Anaerobic Digestion of Corn Ethanol Thin Stillage for Biogas Production in Batch and by Down-flow Fixed Film Reactor

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Abstract

The anaerobic digestion of corn ethanol thin stillage offers the potential to make corn grain ethanol a more energy efficient and practical option as a biofuel. This thesis focuses on experiments and results obtained from anaerobic digestion of thin stillage at mesophilic temperatures in batch and by down-flow fixed film reactor. Experiments conducted included a series of biochemical methane potential (BMP) assays that investigated the digestion of thin stillage at a variety of organic concentrations and food-to-microorganism ratios. Assays conducted include digestion of thin stillage as the sole carbon source with and without acclimation of biomass, co-digestion of thin stillage (with food waste and thickened waste activated sludge) also with and without acclimation of biomass, as well as thin stillage supplemented with a full trace element stock solution and a cobalt only stock solution. Three sequential BMP assays were also conducted which investigated the potential for the use of digested effluent for substrate dilution, in order to reduce fresh water consumption. Promising results were obtained, but further investigation is required in order to determine the full potential of recycled digested effluent for substrate dilution. Acclimation of biomass proved to be beneficial by increasing biogas production rates. Continuous studies employed two 28L down-flow stationary fixed film (DSFF) reactors to examine the potential of thin stillage in a continuous system. Chemical oxygen demand and volatile solids removal efficiencies greater than 85% were achieved up to an organic loading rate of 7.4 g TCOD/L/d and hydraulic retention time of 5 days.

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Nomenclature

COD	Chemical oxygen demand, mg O_2/L
C:N	Carbon – to – Nitrogen ratio, dimensionless
F/M	Food-to-Microorganism ratio (g $VS_{substrate}$/g $VSS_{inocumul}$), dimensionless
HRT	Hydraulic Retention Time, d
OLR	Organic loading rate, g TCOD/L/d
pH	Concentration of hydrogen ions (H^+) in a solution, dimensionless
SCOD	Soluble chemical oxygen demand, mg O_2/L
SRT	Solids retention time, d
TKN	Total kjeldahl nitrogen, mg TKN-N/L
TS	Total Solids, % mass
V	Volume, mL or L
VFA	Volatile fatty acids, mg/L
VS	Volatile solids, % mass
w	Weight, mg, g orkg

List of Abbreviations

AD	Anaerobic digestion
ASBR	Anaerobic sequencing batch reactors
ABR	Anaerobic baffled reactors
AEB	Anaerobic expanded bed reactors
AF	Anaerobic filter
AFB	Anaerobic fluidized bed reactors
AMBR	Anaerobic migrating blanket reactors
BMP	Biochemical methane potential
BOD	Biological oxygen demand
CDS	Condensed distillers solubles
CH_4	Methane
CO	Carbon monoxide
Co	Cobalt/ Cobalt trace element solution
CO_2	Carbon dioxide
CFRA	Canadian Renewable Fuels Association
CTS	Corn thin stillage
D	Days
DSFF	Down flow stationary fixed film reactor
DDG	Dried distillers grain
DDGS	Dried distillers grain with solubles
EU	European Union
FW	Food waste
GC	Gas chromatograph
GHG	Green house gases
ID	Inner diameter
MM	Supplemental mineral mix/trace element solution
Mo	Molybdenum
MTBE	Methyl tert-butyl ether
mV	Millivolts
NEB	Net energy balance
NEV	Net energy value
NPP	Needle punch polyester
R1	Reactor #1
R2	Reactor #2
RCF	Relative centrifugal force
RFA	Renewable fuels association
RPM	Revolutions per minute
SSF	Simultaneous saccharification and fermentation
STP	Standard temperature and pressure
TWAS	Thickened waste activated sludge
UASB	Up-flow anaerobic sludge blanket reactors
WDGS	Wet distillers grains with soluble

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Chapter 1

Introduction

1.1 Background

At present, global energy consumption is approximately 13 Terawatts (1 TW = 10^{12}W = 3.2 Exa joules/year), an estimated 80% of which is supplied by the burning of fossil fuels (Rittmann, 2008). With time, society will have to face the challenges of a larger (and growing) global energy demand, increasingly limited resources, including a declining availability of oil reserves, as well as the environmental consequences of our energy consumption (Rittmann, 2008). These challenges, coupled with the desire for energy security and independence in North America, have spurred research and application of renewable energy technology (Shapouri et al., 2005, 2010 Rajeshwari et al., 2000).

Ethanol production from corn, although not a new technology, is a process that can help to address both energy and environmental concerns. Considered a carbon neutral fuel, its ability to provide a homegrown energy alternative, to create positive impacts on rural areas of North America, and its easy substitution into already existing infrastructure makes it an alternative energy choice for today.

A major criticism of ethanol as a biofuel is a supposed small positive net energy balance (NEB). This has been a source of debate for many years (Chambers et al., 1979) and there are strong arguments on both sides of the issue. However, in order for any biofuel to become a sustainable option, a holistic approach must be taken and all potential energy savings must be studied. For instance, an immediate solution to improve the net energy balance of corn grain ethanol is to find alternative uses for ethanol production process streams (Agler et al., 2008).

Ethanol production in the United States has increased significantly in recent years, from approximately $5x10^6 m^3$ in 1997, to $15x10^6$ m^3 in 2005 (Agler et al., 2008). This trend is expected to continue, with demand projected to reach $19x10^6$ m^3 by 2012 (Bothast and

Schlicher, 2005). Corn grain ethanol can be produced by either the wet milling process or by the dry grinding process. However, growth in this field is anticipated to be primarily in dry grind ethanol plant construction, due to the lower capital investment required when compared to wet mill ethanol plants (Agler et al., 2008). Despite some criticism, ethanol is supported strongly by political lobbies (Hill et al., 2006), and both Canada and the United States have mandated the increased use of ethanol in fuels.

In a dry grind ethanol production facility, the marketing of co-products provides income to offset the cost of processing. In comparison to a wet milling ethanol production facility, the co-products from a dry grind plant are limited. The only co-products of a dry grind ethanol production facility are carbon dioxide, which is typically sold to the beverage industry for carbonation purposes (Bothast and Schlicher, 2005) and dried distillers grain with soluble (DDGS), which is sold as animal feed (Rausch and Belyea, 2005). With an increase in ethanol production expected in the near future, corresponding increases in the availability of DDGS for feed will drive prices down and could alter the economic viability of dry grind ethanol plants (Raush and Belyea, 2005). In order to combat this, alternate methods to expand co-product use will have to be explored.

In a dry-grind ethanol production facility, approximately one third of energy consumption is as a result of water evaporation in the production of DDGS. Thin stillage, an intermediate co-product, is itself dried as part of the process to create DDGS (Wilkie et al., 2000). The process of evaporating water from the thin stillage is both costly and difficult but could potentially be avoided, at least in part, through anaerobic digestion (AD).

The energy required to evaporate thin stillage is equivalent to 10% of the energy content of the ethanol produced, and has a significantly negative impact on the energy balance (NEB) of corn grain ethanol production (Wilkie et al., 2000). Anaerobic digestion of thin stillage will offer not only the benefit of reduced energy input for co-product drying, but also energy recovery through the production of methane, hopefully resulting in an improved net-energy balance for ethanol production.

The following report examines in detail the processes of dry grind ethanol production and anaerobic digestion metabolism. The report also looks at various anaerobic digestion

technologies and the potential applications, with a focus on thin stillage as a substrate for methane production and energy recovery by AD.

1.2 Purpose

To investigate whether or not thin stillage, a by-product of the dry grind corn ethanol production process, is a suitable substrate for anaerobic digestion at mesophilic temperatures as the sole substrate; or as a co-substrate for biogas production.

1.3 Research Objectives

The primary objective of this research was to determine if corn ethanol thin stillage from a dry grind ethanol production facility is a suitable candidate for mesophilic anaerobic digestion as a primary substrate and as a co-substrate.

Objectives specific to biochemical methane potential (BMP) assays were to:

- Determine a suitable range of concentrations and specific food-to-microorganism (F/M) ratios for mesophilic anaerobic digestion of thin stillage as the sole carbon source.
- Evaluate and compare the effects of co-digestion of thin stillage with food waste (FW), and thin stillage with thickened waste activated sludge (TWAS) to the digestion of thin stillage alone.
- Compare the results of the anaerobic digestion of thin stillage supplemented with various nutrients via a trace element solution, to the digestion of thin stillage as the sole carbon source with no supplements.
- Assess and quantify the effect of biomass acclimation on biogas production, biogas production rate, and the removal of chemical oxygen demand and solids.
- Investigate the potential for the use of digested effluent as dilution water to minimize the use of fresh water for substrate dilution.

Objectives of the continuous experiments utilizing a down flow stationary fixed film (DSFF) reactor included:

- To determine the methane potential of thin stillage in a high rate down flow stationary fixed film anaerobic digester.
- Evaluate a range of operating conditions by varying the volumetric and surface loading rates, and hydraulic retention times (HRTs) of the DSFF reactors.

1.4 Thesis Layout

This thesis is divided into 5 chapters, followed by appendices of raw data. Chapter 1, the introduction, is followed by a literature review in Chapter 2, which describes ethanol production processes, anaerobic digestion metabolism, parameters and reactor configurations, followed by a summary of the research presented in published literature relevant to this thesis. Chapter 3 summarizes the materials and methods employed for the presented research, detailing the set up and operation of BMP assays and semi-continuous reactors. It also describes the analytical methods used for sample analysis. Research results are summarized and discussed in Chapter 4, which is followed by conclusions and recommendations for future work in Chapter 5.

Chapter 2

Literature Review

2.1 History of Ethanol

The production of ethanol from corn is an established technology that was first introduced in the United States in the early 1900's. The earliest automobile, the Ford Model T, was designed with an adjustable carburetor that allowed the vehicle to run on either gasoline or ethanol (Kovarik, 1998). Up until the early 1930's, the fuel of choice for vehicles was ethanol, but this changed near the conclusion of the Second World War, when ethanol was phased out in favour of gasoline due to the abundant and cheap supply of petroleum (Bothast and Schlicher, 2005).

In the 1970's, oil embargoes by the Organization of Petroleum Exporting Countries (OPEC) exposed the vulnerability of western countries dependence on foreign oil. As a result, ethanol soon resurfaced as a gasoline extender. In the 1980's, when lead was phased out as an octane booster, ethanol received further attention due to its high octane content (Bothast and Schlicher, 2005, Shapouri et al., 2005, Schaefer, 2006).

The ethanol industry received even more notice in 1990, when the United States Congress passed the Clean Air Act (CAA; Bothast and Schlicher, 2005, Schaefer, 2006). This Act mandated the use of oxygenated fuels to reduce ground level ozone and carbon monoxide (CO) levels, and resulted in the widespread use of petroleum based fuel oxygenate Methyl tert-butyl ether (MTBE). However, MTBE has recently being recognized as a contaminant to ground water, and its use is currently being phased out in the United States (Hill, 2007), and complete elimination of MTBE as a fuel oxygenate is expected in the near future (Shapouri et al., 2002, Bothast and Schlicher, 2005, Rass Hansen et al., 2007).

For gasoline producers looking to meet the oxygen requirements of the Clean Air Act, ethanol has become a popular alternative to MTBE. As a fuel additive, as little as a 10% blend of ethanol in gasoline acts as an octane enhancer and improves combustion, thereby

reducing CO emissions and ground level ozone (Rass Hansen et al., 2007). In addition, due to the higher relative oxygen content of ethanol in comparison to MTBE, only half the volume is required to produce the same level of oxygen in gasoline (Bothast and Schlicher 2005). The advantages that ethanol offers as a fuel oxygenate, combined with the expected phase out of MTBE, clearly demonstrates another opportunity for increased growth in the ethanol industry.

2.2 World Ethanol Production

In addition to its practical applications as a fuel oxygenate and octane enhancer, there are several other reasons that ethanol production has increased in recent years. These include energy security concerns, new government gasoline standards and government incentives (Shapouri et al., 2005, 2010). In 2005, world ethanol production was estimated at 46 billion liters per year, a figure that grew to 65 billion liters in 2008. This is equivalent to an almost 30% increase in production over a 3 year period (RFA 2010, Rass Hansen et al., 2007). As bioethanol production is already taking place in many countries around the world and production is still getting underway in many others, studies have suggested that total production will increase at an annual rate of 6.5% until 2020, leading to an annual production of more than 120 billion liters a year (Rass-Hansen et al., 2007).

According to statistics reported by the Renewable Fuels Association, five countries (United States (US), Brazil, European Union (EU), China and Canada) account for 98% of world ethanol production. Of the five, Brazil and the United States are by far the largest producers of ethanol. These two countries have invested significant resources into production and collectively supplied roughly 90% of the total world production in 2008 (RFA, 2010). According to 2008 Renewable Fuels Association statistics, Canada's ethanol production was 900 million liters per year accounting for approximately 1.4% of world production. Table 1 is a summary of Canadian ethanol producers, location, feedstock and capacity of production current to November 2010 (Canadian Renewable Fuels Association, 2010).

Table 2.1 - Canadian ethanol plants: Location, feedstock and production capacity

Ethanol Plant	City	Province	Feedstock	Capacity (Mmly)	Status
Alberta Ethanol and Biodiesel GP Ltd	Innisfail	Alberta	Wheat	150	Proposed
Amaizelingly Green L.P.	Collingwood	Ontario	Corn	50	Operational
Alantec Bioenergy Corporation	Milford	Nova Scotia	Energy beets	n/a	Demonstration Facility
Enerkem Alberta Biofuels	Edmonton	Alberta	MSW	36	Under Construction
Enerkem Inc.	Sherbrooke	Quebec	Various	475,000L/y	Demonstration Facility
Enerkem Inc.	Westbury	Quebec	Wood Waste	5	Demonstration Facility
GreenField Ethanol	Johnstown	Ontario	Corn	230	Operational
GreenField Ethanol	Varennes	Quebec	Corn	155	Operational
GreenField Ethanol	Tiverton	Ontario	Corn	27*	Operational
GreenField Ethanol	Chatham	Ontario	Corn	195*	Operational
Growing Power	Hairy Hill	Alberta	Wheat	40	Proposed
Husky Energy Inc.	Lloydminster	Saskatchewan	Wheat	130	Operational
Husky Energy Inc	Minnedosa	Manitoba	Wheat and Corn	130	Operational
IGPC Ethanol Inc.	Aylmer	Ontario	Corn	162	Operational
Iogen Corporation	Ottawa	Ontario	Straw from wheat, barley & oats	2	Demonstration Facility
Kawartha Ethanol	Havelock	Ontario	Corn	80	Operational
NorAmera BioEnergy Corp.	Weyburn	Saskatchewan	Wheat	25	Operational
North West Terminal Ltd.	Unity	Saskatchewan	Wheat	25	Operational
Permolex International, L.P.	Red Deer	Alberta	Wheat	42	Operational
Pound-Maker Agventures Ltd.	Lanigan	Saskatchewan	Wheat	12	Operational
Suncor St. Clair Ethanol Plant	Sarnia	Ontario	Corn	400	Operational
Terra Grain Fuels Inc.	Belle Plaine	Saskatchewan	Wheat	150	Operational

* Volumes include industrial alcohol production

2.3 Renewable Fuel Standards

In many countries, demand for ethanol is a result of laws and regulations mandating blending of biofuels with gasoline in some proportion. Presently, about 2% of transportation fuels in the United States are supplemented by corn-based bioethanol, and the Department of Energy has set a goal of replacing 30% of the transportation fuels with bioethanol and biodiesel by 2025. Many other countries have also developed plans to increase ethanol utilization, such as the European union, which had established a goal of

5.75% by 2010 (Rass-Hansen et al., 2007) and has further increased their goal to achieve a 20% cut in greenhouse gas emissions compared to 1990 levels by 2020, by increasing to 10% renewable fuel content (Lawson, 2010).

