFAs, which become precursors for the production of methane. Although these acids are key components of the anaerobic digestion process, they can pose potential problems at relatively high concentrations. When VFA concentrations become too high in an anaerobic digester, the process becomes inhibited and acidifies; and, VFAs have been identified as key culprits of offensive odours (Crolla et al., 2013; Page et al., 2015). Digestate with high VFA concentrations are considered partly digested and non-stable, and the land application of such digestates can lead to odour issues as these VFAs get degraded by soil bacteria. Figure 4.10 illustrates the average short chain VFA concentrations of digestate from the corn thin stillage and waste grease co-digestion trials using both single and two-phase systems, as compared with raw substrates. The short chain VFAs considered for this study were acetic, propionic and butyric acids.

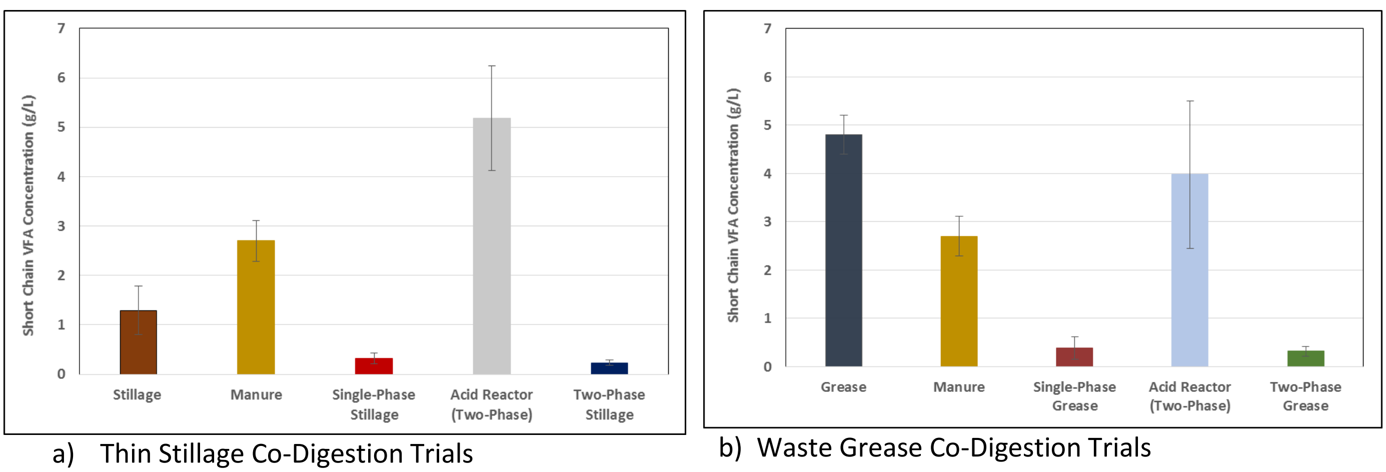


Figure 4.10 Digestate Short Chain VFA Concentrations as Compared to Raw Substrates for both Single and Two-Phase Digestion for Corn Thin Stillage and Waste Grease Co-Digestion Trials

(Notes: i) Excludes trials VIII and IX where digesters experienced upset; ii) Short chain VFA concentration is sum of acetic, propionic and butyric acid concentrations in sample)

Average VFA concentrations for single-phase and two-phase co-digestion of thin stillage with dairy manure were 0.32 ± 0.11 g/L and 0.24 ± 0.05 g/L, respectively, which are significantly (*p*<0.05) lower than the raw substrate VFA concentrations (1.29 ± 0.49 g/L for stillage and 2.70 ± 0.41 g/L for dairy manure). These digestate concentrations are well below the 500 mg/L upper limit suggested by Gerardi (2003) and Labatut and Gooch (2012) as an indicator of a well operating digester. Similar observations were made with the grease co-digestion. Average VFA concentrations for the single-phase and two-phase grease co-digestion trials were 0.39 ± 0.23 g/L and 0.32 ± 0.10 g/L, respectively, while VFA concentrations of the raw substrates were much higher (4.81 ± 0.40 g/L for waste grease and 2.70 ± 0.41 g/L for manure). The digester systems operating under Trials I to VII conditions resulted in what can be considered stable digestate.

For both stillage and grease digestion, digester operating conditions outlined in Trials VIII and IX resulted in digester upset, particularly for the two-phase system. VFA concentrations of the digestate supported that digester upset occurred. The single-phase digestion trials (both stillage and waste grease) demonstrated total short chain VFA concentrations were just under 1.0 g/L (Tables 4.6 and 4.8), while VFA concentrations in the two-phase systems were over 1.0 g/L (Tables 4.7 and 4.9). Labatut and Gooch (2012) suggest a VFA concentration above 1500-2000 mg/L may begin to inhibit the digestion process.

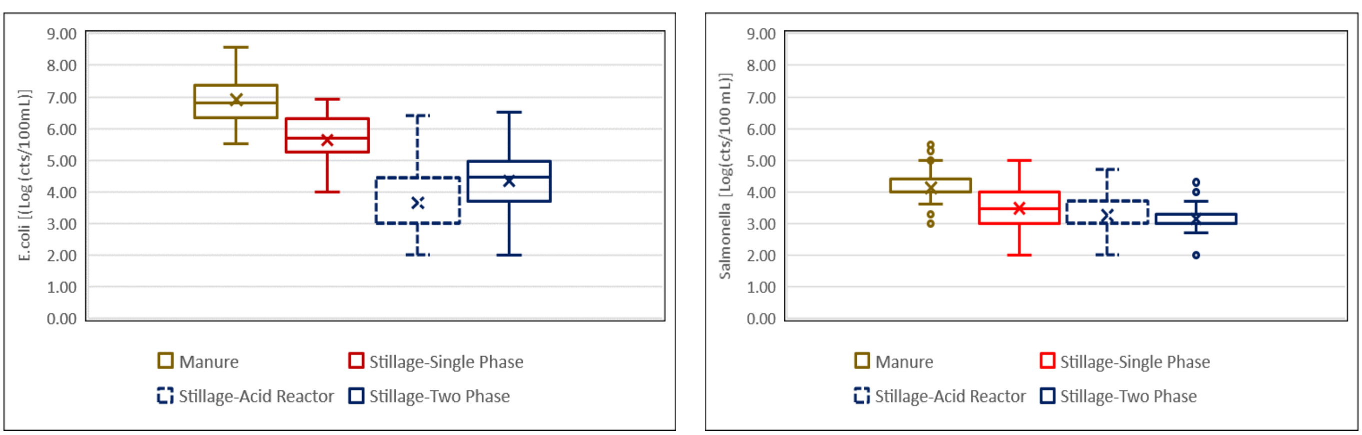
Figure 4.10 also illustrates the high VFA concentrations for the acid reactors in the two-phase systems. The acid reactor for the thin stillage two-phase system averaged a VFA concentration of 5.18 ± 1.05 g/L, while that of the waste grease two-phase system averaged a VFA concentration of 3.98 ± 1.52 g/L. This demonstrates the acid reactors were working well in producing acids for subsequent methane production.

Preliminary BMP assays determined at least 25% of the feed must be co-substrate (thin stillage or waste grease) to maintain acid accumulation (3 g/L short chain fatty acids) and lower pH in the acid reactor.

4.3.2.3 Pathogen Indicators

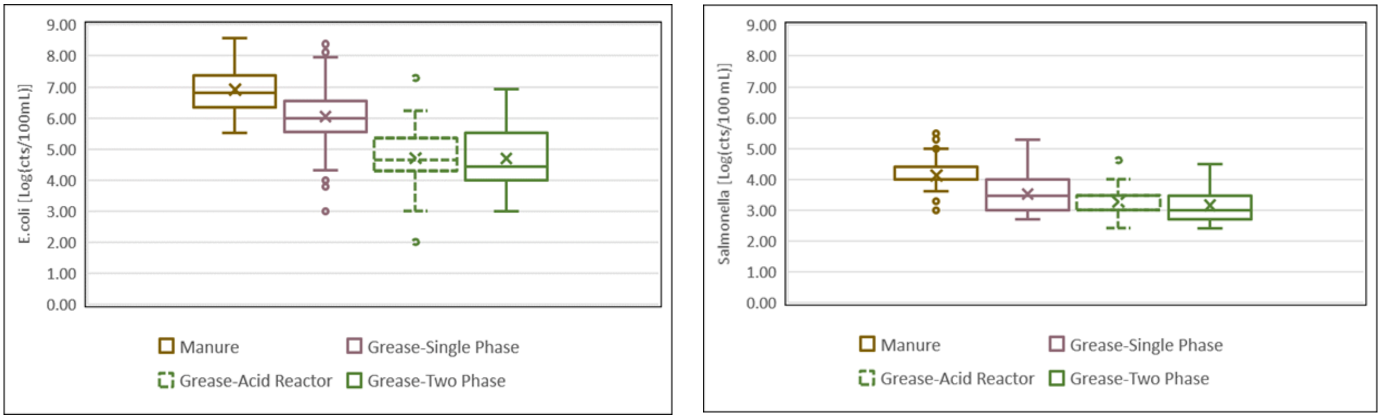
Although pathogens in manure are not regulated under O. Reg. 267/03 for land application, the fate of pathogens in anaerobic digesters is interesting on a treatment perspective. Pathogen indicators monitored for this study were *E.coli* and *Salmonella*, and all feedstocks and digestate samples were analysed for these parameters. Figures 4.11 a) and b) illustrate the Box and Whisker plots for *E.coli* and *Salmonella*, respectively, for corn thin stillage co-digestion trials. Figures 4.12 a) and b) illustrate Box and Whisker plots for *E.coli* and *Salmonella*, respectively, for waste grease co-digestion trials. The Box and Whisker plots illustrate mean and median values of log bacteria concentrations, along with the degree of dispersion and skewness in the data.

When compared to raw dairy manure, there was a 1.3 log CFU/100 mL reduction (from mean values) for *E.coli* in the digestate from the single-phase thin stillage co-digestion, while a 0.8 log CFU/100 mL reduction was observed for *Salmonella*. The low pH (5.64 ± 0.25) in the thin stillage acid reactor allowed for a 3.3 log CFU/100 mL reduction in *E.coli* (compared with raw manure) and a 0.9 log CFU/100 mL reduction in *Salmonella*; however, when conditions became more favourable in the methanogenesis reactor, mean *E.coli* concentrations increased by almost 1 log CFU/100 mL resulting in a digestate with a 2.6 log CFU/100 mL reduction in *E.coli* compared to raw dairy manure. *Salmonella* concentrations remained at 0.9 logs CFU/100 mL lower than raw dairy manure for the two-phase system.



a) *E.coli* Box and Whisker Plots b) *Salmonella* Box and Whisker Plots

Figure 4.11 Box and Whisker Plots for E.coli and Salmonella in Digestate from Single and Two-Phase Co-digestion of Corn Thin Stillage with Liquid Dairy Manure



a) *E.coli* Box and Whisker Plots b) *Salmonella* Box and Whisker Plots

Figure 4.12 Box and Whisker Plots for E.coli and Salmonella in Digestate from Single and Two-Phase Co-digestion of Waste Grease with Liquid Dairy Manure

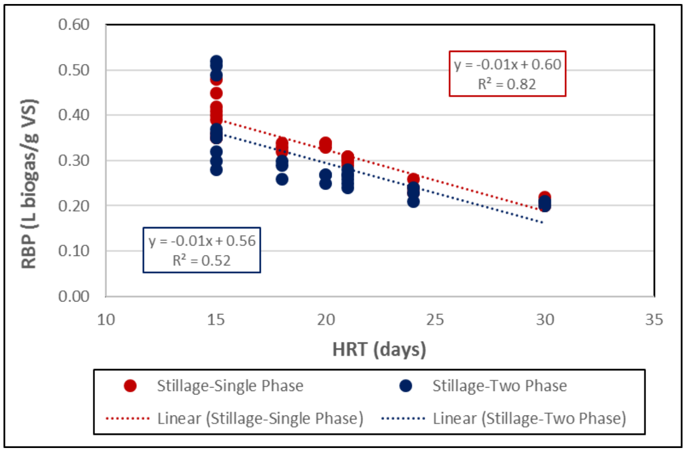
Similar reductions for pathogen indicators were observed from co-digestion trials using waste grease. For single-phase digestion, digestate was 0.9 logs CFU/100 mL lower in *E.coli* (mean values) than raw dairy manure, while *Salmonella* was 0.6 logs CFU/100 mL lower. In the two-phase system, digestate from both the acidogenesis and methanogenesis reactors demonstrated means 2.2 logs CFU/100 mL lower in *E.coli* and 1.0 log lower in *Salmonella* than raw dairy manure. pH of the acid reactor was on average 5.12 ± 0.19.

Essentially 1 to 3 log CFU/100 mL reductions in pathogen indicators were observed in digestate when compared with raw dairy manure. The acidogenesis reactor of the two-phase system seemed to help achieve the higher log reductions in *E.coli* but did not appear to have a greater impact on *Salmonella* die-off. These observations in pathogen indicator die-off are consistent with what has been reported in literature (Harikishan and Sung, 2003; Wright et al., 2004; Smith et al., 2005, Crolla et al., 2013).