In May of 2010, the Canadian Renewable Fuels Association released a third party economic impact assessment of renewable fuels investments in Canada. Conducted by Doyletech Corporation, a study of 28 ethanol and biodiesel plants ac32ross Canada suggested that the annual positive economic impact of renewable fuels is $2.013 billion dollars. The governments analysis of the regulations tabled also considered the cost advantage of ethanol over gasoline, and "could save Canadian consumers $1.7 billion dollars over the next 25 years".

As inscribed in the Copenhagen Accord, Canada has set a target of reducing green house gas (GHG) emissions to 17% below 2005 levels by 2020 (Environment Canada, 2010). In September 2010, the Canadian government announced its new regulations requiring ethanol and biodiesel blended transportation fuels in Canada in order to reach this target. The regulations proposed by the government require an average renewable fuels content of 5% in gasoline, in effect as of December 2010. The Canadian Renewable Fuels Association and the Canadian Government are presently working on preparing a final analysis of a 2% biodiesel and heating oil requirement, which is to be implemented by 2011. Once fully put into practice, Canada's renewable fuel content regulations will reduce green house gas emissions by up to four megatons by 2012, which is the equivalent of removing 1 million cars from the road. These regulations are suggested to achieve green house gas reductions, deliver new jobs, rural growth and foster a vibrant renewable fuels industry in Canada (CFRA, 2010).

2.4 Ethanol Production

Ethanol, or ethyl alcohol, is a renewable fuel, which can be derived from agricultural resources. Considered a carbon neutral, the production of feedstock(s) for ethanol production, combined with the actual production and combustion of ethanol as a fuel, will

in theory, have no net impact on the CO_2 levels in the atmosphere. Figure 2.1 illustrates the lifecycle of emission for the production of a carbon neutral biofuel, such as ethanol.

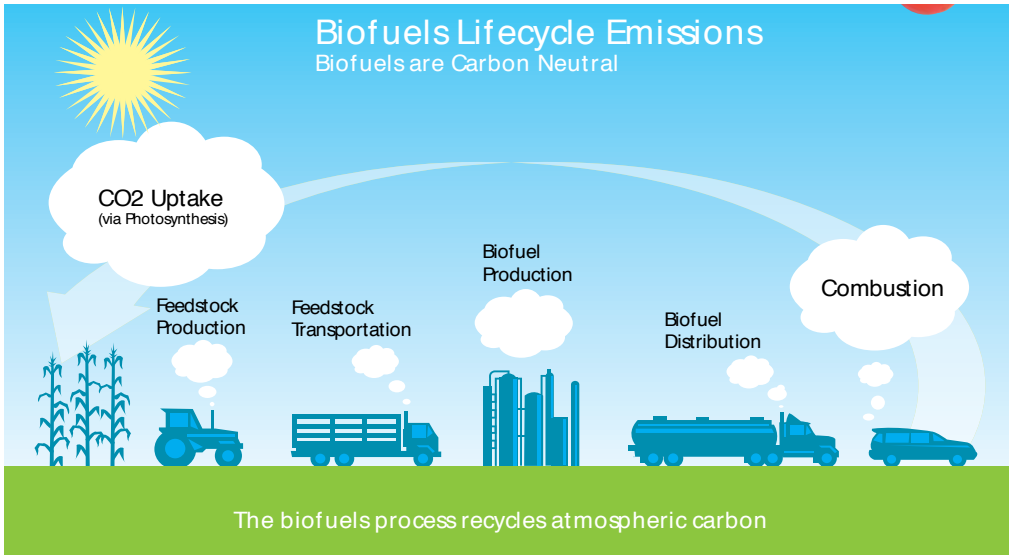


Figure 2.1 – Biofuels Lifecycle Emissions (RFA, 2010)

There are a variety of feedstocks that can be used for bioethanol production, including sugar-based and starch-based feedstocks, as well as whey from the dairy products industry or cellulose based agricultural residues. Sugar-based feedstocks, which include crops such as sugar beets and sugar cane, are the most easily fermented. Brazil has achieved great success in the production of ethanol from sugarcane fermentation at reasonably low costs, while in the European Union the preferred types of biomass for ethanol production are straw and other cellulosic agricultural wastes. Similarly, in Asia the favoured feedstocks for bioethanol production are rice straw, wheat straw and corn stover (Kim and Dale, 2004). Starch based feedstocks predominate in North America and include grains such as corn, wheat, rice and barley. The starch contained in these feedstocks is readily converted to glucose and then fermented into ethanol (Wilkie et al., 2000).

Of the starch based feedstocks, corn is considered to be the most cost-effective option for ethanol production in the United States (Nichols et al., 2006). The only other feedstock used more commonly for ethanol production is sugar cane, but as corn is the principal feedstock used to produce ethanol in North America, it will be the focus of the following information.

2.4.1 Corn Composition

Corn kernels are comprised of the pericap, endosperm, germ and tip cap. Energy is stored in the corn in the form of starch and oil, which are separated in the endosperm and germ respectively. Corn kernels contain approximately 70% starch, 9% protein, 4% fat and oil and 9% fiber on a dry mass basis. The majority of carbohydrates in corn are stored as starch, as insoluble and partially crystalline granules in the endosperm (Bothast and Schlicher, 2005).

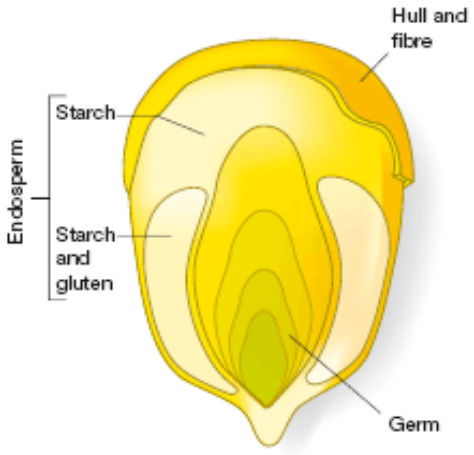


Figure 2.2 – Composition of a corn kernel

(Source: http://annualreports.tateandlyle.com/2010/ara/businessreview/whatwedo/ouroperations.html)

Corn starch is made up of individual glucose molecules linked by alpha 1-4 and intermittent 1-6 linkages. The alpha 1-4 linkages produce chains of glucose that comprise amylose molecules, the alpha 1-6 linkages serve as branching points to produce amylopectin, a branched chain molecule. Typically starch contains 27% amylose and 73% amylopectin (Bothast and Schlicher, 2005).

2.4.2 Corn Grain Ethanol Production

The conversion of corn into ethanol can be accomplished by two methods: dry grinding and wet milling. Wet milling fractionates the corn kernel, at a moisture content of 40-45% (Johnston and Singh, 2004), into its primary components including germ, fiber and starch,

resulting in several process streams and co-products. The wet milling processe produces 9.5 liters (1 liter = 0.264 gallons) of ethanol per bushel of corn, as well as 0.73kg corn oil (1kg = 2.2lbs), 1.18kg of gluten meal and 6.12kg of gluten feed (Bothast and Schlicher, 2005). Wet mills require a very large capital investment and are typically corporately owned. The popularity of dry grind facilities has increased dramatically in recent years due to lower capital costs and incentives for farm owned co-ops, accounting for 70% of all ethanol production in 2005, which increased to 86% by the end of 2008 (Rausch and Belyea, 2005, Mueller, 2010).

The dry grind ethanol production process consists of five steps: grinding, cooking, liquefaction, saccharification and fermentation (Rausch and Belyea, 2005). Resulting products are ethanol, carbon dioxide that is sold as a food and industrial product and distillers dried grain with solubles (DDGS), a high quality livestock feed (Bothast and Schlicher, 2005, Kim et al., 2008). Each bushel of corn processed in a dry grind ethanol plant produces 10.6 liters of ethanol, along with 7.7kg of DDGS and carbon dioxide as co-products (Bothast and Schlicher, 2005, Kim and Dale, 2004). Due to the predominance of dry grind ethanol facilities, this report will focus on its processes and by-products only.

In the dry grind process, the entire corn kernel is ground into coarse flour, then slurried with water to form a 'mash'. Approximately 83 liters (22 gallons) of mash are generated per bushel of corn processed. Since starch cannot be metabolized by yeast without pre-treatment, it must be first degraded to simple six carbon sugars before fermentation. The corn mash pH is adjusted to 6.0 and an enzyme called alpha amylase is added. The enzyme breaks down the starch present by quickly hydrolyzing the alpha 1-4 bonds, producing soluble dextrins. At this point the mash is heated and maintained at an elevated temperature for several minutes, after which the temperature is allowed to drop to 80 – 90 degrees Celsius. More alpha-amylase enzyme is added and the mash is liquefied for a minimum of 30 minutes, greatly reducing the size of the starch polymers.

The dextrinized mash is then cooled and the pH is adjusted to 4.5. Glucoamylase enzyme is subsequently added, which converts the liquefied starch into glucose. Enough enzyme is added to maintain continual saccharification of starch to glucose throughout the fermentation process, and ensures that saccharification does not limit the rate of ethanol

production. It is possible to reduce the amount of glucoamlyase enzyme used in this process by saccharifying the liquefied starch at 65 °C prior to fermentation.

Once the mash is cooled to 32°C it is transferred to a fermenter where yeast is added and the fermentation process is carried out. Depending on the operation, the dextrinized mash may be deficient in readily available nitrogen and a supplemental source, such as ammonium sulfate or urea, may be added as a nitrogen source for the yeast. Recently dry grind ethanol plants have taken to adding proteases, which break down corn protein into free amino acids, which are a supplementary source of nitrogen for yeast growth (Bothast and Schlicher, 2005).

The conventional fermentation process is a batch process that lasts between 48 and 72 hours, resulting in ethanol with a concentration of between 10 and 12% (v/v) and a final pH near 4.0. A low final pH is important for increasing the activity of the glucoamylase and inhibiting the growth of contaminating bacteria. Often, saccharificaion and fermentation are accomplished simultaneously in a process called simultaneous saccharification and fermentation (SSF). SSF offers the advantages of lowering the possibility of microbial contamination, reducing the initial osmotic stress of yeast by avoiding a concentrated glucose solution and is typically more efficient than individual saccharification and fermentation.

Once saccharification and fermentation of the mash is complete, the ethanol is recovered from the solids and water through distillation. Since the vaporization temperature of alcohol is 78°C and water is 100°C (at sea level), heating in a distillation column allows easy separation of ethanol and water. Conventional distillation methods can produce 95% pure ethanol and other processes, such as molecular sieves, remove the 5% of water that remains after distillation. The 100% ethanol produced is then blended with a denaturant such as gasoline, to make it unfit for human consumption and therefore not subject to beverage alcohol tax (Bothast and Schlicher, 2005). Figure 2.3 provides a graphical representation of the full dry grind ethanol production process, including stillage processing, which is covered in more detail in the following section.

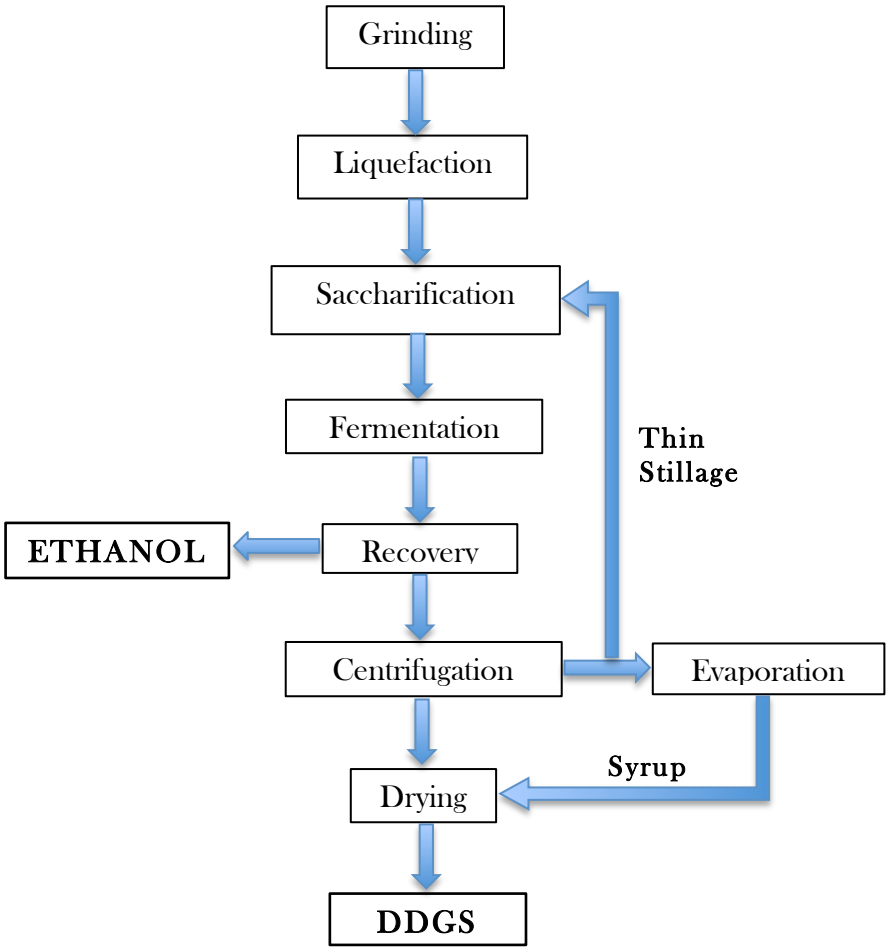


Figure 2.3 – The dry grind ethanol production process

2.4.3 Stillage Processing

Regardless of the feedstock used to produce ethanol, stillage, also known as distillery wastewater, is an aqueous byproduct from the distillation of ethanol following fermentation of carbohydrates (Wilkie et al., 2000). Stillage exhibits significant pollution potential and up to 20L of stillage, with a chemical oxygen demand (COD) potentially greater than 100g/L, can be generated for each liter of ethanol produced (Wilkie et al., 2000, Schaefer and Sung, 2006).

The combined suspended solids and soluble liquids remaining after ethanol distillation is known as whole stillage, which contains the fiber, oil and protein of the grain and any

starch or sugars that were not fermented (Kim et al., 2008). Whole stillage can be marketed as a valuable livestock feed ingredient, but it is typically processed before being sold (McAloon et al., 2000). Thin stillage is first separated from the insoluble portion of the whole stillage using either centrifuges or presses/extruders.

A portion of thin stillage may be used as make-up water for upstream processes (cooking and fermentation), which will help to reduce the volume of stillage produced, as well as reduce consumption of water, steam and some chemicals. However, stillage strength will actually increase with its increased use in backset water, as the amount of COD produced will not change and accumulation of fermentation products and non-fermentable sugars can inhibit the fermentation process (Sun et al., 2010). Typically, between 15 and 30% of the thin stillage is recycled as backset for upstream processes, although a maximum recycle of 50% has been identified (Wilkie et al., 2000, Bothast and Schlicher, 2005).

The remaining thin stillage is concentrated by evaporation to condensed distillers solubles (CDS), also known as syrup (Ganesan et al., 2006). The syrup is subsequently mixed with the solid portion to create the feed product, wet distillers grains with solubles (WDGS). WDGS typically has a moisture content of 65%, and can be directly used as a livestock feed supplement product, but has a short shelf life of 1-2 weeks. To increase shelf life, WDGS is dried to a moisture content of 10-12%, producing dried distillers grains with solubles (DDGS). Drying WDGS is a very energy intensive process and consumes approximately 1/3 of the energy requirements for the entire plant. Some have even estimated that the production of DDGS consumes as much energy as is required by the entire ethanol production process (Drosg et al., 2008). Despite the high-energy requirements, most plants still opt to dry the WDGS to DDGS, as it is a high quality feed product that is essential to the profitability of the plants (Bothast and Schlicher, 2005). However, with rising energy prices bioethanol plants will need to optimize energy consumption in order to avoid a negative impact on the costs of ethanol production (Drosg et al., 2008).

In Canada, 3,383,000 acres of corn was harvested in 2007/2008, producing an average of 135.6 bushels per acre, and a total (average) of just over 458 million bushels of corn (Statistics Canada, 2009). According to 2008 statistics from the Renewable Fuels

Association, Canada produced almost 900 million liters of ethanol. If we assume the same statistics in Canada for dry-grind ethanol production as previously mentioned for the United States (86% of all ethanol production), this corresponds to 774 million liters of Ethanol produced by the dry-grind process. This means that approximately 73 million bushels of corn are being used for dry grind ethanol production, or 16% of all corn harvested in Canada. This level of production also results in the production of about 562 million kilograms of DDGS in Canada alone.

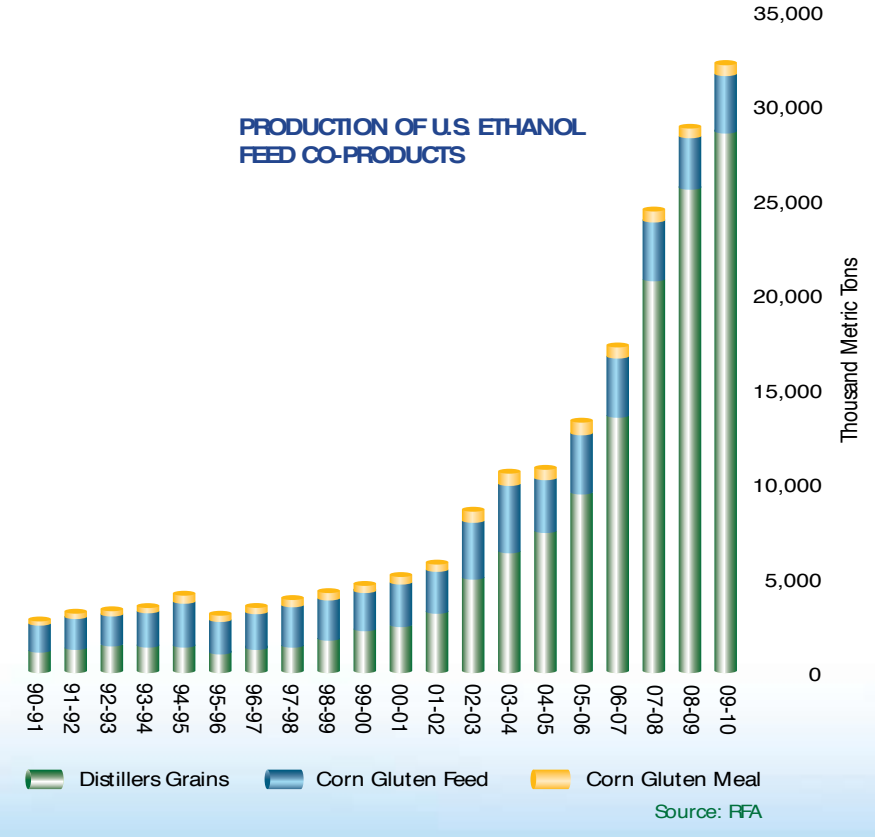


Figure 2.4 – Production of U.S. Ethanol feed co-products (RFA, 2010)

DDGS production has grown significantly over the last decade as shown in Figure 2.4, and increasing growth trends in the dry grind ethanol industry are anticipated to continue. This will result in a greater amount of thin stillage being produced and equally more DDGS available. As conventional markets for DDGS become saturated, driving the price

downwards, it will become necessary to identify additional markets and alternative methods to address co-product streams in order to maintain the economic stability of dry grind corn ethanol production plants (Singh et al., 2005). In particular, the exploitation of thin stillage should be examined due to its high pollution potential, which makes it unsuitable for discharge to a waterway or application to land, and the large energy investment required to produce DDGS.

2.5 Ethanol Industry Energy Balance

Often, we think of ethanol as a way to reduce carbon dioxide emissions and our dependence on foreign energy supplies. Abundant domestic fuels such as coal and natural gas can be used to convert corn into a liquid fuel, effectively reducing the need for imported petroleum (Shapouri et al., 2005). One of the most important issues from an environmental perspective is to justify the use of a biofuel on an energy-savings basis. This has become one of the most debated topics related to corn based ethanol production.

The energy balance of ethanol first surfaced in the mid 1970's when it was receiving attention as a gasoline extender (Nichols et al., 2006, Shapouri et al., 2005). At the time, studies analyzing the energy benefits of substituting ethanol for gasoline concluded that the energy content of ethanol was typically slightly less than the fossil fuel energy required to produce it, the difference being identified as the Net Energy Value (NEV) (Shapouri et al., 2004). However, advances in agronomics, fertilizer production and application and the ethanol production process itself has significantly decreased the energy inputs required to half of what was necessary in the 1970's (Nichol et al., 2006, Hill et al., 2006, Hill, 2007, Wang et al., 2007, Bothast and Schlicher, 2005).

A NEV greater than one indicates that more energy, captured via photosynthesis in the growth of the feedstock, is retained in ethanol than all of the fossil fuels inputs required to produce it. A recent report by Farrell et al. (2006) identified a rather small NEV of 5% (NEV = 1.05) renewable energy content in ethanol and a moderate GHG emission reduction of approximately 13%. Another study, conducted by Hill et al. in 2006, also found that corn grain ethanol results in a small energy gain, providing 25% more energy (a

NEV of 1.25) than the inputs required for its production. In contrast, gasoline is reported to have a negative NEV, requiring 20% more energy inputs than it provides in the end (Nichol et al., 2006, Wang et al., 2007, Bothast and Schlicher, 2005).

Wang et al. (2007) estimated that corn ethanol offered an average reduction in green house gas (GHG) emissions of 19% in 2007 and projected that number to be 21% by 2010. It was also suggested by the authors that ethanol plants fueled by natural gas could achieve an even greater reduction in GHG emissions ranging between 28% and 39%. This would also suggest that supplementing ethanol production plant energy with other energy sources, such as biogas produced from anaerobic digestion of process residuals could also potentially help to reduce GHG emission and improve the NEV. Agler et al., (2008) reported that with a reduction in the mass of solids for drying (resulting from anaerobic digestion of a portion of the thin stillage), methane production results in a 51% reduction in natural gas consumption and improves the NEV from 1.25, as calculated by Hill et al. (2006), up to 1.70. Shapouri and McAloon (2004) reported the highest average NEV ratios that could be found in published literature, of 1.57 and 1.77 for the wet milling and dry grind ethanol production respectively (Shapouri and McAloon, 2004, Nichol et al., 2006, Agler et al., 2008).

The boundaries used in determining the NEV of ethanol can have a great effect on the value, which is exemplified by the range of NEV's reported. The inclusion of co-products is essential to a complete energy balance. The co-products of ethanol production, such as DDGS, have positive economic value by displacing a portion of other animal feed products that require energy to make. Therefore, increases in corn ethanol production will lead to more co-products that can displace whole corn and soybean meal in animal feed, and the energy saved will partly offset the energy required for ethanol production (Wang et al., 2007).

In some cases as described by Farrell et al. (2006), when co-products are not included in the energy balance for corn grain ethanol production, a negative NEV is reported. A paper published by Pimental and Patzek (2005), lists studies that conclude that corn-based ethanol does not provide a positive NEV, but the sources listed are not necessarily convincing. Out of 16 studies referenced 5 are published by the first author or a colleague,

one is by "Citizens for Tax Justice", one is published by a gasoline company, and another is a commentary by a policy analyst that does not offer any scientific data, and yet another is from 1989 (one could argue that the data presented is likely out of date).

Ethanol critics may always find a way to argue that the non-renewable energy inputs required for producing ethanol are greater than the energy value present in ethanol, and although the energy balance is certainly of concern, it is not the only issue at hand. Shapouri makes a case that what is really important is that the production of ethanol results in a net gain in a more desirable form of energy, in order to address energy security issues (Shapouri et al., 2005, 2010). That being said, it is clearly an important issue to resolve and any increase to the NEV of ethanol will make it a more practical and attractive alternative. This, as previously discussed can be addressed, at least in part, by anaerobic digestion of co-products such as thin stillage for energy recovery.

2.6 Addressing disposal of corn ethanol thin stillage

The characteristics of stillage generated by corn ethanol production are highly variable, and depend on the corn and ethanol production processes used. In general, distillery wastewaters such as thin stillage have a high organic content (COD greater than 100g/L), strong acidity (pH 3.5 – 4.5) and high quantities of inorganic substances, such as nitrogen, potassium, calcium and phosphates (Mohana et al., 2009). Other important characteristics of stillage include colour, presence of heavy metals and organic priority pollutants (Wilkie et al., 2000).

The significant pollution potential of stillage makes disposal exceptionally difficult. If discharged into waterways, the high COD, total nitrogen and phosphorus content could result in eutrophication of these natural bodies of water. Similarly, disposal of distillery wastewater on land is problematic, as it has been observed to be harmful to vegetation, reportedly reducing soil alkalinity, manganese availability and consequently seed germination (Mohana et al., 2009, Wilkie et al., 2000). Distillery wastewater also has the potential to significantly effect groundwater quality if applied to land by altering its physiochemical properties, such as pH, colour, and electrical conductivity, due to the

organic and inorganic ions present in the wastewater. In a study by Mohana et al. (2009), which investigated land application of alcohol distillery wastewater, changes in soil microbial populations were observed, resulting in greater fungi populations, and decreased nitrogen-fixing bacteria. Effluent treated by anaerobic digestion showed reduced results in comparison to the application of raw distillery effluent.

Due to the issues related to disposal, the evaporation and application of thin stillage to DDG, which is subsequently sold as animal feed (DDGS), is the currently accepted practice in the ethanol industry. The application of evaporated CTS (syrup) to DDG provides a way for ethanol producers to dispose of residual organic wastes and achieve a 'zero discharge' operation. However, this process is very energy intensive and syrup does not actually add any appreciable nutritional value to the final animal feed (Schaefer, 2006).

In actuality, the application of syrup to DDG to make DDGS can cause problems for farmers. A particular characteristic of corn ethanol thin stillage is its high phosphorus content. Syrup, generated from the evaporation of thin stillage, contains the greatest amount of phosphorus of all ethanol production waste streams. Phosphorus levels in corn ethanol thin stillage characterized by Agler et al. (2008) were reported to be approximately 4,410 mg/L, and Rausch and Belyea (2005) reported a phosphorus concentration of 15,200 mg per kg (dry basis) for syrup. The high phosphorus concentration of syrup is problematic because its addition to DDG to produce DDGS results in an animal feed with equally high phosphorus concentrations. Since the disposal of phosphorus containing wastes is regulated, increased levels of phosphorus in the animals' excrement as a result of consumption of DDGS can be problematic for farmers. Therefore, in order for a farmer to be able to use DDGS as animal feed, sufficient land must be available for waste disposal, potentially posing market limitations for DDGS (Rausch and Belyea, 2005).

The long-term physiological effects on animals fed with DDGS are also a concern due to high concentrations of other elements present in syrup, such as sodium, sulfur and potassium. Potassium, for example, has a laxative effect and was found to be present at levels of 1.5% (approximately 5.50×10^3 mg/L) in syrup by Agler et al. (2008). When found at high levels, potassium will also limit DDGS animal feed formulations (Wilkie et al., 2000).

Anaerobic digestion of thin stillage is a practical alternative to drying and application to DDG, which has previously received minimal attention. AD of thin stillage offers the ability to help maintain the economics of ethanol production by producing and recovering energy, as well as acting as a first step in reducing the pollution potential of thin stillage. Any reduction in the volume of syrup added to DDG would result in decreased levels of phosphorus, potassium and other elements present in DDGS. This could potentailly alleviate problems related to disposal of animal wastes. The quality of animal feed co-product would also be improved because of increased protein concentrations as a result of lower concentrations of salts (Agler et al., 2008). In turn, these improvements may reduce market limitations for the sale of DDGS.