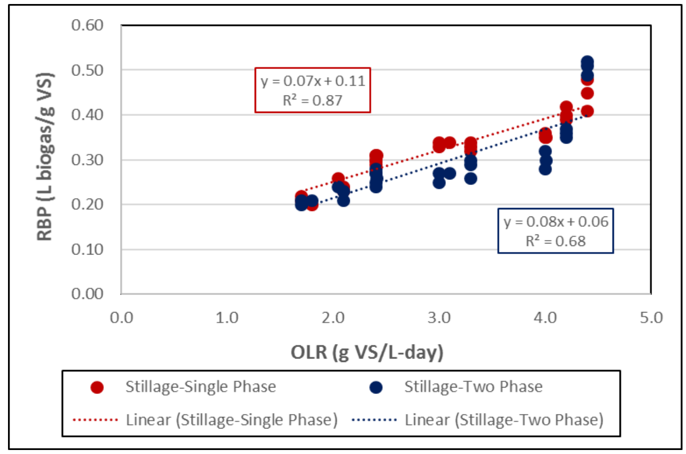
4.3.2.4 RBP Yields

RBP trials were conducted on digestate samples collected during the steady state period for the various OLR trials. RBP yields in this study were not only used to determine the degree to which the substrates were digested but also the organic matter stability of the digestate for subsequent land application. The RBP assays were incubated for 60-days and the cumulative biogas yield calculated. The RBP yields for the various OLR trials are presented in Tables 4.6, 4.7, 4.8 and 4.9 for the single-phase stillage, two-phase stillage, single-phase grease and two-phase grease digestion trials. Figure 4.13 illustrates the correlation of RBP yields with respect to HRT of digestion, digester OLR and VS reduction during digestion of the corn thin stillage trials. Both the single and two-phase stillage digestion trials show a good correlation (Pearson r of 0.90 and 0.72, respectively) of RBP with HRT (Figure 4.13 a)) indicating RBP are lowest when HRTs are highest. The Pearson r for the two-phase stillage digestion is slightly skewed due to the two digester trials (VIII and IX) that resulted in digester upsets. Figure 4.13 b) demonstrates that RBP increases with OLR, which is to be expected as OLR are inversely proportional to HRT. A Pearson r does not show a distinct correlation of RBP with %VS reduction; however, it can be stated that RBP are lowest when %VS reductions are highest (Figure 4.13 c)).

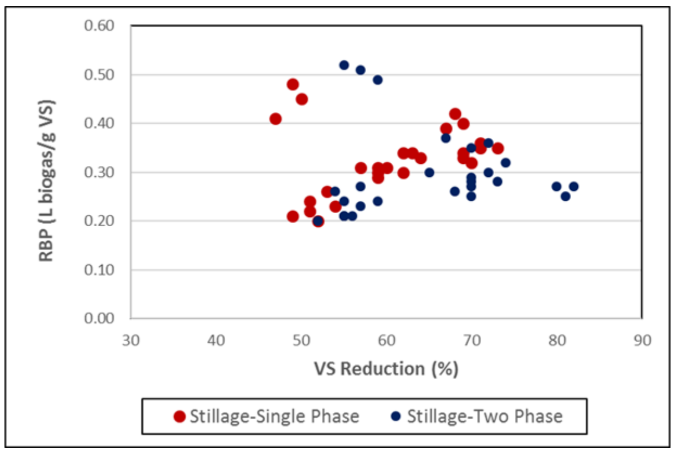
When digesters were operating well, RBP yields ranged from 0.22 to 0.35 L biogas/g VS and 0.21 to 0.29 L biogas/g VS for single-phase stillage and two-phase stillage digestion trials, respectively. RBP yields increased to over 0.4 L biogas/g VS when digester upset occurred. The RBP yields from this study demonstrated the digestion trials with corn thin stillage produced stable digestate with low residual biogas production. Lower RBP yields were obtained with the two-phase system, suggesting a more stable digestate.



a) RBP with Respect to HRT of Digestion



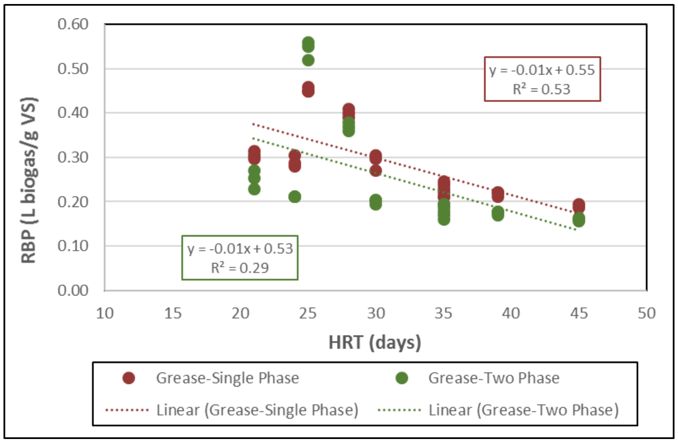
b) RBP with Respect to OLR of Digester



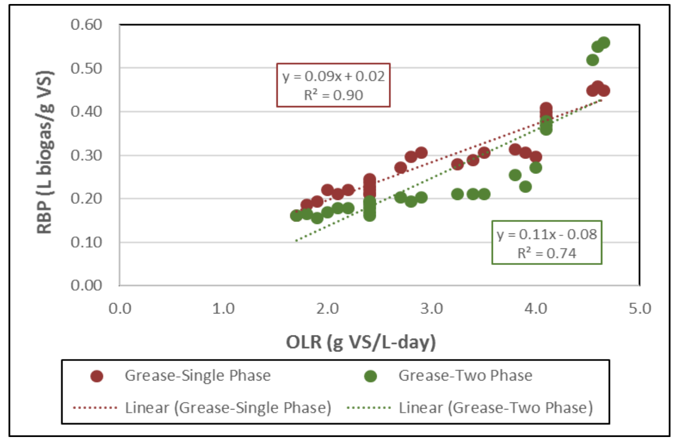
c) RBP with Respect to VS Reduction during Digestion

Figure 4.13 Correlation of RBP with Respect to HRT of Digestion, OLR of Digester and VS Reduction during Digestion – for Co-digestion Trials using Corn Thin Stillage

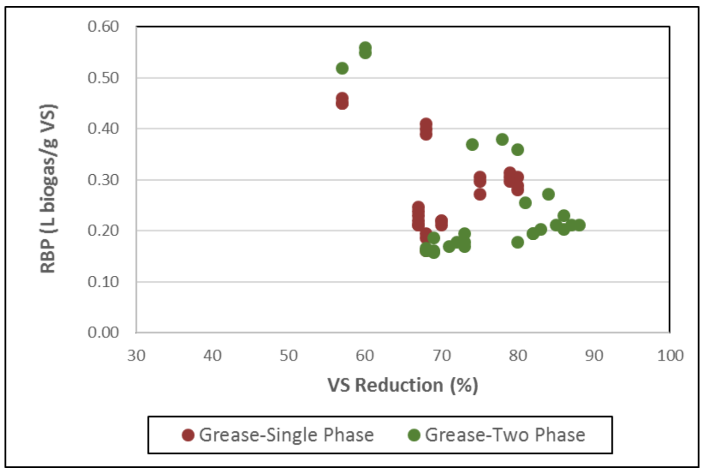
Figure 4.14 illustrates the correlation of RBP yields with respect to HRT of digestion, digester OLR and VS reduction during digestion of the waste grease trials. Both the single and two-phase grease digestion trials show a negative correlation (Pearson r of 0.73 and 0.54, respectively) of RBP with HRT (Figure 4.14 a)) indicating RBP are lowest when HRTs are highest. The Pearson r's for the single and two-phase grease digestion trials are somewhat skewed due to the two digester trials (VIII and IX) that resulted in digester upsets. RBP are lowest when %VS reductions are highest. Figure 4.14 b) demonstrates that RBP increases with OLR, while Figure 14.14 c) demonstrates RBP are lowest when %VS reductions are highest.



a) RBP with Respect to HRT of Digestion



b) RBP with Respect to OLR of Digester



c) RBP with Respect to VS Reduction during Digestion

Figure 4.14 Correlation of RBP with Respect to HRT of Digestion, OLR of Digester and VS Reduction during Digestion – for Co-digestion Trials using Waste Grease

For digesters that did not experience an upset, RBP yields ranged from 0.19 to 0.31 L biogas/g VS and 0.17 to 0.26 L biogas/g VS for single-phase grease and two-phase grease digestion trials, respectively. RBP yields increased to over 0.4 L biogas/g VS when digester upset occurred. The RBP yields from the study demonstrated the digestion trials with grease produced stable digestate with low residual biogas production. Lower RBP yields were obtained with the two-phase system, suggesting a more stable digestate.

Data presented in literature has demonstrated RBP to be an effective measure of digestate quality; assessing whether residual VS of the digestate can be further degraded. Schievano et al. (2008) determined the average RBP from the digestate of a full-scale biogas plant in Italy was 261.7 ± 89.1 mL biogas/g TS (co-digestion of swine manure with energy crops, agri-food industry waste and source separated municipal solid waste). Thygesen et al. (2014) determined the RBP from four Danish biogas plants (manure and co-substrates) to range from 156 – 240 L CH_4/kg VS and reported these yields to be in accordance with BMPs from untreated cattle manure (148-223 NL CH_4/kg VS) and non-herbaceous plant biomass (147-250 NL CH_4/kg VS). The authors found the lowest RBP in samples from digesters with long HRTs, while relatively higher residual RBP were obtained from samples from digesters with short HRTs. Banks et al. (2013) presented RBP data from 59 German biogas plants that showed no relationship between VFA concentrations and RBP. The study demonstrated RBP increased from digestate samples with plants operating at increased OLRs. Plants with short HRTs (less than 50 days) tended to result in higher RBPs.

4.3.2.5 Nutrients

During anaerobic digestion, organic matter is degraded and organic C is converted to biogas. In doing so, nutrients in organic matter are transformed into their inorganic (soluble) form. That is, organic N is transformed to NH_4^+-N and organic P is converted to PO_4^{3-} -P. Essentially, the total nutrients content remains the same during digestion but only their respective forms change. Nitrogen data is presented in Tables 4.6 to 4.9 and NH_4^+-N concentrations consistently increased throughout the digestion trials (compared to the raw feed substrates). The NH_4^+-N/TKN ratios in digestate ranged from 0.6 to 0.8, while the ratio is closer to 0.2 for raw dairy manure. A similar observation was made with P. The PO_4^{3-} -P/TP ratios in digestate ranged from 0.5 to 0.8, slightly higher than those of the raw substrates. The increased concentrations of inorganic nutrients in the digestate can mean increased crop yields since mineral nutrients are readily available for crop uptake soon after land application. The uptake of nutrients right after land application can also translate to less nutrient migration to tile drains or surface waters and ultimately decreasing the potential of negative impacts on the environmental. Crolla et al. (2013) thoroughly discusses increased crops yields and environmental impacts associated with the land application of digestate.

4.4 Conclusions

The co-digestions of corn thin stillage with liquid dairy manure and waste grease with dairy manure demonstrated to enhance biogas production. Literature suggested two-phase digestion systems may not be suitable for dairy manure; however, these studies did not consider co-digestion with substrates rich in soluble organic matter. The use of a two-phase digestion system demonstrated to be effective with the co-digestion. The main conclusions from this study include:

1. Methane yields increased by over 22% using two-phase digestion systems compared with single-phase, for both corn thin stillage and waste grease co-digestion. Based on current FIT contracts of 18 to 20¢/kWhe, the increased electricity and heat production could make the two-phase system economically attractive for producers.
2. OLRs over 4.4 g VS/L led to digester upset. It is recommended that co-digestion of corn thin stillage with dairy manure and co-digestion of waste grease with dairy manure should not be operated at OLRs of over 4.2 g VS/L·day.
3. The sudden peak and continued rise of IA/PA was an effective indicator of an impending digester upset. IA/PA values are typically specific to the digester substrate makeup.
4. A proportion of at least 25% co-substrate in the feed was effective at maintaining the acid reactor at lower pH values and maintaining VFA concentrations above 3.0 g/L. Lower proportions of co-substrate in the feed are not recommended as the buffering capacity of manure will make acidification of the acid reactor difficult.
5. Significant VS reductions of 51 to 81% were obtained in the co-digestion of corn thin stillage and waste grease with liquid dairy manure. Final digestate VS concentrations were over 50% lower than raw dairy manure.
6. 1 to 3 log CFU/100 mL reductions in pathogen indicators (*E.coli* and Salmonella) were observed in the digestate when compared with raw dairy manure. The unfavorable accumulated acids and low pH in the acid reactor (two-phase system)

helped achieve the higher log reductions in *E.coli* but did not appear to have a greater impact on *Salmonella* die-off.