2.7 Advantages and Disadvantages of Anaerobic Digestion

Traditionally, anaerobic digestion has been employed to stabilize sludge from municipal and industrial wastewaters (Manariotis et al., 2010). However, due to recent political pressure to shift towards renewable energy alternatives and rising energy costs, AD has become not only a way to treat wastewater, but also to recover and/or produce useable energy (Agler et al., 2008). Rapid industrialization has also resulted in a large quantity of high-strength organic wastewaters in need of treatment. If approached properly, anaerobic digestion of these effluents offer the potential for a perpetual source of energy. Recent attention has been given to anaerobic digestion for the production of biogas for this very reason (Rajeshwari et al, 2000).

Anaerobic digestion offers several advantages over conventional aerobic digestion that make it attractive for the treatment of high strength wastewater. Additionally, the Carbon-Nitrogen-Phosphorus (COD:N:P) ratios of winery and distillery wastewaters are typically unbalanced, making anaerobic digestion the more suitable option (Acharya et al., 2008). Table 2.2 summarizes the motivation for the use of anaerobic treatment processes.

Table 2.2 - Advantages and disadvantages of anaerobic treatment of wastewaters

Advantages	Disadvantages
• Less energy required, resulting in lower operating costs • Less biological sludge production due to lower microbial growth rate, also increased sludge stabilization and dewaterability • Less nutrients required • Potential for energy recovery through methane production • Rapid response to substrate addition after periods of dormancy • With acclimation most organic compounds can be transformed • Smaller reactor volume required • Technology requires simple construction, operation and maintenance	• Longer start up period required to develop the necessary biomass inventory • May require alkalinity or specific ion additions • Lower removal efficiency: effluent may require polishing steps following AD to meet discharge requirements • Biological nitrogen and phosphorus removal is not possible • Sensitive to changes in temperature, which can cause reduced reaction rates • May be more susceptible to system upsets due to toxic compounds • Potential production of odours and corrosive H_2S gas

*Table adapted from Metcalf and Eddy, Manariotis et al., 2010, and R.E. Speece 1996

When oxygen is the terminal electron acceptor a significantly greater quantity of energy is available to microorganisms, as is the case in aerobic digestion. Anaerobic pathways have been shown to yield seven times less available energy than aerobic processes from the same substrate for this reason. Consequently, the greater available energy in aerobic processes results in increased biomass yield, and therefore waste biomass disposal concerns. In comparison, anaerobic systems produce methane gas, which is unavailable for biomass synthesis, resulting in only 5-20% as much waste biomass, significantly reducing financial and disposal site requirements (Speece, 1996).

The small catabolic energy yield in methanogenesis has resulted in highly efficient and synergistic relationships between methanogenic bacteria. The ultimate products of biological metabolism in anaerobic systems are carbon dioxide (CO_2) and methane (CH_4), the latter of which has inherent economic value as a source of energy. Methane production is a major consideration in the selection of anaerobic digestion technology for wastewater treatment.

The following equations illustrate the conversion of hexose and the consequent energy produced, through aerobic and anaerobic processes respectively:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ ($\Delta G° = -2,870 kJ/mol$) Eqn. 2.1

$C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$ ($\Delta G° = -390 kJ/mol$) Eqn. 2.2

Despite a low energy yield, methanogenesis results in a great deal of potential energy stored in the methane gas produced ($\Delta H° = 816 kJ/mol$), which can then be harvested and exploited in the presence of oxygen for human uses and other physical processes (Schink, 1997).

2.8 Anaerobic Metabolism

Anaerobic digestion is a complex interdependent process involving several diverse microbial populations, working collectively to convert organic compounds into methane (CH_4) and carbon dioxide (CO_2). According to the accepted schematic of anaerobic digestion metabolism as described by Gujer and Zehnder (1983), six distinct processes can be identified:

1. Hydrolysis of biopolymers
    a. Hydrolysis of proteins
    b. Hydrolysis of carbohydrates
    c. Hydrolysis of lipids
2. Fermentation of amino acids and sugars
3. Anaerobic oxidation of long chain fatty acids and alcohols
4. Anaerobic oxidation of intermediary products such as volatile acids, except acetate
5. Conversion of acetate to methane and carbon dioxide
6. Conversion of hydrogen to methane and carbon dioxide

The conversion of complex substrates via AD is the result of the collaborative exertion of a minimum of four different bacterial groups. These four bacterial groups pass substrates from one group of microorganisms to another in order to achieve full digestion and formation of end products. The bacterial groups involved in AD include primary fermenting bacteria, secondary fermenting bacteria, and two types of methanogens (Mohana et al., 2010, Schaefer, 2006). Figure 2.5 illustrates the flow of the anaerobic digestion process from complex organic compounds to the final products of CH_4 and CO_2. A more detailed description of the four main stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis) of anaerobic digestion follows.

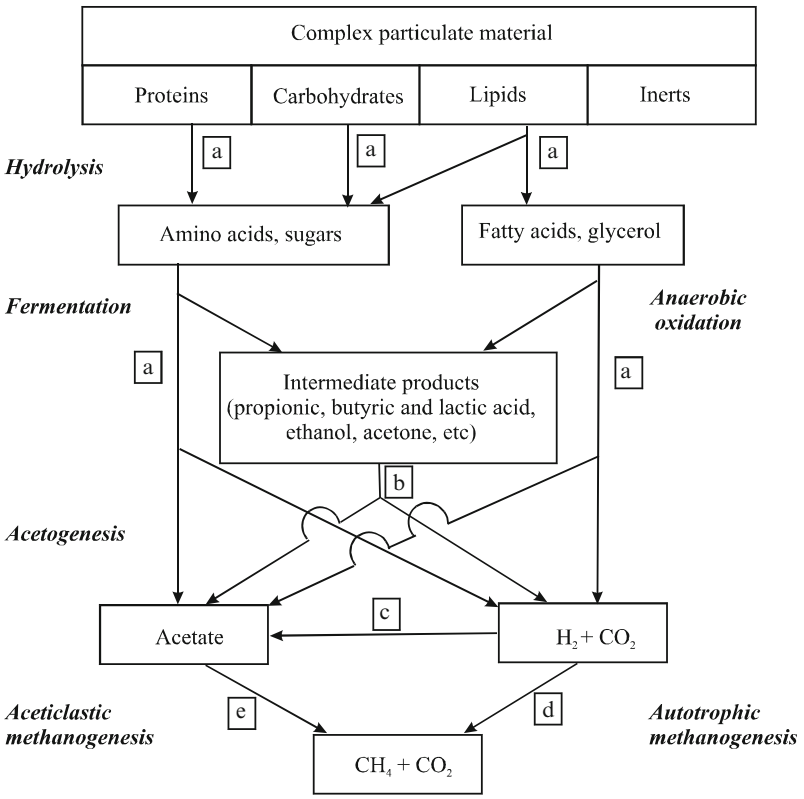


Figure 2.5- Anaerobic Metabolism of Complex Organic Materials (Manariotis et. al., 2010)

2.8.1 Hydrolysis

Hydrolysis is the first stage of anaerobic digestion, converting complex organic materials into their constituent parts through the work of primary fermentative bacteria. Since methanogenic and acetogenic bacteria are unable to directly use polymers, they must first be converted into soluble monomers before the process can proceed. Polysaccharides are converted into simple sugars such as glucose, proteins are transformed into amino acids, nucleic acids become purines and pyrimidines, and lipids become fatty acids and glycerol by the action of the hydrolytic bacteria (Schaefer, 2006).

Hydrolysis is catalyzed by the action of extracelluar hydrolytic enzymes, such as celluases, proteases, and lipases. Primary fermentative bacteria excrete these enzymes in order to break down large substrate molecules that would be unable to pass through cell membranes otherwise. If the feedstock is complex, the hydrolytic phase is relatively slow as it is the sum of individual compounds, which have different rates of hydrolysis (Manariotis et al., 2010). Considered to follow first order kinetics, hydrolysis is typically considered the rate-limiting step in anaerobic digestion of complex wastes (Schaefer, 2006).

2.8.2 Acid Forming Steps: Acidogenesis and Acetogenesis

Hydrolysis is immediately followed by the acid forming stage, in which two groups of secondary fermentative bacteria convert the monomers produced by hydrolysis into several intermediary products. Biological and chemical oxygen demand (BOD and COD) is reduced through these acid-generating pathways.

Acidogenic bacteria turn the products of hydrolysis into volatile fatty acids (VFAs) (butyric, propionic, formic, lactic or succinic acids), keytones (methanol, ethanol, glycerol or acetone) as well as alcohols. The types and concentrations of end products produced by the secondary fermentative bacteria in the acidogenic phase depend on culture conditions, such as pH and temperature. Acetogenesis occurs through carbohydrate fermentation, of which acetate is the main end product. The possible combination of end products includes acetate, carbon dioxide, and hydrogen, which are the only precursors that can be used in methanogenesis.

The conversion of organic material to organic acids in the acid forming stages naturally causes the pH of the system to drop. This is beneficial for the acidogenic and acetogenic bacteria that prefer a slightly acidic environment, in the range of 4.5 – 5.5, but can be problematic for bacteria in the final stage of methanogenesis.

2.8.3 Methanogenesis

Bacterial methanogenesis is a ubiquitous process in most anaerobic environments. The generation of CH_4 is performed by two unique groups of strictly anaerobic *Archaea* (Rittmann, 2008). The first group of Archaea are aceticlastic methanogens, which are

responsible for biogas production directly from acetate, and the second group are hydrogen scavenging hydrogenotrophic methanogens. It has been well established that acetate is the major methanogenic precursor in most anaerobic ecosystems (Horan, 2003). It has been reported that aceticlastic methanogens are responsible for 70% of methane production, while hydrogenotrophic methanogens account for the remaining 30% (Sawayama et al., 2004).

Hydrogen (H_2) plays an integral role in anaerobic digestion as an intermediary. Long chain fatty acids produced by lipid hydrolysis, are oxidized by acteogenic bacteria in the acid forming steps to acetate or propionate and hydrogen gas is produced. Hydrogen present under standard conditions results in inhibition of oxidation, and the reaction can only proceed if the partial pressure of hydrogen is low enough (<100Pa) to thermodynamically allow the reaction to occur (Schink, 1997, Rittmann, 2008). Hydrogen scavenging methanogenic bacteria that consume hydrogen are essential for any anaerobic system. Their action lowers the H_2 partial pressure, ensuring a thermodynamically favorable conditions resulting in full conversion of acids present in the system.

Methanogens are very sensitive to system changes and prefer a slightly alkaline environment. They are most effective in the pH range of 6.5 - 8.0 (Horan, 2003). Methanogenesis is the rate controlling process as methanogens have much slower growth rates that acidogenic and acetogenic bacteria. Therefore, the global kinetic rate of the process is driven by the kinetics of the methanogenic (Davis and Cornwell, 1998).

2.9 Anaerobic Process Parameters

In order to maintain the stability of an anaerobic digester, the complex and interdependent bacterial consortium necessary for AD must exist in equilibrium. Disruptions in environmental conditions can cause the system to shift away from equilibrium, resulting in the build up or depletion of intermediaries, ultimately inhibiting bacterial populations, or shutting down the entire system.

Several digestion parameters affect the physical system and consequently the rate of digestion and production of biogas (CH_4 and CO_2). The following parameters must be

monitored and maintained at acceptable levels to ensure process stability: temperature, pH and alkalinity, carbon-to-nitrogen (C:N) ratio, hydraulic retention time (HRT), solids retention time (SRT), organic loading rate (OLR), nutrients, and presence of toxic inhibitors (ammonia, sulfate and sulfites). Deviations from the acceptable ranges for these parameters can result in digester failure and it is essential to understand the importance of each parameter.

2.9.1 Temperature

Rate of digestion is strongly linked to system temperature and as a result is one of the most important parameters to maintain. Anaerobic bacteria have the ability to survive in a wide range of temperatures: from freezing up to 70°C, and are typically divided into the following classifications: psychrophilic (0°C to 20°C), mesophilic (20°C to 40°C) and thermophilic (50°C to 65°C) (Sakar et al., 2009). Typically, anaerobic digestion is carried out within the mesophilic or thermophilic ranges, with optimum temperatures of 35°C and 55°C for mesophilic and thermophilic digestion respectively.

Both mesophilic and thermophilic temperature ranges have advantages and disadvantages associated with them. Thermophilic digestion allows higher loading rates, achieves a higher percentage of pathogen destruction, and greater substrate degradation (Parawira, 2004). However, thermophilic systems are more sensitive to toxicity and environmental changes than mesophilic systems. From an energetics perspective, thermophilic is also less favourable than mesophilic systems, as they require a greater heat input for the process if the feedstock to be digested is initially cool. This may not be accurate if the feed is incoming at a higher temperature which would not require the addition of heat prior to the addition to a thermophilic digester.

Mesophilic bacteria are thought to be more robust and able to tolerate greater environmental changes in comparison to thermophilic bacteria. Despite the longer retention times required, mesophilic bacteria have demonstrated considerable stability in many different applications, which makes them the bacterial population of choice in most anaerobic digestion facilities.

2.9.2 pH and Alkalinity

System changes in pH are observed in response to biological conversion during the different stages of anaerobic digestion. It is a primary indication of system health and a stable pH indicates digester equilibrium and stability. An acceptable pH range for anaerobic digestion is anywhere between 5.5 and 8.5, however the optimal pH for growth of anaerobic bacteria lies between 6.5 and 7.8 (Sakar et al., 2009, Horan, 2003). Methanogenic bacteria are especially sensitive to extremes in pH, and in order to maintain efficient methanogenesis a suitable and stable pH should be maintained within a digester (Horan, 2003). The major chemical and biochemical reactions, as described by Horan (2003), which influence the pH of a system are:

- Volatile fatty acid production and consumption
- Ammonia release and consumption
- Sulphide release due to reduction of sulphate or sulphite

The accumulation of acids in a reactor can be particularly problematic, resulting in a declining pH and unstable digester. A sharp increase in the volatile solids (introduced to a digester as fresh waste) will result in these conditions. As a consequence of increased availability of volatile solids, there is an increase in the activity of acidogenic bacteria present in the system. The increased activities of acidogenic bacteria will accordingly producing larger amounts of VFAs and lower the pH of the system. If the pH falls into a range that is lethal to methanogens (pH < 6), the system creates a positive feedback loop. As methanogens are responsible for the consumption of volatile fatty acids to maintain a balanced system, a decrease in their population will result in further accumulation of acids. Conversely, profuse methanogenesis can result in an increased pH (pH > 8). An accumulation of ammonia resulting from overactive methanogenesis, will result in acidogenesis being inhibited and ultimately methanogenesis will be as well, due to the reduced VFAs available for conversion to biogas (Sawayama et al., 2004).

Monitoring pH during start up of an anaerobic reactor is especially important for a healthy digester system. Fresh waste must go though acidogenesis and acetogenesis stages before methane formation can begin. This results in an initial dip in pH levels, which can be

easily combated with the addition of bicarbonate alkalinity to buffer the system. Alkalinity refers to the ability of a system to resist changes in pH. This is important to an anaerobic system, as when acids are produced or added to the system, alkalinity present will contribute hydroxide ions, helping to neutralize the acids present (Sakar et al., 2009). Another advantage to adding alkalinity to a digester system is its tendency to cause particulate organics to swell, making them more susceptible to enzymatic activity (Baccay and Hasimoto, 1984).

2.9.3 Carbon-to-Nitrogen Ratio

The carbon-to-nitrogen (C:N) ratio is a measure of the organic carbon and nitrogen present in the feedstock. For an aerobic system, the optimum range for the C:N ratio is between 20-30, however anaerobic systems are reported to have lower nitrogen requirements. The most commonly practiced and recommended C:N ratio for anaerobic digestion is 100:2.5, or a ratio of 40 (Horan, 2003). If there is too much nitrogen present (a low C:N ratio), toxic conditions can result due to the accumulation of ammonia in the system, leading to pH values greater than 8.5. Conversely, too much carbon (or a high C:N ratio), results in the rapid consumption of nitrogen by methanogenic bacteria and consequently lower biogas production (Hartmann et al., 2002).

2.9.4 Hydraulic Retention Time

The hydraulic retention time (HRT), also known as the residence time, is the period of time that the digester liquid remains in the reactor (Sakar et al., 2009). It represents the amount of time the mixed liquor remains in the reactor and is calculated based on the volumetric loading rate at which a reactor is operated. Theoretically, longer retention times yield more complete degradation of a feedstock. Reaction rate, however, is known to decrease with increasing retention time, and an optimum time should be determined based on time and cost effectiveness. Appropriate retention times will differ for each substrate and for most dry processes ranges from 14 to 30 days, while wet processes can be as little as 3 days.

The shorter the retention time is, the smaller the digester that can be employed, resulting in cost savings in operation and building materials. Shorter retention times will increase

production rate per unit reactor volume, but result in a lower final degradation. Research has indicated that a reduction of 64 – 85% of volatile solids in a reactor could be achieved in as little as 10 hours, but typically retention times of ten days are required for complete digestion (Lin et al., 1997, Vlyssides and Karlis, 2003, Horan, 2003).

2.9.5 Solids Retention Time

The solids retention time (SRT) of a reactor is a measurement of the concentration of bacteria maintained within the system with time. Higher SRTs result in greater populations of biomass being retained within the reactor, and consequently has impacts on the type and size of reactor required (Horan, 2003). In a completely mixed reactor with no solids recycle the SRT is equal to the HRT, while more advanced reactor configurations such as sludge recycle systems like the anaerobic contact reactor, fixed film reactors or upflow anaerobic sludge blanket reactors are able to un-couple the SRT from the HRT. The greater the amount of sludge retained within a reactor, the higher the loading potential of the system will be (Lettinga, 1995).

2.9.6 Organic Loading Rate

The organic loading rate of a reactor describes the amount of volatile solids introduced over a period of time. A higher loading rate introduces a greater amount of volatile solids per unit time, and if too high, can stress a digester into shock by a quick and sever drop in pH. As previously discussed, methanogenic bacteria reproduce at a rate slower than that of the acid-producing bacteria, there is a danger of rapid production of acids when a greater amount of volatile solids are available for consumption by acid forming bacteria.

2.9.7 Food to Microorganism (F/M) Ratio

Another key factor controlling anaerobic digestion is the food-to-microorganism (F/M) ratio (Burke, 2001). The F/M ratio expresses the ratio of substrate (F) to the amount of inoculum (M) present in a system (Koksoy and Sanin, 2010). It can be described in the units of grams (g) of VS or COD of substrate per g volatile suspended solids (VSS) of the bacterial population (inoculum) present. It is an important parameter to use to evaluate the potential volatile solids loading, as at a given temperature bacteria are only capable of

consuming a limited amount of food each day (Pranshanth et al., 2006, Koksoy and Sanin, 2010). In a biological treatment system, efficiency can be improved either by increasing the biomass present or decreasing the amount of substrate provided thereby lowering the F/M ratio. Research has indicated that too high an F/M ratio results in toxic conditions, while too low an F/M ratio also may inhibit digestion (Pranshanth et al., 2006).

Pranshanth et al. (2006) studied the performance of F/M ratios between 0.18 and 2.0 g COD/g VSS for the digestion of synthesized wastewater (cellulose, sucrose, and peptone). The optimum ratios in batch mesophilic systems were determined to be between 0.57 and 0.68 g COD/g VSS. Koksoy and Sanin (2010) also investigated the effect of F/M ratios at mesophilic temperatures. F/M ratios of 0.5, 2.0, 5 and 10 (g VSS_{WAS} /g VSS_{INOC}) were examined with sonicated and un-sonicated waste activated sludge (WAS) as the substate. High F/M ratios initially experienced a lag period, but ultimately the cumulative biogas production surpassed that of the lower F/M ratios. However, the authors noted a decrease in specific methane production as the F/M ratio is increased from 0.5 to 2.0, and that on a volume of CH_4/g COD_{added}, the higher F/M ratios are disadvantaged despite increased COD removal efficiencies. Stover et al. (1983) drew similar conclusions based on batch tests examining the digestion of corn thin stillage at various F/M ratios. They concluded that the quality of methane gas appears to be a function of the F/M ratio or SRT operating conditions of the system, and increased in CO_2 levels were noted with decreased F/M ratios. This would appear to indicate that both too high and too low F/M ratios inhibit methane production.

2.9.8 Nutrients

Methanogenic bacteria have a wide spectrum of micro and macro-nutrients required for healthy growth. Appropriate concentrations and ratios of these nutrients are required for reactor stability and proper bacterial metabolism. Studies by Agler et al. (2008), and Schaefer (2006), have illustrated the need for supplemental nutrients for the digestion of corn thin stillage. In general, methanogenic bacteria require the micro and macro-nutrients described in Table 3.1, on the following page.

2.9.9 Sources of Inhibition

There are a variety of sources of potential inhibition for mesophilic anaerobic digestion. These include the accumulation volatile fatty acids or alcohols, potentially caused by hydraulic or organic overloading, temperature fluctuations or the presence of toxins. The accumulation of VFAs is typically an indication of microbial stress, however at a neutral reactor pH, only propionate has been shown to have adverse effects on digestion. Acetic and butyric acids have not been shown to have toxicity effects on methanogenic bacteria up to a total concentration of 10,000mg/L, while propionate concentrations higher than 1000mg/L have been indicated to be unfavourable for digestion (Eskicioglu et al., 2011).

Increased ammonia levels are also reported to inhibit methanogenesis. Sawayama et al. (2004) studied the effect of ammonium on methanogens immobilized on carbon felt in a fluidized bed reactor. The authors reported that ammonium concentrations of greater than 6000mg N/L negatively impacted reactor biogas production. Methanogenic activity was reported to decrease by 10% at ammonium concentrations of between 1670 mg N/L and 3720mg N/L and by 50% for 4090 to 5550 mg N/L. Between 5550mg N/L and 6000mg N/L, methanogenic activity was observed to decrease to nearly zero.

Organic or inorganic toxins, and changes in substrate are also possible sources of inhibition (Ahring et al., 1995). Substances that can potentially cause inhibition and reduced reactor performance include presence of sulfate/sulfide, furfural, and phenol (Schaefer, 2006).

Table 2.3 - Functions of micro and macro nutrients in anaerobic digestion*

Nutrient	Function	Comments
Macro-nutrients		
Carbon, C	Energy and cell material	Cell building material and primary source of energy.
Nitrogen, N	Protein synthesis	Primary nutrient required for microbial synthesis (proteins)
Phosphorus, P	Nucleic acid synthesis	Typically, P requirements are lower than that of N & C. P aids in the synthesis of nucleic acids.
Potassium, K	Cell wall permeability	K increases cell wall permeability by helping cellular transport of nutrients and cation balancing.
Sulfur, S	Various enzymes	Sulfur will generally take the non-reduced form of sulfates or the reduced form of sulfides. Sulfates may inhibit methanogenesis because methanogens can only use the fully reduced sulfide form of sulfur. The presence of sulfide has been shown to have stimulatory growth effects and is required for numerous enzymes.
Micro-nutrients		
Cobalt, Co	Corrinoids, CODH	Co is present in specific enzymes, such as carbon monoxide dehydrogenase (CODH), which plays an important role in acidogenic activity and corrinoids.
Copper, Cu	SODM, hydrogenase	Copper has been found in many methanogenic bacterial strains, it is thought to be a component of super dimutase (SODM) as well as hydrogenase. As of yet, copper has not been found to stimulate microorganisms.
Iron, Fe	CODH, precipitates suphides	Present in methanogenic tissue in the highest content of any other heavy metal. The large reduction capacity of iron allows it to play many roles in anaerobic digestion and is found in and helps to activate many enzymes. Fe may also form sulfide precipitates and promote the excretion of extracellular enzymes.
Molybdenum, Mo	FDH, inhibits sulfur reducers	Mo is present in the common formate dehydrogenase enzyme (FDH), as well Mo may also inhibits sulfur reducing bacteria, resulting in limited formation of necessary sulfides
Nickel, Ni	CODH, F_{430}, essential for sulfate reducing bacteria, aids metabolism of CO_2 and H_2.	Anaerobic bacteria are often dependent on Ni when CO_2 and H_2 are the exclusive energy sources. Most Ni is cells is taken up by a compound called the F_{430} (factor 430), which is present in every bacterium ever examined. As well, CODH is a Ni protein and may aid sulfur-reducing bacteria.
Selenium, Se	Fatty acids metabolism, FDH	Se is a component of several anaerobic enzymes and certain nucleic acids. FDH is a common enzyme that contains Se. Enzymes that are dependent on Se are often very reactive at neutral pH, have low redox potential and may help to metabolize fatty acids.
Tungsten, W	FDH, metabolism of CO_2 and H_2.	W is also a component of the FDH enzyme and may aid in the metabolism of CO_2 and H_2.
Zinc, Zn	FDH, CODH, hydrogenase	Zn, as in Cu, is present in fairly large quantities in many methanogens. It may be part of the FDH, CODH and hydrogenase enzymes, but has not yet been proven to be an essential metal.

*Adapted from Kayhanian and Rich, 1995.

2.10 Anaerobic Co-digestion

Co-digestion involves the anaerobic treatment of more than one type of waste in a single step. Combining different types of wastes for anaerobic digestion offers several advantages, allowing treatment of a broader range of organic waste types. Improved process economy and positive effects on the anaerobic digestion process, including improved methane yields, and improved reactor stability are the main advantages of co-digestion (Hartmann et al., 2002). In most cases, co-digestion improved biogas yields due to synergy developed in the digestion medium and a source of potentially missing nutrients provided by the co-substrate (Mata-Alvarz et al., 2000).

The key to successful co-digestion lies in maintaining a balance of several parameters in the mixture of substrates. While some qualities of each substrate can be beneficial, there are also other properties that may hinder or be inhibitory for the anaerobic digestion process. Other drawbacks include slurry transportation costs when co-digesting solid waste and problems arising from varying policies of the waste-generators (Mata-Alvarez et al., 2000). As previously discussed, an appropriate C:N ratio and pH are integral for efficient anaerobic digestion and co-digestion offers the potential to solve these issues in a practical and economical way. An inappropriate C:N ratio can be corrected for by digestion with a nutrient rich waste that will help to reach the desired ratio. The pH of a system can also be addressed by co-digestion with a waste that offers a high buffering capacity, which will help to protect the system from failure due to a drop in pH if VFA concentrations begin to rise. Other advantages include offsets in the inhibitory effects of ammonia and sulfide, for substrates co-digested with clay and iron compounds respectively and the potential to lower the TS concentration of a waste by co-digestion with another waste that has a lower TS concentration (Hartmann et al., 2002).

2.11 Conventional Anaerobic Systems

The oldest and most simple form of anaerobic digester is the unmixed, uncovered lagoon. Modern lagoon designs include a cover, which facilitates the capture of biogas. These lagoons typically handle a solids content of less than 2% and operate at ambient temperatures. Hydraulic retention times range from 35 days in warmer climates, to 60 days in more northern locations and biogas production tends to vary with the season (Wilkie, 2005). Although anaerobic lagoons have been used in the past for high-strength industrial wastewaters, they are impractical for distillery waste treatment, as they require exceptionally long retention times, require large areas of land and are often un-lined, which may result in contamination of groundwater. Additionally, souring is a frequent phenomenon and odour control is problematic (Mohana et al., 2009).

Continuously stirred tank reactors (CSTR's) make use of mixing to ensure good contact between the organic matter and reactor biomass and are the simplest reactor with the ability for gas collection (Mohana et al., 2009). Despite the reduction in HRT required from weeks in lagoons to days, CSTR's are still considered "low-rate" systems and the hydraulic retention time is equal to the solids retention time. The HRT in a CSTR is determined by the specific growth rate of the slowest growing microorganisms in the system, and generally means that a long HRT is required before an acceptable level of degradation has been reached (Mohana et al., 2009). Despite being considered low rate systems, CSTR's are able to achieve good removal efficiencies for suitable wastewaters.

Research conducted on the treatment of tequila distillery wastewater (vinasses) with a CSTR was able to achieve 90% COD removal at a 5 day HRT and OLR of approximately 6 g TCOD/L/d (Méndez-Acosta et al., 2010). Rićon et al. (2006) investigated the performance of a CSTR treating two-phase oil mill solids wastes at low organic loading rates and mesophilic temperatures. The influent substrate concentration was high at 162 g total chemical oxygen demand (TCOD) and 126g total volatile solids. Organic loading rates investigated ranged from 0.75 to 3.0 g TCOD/L/d, and achieved removal efficiencies of 97.0 – 95.6%, respectively. However, based on data presented, the HRTs required to achieve these removal efficiencies were exceptionally long (between 216 and 54 days).