7. RBP yields demonstrated to be a good parameter for measuring digestate stability. Two-phase digestion contributed to more stable digestate as lower RBP yields were obtained. RBP yields over 0.4 L biogas/g VS were obtained when digestion was incomplete due to digester upset. RBP yields were highest with low HRTs and lowest with high HRTs. Digestion processes that achieved VS reductions of 60 % or lower had digestates with the highest RBP yields.
8. The NH_4^+-N/TKN ratios in digestate ranged from 0.6 to 0.8, while the ratio is closer to 0.2 for raw dairy manure. A similar observation was made with P. The PO_4^{3-} -P/TP ratios in digestate ranged from 0.5 to 0.8, slightly higher than those of the raw substrates. Increased concentrations of inorganic nutrients in the digestate can mean increased crop yields.

Acknowledgements

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Chapter 5 - Evaluation of the Operation and Digestate Quality of Ontario On-farm Digesters

Abstract

Seven on-farm anaerobic digesters were studied for digester performance and digestate quality. Digestate characteristics were in accordance with performance criteria suggested in literature. Residual biogas potential (RBP) yields were effective at evaluating the stability of digestate and the U.K. PAS 110:2014 limit of 0.45 L biogas/g volatile solids (VS) (28 days incubation) was assessed too lenient for the Ontario systems studied. A limit of 0.25 L biogas/g VS after 28 days of incubation or 0.45 L biogas/g VS after 60 days of incubation are recommended. VS reductions for on-farm digester systems ranged from 56 to 76%; higher than the Nutrient Management Act O. Reg. 267/03 regulation of 50% reduction. However, digesters using grease or food based co-substrates can easily achieve the 50% VS reduction; however, when using co-substrates that are harder to digest, like cellulosic energy crops, a 50% reduction in VS may be more challenging to achieve consistently. *E.coli* and *Salmonella* were typically 1 to 3 logs CFU/100 mL lower than raw manure and increased hydraulic retention time (HRT) did not demonstrate a significant impact on the bacterial log reductions. Intermediate alkalinity (IA)/partial alkalinity (PA) proved to be a valuable tool in determining potential digester upset and has been recommended as a standard performance parameter for on-farm systems.

Keywords: residual biogas production, digestate stability, digester performance, VS reduction, pathogen die-off, biogas yields

5.1 Introduction and Literature Review

In Ontario, Canada, on-farm anaerobic digesters may be regulated under the Nutrient Management Act Ontario Regulation 267/03 (O. Reg. 267/03) and in October 2013 new amendments were made. The province currently has over 40 full scale on-farm anaerobic digesters co-digesting manure and organic rich substrates for methane and electricity/heat generation that is sold to the Ontario electrical grid. Most of the digesters are co-digesting waste grease (due to high biogas yields); however, as the supply of this grease is being depleted other co-substrates like food waste and energy crops are also being implemented. The on-farm biogas-electricity industry in Ontario is relatively young (just over 10 years) and the objective of this study was to evaluate design criteria, management, and performance criteria for anaerobic digestion process monitoring, and potential digestate output characteristics testing. The current requirements for digester approvals under the Nutrient Management Act (O. Reg. 267/03) regulations include a minimum retention time of 20 days or a system designed by a Professional Engineer to reduce VS by at least 50 percent in less than 20 days. As the design of Ontario digester systems change and the use of alternative co-substrates increases, there is a need to thoroughly evaluate alternative criteria for ensuring proper operation and digestate quality from on-farm anaerobic digesters.

The biological activity of a digester can be determined by assessing the extent to which the readily degradable organic matter has been consumed (Gerardi, 2003; Banks et al., 2013). Banks et al. (2013) go on to describe that within the context of compost or stored agricultural residues, stable digested products are characterised by being rich in recalcitrant organic molecules that have reduced levels of residual metabolic activity. Residual biogas production, VFA concentrations and reduced VS, with minimum HRTs, have all been proposed as potential parameters that can be used to assess the stability of organic wastes; however, the relationship between these parameters and the scientific support for their use related to AD digestate needs to be further assessed (Siebert et al., 2008; PAS 110, 2010; Walker et al., 2010; Eastern Research Group, 2011). Further, the relationship needs to be assessed between these parameters, the operational productivity of an anaerobic digester and environmental risk management.

The overall objective of this study was to assess how well monitoring parameters can be used to evaluate digestate quality and ultimately digester performance of seven Ontario on-farm digesters, and how these monitoring parameters vary with different digester operating conditions. The information gathered from this study will help in better understanding different digester designs, management and performance, which will help inform future policy development of regulatory approvals for on-farm anaerobic digesters.

5.1.1 European Certification Programs for the Operation of Anaerobic Digesters and Digestate Quality Requirements

European certification programs concerning the operation of biogas plants and digestate quality vary by country. In general, the certification programs are voluntary quality assurance systems that take into account national regulations, set lists of permissible feedstocks and outline digestate quality standards required to meet stability and hygienic requirements (Saveyn and Eder, 2014). Many of the protocol requirements include documenting reactor temperature, pH, HRT and OLRs, along with detailed records of types and sources of feedstocks added. Some standards identify a minimum number of annual digestate samples and report of analysis that demonstrates digestate quality. This section provides a brief summary of some of the various certification programs in Europe describing digester operation and digestate quality criteria.

For the purpose of commercially marketing a biofertilizer product in the UK the British Standards Institution's Publicly Available Specification (BSI PAS 110) aims to set criteria for digestate quality from anaerobic digesters that accept source-segregated biowastes (which can include both green bins and food-processing waste). An alternative set of quality criteria are provided in the PAS 110 for digestate made only from manure, processed and unprocessed crops, crop residues, glycerol, and animal bedding. The PAS

110 also includes setting controls on input materials and the management of anaerobic digestion systems, along with specifying minimum quality objectives of whole digestate, separated liquor and separated fibers (PAS 110, 2014). Test parameters outlined in the PAS 110 define upper and lower limits of various parameters which include: pathogen indicators (*E.coli*, *Salmonella*), metals, VFAs, residual biogas production, physical contaminants (e.g. glass, stones, and plastics), TS, VS, nutrient concentrations and pH (Walker et al., 2010; PAS 110, 2014).

The German RAL GZ 245 (biowaste) and 246 (renewable energy crops) are voluntary standards which monitor and assess performance of many German composting sites and a large number of digesters (Saveyn and Eder, 2014). RAL is the German Institute for Quality Assurance and Certification. Under the RAL GZ 245 standards suitable raw materials for digestate products from biowaste include organic municipal waste, residues from the food and animal feed industry and organic industrial waste, and mineral composting additives. Sewage sludge is not included as an input material. The RAL GZ 246 standards define input materials for digestate products from renewable energy crops as manure, slurry and dung from animal origin, energy crops and diversified materials, like green-waste from landscaping and straw. The minimum quality criteria for digestate products under the RAL GZ standards include minimum VS percentage of TS, limits for heavy metals and pathogens, physical contaminants and the degree of fermentation (total VFAs limit) (Siebert et al., 2008; Saveyn and Eder, 2014).

The SPCR 120 (SP Technical Research Institute of Sweden) is a Swedish voluntary certification program for anaerobic digestion systems that outlines quality assurance for both the process and digestate quality. The program outlines that feedstocks used to produce certified digestate should be clean, source separated and easily biodegradable; while manure substrates are allowed, sewage sludge is not permitted among input materials (Saveyn and Eder, 2014). Digestate certified under this program will carry a waste status label of Swedish Waste Management (SPCR 120, 2009; Al Seadi and Lukehurst, 2012; Saveyn and Eder, 2014). The digestate quality criteria under this program include guidelines for metals content, requirements for disease control, and limits for visible impurities (e.g. plastics, glass, and composites), weed seeds and plant parts (Saveyn and Eder, 2014).

The Swiss Quality Guidelines for Compost and Digestate outlines minimum quality requirements for final products, focussing on parameters such as nutrient content (N, P, K), heavy metals, organic matter, hygiene and xenobiotic compounds. These quality requirements were first established by the Research Institute of Agrochemistry and Environmental Hygiene in 1995 and are still being used in the 2010 standards (Fuchs et al., 2008; Al Seadi and Lukehurst, 2012). The guidelines are designed to ensure high quality products and recommends best practices for their uses in agricultural applications as soil amendments. In doing so, the guidelines recommend suitable substrates for digestion and compost and outlines sampling regimes and analytical parameters to ensure the quality of the finished products (Al Seadi and Lukehurst, 2012).

Other European countries including Belgium, Denmark, Greece, Italy and The Netherlands have also adopted guidelines or quality standards outlining the use of appropriate substrates for digestion, digestate quality and stability, and recommended end-uses (Al Seadi and Lukehurst, 2012; Saveyn and Eder, 2014; Nordberg, 2015). Typically, in Europe guidelines and quality standards are voluntary programs designed to assure product quality primarily for those products being sold outside of the farm unit.

5.1.2 U.S. and Canadian Standards and Regulations for Digester Operation

In comparison to Europe, the current U.S. and Canadian biogas industries are relatively young. In the U.S., anaerobic digesters are obligated to comply with local, state and federal regulatory requirements. These regulations can include federal air regulations, federal solid waste regulations and federal water regulations. Due to some inconsistencies within these regulations, the American Biogas Council (ABC) is developing its own Digestate Standard Testing and Certification Program. Development of this program started in 2014 and should be fully launched in early to mid-2017. The program is a voluntary, industry-led initiative that identifies suitable testing methods and quality management systems for characterizing digestate products. The standard focuses on outlining suitable substrates for digestion and the characterization of digestate for post-digestion application. Characterization of the digestate includes, but not limited to, pH, conductivity, nutrients (N, P, K), organic matter, particle size, maturity (bioassay), stability (respiration) and trace metals (ABC Task Force, 2016). The ABC standard and certification program considers digestate standards from other countries and U.S. EPA regulations, resulting in standardized testing procedures and technical data sheets for digestate physical, chemical and biological characterization (McDonald and McCrae Kessler, 2016).

In Canada, agricultural anaerobic digesters are regulated by provincial governments, typically Ministries of Agriculture and/or Environment. In Ontario, on-farm anaerobic digesters can fall under the jurisdiction of OMAFRA (Nutrient Management Act, O. Reg. 267/03) and/or MOECC (Environmental Protection Act. R.S.O. 1990, c.E. 19, as amended and O. Reg. 347). The Nutrient Management Act regulates the operation of the on-farm anaerobic digester in terms of minimum HRT and VS reductions, categories of off-farm materials allowed as co-substrates with manure, restrictions on annual and daily acceptable volumes of off-farm materials, allowable metals contents in off-farm materials and, setbacks to dwellings, residential areas and commercial or institutional uses. Under the MOECC an Environmental Compliance Approval (ECA) will be issued for the acceptance and use of off-farm materials and use of the digestate post-digestion.

5.1.3 Residual Biogas Potential Test

PAS 110 describes the RBP test as a parameter that can be used to measure digestate stability of whole digestate, separated liquor and separated fibers (PAS 110, 2014). The RBP test is a lab method that was developed by a consortium made up of the University of Southampton, the Open University and the consultancy WRc Plc in an inter-laboratory comparative study. The test involves measuring the residual biogas production (to completion) from the incubation (35°C) of a digestate sample (Walker et al., 2010). The samples can be incubated with or without inoculum and have an incubation period of 28 to 50 days; RBPs without inoculum typically exceed 50 days (Walker et al., 2010; REA et al., 2013). The residual biogas production from the digestate samples were compared with results from a virgin comparator (cattle slurry) and the original PAS 110 requirements set the RBP limit to 0.25 L biogas/g VSadded under an incubation period of 28 days (PAS 110, 2010). Cattle slurry was chosen as the virgin comparator as it was considered to have already undergone natural digestion (REA et al., 2013). In the revised version of PAS 110 the RBP limit is set at 0.45 L biogas/g VSadded (PAS 110, 2014). In 2013 a proposal was submitted to the PAS Review Steering Group by the AD Operators Working Group (composed of various anaerobic digester operators in the UK) and recommended the revision of the RBP limit of 0.25 L biogas/g VSadded in PAS 110:2010 (REA et al., 2013). The proposal argued the defined limit would be too low for many digestates, particularly food-based ones. The authors suggested the limit be revised and pig slurries a more appropriate virgin comparator as these slurries were suggested to be much more representative of food-based digestates, both in physical appearance and nutrient content. The proposal presented the RBP of six pig slurries, where four of the six samples had values equal or above 0.5 L biogas/g VSadded, with one peaking at 0.9 L biogas/g VSadded (REA et al., 2013).

A comprehensive review of the RBP test was conducted by Banks et al. (2013) for the Waste and Resources Action Programme (WRAP), where the RBP values were determined for digestate samples from 24 full-scale anaerobic digesters in the UK (treating wastes from commercial and municipal sources). Seventy five percent (75 %) of the digestate samples tested passed the PAS 110:2010 limit of 0.25 L biogas/g VSadded, and those that failed had clear indications of process instability (Banks et al., 2013). The study therefore suggested that the test is a satisfactory method for demonstrating the finished digestate stability and the test procedure gives reproducible and repeatable results. The review goes on to suggest the test duration could potentially be reduced to 10 days with a corresponding residual biogas production of 0.2 L biogas/g VSadded. The Banks et al. (2013) review also suggested the RBP test should only be conducted on whole digestate or separated digestate fiber fractions, as the TS content of these substrates more closely resembles the dry matter content of the virgin comparator of cattle slurry that was used to set the RBP biogas limit. Due to the long incubation period of the test, Banks et al. (2013) suggested it may be more suitable for RBPs to be conducted periodically, at the start of the operation of a digester system or with changing digester operating parameters. The review concluded that pre-screening samples with a VFA content greater than 0.43 g COD/g VS lead to a higher potential of substrates failing the RBP limit of 0.25 L biogas/g VS. The revised version of PAS 110 states the pre-screening value to be 0.774 g COD/g VS for potential failure of the RBP limit of 0.45 L biogas/g VS (PAS, 2014).

In Denmark digester HRTs are typically 15 days, and because these shorter HRTs may not allow for full digestion of organic matter Hansen et al. (2006) conducted a study to quantify potential methane production during the storage of anaerobically digested municipal organic waste. The study involved conducting residual methane trials on digestate from pilot-scale reactors (HRT = 15 days) digesting source separated organic waste (diluted to 5% dry matter). The batch tests were conducted under various temperatures (5, 10, 15, 22, 28, 35 and 55°C) and incubated for 125 days; no inoculum was used. The study results demonstrated slow methane production at temperatures below 28°C and methane potential yields were determined to be approximately 95 Nm^3 CH_4/Mg VS (NL CH_4/kg VS), 140 Nm^3 CH_4/Mg VS and 225 Nm^3 CH_4/Mg VS at incubation temperatures of 28, 35 and 55°C, respectively. The authors also determined that 80% of the degradable waste in the digestate was degraded during the batch residual methane tests when incubated at 55°C. The study concluded that covering storage tanks and collecting the biogas not only contributes to the overall biogas yield of the plant but reduces potential methane emissions during subsequent storage. These residual methane yields are similar to those determined by Paavola and Rintala (2008) who investigated the methane potential of digestate from lab-scale reactors (HRT = 20 days; 35°C; 55°C) co-digesting dairy manure with household source-separated organics. The authors determined the digestate residual methane yields to range from 140 to 160 mL CH_4/g VS when assayed for 200 days at 35°C; no inoculum was added.

Lindorfer et al. (2008) measured the residual methane potential from the digestate of a full-scale two stage digester system in Austria co-digesting swine manure, energy crops and residues from vegetable processing. The digester system was composed of two CSTRs operating at mesophilic temperature (39 °C) and a total HRT of 130 days. The residual methane potential of the digestate was reported to be 1.54 L CH_4/kg fresh matter (after approximately 95 days of incubation at 35 ± 1 °C), and with 3.94 % VS this yield can be converted to 39 L CH_4/kg VS; considerably lower than the RBP limits suggested by PAS 110. It is presumed that the long HRT of 130 days of the digester biogas plant was an important contributor to producing a digestate with a low residual methane potential. Lehtomaki et al. (2008) conducted lab trials in determining residual methane potentials of digestate from mesophilic (35 ± 1°C) one-stage and two-stage grass silage leach beds (HRT = 50 days), with leachate recirculated through a UASB reactors. The digestate residual methane potentials ranged from 0.141 to 0.204 m^3 CH_4/kg VS, when assays were incubated at 35°C for up to 100 days.

Schievano et al. (2008) conducted a study that monitored a full-scale biogas plant in northern Italy, which co-digested swine manure with energy crops, agri-food industry waste and source separated municipal solid waste and consisted of a series of four thermophilic CSTRs (HRT = 40-50 days). The monitoring program included measuring the RBP of the digestate and when incubated at 37 ± 1 °C for 60 days was determined to average 261.7 ± 89.1 NmL biogas/g TS. The study also showed that there was a strong linear regression between biogas potential and VS concentration of the digestate. The authors state that this was unexpected as RBP depends not only on VS content but also VS composition and quality. The RBP reported in this study is in the same magnitude as the PAS 110 limits.

Another study of full-scale Italian biogas plants was conducted by Menardo et al. (2011), who investigated residual biogas and methane yields from digestate samples of four mesophilic (41 °C) biogas plants in the Piemonte region of northern Italy. All four of the biogas plants co-digested animal manure with energy crops; however, two of the digesters also received food industry by-products (e.g. slaughterhouse waste, tomato skins or glycerol) as part of the feeding mixture. The OLRs of the biogas plants ranged between 0.85 to 2.25 kg VS/m^3 and HRTs varied between 51 and 105 days. Study results demonstrated residual biogas yields ranging from 20.75 ± 5.81 NL biogas/kg VS (2.88 ± 1.15 NL CH_4/kg VS) to 81.67 ± 4.35 NL biogas/kg VS (37.63 ± NL CH_4/kg VS). No inoculum was added for the residual biogas yield tests in this study. The reported values are significantly lower than residual biogas/methane yields reported in other literature references described in this review; however, the authors propose these discrepancies may be due to the chemical characteristics of the digestate samples (which were high in cellulose and lignin) and the specific operating conditions of the biogas plants studied (long HRTs and low OLRs). Menardo et al. (2013) used the Pearson "R" correlation coefficient analysis to demonstrate digestate biogas yields correlated best with OLRs of the biogas plants and the VS (and TS) content of the samples, and found HRT did not significantly affect residual biogas yields.

A study conducted by Thygesen et al. (2014) investigated residual BMPs of the concentrated digestate from eight Danish full scale biogas plants. The mesophilic biogas plants investigated were all manure (dairy or swine) based digesters with some digesters also co-digested food processing waste; digester HRTs ranged from 16 to 30 days. The residual methane potential of the concentrated digestate samples ranged from 156 – 240 NL CH_4/kg VS and Thygesen et al. (2014) reported these yields to be in accordance with BMPs from untreated cattle manure (148-223 NL CH_4/kg VS) and non-herbaceous plant biomass (147-250 NL CH_4/kg VS). The lowest residual BMPs were found in samples from digesters with long HRTs, while relatively higher residual BMPs were obtained from samples from digesters with short HRTs and added co-substrates.

Banks et al. (2013) presented data obtained from Professor Bernd Linke from the Leibniz-Institut für Agrartechnik in Germany showing the performance of 59 German biogas plants with respect to VFA concentrations, OLRs, HRTs, and digestate VS concentrations in relation to RBP. The RBP tests were conducted at 37°C and 22°C for 60 days of incubation. The data showed no relationship between VFA concentrations and RBP. The study demonstrated RBP increased from digestate samples with plants operating at increased OLRs. Plants with short HRTs (less than 50 days) tended to result in higher RBPs. Increased RBP yields were observed when digestate VS concentrations were higher; however, no good correlation between RBP and digestate VS could be determined.

The literature reviewed has demonstrated RBP to be an effective measure of digestate quality; assessing whether residual VS of the digestate can be further degraded for biogas production post the anaerobic digester. The literature also supports the PAS 110 limit to be the appropriate magnitude for RBP of digestates from biogas plants with moderate HRTs.

5.1.4 Volatile Solids Reduction

VS are a measure of organic matter and converted to biogas (CH_4 + CO_2) under anaerobic conditions. The degradation of organic matter (VS) in anaerobic digesters depends primarily on the system configuration and, the physical and chemical characteristics of the substrates (Labatut and Gooch, 2012). Burke (2001) states that the percentage conversion of VS to biogas published in literature is quite variable and can range from 40 to 55% VS reduction in mesophilic manure digesters with loading rates of 2 to 6 kg VS/m^3/day. Labatut and Gooch (2012) suggest VS degradation in manure-only digesters ranges between 30 and 42%, with percent removals considerably higher when co-digesting manure with high strength substrates. Al Seadi et al. (2013) are less conservative and suggest at least 50% of the dry matter content is converted to biogas in manure based digesters, whereas for energy crops the dry matter content can be reduced by as much as 90%. Lindorfer et al. (2008) reported VS reductions of 88.2 ± 1.6% in a full-scale system co-digesting swine manure and energy crops (1:1). Lab studies conducted by El-Mashad and Zhang (2010) demonstrated 30-day batch digestion trials of dairy manure to reduce VS by 41-45%, while digestion trials using food waste alone reduced VS by 82% and the co-digestion of dairy manure with food waste reduced VS by 60-68%. Similar performances were observed in a study conducted by Rico et al. (2011) who found VS removals of 30-36% from the digestion of the liquid fraction of dairy manure in a mesophilic pilot CSTR (HRT of 20 days; OLR of 2.0 kg VS/m^3·day), while Lehtomaki et al. (2008) observed VS reductions of 42-55% in a two-stage leach bed reactor digesting grass silage.

Banks et al. (2013) report Muller et al. (1998) to have observed biological activity diminished at VS levels between 20 and 25% of TS of MSW, but the authors state it would be inappropriate to generalize that VS of 20-25 % TS will ensure minimal biological activity in the digestate. The authors go on to state that this would only be true if the VS content was degraded during digestion by more than 60% and subsequently stabilizing the digestate. Banks et al. (2013) identify that there is a general consensus among the European digestate certification programs that using a percentage in VS destruction alone is not an ideal indicator of digestate quality. In practice, the degree of VS destruction is highly dependent on the type of digestion substrates; VS destruction can be higher for grease and food residues compared to livestock manures (Burke, 2001; Siebert et al., 2008; Banks et al., 2013). PAS 110 does not identify specific criteria for VS content of the digestate and only requires that the final VS concentration of the digestate material be reported. The German RAL GZ 245 and 246 standards state the VS of digestate should represent a minimum of 30% of TS (Siebert et al., 2008; Saveyn and Eder, 2014); acknowledging the need for sufficient organic matter when using digestate as a soil amendment. The Swedish SPCR 120 standard requires solid digestate to contain VS that is at least 20% of TS (Saveyn and Eder, 2014). Under Ontario's Nutrient Management Regulations (O. Reg. 267/03, 2003) the 50% required VS reduction during digestion is only stipulated for systems with HRTs less than 20 days, otherwise a HRT of 20 days is sufficient.

5.1.5 Volatile Fatty Acids

VFAs are intermediates during the production of methane under anaerobic conditions. Methane can be produced by acetoclastic methanogenesis (conversion of acetate to methane) and hydrogenotrophic methanogenesis (conversion of hydrogen and carbon dioxide to methane). VFAs can vary in length but most VFAs produced during anaerobic digestion are short-chain, low molecular weight acids, which include mainly acetic acid/acetate (2 carbons), propionic acid/propionate (3 carbons), butyric acid/butyrate (4 carbons), valeric acid/valerate (5 carbons) and caproic acid/caproate (6 carbons) (Gerardi, 2003; Labatut and Gooch, 2012). Of these acids, acetate is predominant during anaerobic digestion and approximately 85% of the VFA content is acetate (Gerardi, 2003). Labatut and Gooch (2012) and Murphy and Thamsiriroj (2013) suggest 65 to 70% of the methane produced during anaerobic digestion is from acetate and the balance is produced via hydrogen. Propionate is an important precursor to the production of acetate and hydrogen, where approximately 30% of the electron flow that is directly related to methane production is attributed from propionate (Labatut and Gooch, 2012). Banks et al. (2013) present the optimum ratio of acetate to propionate to be 2:1. A useful indicator of possible process upset is an accumulation of acetate followed by propionate (Murphy and Thamsiriroj, 2013). Propionate, acetate and hydrogen are more sensitive to digester upset than biogas production and therefore the periodic monitoring of VFAs is important for the detection of possible impending digester problems (Labatut and Gooch, 2012).

Some of the literature reviewed suggests ideal VFA concentrations for optimum digester performance. Gerardi (2003) reports VFA concentrations below 500 mg/L to be optimum, while concentrations approaching 2000 mg/L may suggest digester upset. These values are corroborated by Labatut and Gooch (2012) who state that a properly designed and operated digester would typically have a total VFA concentration below 500 mg/L (as acetic acid), while higher concentrations may be an indication of an undersized system for the specific loading rate. The authors go on to suggest concentrations over 1500-2000 mg/L may in fact begin to inhibit biogas production. Banks et al. (2013) report that a German publication, *Bavarian State Research Center for Agriculture on 'Ensuring process stability in agricultural biogas plants'*, suggests the most common VFAs in digestate are acetic, propionic and iso-butyric acids and practical experience has shown total VFAs should be below 4000 mg/L, with acetate concentrations below 3000 mg/L, propionate below 1000 mg/L and iso-butyrate much lower than 500 mg/L. Banks et al. (2013) also reported from the same institute on *'Biogas plants – laboratory parameters and process control'* that the acetate concentration in digestate should be around 1000 mg/L and propionate should be much lower and other VFAs should be even lower by a factor of 10. Finally, from another German publication (Biogasbericht Südtirol, 2008) where 30 biogas plants were studied, Banks et al. (2013) summarized the report findings to suggest many of the biogas plants monitored had stable operations even with total VFA concentrations above 1500 mg/L, and plants with high organic loading rates tended to have higher VFA concentrations in the digestate. The report goes on to suggest that VFA content becomes critical when alkalinity is below 8000 mg/L and the biological balance of the digestion process can be at risk from an accumulation of VFAs. Gerardi (2003) and Murphy and Thamsiriroj (2013) state that a VFA/alkalinity ratio greater than 0.3-0.4 may indicate an unstable process, while a VFA/alkalinity ratio greater than 0.8 indicates the process in failing due to acidification and inhibition of methanogenesis.