Some systems, called contact CSTR's, have been designed with sludge recycle in order to increase the organic load and reduce the HRT. Despite the improvements the contact CSTR offers, for wastes with high concentrations of solids, or very high dissolved organic concentrations for which thickening is problematic, and it is more realistic to operate with an HRT equal to the SRT and a CSTR without recycle is considered more suitable (Metcalf and Eddy 2003).

2.12 High Rate Anaerobic Systems

High rate anaerobic systems address the shortcomings of the conventional low rate systems. Washout of biomass has been identified as one of the most prevalent problems of industrial anaerobic digestion plants. The major advantage of anaerobic digestion, the low yield of biomass, is also its major disadvantage when attempting to preserve slow growing methanogens and increase biomass inventory (Speece, 1996).

Modern high-rate bio-methanation processes address this issue by centering on a long solids retention time. Accomplished via bacterial immobilization, these systems reduce biomass washout and have an ability to retain a high volume of viable biomass (Mohana et al., 2009, Acharya et al., 2008, Rajeshwari et al., 2000, Agler et al., 2010). As a result, these reactors, also known as second-generation reactors, can handle high organic loading rates up to 24kg COD/m^3/d and short hydraulic retention times (Rajeshwari et al., 2000).

Poor immobilization rates have adversely affected the credibility of anaerobic processes (Speece, 1996). Bacterial immobilization or the accumulation of an increased quality of biomass in second-generation reactors can be accomplished through a variety of methods. These methods include: sedimentation, floc agglomeration, biomass attachment to media and biomass recycling. These high-rate systems offer several advantages over low rate AD systems, including smaller reactor volumes, low biomass washout, increased preservation of acclimated biomass resulting in more active biomass overall (Manariotis et al., 2010), good shock loading capabilities and an ability to quickly return to normal biogas production rates after long periods of dormancy (Mohana et al., 2009).

Several types of anaerobic reactors are employed for high rate digestion, including:

- Anaerobic Sequencing Batch Reactors (ASBR)
- Up-flow Anaerobic Sludge Blanket Reactors (UASB)
- Anaerobic Baffled Reactors (ABR)
- Anaerobic Migrating Blanket Reactors (AMB)
- Anaerobic Filter Reactors (AF)
    - Anaerobic Expanded Bed (AEB) and Anaerobic Fluidized Bed Reactors (AFB)
- Anaerobic Fixed Film Reactors
    - Upflow reactors
    - Downflow stationary fixed film (DSFF) reactors

2.12.1 Anaerobic Sequencing Batch Reactors

Anaerobic sequencing batch reactors (ASBR's) are considered non-attached or suspended biomass systems. They are operated in a sequence of four operations: feed, reaction, settle and decant (Donoso-Bravo et al., 2009). Mixing occurs during both the feeding and reaction stages, and settling allows biomass perseveration before the effluent liquid is decanted from the system and the process starts again. Similar to anaerobic contact reactors, the efficiency of an ASBR depends on the settling ability of the sludge granules produced (Speece, 1996, Metcalf and Eddy 2003).

ASBR'S offer great flexibility in operation, and can be run in batch, fed-batch or both, depending feeding and reaction time variables (Donoso-Bravo et al., 2009). However, treatment of distillery waste using batch reactors has not been widely examined (Mohana et al., 2009). Research by Ruiz et al. (2002) examined the potential for digestion of winery wastewater using ASBR's. Successful digestion was achieved at an OLR of 8.6g TCOD/L/d and HRT of 2.2 days, with a soluble COD removal efficiency greater than 98%. Donoso-Bravo et al. (2009) also examined the potential of a variety of ASBR configurations for digestion of a synthetic wine distillery wastewater (phenol, glucose as a co-substrate, sodium bicarbonate as a buffer, and micro and macro nutrient solutions). Configurations studied included one-phase batch operation, one-phase batch fed operation and two-phase batch and batch fed. One-stage reactors were found to provide the best results, achieving 100% removal of phenol in 10 days.

2.12.2 Up-flow Anaerobic Sludge Blanket Reactors

Up-flow anaerobic sludge blanket (UASB) reactors developed in the late 1970's by Lettinga and coworkers can be considered the one of the most important developments in anaerobic treatment technology (Lanting and Gross, 1985, Manariotis et al., 2010). Used extensively for treatment of effluents from food-processing units, tanneries and municipal wastewater, UASB reactors have also become one of the most popular and extensively used reactor designs for the treatment of distillery wastewaters globally (Mohana et al., 2009, Akarsubasi et al., 2006). Consisting of a gas-solids separator, an influent distributor and an effluent draw-off system, UASB reactors guarantee sufficient contact between wastewater and sludge particles even at low OLRs due to the influent system. As such, effluent recycle is not required (Rajeshwari et al., 2000).

The development of active granular or flocculent sludge with good settling characteristics is essential for efficient reactor operation, enabling the reactor to maintain high biomass quantities (Mohana et al., 2009). The formation of the type of floc in the reactor is dependent on many factors including pH, up-flow velocity and nutrients. Feed type can also significantly affect the physical nature of granule or floc formation. High carbohydrate or sugar feedstocks will form good sludge granules while wastewaters high in protein result in a fluffier floc (Manariotis et al., 2010). Reactors with granular sludge can achieve considerably higher OLRs compared to those with flocculent sludge. In reactors with flocculent sludge, suspended matter that is either poorly or non-degradable becomes trapped in the sludge, resulting in a non-reversible and sharp decrease in methanogenic activity. A healthy UASB reactor will have a biomass concentration ranging between 50 and 100g/L at the bottom of the reactor and 5 to 40 g/L in the diffuse layer. The development of good biomass may take months in a UASB reactor, and granulated particles will typically reach 1 – 3mm in size. Maximum loading rates for flocculent bed systems are between 1 and 4g COD/L/d compared to the 10 – 30 g COD/L/d typical of UASB reactors (Rajeshwari et al., 2000).

Advantages of UASB reactors include the absence of mechanical mixing and biomass recirculation, as well as the ability to manage disruptions caused by high loading rates or temperature oscillations (Mohana et al., 2009). Significant disadvantages to UASB systems include slow start up, likelihood of washout of sludge during initial process phases and the need for a skilled operator to maintain appropriate operating parameters (Rajeshwari et al., 2000, Manariotis et al., 2010). Successful start up requires UASB reactors to be operated at low loading rates

(between 4-8kg COD/m^3/d), with COD removal rates carefully monitored. Loading rates can be increased once COD removal efficiencies are greater than 90% (Mohana et al., 2009). Modifications such as the addition of packing material at the top of the UASB tank or a settling tank are strongly recommended by Speece (1996) in order to maintain solids within the system in case of an upset that alters sludge blanket characteristics or density.

Pilot scale UASB studies which evaluated the digestion of alcohol distillery wastewaters at mesophilic temperatures have been conducted by Akarsubasi et al. (2006). The authors reported COD removal efficiencies of two reactors as 90% and 60-80% for organic loading rates of between 6-11kg TCOD/m^3/d and 2.5 – 8.5kg TCOD/m^3/d, respectively. The difference in reactors performance was speculated by the authors to be a result of the higher OLRs applied to the one reactor. Generally UASB reactors treating similar wastewaters can effectively be operated at OLRs in the range of 10-20kg/m^3/d and achieve COD removal efficiencies of between 65 and 95% (Akarsubasi et al., 2006).

Kaparaju et al. (2010) examined the digestion of wheat straw stillage with UASB reactors at thermophilic temperatures. The authors reported a soluble COD removal efficiency 76% at an OLR of 17.1 g COD/L/d. However, the high COD (150g/L) of the substrate necessitated dilution prior to digestion, and the results presented were achieve with feed that was only 25% wheat straw stillage by volume.

Expanded granular sludge bed (EGSB) reactors are a modification of the classic UASB design. In comparison, the EGSB has slightly higher superficial liquid velocities ranging between 5 and 10m/h, while typical values for a UASB reactor are around 3m/h and 1-1.25m/h for soluble and partially soluble wastewaters respectively (Rajeshwari et al., 2000). Higher velocities in the EGSB result in the expansion of a portion of the sludge bed, increasing the contact between the wastewater and sludge, which results in a slightly higher possible loading rate. In contrast to conventional UASB systems, EGSB reactors are outfitted with an innovative gas-liquid-solid separation device, as opposed to an internal settler (Mohana et al., 2009).

Anaerobic treatment of low-strength (550 – 825 mg COD/L) brewery wastewater was investigated with a pilot scale EGSB. Even at 20°C, removal efficiencies greater than 80% were obtained for

OLRs up to 12.6kg/m^3/d at HRTs and liquid upflow velocities of 2.1 – 1.2 hours and 4.4 – 7.2 m/h respectively (Kato et al., 1999).

2.12.3 Anaerobic Baffled Reactors and Anaerobic Migrating Blanket Reactor

Additional variations of anaerobic sludge blanket reactor configurations include the anaerobic baffled reactor (ABR) developed in the early 1980's, and the anaerobic migrating blanket reactor (AMBR), developed in the late 1990's. In the ABR system, baffles are used to direct reactor flow through a series of sludge blanket reactors, and unlike UASB reactors, the presence of granular biomass is not necessary (Manariotis et al., 2010). Long biomass retention times are possible without granulation and as a result of selective pressure appropriate bacterial populations establish themselves in different parts of the reactor. Laboratory, pilot and full-scale research has demonstrated the capabilities of ABR's in treating wastewaters at organic loading rates ranging from 0.4 – 28kg COD/m^3/d (Barber and Stuckey, 1999). The AMBR system is identical to that of the ABR in physical set up, with the exception that mechanical mixing is added for each sludge blanket reactor in the series. In AMBR's the biomass tends to migrate (hence the name) into the final reactor, and in order to prevent the loss of biomass from the primary compartment, flow is reversed at specified time intervals (Manariotis et al., 2010).

2.12.4 Anaerobic Filter

A prime example of sophisticated biomass immobilization reactor design is Young and McCarty's Upflow Anaerobic Filter (AF) developed in the 1960's (Young and McCarty, 1969, Speece, 1996). Initially operated in an up-flow mode with gravel as the packing material, several distinct advantages resulted from the unique design. No effluent or solids recycle is required due to the entrapment of biomass within the filter. The accumulation of high concentration of active solids and the low sludge production nearly eliminates the need for wasting (Young and McCarty, 1969). Packing material in up-flow AF's may be the entire depth of the reactor or as in hybrid designs, in only the upper 50 to 70%. Currently, common packing materials include rock particles, ceramic rings, polyvinyl chloride (PVC), plastic pall rings, corrugated plastic cross flow or tubular models (Rajeshwari et al., 2000, Manariotis et al., 2010). Packing media specific surface areas are typically 100m^2/m^3, with no better performance observed at higher packing densities (Metcalf and Eddy, 2003). With time, clogging and flow short-circuiting can results due to accumulations of solids and

biomass, requiring flushing and draining of the packing material to rectify the problem. As a result, up-flow AF's are thought to be best suited for the treatment of soluble wastewaters or those with low concentrations of suspended solids.

Variations of the AF include the Anaerobic Fluidized bed reactor (AFB) and the anaerobic expanded bed reactor (AEB). The AEB reactor is generally packed with silica sand with a diameter between 0.2 and 0.5 mm. Up-flow velocities and effluent recycle rates are determined by the percentage of bed fluidization desired. The smaller packing size provides a greater specific surface area (in the range of 10,000m^2/m^3) theoretically supporting a greater amount of biomass. Due to the small packing and void volume, expanded bed operation is required to prevent clogging but due to the fact that the bed is not fully fluidization, solids can become trapped and result in some degradation (Metcalf and Eddy 2003, Manariotis et al., 2010).

In an AFB reactor the packing material is typically sand, slightly smaller than the support media of AEB reactors with diameters between 0.2 and 0.8 mm. Full bed expansion is maintained by operation at higher up-flow velocities (10 – 30 m/h) in comparison to AEB reactors (Heijnen et al., 1989). The high up-flow velocities help to alleviate the problem of clogging seen in anaerobic filters. A variety of packing materials have been used for AFBR's, including anion and cation exchange resins, activated carbon and diatomaceous earth. As a result of the high up-flow velocities required for this reactor configuration, high turbulence and thin biofilm development result in minimal solids capture, making AFBR's most suitable for wastewaters with mostly soluble COD (Metcalf and Eddy, 2003). Advantages of AFBR's over AF's include the elimination of bed clogging, low hydraulic head loss, better hydraulic circulation and the greater surface area per reactor volume (Rajeshwari et al., 2000).

2.12.5 Anaerobic Fixed Film Reactors

Stationary fixed film reactors consist of a biofilm support structure or media, and are classified based on the point of distribution for influent wastewater. Upflow fixed film reactors receive influent from the bottom and effluent is wasted from the top of the reactor, while the downflow variety operates in the opposite direction. A variety of support materials can be used in a fixed film reactor including glass beads, sand, PVC and porous materials such as needle punched

polyesters, sintered glass, as well as various others (Rajeshwari et al., 2000, Acharya et al., 2008, Patel and Madamwar, 2000).

The attachment of anaerobic microorganisms in a fixed film system prevents them from being washed out of the reactor, which is exceptionally beneficial for slow growing methanogens. The ability of fixed film reactors to uncouple solid retention and hydraulic retention times (SRTs and HRTs) permits high loading rates and COD removal capabilities (Kennedy and Droste, 1985). One limitation of fixed film reactors is that the active reactor volume is relatively high compared to other high rate reactors due to the volume occupied by the biofilm support structure. Other constraints include the accumulation of excess biomass which may results in hydraulic head losses, reduced carrier specific surface area and increased biofilm weight potentially resulting in biofilm sloughing (Boltz and Daigger, 2010). Clogging of the reactor channels can also occur due to increased biofilm thickness and/or high suspended solid concentrations in the wastewater (Rajeshwari et al., 2000). An increase in biofilm thickness is not necessarily beneficial to treatment efficiency, and Boltz and Daigger (2010) suggest that as biofilm thickness increases, surface area decreases.