The German RAL GZ 245 and 246 standards proposed 1500 mg/L for organic acid concentrations (reduced from 4000 mg/L) as a potential limit for digestate (Banks et al., 2013); however, using VFAs as a measure of the degree of digestion is still under debate. It should be noted that in the German RAL standards the VFA limit is also linked to a requirement for a 20-day minimum retention time in the digester to ensure effective degradation. The PAS 110 includes the determination of VFA concentrations as a pre-screening test, where a VFA cut-off value for RBP tests is suggested to be 0.43 g COD/g VS to meet the RBP limit of 0.25 L biogas/g VS in PAS 110:2010 (PAS 110, 2010) and 0.774 g COD/g VS to meet the recent RBP limit of 0.45 L biogas/g VS in PAS 110:2014 (PAS 110, 2014). The only firm conclusions that can be drawn from these VFA cut-off values is that digestate with a high VFA concentration will almost certainly fail a RBP test, but digestate with a lower VFA concentration won't necessarily pass (Banks et al., 2013). VFA concentrations can be converted to COD using Equation [1] as described in Walker et al. (2010).

$$\text{VFA as COD} = (1.066 \cdot \text{Acetic acid}) + (1.512 \cdot \text{Propionic acid}) + (1.816 \cdot \text{Butyric acid}) + (2.037 \cdot \text{Valeric acid}) + (2.235 \cdot \text{Hexanoic acid}) + (2.306 \cdot \text{Heptanoic acid}) \quad [5\text{-}1]$$

– where COD and each VFA is expressed as g/L

The literature reviewed unanimously agreed that VFA (or organic acid) concentrations in a digester system is not a measure of the stability of the digestate but rather an indication of the stability of the digestion process itself (Gerardi, 2003; Labatut and Gooch, 2012; Banks et al, 2013; Murphy and Thamsiriroj, 2013). Labatut and Gooch (2012) go on to suggest that instead of monitoring a digestion process for a specific VFA concentration, it will be the sudden and steady increase of the VFAs in the digestate effluent that will indicate a potential digester upset.

5.1.6 Fate of Pathogens

Since digestate is often land applied, some standards or quality guidelines have included pathogen indicators criteria for digester systems, for example the PAS 110, German RAL GZ 245/246, Swedish SPCR 120, Swiss Quality Guidelines for Compost and Digestate, USEPA AgStar Protocols for Quantifying and Reporting the Performance of Anaerobic Digestion Systems for Livestock Manures (Eastern Research Group, 2011). The pathogen reduction requirements are dependent on the end use of the digestate and the type of feedstocks used in the digestion process; however, the reduction of pathogen indicators is not indicative of the effectiveness of a digestion process itself. It should be noted that the European and American pathogen standards and quality guidelines are requirements for digestate products sold for off-farm uses. Under the Ontario NMA and ECA (MOECC), digestate is used for on-farm land application and pathogen reductions are not a requirement; however, for the purposes of this study pathogen concentrations are compared with raw manure levels.

Smith et al. (2005) suggest a 2-log reduction for *E.coli* is probably the highest level of bacteria die-off that can be obtained from mesophilic digesters. This observation was corroborated by Crolla et al. (2013) who observed average log reductions of 2.5, 1.3 and 2.3 for *E.coli*, *Salmonella* and Enterococci, respectively, in four on-farm mesophilic digester systems co-digesting liquid dairy manure and waste grease. Manyi-Loh et al. (2014) investigated the inactivation of bacterial pathogens in a mesophilic anaerobic digester treating dairy manure and found 1 log reductions for *E.coli* and *Campylobacter* and up to 2 log reductions for *Salmonella*. Smith et al. (2005) suggest efficient mixing and long retention times are important factors for bacterial reductions in digester systems, while Harikishan and Sung (2003) highlight the importance of operating temperature.

5.2 Materials and Methods

5.2.1 Monitoring of On-farm Digester Performance

Seven on-farm digesters were evaluated within the scope of this project. Table 5.1 lists the digester systems used for the study, where the identities of the sites use an anonymous numeric code. The digesters chosen for the study all operated with manure and food processing by-products as the primary co-substrate. The digesters differed in percent addition of off-farm materials (25% vs. 50%), the frequency of feeding and hydraulic retention time (short vs. long). Digesters operating as single and two-tank systems were also included for management and performance evaluation. The number of sample sites and project duration was limited by budget resources.

The digesters selected for the study were sampled at bi-weekly frequencies (and sometimes weekly) for 6 months. The farms that participated in the study typically received new loads of off-farm feedstocks once a week or every two weeks, and therefore the variability of the feedstock characteristics were captured during the length of this study. A sampling package, which included sterilized bottles, gloves, safety glasses, FedEx boxes and slips, and sampling instructions, was sent to each farm prior to the start of the study. Composite samples, comprised of at least five grab samples, were taken for the raw manure, co-substrates and digestate (directly from the digester tank) by farm personnel and sent to the University of Guelph – Campus d'Alfred lab by FedEx using next day delivery. Samples were packed cold using ice packs. Upon arrival at the lab, samples were placed in the fridge (4 ± 1°C) and analyzed within the holding period of the respective analyses. When possible, two-tank digester systems were sampled at the outlet of the first and second digester tanks. Samples were analyzed for RBP, TS, VS, VFA, pH, alkalinity, IA/PA and pathogen indicators (*E.coli* and *Salmonella*). All lab analyses were conducted at the Campus d'Alfred lab following Standard Methods (APHA, 2005); see Section 5.2.2 for analytical details.

Table 5.1 On-farm Digesters used for Performance Evaluation Study

Digester ID	No. of Head Associated with Digester	Hydraulic Retention Time (days)	% Off-farm	Number of Tanks	Co-substrates	Description
Farm A (7012)[1]	316	80	50	1	Waste Grease	Long HRT High co-substrate loading
Farm B (7006)	297	35	50	1	Waste Grease DAF	Short HRT High co-substrate loading
Farm C (7021):	290		25	2	Waste Grease Bread Crumbs Corn Silage	Intermediate HRT Intermediate co-substrate loading
Digester 1		25				
Digester 2		25				
Farm D (7005)	245	30	50	1	Waste Grease Food Waste Corn Silage	Short HRT High co-substrate loading
Farm E (7062):	235		25	2	Waste Grease Chicken Bin Onions	Long HRT Intermediate co-substrate loading
Digester 1		32				
Digester 2		32				
Farm F (7073)	720	64	25	1	Waste Grease	Long HRT Intermediate co-substrate loading
Farm G (7087)	250	45	25	1	Waste Grease	Intermediate HRT Intermediate co-substrate loading

1 Anonymous numeric code identifier for each digester

Information on the operation of the digester systems was collected from the operators, which included: frequency and quantity of input material additions, operating parameters (i.e. hydraulic retention times, temperature, mixing), pre-treatment, biogas/electricity production and composition (where possible), and performance parameters currently being measured. Specifications of the digester system were also required from the producer: number of tanks, tank volumes and dimensions, size of generator. Since the focus of the study was to evaluate digestate quality, biogas production was collected not to determine the efficiency of biogas yield but used instead to assess whether the digester was operating under steady-state conditions.

5.2.2 Lab Analyses for Digester Samples

The raw manure, co-substrate and digestate samples collected at the various digester systems were analyzed for TS, VS, VFA, pH, alkalinity, and pathogen indicators (*E.coli* and *Salmonella*) following Standard Methods (APHA, 2005). Digestate samples were also analyzed for RBP and IA/PA. RBP was analyzed using a modified method as outlined by Walker et al. (2010) and Banks et al. (2013). Refer to Table 5.2 for a description of the analytical methods used.

OLRs for the digester systems were calculated using substrate feeding volumes, VS concentrations of the substrates and digester volumes. HRTs were calculated using substrate feeding volumes and digester volumes. The percent reduction of VS in the digester systems was calculated as the percent difference of the VS digester feed mixture concentration and the VS concentration of the digestate.

Table 5.2 Description of Analytical Methods Used for Digester Samples

Lab Analyses	Description
TS	• Adapted from Standard Method 2540 B. Total Solids dried at 103-105°C (APHA, 2005)
VS	• Adapted from Standard Method 2540 E. Fixed and Volatile Solids ignited at 550°C (APHA, 2005)

VFAs	• Adapted from method as outlined in Adorno et al. (2014) • Varian GC 3800 with FID for VFA detection • Varian Capillary Column CP-FFAP CB (25m, 0.32mm, 0.3 µm) for detection of acetic, propionic, butyric and iso-butyric acids • H_2 gas and Air used for sample combustion, while He was used as a carrier gas and N_2 gas as make-up • FID Temperature: 300°C; make-up N_2 at 20 mL/min • H_2 gas flow: 40 mL/min • Air flow: 250 mL/min • Column flow: 1.0 mL/min • Injector volume: 1 µL • Injector temperature: 260°C; split 20 • Oven temperature: 80°C (1 min) 20°C/min; 120°C (1 min) 20°C/min; 205°C (2 min); 10.25 min of chromatographic time
pH	• Adapted from Standard Method 4500-H^+ B. (APHA, 2005)
Alkalinity	• Adapted from Standard Method 2320 B. Titration Method (APHA, 2005)
IA/PA	• Adapted from Standard Method 2320 B. Titration Method (APHA, 2005) • PA is determined for a 0.1 N H_2SO_4 titration to an endpoint of 5.75 • TA is determined for a 0.1 N H_2SO_4 titration to an endpoint of 4.50 • IA = TA – PA
E.coli	• Adapted from Standard Method 9222 Membrane Filter Technique for Members of The Coliform Group (APHA, 2005) • Difco m-FC Basal Medium with BCIG • Agar plate and filter incubation at 44.5 ± 0.2°C for 24 ± 2 hours
Salmonella	• Adapted from Standard Method 9222 Membrane Filter Technique for Members of The Coliform Group (APHA, 2005) • Difco SS Agar • Agar plate and filter incubation at 35 ± 2°C for up to 48 ± 2 hours
RBP	• Adapted from Walker et al. (2010) and Banks et al. (2013) • Replicated assays were conducted using 500 mL Wheaton serum bottles (150 mL digestate + 75 mL inoculum; 2:1 ratio), agitated at 100 rpm and incubated under mesophilic conditions (37 ± 2 ºC) for 60 days • Inoculum source was digestate from Farm B that had undergone lab incubation and high rate feeding for 2 weeks prior to use • Biogas measurements were taken regularly using a water displacement manometer to measure biogas accumulation in the serum bottle headspace • Biogas composition measurements (%CH_4 and %CO_2) were taken only periodically to confirm methane production during incubation. A GC was used to measure biogas composition.

5.2.3 Analysis of Experimental Data

To normalize the biogas and methane data from the full scale systems, biogas yields (L biogas/g VS) and methane yields (L CH_4/g VS) were calculated. Daily biogas and methane production were divided by the VS being fed to digesters, thereby expressing biogas and methane production in terms of available organic matter. The percent VS reduction was calculated as the percent difference of the VS concentration at the start and end of the digestion period, under steady state conditions.

Standard statistical procedures were implemented. In addition to standard deviation and mean averaging, single factor ANOVA, with one-sided t-test were used to determine if significant differences exist between digestion trials. Statistical significance was established at the $p < 0.05$.

Pearson r regression analysis was used to determine correlations between two variables. Essentially the Pearson coefficient is the covariance of the two variables divided by the product of their standard deviations.

5.3 Results and Discussion

5.3.1 Residual Biogas Potential

RBP trials were conducted on all bi-weekly digestate samples collected from six of the digester systems studied. Average residual biogas yields (along with standard deviations), after 28 and 60 days of incubation at $37 \pm 2\ ^{o}C$, are summarized in Table 5.3 for each of the on-farm digester systems. RBP from Farm G could not be conducted as the duration of the incubation tests would extend beyond the closure date of the Alfred Campus lab closure.

Table 5.3 Average Residual Biogas Production (RBP) of Digestate Samples from Six Ontario On-farm Digesters – after 28 and 60 days of incubation

Digester ID[1]	% Off-farm	Organic Loading Rate (g VS/L· day)	Residual Biogas Production – 28-day Incubation (L biogas/g VS)	Residual Biogas Production – 60-day Incubation (L biogas/g VS)	% VS Reduction during RBP Incubation
Farm A	50	3.2 (0.8)[2]	0.18 (0.04)	0.21 (0.05)	23
Farm B	50	4.3 (0.8)	0.23 (0.04)	0.29 (0.05)	26
Farm C:					
Digester 1	25	3.4 (0.9)	0.27 (0.02)	0.31 (0.02)	34
Digester 2			0.20 (0.03)	0.26 (0.04)	29
Farm D	50	3.9 (0.8)	0.21 (0.04)	0.31 (0.05)	37
Farm E:					
Digester 1	25	3.1 (0.4)	0.19 (0.06)	0.24 (0.05)	28
Digester 2			0.08 (0.01)	0.12 (0.02)	21
Farm F	25	2.0 (0.4)	0.21 (0.03)	0.26 (0.04)	24

1 Residual biogas production for Farm G could not be completed (lab closure).
2 Numbers in brackets indicate standard deviation on average value.

At 28 days of incubation, the average RBP yields of the digestate samples from all of the digester systems met the 0.25 L biogas/g VSadded and 0.45 L biogas/ g Vsadded respective limits set by the PAS 110:2010 and PAS 110:2014 standards (PAS 110, 2010; PAS 110, 2014). At 60 days of incubation, the average RBP yields increased for all digester systems, with only Farms B and D slightly exceeding the older, more stringent PAS 110:2010 limit. The elevated average residual biogas yield for Farms B and D could be a result of both these systems having higher OLRs and Farm D also experienced a mixer breakdown during the study period. Nonetheless, the average RBP yields for all biogas systems had no difficulty meeting the current PAS 110:2014 limit of 0.45 L biogas/g Vsadded. Figure 5.1 illustrates the average residual biogas yields from the digester systems and demonstrates that biogas production was exponential at the onset of the RBP tests but started to plateau at about 20 days into the test. The average RBP yields for Farms A and E were significantly lower (p<0.05) than the other systems, with the lowest RBP yield being from Farm C's second tank at 0.12 L biogas/g VS after 60 days of incubation.

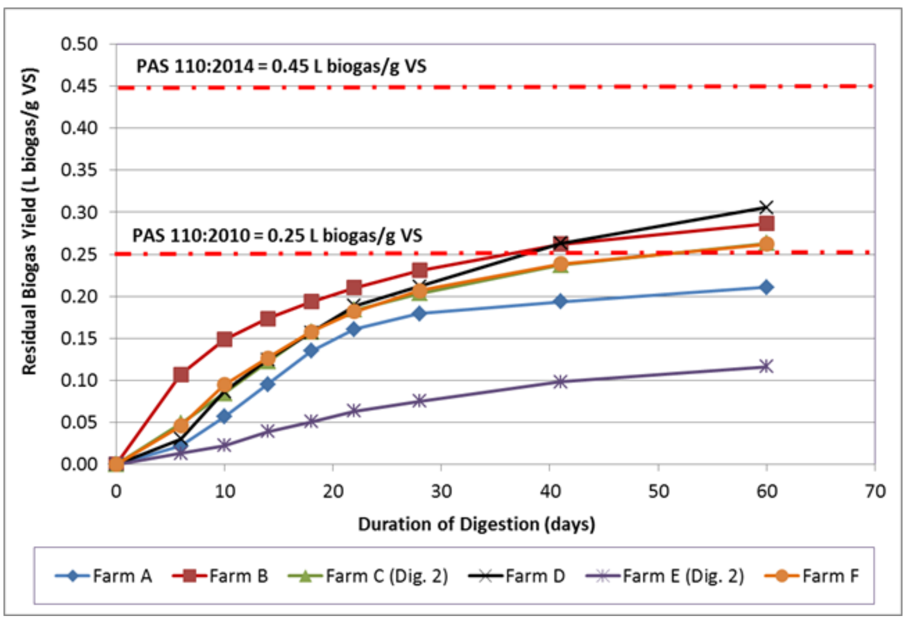
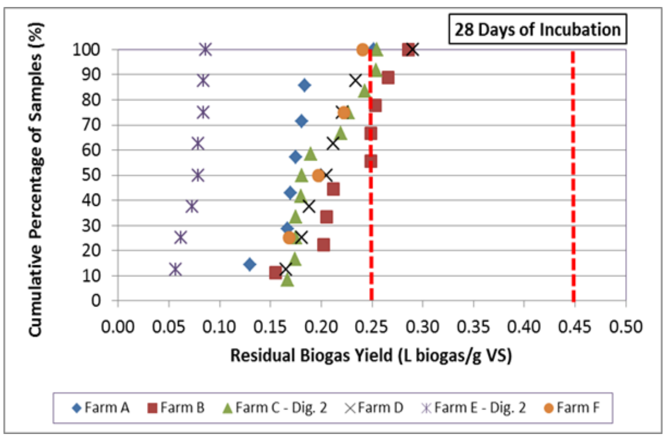


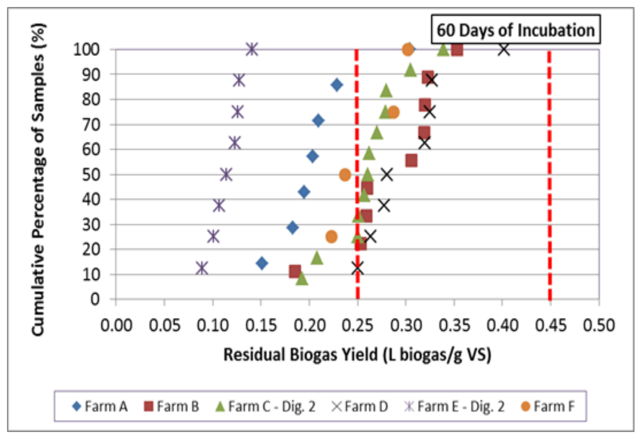
Figure 5.1 Average Residual Biogas Yields for Digestate from Farms A, B, C, D, E and F.
Note: Error bars representing standard deviations on averages were not included for visual clarity of graph. Refer to Table 3 for standard deviations on average RBP values.

When reviewing individual digestate samples from the digester systems, Figure 5.2a) illustrates that 100% of the samples tested for RBP yields from Farms A, C, E and F met the older, more stringent PAS 110:2010 standard at 28 days of incubation, while 80% of the samples from Farm B and 90% of the samples from Farm D met the standard. At 60 days of incubation (Figure 5.2b)), all the digestate samples from Farm E demonstrated RBP yields less than the PAS 110:2010 standard and typically 50% of the samples from the other five farms met the standard. The RBP yields from all digestate samples met the current PAS 110:2014 standard after 60 days of incubation.

Figures 5.3 a), b), c) and d) illustrate the correlation of RBP yields with OLRs, HRTs, %VS reduction and VS concentration of the digestate, respectively. A Pearson R^2 regression analysis does not show a distinct correlation of RBP with OLRs, HRTs, %VS reduction and digestate VS concentration; however, it can be stated that RBP yields were highest when %VS reductions and digester HRTs were shortest.



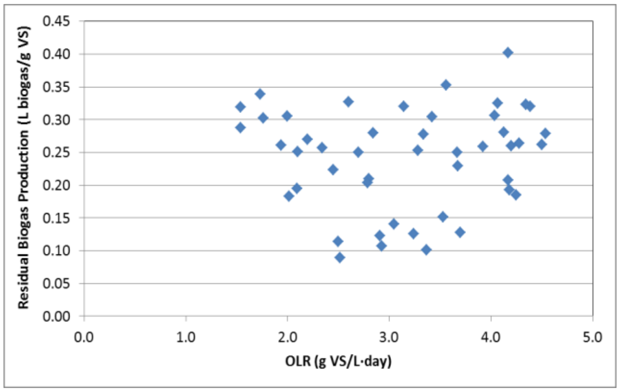
a) RBP Yields after 28 days of Incubation



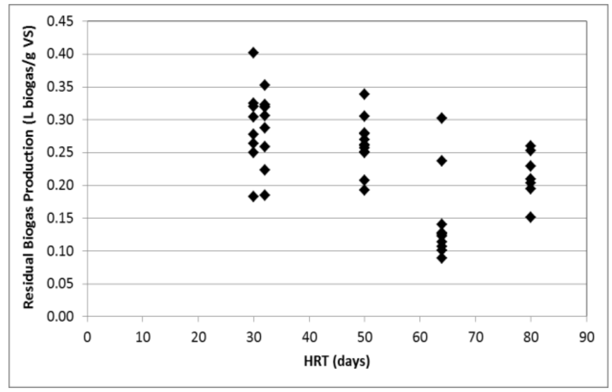
b) RBP Yields after 60 days of Incubation

Figure 5.2 Cumulative Percentage of Samples Meeting PAS 110:2010 (0.25 L biogas/g VS) and PAS 110:2014 (0.45 L biogas/g VS) RBP Limits at both 28 and 60 days of Incubation

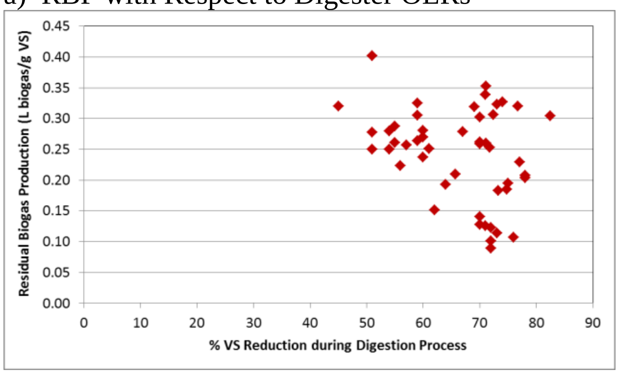
RBP yields were effective at evaluating the stability of digestate and essentially the U.K. PAS 110:2014 limit of 0.45 L biogas/g VS (28 days incubation) was easily achieved. However, this standard may be too lenient for the Ontario systems studied. A limit of 0.25 L biogas/g VS after 28 days of incubation or 0.45 L biogas/g VS after 60 days of incubation are recommended as better standard limits for evaluating digestate stability.



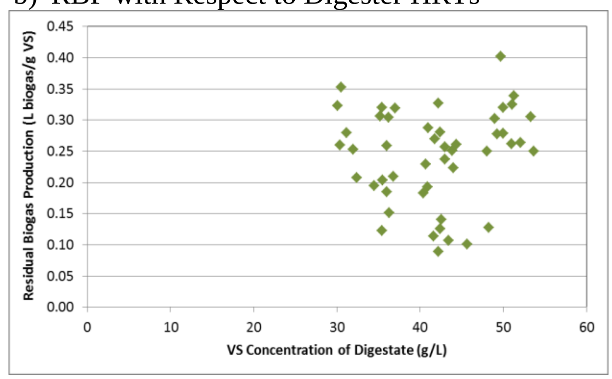
a) RBP with Respect to Digester OLRs



b) RBP with Respect to Digester HRTs



c) RBP with Respect to %VS Reduction during Digestion



d) RBP with Respect to VS Concentration of Digestate

Figure 5.3 Correlation of Residual Biogas Production with Respect to Digester OLRs, HRTs, %VS Reduction and VS Concentration of Digestate

5.3.2 Volatile Solids

Table 6.4 summarizes the average VS reductions in the seven on-farm digester systems studied. The % VS reductions ranged from 56 to 76%, which are slightly higher VS reductions than reported in literature for mesophilic digesters (Burke, 2001; Al Seadi et al., 2013; Banks et al., 2013). The higher VS reductions observed in these digester systems can be partially explained by the readily degradable grease waste that comprised a portion of the digester feed. It should also be noted that the VS reductions encompass the entire digester systems, which for many of the ones studied includes pasteurizers and/or hydrolysers, which can also contribute to VS reductions.

Table 5.4 Average Volatile Solids Reduction in Digester Systems

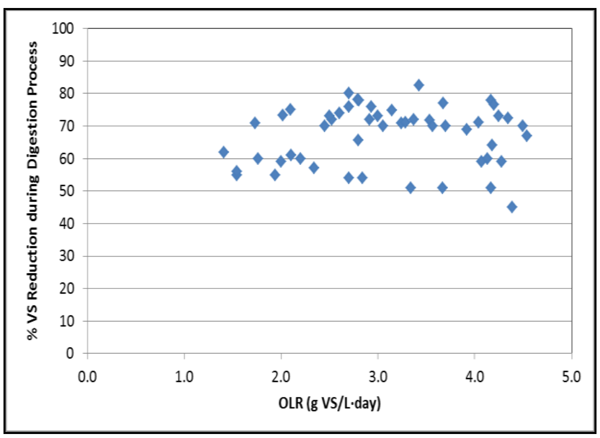
Digester ID	Hydraulic Retention Time (days)	Organic Loading Rate (g VS/L· day)	VS of Digestate (g/kg wet)	% VS Reduction	VS/TS in Digestate
Farm A	80	3.2 (0.8)[1]	37.1 (2.1)	76	0.73
Farm B	35	4.3 (0.8)	46.8 (3.6)	72	0.80
Farm C	50[2]	3.4 (0.9)	46.9 (8.6)	69	0.73
Farm D	30	3.9 (0.8)	48.1 (3.8)	56	0.77
Farm E	64[2]	3.1 (0.4)	42.8 (3.7)	71	0.71
Farm F	64	2.0 (0.4)	44.6 (2.7)	60	0.75
Farm G	45	2.7 (0.6)	25.6 (6.1)	74	0.70

1 Numbers in brackets indicate standard deviation on average value.
2 Cumulative HRT of two tank digester system

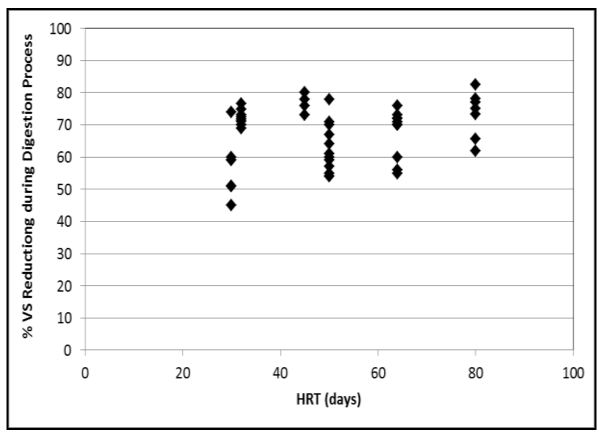
The digester systems at Farm A and Farm B were similar in operation in that they both had digester feeds composed of 50% grease waste/DAF and were comparable in design; however, Farm A had a slightly lower ORL and more than twice the HRT. Despite the longer HRT, the % VS reductions in the two digester systems were not considered significantly different (p>0.05), with 76% VS removal at the Farm A digester system versus 72% VS removal at the Farm B system. Figures 5.4 a) and b) illustrate the correlation of % VS reduction with respect to OLRs and HRTs, respectively. A Pearson R^2 regression analysis does not show a distinct correlation of % VS reduction with OLRs or HRTs; however, the figures do show that the lowest % VS reductions occurred at the highest OLRs and the shortest HRTs.