A recent study carried out by Acharya et al. (2008) evaluated the digestion of distillery spent wash with an TCOD value between 110, 000 – 190,000mg/L and total solids ranging from 110,000 – 190,000mg/L, in an anaerobic upflow fixed film reactors under mesophilic conditions. Three different types of reactor support materials were examined: charcoal, coconut coir, and nylon fibers. Distillery spent wash with a pH of 4.5 was added to the reactors without neutralization or pretreatment. Reactors with nylon packing material were unable to reach hydraulic retention times lower than 20 days, while reactors with charcoal and coconut coir achieved HRTs of 8 days and an organic loading rate (OLR) of 31kg COD/m^3/d and 6 days and an OLR of 23.25kg COD/m^3/d, respectively. The following table summarizes the findings of Acharya et al. (2008):

Table 2.4 - Summary of findings from Acharya et al., 2008

Reactor Support Material	HRT (d)	OLR (kg COD/m³/d)	Total Solids (g/L)	% COD Reduction	Biogas Production (m³/m³/d)
Charcoal	30	6.2	125.10 ± 3.50	80	2.4
	12	15.5	70.5 ± 2.15	73	6.2
	8	23.25	43.50 ± 1.50	16	0.5
Coconut Coir	30	6.2	42 ± 3.50	80	2.9
	8	23.25	106.5 ± 10.15	60	7.25
	6	31	123 ± 11.50	50	3.5
Nylon	30	6.2	70.0 ± 5.85	62	1.00
	20	9.3	123.6 ± 30.50	30	0.2

Based on the results presented by Acharya et al. (2008) it appears that the choice of media has a significant impact on the performance of fixed film reactors. Porous media is understood to enhance biofilm development considerably as compared to smooth media. Results from this study indicated that even at long retention times, the reactor packed with nylon fibers was not stable. The authors noted that during reactor failure there was significant loss of biofilm for the reactors with the nylon fibers as support material in comparison to those with the charcoal or coconut coir.

Patel and Madamwar (2000) also evaluated a variety of carrier materials for use in an upflow fixed film reactor. Treating a low pH petrochemical waste at mesophilic temperatures, support materials evaluated were bonechar, charcoal, bricks, polystyrene beads, and polyurethane foam. Steady state data revealed that all carrier materials were able to facilitate good biomass support and at an HRT of 15 days, 95% COD removal was observed with high methane yields. However, with decreased HRTs and increased organic loading rates, only the bonechar support material was observed to provide the acceptable reactor performance. Even at an HRT of 2.5 days and an OLR of 21.7kg COD/m^3/d, the reactor with bonechar support material was able to achieve 95% COD removal. Similar to the conclusions drawn by Acharta et al. (2008), Patel and Madamwar (2000) concluded that the performance of the bonechar support material was due to its roughness, porosity and large surface area.

In the early 1980's, advanced anaerobic Downflow Stationary Fixed Film Reactors (DSFF) were developed in order to address the operational issues experienced in classical upflow fixed film

systems. The downflow mode was found to reduce plugging, which had been problematic in other reactor configurations. Numerous studies have been conducted to determine operational limits and optimal reactor configurations (Kennedy and Droste, 1985, Kennedy and van den Berg, 1982, van den Berg and Kennedy, 1981, van den Berg et al., 1985).

Designed with vertically oriented stationary packing media, unlike the random packing of the up-flow variety, the downflow configuration is better able to handle concentrated and high solids wastewaters and the accumulation of inert solids and biomass with less chance of media clogging. Fixed film reactors utilize a biofilm support media for biomass attachment and offer simplicity in construction, elimination of mechanical mixing, better stability and capability to withstand toxic shock loads, high loading rates and periods of starvation (van den Berg et al., 1985).

One of the most serious problems which face the application of fixed film reactors to full-scale anaerobic processes, is reactor start up. Typically time-consuming and difficult, start up depends greatly on the slow growth rate of acetate utilizing methanogens (Kennedy and Droste, 1985). Many factors affect the biofilm formation rate, including reactor environmental conditions such as pH, temperature, and macronutrient availability (Boltz and Daigger, 2010). Young and McCarty (1969) suggested that due to the slow growth of methanogens and occasional difficulty maintaining pH during start up, heavy seeding or more than one seeding is best for rapid start up.

Kennedy and Droste published research on the startup of anaerobic down flow stationary fixed film reactors in 1985. The effect of influent concentrations, various support materials, and surface to volume ratios were examined to characterize their effect on biofilm development. Support medias employed included: glass, polyvinylchloride (PVC), potters clay, red drain-tile clay and needle punched polyester (NPP). Of the five materials tested, the authors concluded that NPP and red drain-tile were the most effective as biofilm support media. They determined that support materials with roughened surfaces provided the best start up results, and performance was based on the ease of which bacteria becomes entrapped and attached to the media.

Results from experiments evaluating the effect of surface-to-volume ratios indicated that although increase ratios provided increased space loading rates and COD removal efficiencies, start-up required more time. Kennedy and Droste attributed the increased start up time for these reactors to the increased dependence on methanogenic biofilm accumulation. Performance of the reactors

under various substrate-loading conditions showed that biofilm yields and rates of biofilm accumulation were found to be faster with low influent wastewater concentrations. The authors also state that the biofilms that developed had similar specific activities, indicating minimal diffusional resistance for biofilms up to 0.18cm. Typical times required for start up of a fixed film reactor have been found to be between 75 and 90 days (Droste and Kennedy, 1988, Samson et al., 1985).

Despite potentially difficult start up, fixed film reactors have proved to be practical and efficient systems for treating a wide variety of wastes (Kennedy and van den Berg, 1982, Kennedy et al., 1988, Bories et al., 1988, Patel and Madamwar, 2000).

In 1988, Bories et al. conducted anaerobic digestion of concentrated wastewater produced by the alcohol distillation from sugarcane molasses was conducted at pilot and industrial scales using a fixed film reactor. Pilot plant experiments employed a $10m^3$ fermenter packed with PVC (plastic) media with a specific area of $230m^2/m^3$ and porosity index of 95%. The fermenter operated in a down flow mode at mesophilic temperatures, with biomass recirculation. Cane molasses stillage was collected immediately after distillation and no modification was made to the stillage composition or pH. Fermentation tests were carried out on the fixed film pilot reactor with gradually increasing loading rates, up to 20kg COD/m^3/d. The optimum COD removal efficiency was found to be between 71 – 73.8% at loading rates up to 11.4kg COD/m^3/d, and decreased slightly to 61.9% at the highest OLR of 20.4kg COD/m^3/d. The authors note that the loading rate was increased up to 20.4kg COD/m^3/d without disturbing the system. The following table summarizes the finding of Bories et al., 1988:

Table 2.5 – Summary of findings for Bories and Bazile, 1988

HRT (d)	OLR (kgCOD/m^3/d)	% COD Reduction	Biogas Production (m^3/m^3/d)	Methane Yield (L CH_4/kg COD)
4.9	9.2	72.1	4.5	22.3
4	11.4	71.6	5.6	22.5
3.4	14.2	71.3	6.3	21.3
2.7	20.4	61.9	8	21.4
2.8	19.4	60.4	7.3	20.7
3.2	17.4	62.4	6.8	21.8

In a study published in 2000, Lomas et al. investigated the efficiency of DSFF pilot scale reactor over a period of 6 months, focusing on the effect that internal recirculation had on effluent quality and biogas production. Considered one of the main operational parameters of a DSFF reactor, recirculation is generally used for one of two reasons: (1) to increase in the influent alkalinity and (2) to modify the flow (Lomas et al., 2000). Lomas et al. state that earlier studies have shown that the operation of recirculation within a DSFF reactor at rates four times higher than the influent flow, provide similar hydraulic conditions to a completely mixed reactor. Well-mixed reactors provide more uniform conditions for microbial growth, and increase the active areas in the filling zone. This allows the absorption of sudden organic overloads and the effect of acidgenic bacteria is reduced, before affecting system pH. Down-flow recirculation favours mixing, due to the crosscurrent of biogas and liquid, which also prevents obstructions.

High rate anaerobic digestion using fixed film reactors has illustrated high loading rates at low HRTs, giving promise for the treatment of high strength wastewaters such as corn stillage. From an economic perspective, the shorter the HRT the smaller the reactor volume required, making the conversion of waste into biogas to supplement energy requirements in an ethanol production plant an affordable, and practical option.

The distillery process results in the generation of a strong organic effluent, which due to its high strength appears to have the maximum potential in comparison to other industry effluents (Rajeshwari et al., 2000). UASB and fixed film reactors have been commonly used for treatment of distillery effluents due to their ability to withstand high OLRs, but require an aerobic treatment step in order to achieve acceptable COD concentrations for final discharge to a water way. Reactor characteristics such as efficiency (loading rates and COD reductions), biomass retention, cost, and operation and maintenance requirement, UASB and fixed film reactors appear to be the most suitable (Rajeshwari et al., 2000).

2.13 Research Studies on Anaerobic Digestion of Thin Stillage

Despite the large and growing number of corn grain ethanol production plants, there appears to be a limited amount of research that has been conducted on the anaerobic digestion of corn thin and whole stillage. The earliest research was published in the mid 1980's and only since 2006 has there been addition research published concerning the digestion of corn ethanol thin stillage specifically.

In 1983 Stover et al. published a paper that examined the anaerobic treatment of fuel alcohol (corn grain ethanol) wastewater using bench scale complete mix continuous flow anaerobic activated sludge systems. Waste sludge collected from the continuous systems operated was also used to evaluate batch treatment kinetics at various F/M ratios. Thin stillage used by Stover et al. (1983) had a TCOD of 64,500mg/L and was subjected to pretreatment by gravity settling. Decanted supernatant was subsequently used for the biological treatment studies.

Thin stillage supernatant degradation was evaluated under HRTs ranging from 2 to 5 days, and alkalinity was added in order to maintain a neutral pH throughout the experiments. The substrate was diluted to one third of its full strength for all HRTs with the exception of the 5 days HRTs, which were carried out with 2/3 strength and full strength stillage. Sludge recycle was employed for all studies except the 2 and 4 day HRTs which were operated as once through systems, while all others were operated as sludge recycle systems to obtain the desired SRT. Table 2.6 summarizes the operation conditions investigated by Stover et al., (1983).

Table 2.6 – Summary of findings for Stover et al., 1983

HRT (days)	SRT (days)	OLR (g SCOD/L/d)	SCOD Removal (%)
2	2	3.25	9.2
4	4	1.30	76.9
5.3	6	1.69	72.4
5.3	10	1.75	90.9
5.3	20	2.31	96.2
5.0	30	3.36	92.9
5.0	30	5.72	98.0

The results presented by Stover showed promising soluble COD removal, but organic loading rates (OLRs) achieved are not exceptionally high. Calculations based on the data presented indicate that OLRs in this study ranged from 1.30 g SCOD/L/d to 5.72 g SCOD/L/d as summarized in Table 2.6. Biogas production from the continuous systems was not presented due to measurement problems. Overall this research showed promise for the AD of thin stillage, but indicated the need for a high SRT and/or substrate dilution.

In an article published the following year, Stover et al. (1984) continued to investigate the potential of methane gas production from anaerobic treatment of thin stillage using a complete mix continuous flow anaerobic activated sludge systems, as well as an upflow fixed film reactor system. Both reactor configurations were found to be capable of achieving high treatment efficiencies. Biogas production results from these experiments were promising, resulting in a range of 0.25 to 0.37$m^3CH_4/kg/COD_{removed}$, calculated based on data presented. The authors also demonstrated the importance and potential of energy recovery on the overall ethanol production process. Based on 97,850 BTU required per production gallon consumed, the authors attributed 28,400 BTU to the evaporation of thin stillage. Their calculations estimated that if anaerobic digestion of thin stillage was employed as an alternative to evaporation, 60% of the daily BTU requirements of the ethanol plant could be supplemented with methane combustion, illustrating great potential for AD of thin stillage to help balance the net energy value of corn grain ethanol.

In 1985, Stover et al. investigated the shock loading capabilities of anaerobic systems treating thin stillage under mesophilic conditions. Again, reactor configurations employed were a bench scale continuous flow anaerobic suspended growth (activated sludge) system and anaerobic upflow fixed film reactors, filled with plastic packing material, giving a specific surface area of $138m^2/m^3$ ($42ft^2/ft^3$). The fixed film reactor was subjected to shock loading in the form of a doubled organic loading rate for 24 hours, while being maintained at mesophilic conditions. During the period that the OLR was doubled, there was an observed positive impact on biogas production, which increased from 52L/d up to 75L/d. The production rate immediately returned to 52L/d when the original OLR was reestablished. Similarly, effluent COD and volatile acids (VA's) were all observed to increase during the shock loading but returned to normal values within 24 hours of retuning to the initial loading rate. Temperature shock experiments were also carried out, in which the fixed film reactor temperature was dropped to 10°C for four days. Biogas production rates

decreased by slightly more than half, while effluent COD and Vas were observed to increase. All parameters returned to values similar to those observed before the decrease in temperature within one to two days once the temperature was brought back up to near 36°C. The potential for dormancy was also investigated for the fixed film reactor under two different scenarios. In the first, feeding was stopped for 16 days and the reactor temperature was dropped to between 20 and 25°C. Feeding was then resumed for one week, followed by a second period in which no feed was provided for another 11 days, while the reactor was maintained at mesophilic temperatures. In both cases, the reactor responded with vitality to resumed feeding within one day. Similar results were reported for shocking loading and dormancy tests conducted on the activated sludge system. These results indicate that enhanced biomass retention systems offer the ability to withstand shock loadings, as well as go offline when not required (at least for a short period) and return to operation quickly and efficiently with little to no effect on performance.

There are several conclusions and observations that were re-iterated by Stover et al. in these three papers. It appears in all cases, that digestion was carried out with thin stillage that was diluted and that the addition of alkalinity is required to maintain a stable pH. The authors concluded that although the shock loading capabilities of both reactor configurations was excellent, pH was one of the most critical parameters that could result in operational issues if not carefully monitored. As well, the importance of a suitable F/M ratio was stressed in order to achieve the production of a high quality biogas and suitable VA concentrations. The authors also conclude that anaerobic sludge should still be suitable for cattle feed when dried and mixed with the grain solids. Although this would impact the improved energy balance of the ethanol plant as drying would still be required, it does give ethanol producers another option to achieve a zero discharge plant, while still recovering some energy via AD.

Research by Lanting and Gross also published in 1985 investigated the potential of mesophilic anaerobic digestion for pretreatment of corn ethanol production wastewater as a method to remedy compliance problems that were being experienced with the facilities aerobic trickling filters. A pilot study using a 6m^3 UASB biothane reactor was conducted, and achieved 76% COD removal at a volumetric loading rate of 9.3kg COD/m^3/d. Shock loading studies conducted indicated that the UASB pilot could handle loadings three to four times the average flow (Schaefer, 2006). However, the TCOD of the thin stillage used for these experiments was quite low, at

approximately 3,600mg/L. Conclusions drawn by the authors were that the thin stillage wastewater was nutrient deficient, and that the addition of nitrogen and phosphorus and caustic (to control pH) are required.