All digesters demonstrated a significant (p<0.05) decrease in average VS concentration between the digester feed and digestate, regardless of whether the digester feed was composed of 25% or 50% off-farm substrates (Figure 5.5). VS concentrations of digestate from all seven digester systems were significantly (p<0.05) lower than raw manure, almost half the concentration of raw manure. The digester systems were effective at reducing the organic matter of the digestate material, thereby confirming substrate treatment and reduction of organic loading during the subsequent land application of the digestate.

VS reductions for on-farm digester systems ranged from 56 to 76%, higher than the O. Reg. 267/03 regulation of 50% reduction. However, digesters using grease or food based co-substrates can easily achieve the 50% VS reduction; however, when using co-substrates that are harder to digest, like cellulosic energy crops, a 50% reduction in VS may be more suitable. A range in VS reductions is better suited to evaluating digester stability and it was recommended that systems using easy to digest substrates should meet VS reductions of greater than 60%.



a) VS Reduction with Respect to OLR



b) VS Reduction with Respect to HRT

Figure 5.4 Correlation of VS Reduction with Respect to OLRs and HRTs

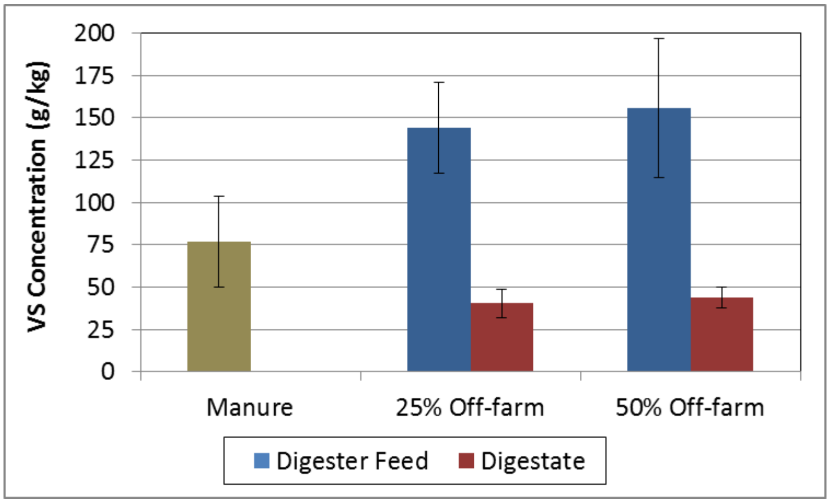


Figure 5.5 Average VS Concentrations in Digestate as Compared to Digester Feed and Raw Manure (average ± standard deviation)

5.3.3 Volatile Fatty Acids

All feedstocks and digestate samples were analysed for VFA concentrations: acetic, propionic and butyric acids. The average digestate VFA concentrations for six of the farm digester systems studied are presented in Table 5.5, along with pH and IA/PA values. Most of the farm digester systems were well under the 1500-2000 mg/L VFA concentration range proposed to be the threshold indicating potential digester upset (Gerardi, 2003; Labatut and Gooch, 2012; Banks et al., 2013). The average VFA concentrations for the digestate from Farm G were considerably higher than the average VFA concentrations for the other digesters. During the study Farm G experienced heating problems with the digester system which may have contributed to the elevated VFA concentrations in the digestate due to some digester upset.

Banks et al. (2013) also present the optimum ratio of acetic to propionic acid to be 2:1; most of the digestate samples met this requirement. During the investigation period of this study, Farm D experienced operational difficulties, including a broken digester mixer and difficulty maintaining operating temperature, which resulted in a stressed digestion system with slightly elevated VFA concentrations.

Table 5.5 Average volatile fatty acid (VFA) concentrations and IA/PA in digester systems

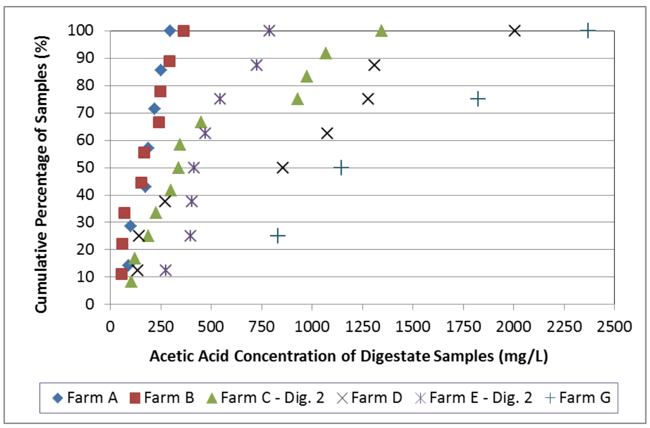
Digester ID	% Off-farm	pH of Digestate	Butyric Acid in Digestate (mg/kg wet)	Propionic Acid in Digestate (mg/kg wet)	Acetic Acid in Digestate (mg/kg wet)	Average IA/PA (Digestate)
Farm A	50	7.99 (0.16)[1]	62 (12)	72 (26)	186 (62)	0.20 (0.03)
Farm B	50	7.52 (0.16)	49 (4)	71 (42)	178 (106)	0.22 (0.05)
Farm C	25	7.96 (0.25)	83 (43)	197 (151)	551 (347)	0.16 (0.05)
Farm D	50	7.92 (0.23)	172 (88)	366 (245)	885 (567)	0.32 (0.12)
Farm E	25	8.64 (0.09)	142 (125)	148 (64)	503 (176)	0.11 (0.03)
Farm F	25	7.56 (0.20)	N/A[2]	N/A	N/A	0.24 (0.05)
Farm G	25	7.41 (0.65)	476 (358)	1060 (269)	1543 (690)	0.27 (0.03)

1 Numbers in brackets indicate standard deviation on average value.
2 VFA analysis on Farm F digestate samples could not be conducted (lab closure).

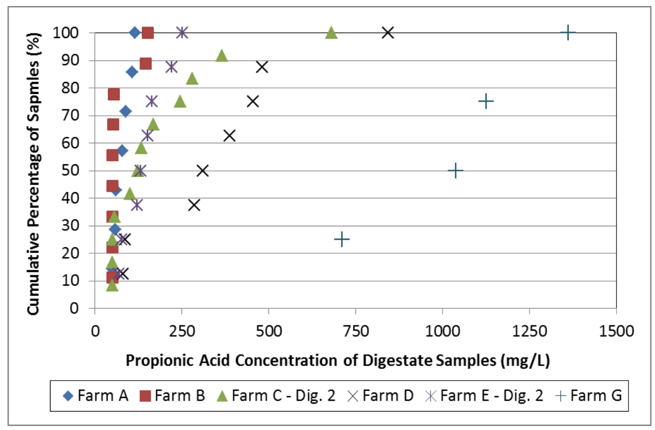
IA/PA ratios should be kept below the range of 0.3 to 0.4 for stable digester performance (Gerardi, 2003; Martin-Gonzalez et al., 2013; Murphy and Thamsiriroj, 2013) and all of the digester systems met this requirement. Although the average digestate IA/PA for Farm D was within the recommended range it was slightly higher than the rest of the digester systems. The use of VFA concentrations and IA/PA ratios was successful in identifying digester upset, while average pH values were normal and showed no sign of upset.

Figures 5.6 a), b) and c) illustrate the cumulative percentage of samples with increasing acetic acid, propionic and butyric acid concentrations, respectively. The majority of digestate samples collected from the digester systems had acetic acid concentrations below 500 mg/L; however, over 50% of the digestate samples from Farms D and G had elevated acetic acid concentrations.

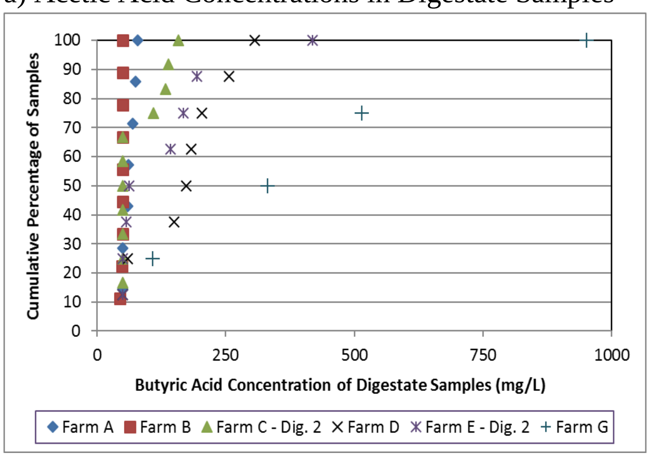
Propionic and butyric acid concentrations were also low for most of the digestate samples from digester systems at Farms A, B, C, and E; however, concentrations were higher for digestate samples from digester systems at Farms D and G. The digester operational upsets experienced at Farms D and G during the study period may have contributed to VFA accumulation within the digester systems. A regression analysis shows a mild correlation of IA/PA with acetic acid concentrations (Figure 5.6 d)) with a R^2=0.3017, indicating IA/PA increases with increasing accumulation of acetic acid concentrations in the digester systems.



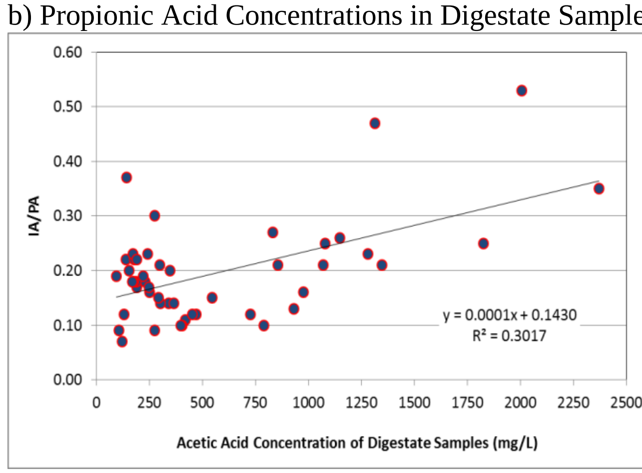
a) Acetic Acid Concentrations in Digestate Samples



b) Propionic Acid Concentrations in Digestate Samples



c) Butyric Acid Concentrations in Digestate Samples



d) IA/PA with Respect to Acetic Acid Concentration

Figure 5.6 Cumulative Percentage of Samples with Increasing VFA Concentrations in Digestate and Correlation of IA/PA with Respect to Acetic Acid Concentrations

5.3.4 Fate of Pathogen Indicators

The pathogen indicators used for this study were *E.coli* and *Salmonella*, and all feedstocks and digestate samples were analysed for these parameters. Typically, a 1 to 3 log reduction in pathogen indicators was observed for the seven digester systems (Table 5.6) and is typical of what has been reported in literature (Harikishan and Sung, 2003; Smith et al., 2005, Crolla et al., 2013).

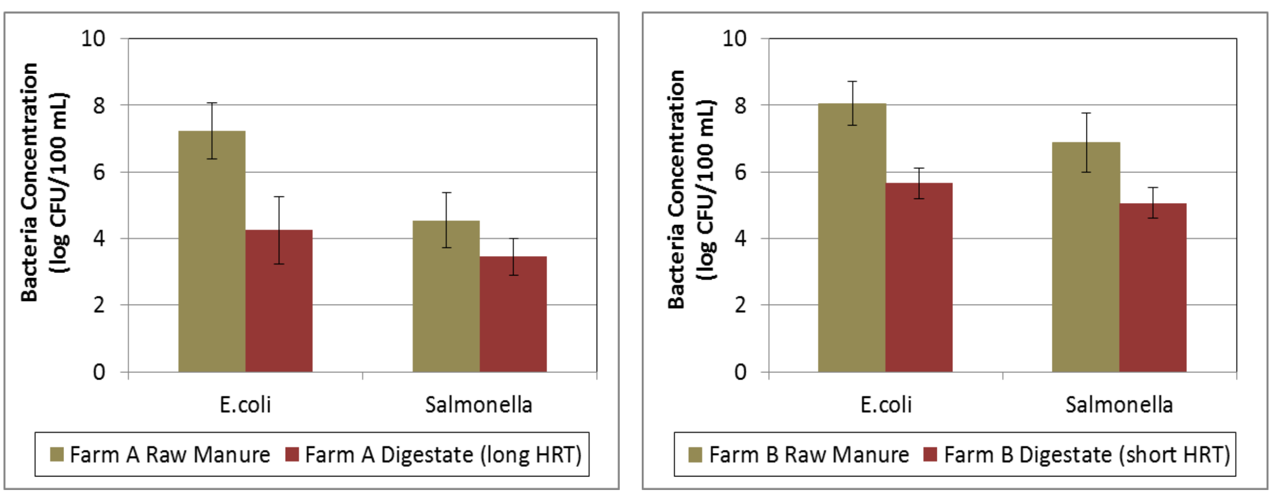
HRT did not seem to have a significant impact on the log reduction of *E.coli* or *Salmonella*, which is contrary to what was proposed by Smith et al. (2005). Figure 5.7 illustrates that a higher log reduction was observed for *E.coli* with a long HRT, while at a shorter HRT there was a higher log reduction for the Salmonella indicator. For all digester systems studied, digestate samples were consistently lower in both *E.coli* and *Salmonella* concentrations when compared with raw manure.

At the Farm C two-tank digester system a log reduction in *E.coli* was observed in each of the digester tanks, while *Salmonella* was reduced by a half log in each tank (Figure 5.7). Increased pathogen die-off may be observed in a two digester system if the tanks are operated as two-stage systems, where elevated VFA concentrations in the first tank could contribute to bacterial inactivation (Harikishan and Sung, 2003).

Table 5.6 Geometric Mean of Pathogen Indicators in Digester Systems

Digester ID	HRT (days)	Raw Manure *E.coli* (CFU/100mL)	Raw Manure *Salmonella* (CFU/100mL)	Digestate *E.coli* (CFU/100mL)	Digestate *Salmonella* (CFU/100mL)	*E.coli* (Log Difference[1])	*Salmonella* (Log Difference)
Farm A	80	1.69 E+07 (0.83)[2]	3.52 E+04 (0.84)	1.78 E+04 (1.02)	2.85 E+03 (0.55)	**3.0**	**1.1**
Farm B	35	1.12 E+08 (0.66)	7.75 E+06 (0.89)	4.58 E+05 (0.46)	1.16 E+05 (0.46)	**2.4**	**1.8**
Farm C: Dig. 1	50	8.76 E+06 (0.69)	1.82 E+05 (0.80)	2.45 E+05 (0.47)	7.60 E+03 (0.80)	**1.6**	**1.4**
Dig. 2				5.15 E+04 (0.53)	8.34 E+03 (0.54)	**2.3**	**1.4**
Farm D	30	2.35 E+06 (0.67)	1.34 E+04 (0.73)	6.60 E+04 (0.45)	8.84 E+03 (0.70)	**1.6**	**0.2**
Farm E: Dig. 1	64	3.67 E+06 (0.81)	5.67 E+04 (0.33)	1.18 E+05 (0.33)	3.27 E +04 (0.77)	**1.5**	**0.2**
Dig. 2				2.09 E+03 (0.87)	1.37 E+04 (0.63)	**3.2**	**0.6**
Farm F	64	3.22 E+07 (0.40)	2.92 E+06 (0.41)	5.65 E+05 (0.24)	3.60 E+05 (0.35)	**1.8**	**1.0**
Farm G	45	4.27 E+05 (0.88)	7.28 E+04 (0.50)	8.18 E+04 (0.65)	1.40 E+04 (0.34)	**0.7**	**0.7**

1 Log difference between Raw Manure and Digestate pathogen indicator concentrations.
2 Numbers in brackets indicate log standard deviation on geometric mean values.



a) Farm A with a Long HRT **b) Farm B with a Short HRT**

Figure 5.7 Average Bacteria Concentrations in Digestate of Farm A (long HRT) and Farm B (short HRT) Digester Systems, as Compared to Raw Manure (log geometric mean ± log standard deviation)

5.4 Conclusions

The seven on-farm anaerobic digesters studied demonstrated digestate characteristics to be in accordance with some of the performance criteria suggested in literature. Currently under the Nutrient Management Regulations (O. Reg. 267/03) the only performance criteria for digesters is a 20-day HRT and 50% VS reduction; however, these requirements may be too lenient. OMAFRA and MOECC are currently reviewing the data presented in this study and considering implementation of additional regulated requirements for on-farm digester systems.

The main conclusions from this study include:

1. RBP yields were effective at evaluating the stability of digestate and the U.K. PAS 110:2014 limit of 0.45 L biogas/g VS (28 days incubation) was too lenient for the Ontario systems studied. A limit of 0.25 L biogas/g VS after 28 days of incubation or 0.45 L biogas/g VS after 60 days of incubation are recommended as better standard limits for evaluating digestate stability.
2. RBP yields from digestate of on-farm systems can be significant under conditions of short HRTs or higher OLRs. It is recommended that producers consider covering holding tanks of digestate to capture residual biogas.
3. VS reductions for on-farm digester systems ranged from 56 to 76%, higher than the O. Reg. 267/03 regulation of 50% reduction. However, digesters using grease or food based co-substrates can easily achieve the 50% VS reduction; however, when using co-substrates that are harder to digest, like cellulosic energy crops, a 50% reduction in VS may be more suitable. A range in VS reductions is better suited to evaluating digester stability and it was recommended that systems using easy to digest substrates should meet VS reductions of greater than 60%.
4. *E.coli* and *Salmonella* were typically 1 to 3 logs CFU/100 mL lower than raw manure and increased HRT did not demonstrate a significant impact on the bacterial log reductions.

5. IA/PA proved to be a valuable tool in determining potential digester upset and has been recommended as a standard performance parameter for on-farm systems. This recommendation has just been implemented by OMAFRA as a condition of operation of a newly built on-farm digester system.

Acknowledgements

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Chapter 6 - Overall Conclusions and Recommendations and Future Research

6.1 Overall Conclusions and Recommendations

A comprehensive study evaluating the effectiveness of on-farm anaerobic digestion has been conducted and industry standards specific for Ontario have been developed. The overall objective of this study was to investigate the co-digestion of off-farm substrates with dairy manure to enhance biogas production and digestate quality. The study progressed in three phases: laboratory experiments evaluated the use of various substrates for co-digestion; bench-scale experiments investigated two-phase digestion, suitable operating conditions and digestate quality; and, on-farm studies evaluated operating parameters and digestate quality.

Laboratory Experiments

The main objective of this study was to establish biogas conversions yields for various substrates in the co-digestion with liquid dairy manure. This is the first comprehensive study specific to Ontario based substrates and results are being used by the biogas industry and in OMAFRA's biogas calculator. The major conclusions from this study include:

1. Energy crops like corn silage, switchgrass and wheat straw were effective at increasing biogas yields in co-digestion of dairy manure when compared to digesting manure alone. Woodchips showed significant non-biodegradability and need to be reconsidered as animal bedding as the material will add bulk to an anaerobic digester but have no benefit for biogas production. Design HRTs of greater than 40 days were determined to be essential when co-digesting cellulosic substrates. At optimum biogas productions, VS reductions were just under 50%, suggesting O. Reg. 267/03 requirement of 50% VS reductions in on-farm digesters may not be suitable for cellulosic substrates.
2. Co-digestion of dairy manure with DAF sludge and waste grease produced the highest biogas yields. Co-digestion was most effective when these substrates represented 50% of the feed VS. Design HRTs of at least 25 days were

determined necessary for grease based substrates and VS reductions of 50% were achievable.

3. Grocery food waste, fish offal and corn thin stillage all increased biogas yields when co-digested with manure. Biogas production for these substrates was slow but steady. Design HRTs of 25 to 30 days were determined suitable for industry waste substrates.
4. Box-Wilson Central Composite Circumscribed Models were developed and describe that methane yields optimize with increasing proportion of the feed VS from co-substrates (constant total VS in all assays) and increasing temperatures. Although the models suggest the proportion of feed VS from co-substrate to be positively correlated to methane production, temperature was shown to have a great influence on yields. The models also suggest there is minimal interaction between temperature and proportion of feed VS from co-substrates on methane yields.

Bench-scale Experiments

The overall objective of this study was to determine the use of corn thin stillage and waste grease in the co-digestion of liquid dairy manure for enhanced biogas production and, digestate stability and quality. Literature suggests two-phase digestion systems may not be suitable for dairy manure; however, it was hypothesized that the co-digestion of dairy manure with substrates high in soluble organics would demonstrate the applicability of a two-phased digestion system to agricultural residues. Four bench scale mesophilic anaerobic digesters, operating under single and two-phase systems were operated for over two years. The main conclusions from this study include:

1. Methane yields increased by over 22% using two-phase digestion systems compared with single-phase, for both corn thin stillage and waste grease co-digestion. Based on current FIT contracts of 18 to 20¢/kWhe, the increased electricity and heat production could make the two-phase system economically attractive for producers.

2. Several OLRs were evaluated and digester systems crashed at OLR over 4.4 g VS/L·day. It is recommended that co-digestion of corn thin stillage with dairy manure and co-digestion of waste grease with dairy manure should not be operated at OLRs of over 4.2 g VS/L·day.
3. A proportion of at least 25% co-substrate in the feed was effective at maintaining the acid reactor at lower pH values and maintaining VFA concentrations above 3.0 g/L. Lower proportions of co-substrate in the feed are not recommended as the buffering capacity of manure will make acidification of the acid reactor difficult.
4. Significant VS reductions of 51 to 81% were obtained in the co-digestion of corn thin stillage and waste grease with liquid dairy manure, with final digestate VS concentrations over 50% lower than raw dairy manure. Lower VS concentrations applied to agricultural land will help reduce soil biological activities that contribute to odours and GHG emissions, thereby reducing environmental impacts.
5. Essentially 1 to 3 log CFU/100 mL reductions in pathogen indicators (*E.coli* and Salmonella) were observed in digestate when compared with raw dairy manure. The unfavorable accumulated acids and low pH in the acid reactor (two-phase system) helped achieve the higher log reductions in *E.coli* but did not appear to have a greater impact on *Salmonella* die-off.
6. RBP yields demonstrated to be a good parameter for measuring digestate stability. Two-phase digestion contributed to more stable digestate as lower RBP yields were obtained. RBP yields over 0.4 L biogas/g VS were obtained when digestion was incomplete due to digester upset. RBP yields were highest with low HRTs and lowest with high HRTs. Digestion processes that achieved VS reductions of 60 % or lower had digestates with the highest RBP yields.

On-farm Digester Systems

The agricultural biogas industry in Ontario is young and the objective of this study was to conduct a comprehensive study on the performance of on-farm digester systems. Under the Nutrient Management Regulations (O. Reg. 267/03) the only performance criteria for digesters is a 20-day HRT and 50% VS reduction. The monitoring parameters presented in this study have demonstrated to be useful tools in understanding digester performance and recommendations specific for Ontario digesters are presented. OMAFRA and MOECC are currently reviewing the data and considering implementation of the recommendations as regulated performance requirements for Ontario digesters. The main conclusions from this study include:

1. RBP yields were effective at evaluating the stability of digestate and the U.K. PAS 110:2014 limit of 0.45 L biogas/g VS (28 days incubation) was too lenient for the Ontario systems studied. A limit of 0.25 L biogas/g VS after 28 days of incubation or 0.45 L biogas/g VS after 60 days of incubation are recommended as better standard limits for evaluating digestate stability.
2. VS reductions for on-farm digester systems ranged from 56 to 76%, higher than the O. Reg. 267/03 regulation of 50% reduction. However, digesters using grease or food based co-substrates can easily achieve the 50% VS reduction; however, when using co-substrates that are harder to digest, like cellulosic energy crops, a 50% reduction in VS may be more realistic. A range in VS reductions is better suited to evaluating digester stability and it was recommended that systems using easy to digest substrates should meet VS reductions of greater than 60%.
3. *E.coli* and *Salmonella* were typically 1 to 3 logs CFU/100 mL lower than raw manure and increased HRT did not demonstrate a significant impact on the bacterial log reductions.
4. IA/PA proved to be a valuable tool in determining potential digester upset and has been recommended as a standard performance parameter for on-farm systems. This recommendation has just been implemented by OMAFRA as a condition of operation of a newly built on-farm digester system.

6.2 Future Research

Future research is recommended in the following areas:

1. Operation of the acid reactor (two-phase digestion system) at thermophilic temperatures and its impact on biogas/methane yields and digestate quality. The operation of on-farm single-phase digesters at thermophilic temperatures has proven to be a challenge under Ontario winter temperatures; however, the smaller size of the acid reactor may make thermophilic temperatures more economically viable (on an energy balance perspective).
2. Investigate alternative HRTs for the acid reactor to optimize pathogen die-off. O. Reg. 267/03 requires a pasteurizer as pre-treatment if waste grease is used as a co-substrate. This pre-treatment could potentially be eliminated if sufficient pathogen reductions are obtained in the acid reactor.
3. Compare economic assessments of a two-phase digester system versus a two-stage system. A two-stage digester system involves a two-tank operation; however, neither tank operates as an acid reactor but in fact both tanks are designed for methanogenesis. The second tank is a very slow rate system that captures residual biogas. This economic analysis will provide valuable information for producers in optimizing biogas production.