Agler et al. (2008) examined the potential for anaerobic digestion of thin stillage under thermophilic conditions, with and without the addition of trace nutrients. Two identical high-rate anaerobic sequencing batch reactors were employed to digest thin stillage from a dry grind corn-to-ethanol facility. Reactors were operated for approximately 400 days with HRTs investigated varying from 40 days, down to 7 days. From day 78 of digestion until the end of the operating period, each reactor was augmented with modified trace element solutions based on Zehnder et al., (1980).

The authors reported that reactor failure was observed at an HRT of 7-8 days, corresponding to an OLR of 9.27-10.71 g TCOD/L/day. It was concluded that the best performance was observed at an HRT of 10 days (OLR = 7.5g TCOD/L/day) with a mean total COD removal efficiency due to biodegradation of 74.7% and VS degradation of 80.3% removal of TS. Maximum methane production was calculated to be 0.254 L CH_4/g $TCOD_{fed}$ or approximately 0.280 L CH_4/g $TCOD_{removed}$ at the 10 day HRT. The authors concluded based on the results of augmenting the reactors with trace element solutions, that cobalt is necessary for successful long-term anaerobic digestion under thermophilic conditions. This study also indicated a 30% increase in ammonia nitrogen observed in reactor effluents, which the authors attributed to protein degradation.

Schaefer (2006) operated two continuously stirred tank reactor operated under thermophilic conditions to digest undiluted thin stillage (100g TCOD/L) from a dry grind ethanol production facility. One reactor was fed with thin stillage pre-treated with ultrasound, while the other reactor acted as a control reactor with no pre-treatment of the thin stillage. Both reactors were supplemented throughout experimentation with a trace element solution, also based on Zehnder et al. (1980). Reactors were operated without alkalinity addition for pH control, and HRTs investigated included 30, 20, 15 and 12 days, with failure observed at the 12 day HRT. Semi-steady state conditions were reported for the 15-day HRT after 45 days of successful operation, although the authors noted that signs of digester instability were observed. A significant spike in VFA concentrations was reported when the systems were transitioned to an HRT of 12 days and

the experiments were subsequently terminated. The best volatile solids reduction was observed at the 20 day HRT at 88.5% and 89.8% for the sonicated and control digesters respectively.

The highest COD reductions were observed at the 20 day HRT, and effluent soluble COD was observed to increase at the 15 day HRT, which can likely be attributed to the increase in VFAs present in the system. The maximum methane production was observed at the 15 day HRT for both the sonicated and the control system. Methane yields of 0.616 and 0.621 m^3/kg-VS_{added} and 0.728 and 0.737 m^3/kg-$VS_{removed}$ were observed for the sonicated and control reactors respectively. Interestingly, the control system achieved nearly identical methane yields at both the 15 and 30 day HRTs.

Results presented by Schaefer illustrate that there is potential for anaerobic degradation of high strength thin stillage, but results indicate limited success. The best results were obtained operating at an HRT of 20 days, which is an unrealistic retention time for full-scale operations. Loading rates achieved were lower than those reached by Agler et al. (2008), with a maximum OLR of 6.4 g COD/L/d at a 15 day HRT. Agler et al. (2008) were able to achieve a 10 day HRT and OLR of 7.5 gTCOD/L/d. It was also noted by the author that the sonication pre-treatment achieved limited success, with the only notable advantage being the slightly lower levels of VFAs observed at lower HRTs. However, high volatile solids reductions observed suggest the possibility for improved water recycling within the ethanol production process. The author did not recommend ultrasounds pretreatment for future use with thin stillage.

Most recently research investigating the potential for anaerobic digestion of whole stillage (253,929 mg TCOD/L) from a corn ethanol production plant under thermophilic and mesophilic conditions, was conducted by Eskicioglu et al. (2011). Batch tests with stillage ranging in concentration from 6,348 mg TCOD/L to 50,786 mg TCOD/L were carried out. VS removal efficiencies achieved were between 94 and 83% and TCOD removal efficiencies between 76 and 88% under mesophilic conditions. Under thermophilic conditions, between 82 and 97% VS and between 73 and 94% TCOD removal efficiencies were reported.

Eskiciglu et al. (2011) also operated two semi-continuous CSTR's with full strength whole stillage under mesophilic and thermophilic conditions. SRTs investigated were 60, 45 and 30 days. The authors reported that the thermophilic reactor was unable to handle the organic loading rate of

4.25 g TCOD/L/d at the 60 day SRT, and the mesophilic reactor was only stable at the 60 day HRT with a methane yield of 58L CH_4/kg stillage. Propionic acid concentrations were observed to increase to 2998mg/L in the thermophilic digester after only 30 days of digestion, while the mesophilic digester had negligible amounts after the first 15-day acclimation period. Similar to the increase in propionic acid in the thermophilic reactor, levels were observed to increase to 2400mg/L when the mesophilic reactor was transitioned from the 60 day to the 45 day HRT. In both cases, the accumulation of propionic acid corresponded to a drop in pH (below 6.5) despite the addition of alkalinity as buffer and gradual reductions in biogas production.

2.13.1 Potential of water recycle for ethanol production and anaerobic digestion

A common trend in the published literature concerning digestion of corn ethanol thin stillage or similar high strength wastewaters is the need for long retention times, due to the high strength of CTS (Schaefer (2006), Eskicioglu et al. (2011)) or dilution in order to achieve a concentration appropriate for anaerobic digestion (Kaparaju et al., 2010). As it is, the production of corn grain ethanol is a water intensive process, with consumption estimated to be between 13.2L – 22.7L per liter of ethanol produced by the dry mill production processes (King and Webber, 2008). Additional water consumption for anaerobic digestion of thin stillage may only increase the environmental impact of corn grain ethanol. There appears to be very little literature available on the impact of using digested effluent as a means to reduce fresh water use for substrate dilution prior to anaerobic digestion.

Recent research conducted by Sun et al. (2010) examined the potential for integrating anaerobic digestion into a water-recycled cassava bioethanol process. With similar characteristics to corn ethanol thin stillage (100g TCOD/L and a pH between 3.8 and 4.5) cassava stillage has analogous disposal and treatment issues. In order to address disposal issues, back-set of stillage is commonly used, however the direct use of stillage as back-set water has been found to be adverse to both fermentation time and alcohol yields (Sun et al., 2010, Wilkie et al., 2000). The negative impact of backset has resulted in the adoption of various physical and chemical methods in order to mitigate the effects and enhance recycling performance. Anaerobic digestion of cassava thin stillage by thermophilic and mesophilic UASB digesters prior to use as backset water in the upstream ethanol processes was carried out. The effects on fermentation were observed and the authors found that the use of anaerobic digester effluent had a positive impact on yeast growth. Also, improved

microbial biomass inventory was observed when compared to fermentation of substrate diluted with tap water. It was also concluded that the use of effluent expedited sugar consumption and positively impacted ethanol fermentation. This study indicates a significant potential for water savings and emission reduction in the production of ethanol from cassava.

A study by Nordberg et al. in 2007 investigated the recirculation of process liquid for anaerobic digestion of alfalfa silage in semi-continuous continuously mixed reactors. With 100% recirculation of process liquid used for substrate dilution the reactors were able to operate without the need for an extra supply of water. However, the authors found that this resulted in an accumulation of organic and inorganic compounds and an increase in pH and alkalinity. Initially the increases in pH and alkalinity made it possible for the authors to achieve the desired OLR while maintaining hydrolysis and a stable methane yield, however 100% recirculation eventually led to inhibition. They attributed this to the high concentrations of accumulated organic and inorganic substances. Interestingly, when a 50% process liquid, 50% water ratio was used for substrate dilution there was an observed improvement in process performance. Nordberg et al. (2007) indicated that this meant that an optimal process can likely be obtained by adjusting the degree of process liquid recirculation. The authors were able to make some interesting observations and there appears to be promise for the use of digested process water for substrate dilution to reduce fresh water consumption. However, the hydraulic retention time at which Nordberg et al. achieved these results was rather long at 26 days.

2.13.2 Thesis Research

A limited amount of research concerning the anaerobic digestion of thin stillage has been conducted. There are many approaches that have not been considered, and this thesis attempts to address a few of them. The research presented examines the potential for digestion of thin stillage diluted with clean water or digested effluent. No research into the digestion of thin stillage diluted with effluent has been conducted to the author's knowledge and aims to determine the potential for effluent recycle. Reactor studies presented are conducted with a down-flow fixed film reactor configuration, which is a configuration that has not been applied for the treatment of corn ethanol thin stillage in the past and aims to reduce digestion times and increase organic loading rates.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Corn Ethanol Thin Stillage

The following research was conducted with corn ethanol thin stillage (CTS) obtained from the Greenfield Ethanol Plant located in Tiverton, Ontario Canada. The Tiverton plant is a dry grind corn ethanol facility with a capacity of 26 million liters per year (MMly). Well-mixed samples of thin stillage were preserved at -18°C for long term storage and transferred to 4°C just prior to use.

3.1.2 Mesophilic Anaerobic Inoculum

Mesophilic inoculum was obtained from the effluent line of the anaerobic sludge digesters (an SRT of 15-20 days) treating a mixture of primary sludge (PS) and thickened waste activated sludge (TWAS) at the Robert O. Pickard Environmental Center (ROPEC) sewage treatment plant located in Gloucester, Ontario Canada. ROPEC has preliminary and primary treatment followed by a conventional aerobic activated sludge unit operated at an average solids retention time (SRT) of five days. Ferric chloride is added to waste activated sludge (WAS) for phosphorus removal prior to thickening. Thickened WAS and PS are blended in a 58:42 (v/v) ratio and undergo mesophilic anaerobic digestion to produce a stabilized biosolids product for disposal.

3.2 Experimental Set-up and Protocols for BMP Assays

Biochemial methane potential (BMP) batch assays (Owens et al., 1979), were conducted in order to determine the suitability of corn ethanol thin stillage for anaerobic digestion and biogas production for energy recovery. Prior to the start of each batch test, characterization of inoculum and thin stillage was conducted, verifying total and soluble chemical oxygen demand, total and volatile solids, ammonia concentration, alkalinity and pH, as described in the following section, 3.3 - Analytical Methods.

Identical experimental set-up and sampling proceedures were carried out for each set of batch tests. Five sets of BMP tests were conducted in duplicate, evaluating different conditions for the digestion of thin stillage. The assays conducted were:

- An initial suitability study to evaluate the digestion of CTS as the sole carbon source
- An investigation into the effects of biomass acclimation on CTS digested as the sole carbon source
- A study of the impact resulting from providing trace element solutions on the digestion of CTS as the sole carbon source
- A study of the effects of CTS co-digestion with food waste (FW) and alternately thickened waste activated sludge (TWAS), with and without acclimation of biomass
- A BMP assay to examine the potential for the use of digested effluent for substrate dilution in place of clean water.

3.2.1 Experimental Set-up

Glass Serum bottles (125mL) sealed with butyl rubber stoppers were used as batch digesters for all BMP assays. Inoculum (50% by volume) and thin stillage, titrated to a neutral pH (approximately 6.9) and diluted with either distilled water or a combination of distilled water and BMP effluent, was added to each serum bottle. The volume of thin stillage varied with each food-to-microorganism (F/M) ratio in order to achieve the desired chemical oxygen demand (COD) for each experiment. Equal parts of $NaHCO_3$ and $KHCO_3$ were added to each bottle to achieve an alkalinity concentration of between 4000 and 6000mg/L as $CaCO_3$. Experiment bottles were subsequently sparged with nitrogen gas for 2 minutes to produce anaerobic conditions and prevent exposure to air and then sealed. Assay bottles were brought to atmospheric pressure prior to incubation by inserting a BD 21G1½ needle connected to a manometer and allowing the bottle pressure to equilibrate with atmospheric pressure.

Bottles were incubated at 35 ± 2°C and agitated in a New Brunswick Scientific Controlled Environment Incubator Shaker model G-25 at 100 rpm, in order to keep bacteria in suspension. Biogas production was monitored daily with a BD 21G½ needle connected to a u-tube manometer, while pH, volatile fatty acids (VFAs) and biogas composition (methane and carbon

dioxide) were typically monitored weekly until the conclusion of the experiment. The experiments were deemed complete when the biogas production rates began to approach zero.

Final characterization was conducted at the conclusion of each assay. Analysis of total and soluble COD, total and volatile solids, final pH, VFA concentrations, as well as ammonia and alkalinity of the experiment bottles were completed. All experiments and analyses were carried out in duplicate, for both sample bottles and controls (inoculum only).

3.2.2 Initial Suitability BMP Assay

Initial studies investigated chemical oxygen demands (COD's) corresponding to food-to-microorganism ratios of 0.2, 0.5, 0.7, 1.0, 1.5 and 2.0 (g VS_{feed}/g VSS_{inoc}). Biomass was not acclimatized to thin stillage prior to the start of initial BMP assay in order to facilitate the comparison of COD removal and biogas production between acclimated and non-acclimated biomass at a later time. Experiment bottles were prepared and monitored in duplicate as described in section 3.2.3 - Experimental Set-up. Control bottles were used as the baseline for comparison, which consisted of biomass and buffered water, in a 50:50 ratio by volume. All sample analysis results were adjusted for the contribution of inoculum. Table 3.1 summarizes experiment bottle compositions.

Table 3.1 - Initial suitability BMP assay bottle compositions

F/M Ratio	Inoculum (mL)	Corn Stillage (mL)	Water (mL)	Total Volume (mL)
Control	40	0	40	80
0.2	40	1.2	38.8	80
0.5	40	3	37	80
0.7	50	5.8	44.2	80
1.0	50	8.2	41.8	100
1.5	50	12.5	37.5	100
2.0	50	17	33	100

3.2.3 Acclimation BMP Assay

Following the initial suitability studies, a BMP assay was conducted in order to observe the effect of biomass acclimation on biogas production, COD and volatile solids (VS) removal. After the completion of the initial suitability BMP assay, the mixed liquor from each experiment bottle was well mixed and half was put aside to be used as inoculum for a new set of experiment bottles while the other half was used for final analysis of the initial suitability study. Acclimation studies were carried out on F/M ratios of 0.7, 1.0, and 1.5, which were chosen based on their performance in the initial suitability BMP assay.

Bottle compositions are presented below in Table 3.2. Compositions were essentially identical to those of the initial suitability assay bottles, with the exception that the inoculum for the acclimation study was the final effluent of the corresponding F/M ratio assay bottle from the initial suitability BMP. Since the inoculum used in the acclimation BMP assay was only half of the liquid from the initial suitability study, these conditions represent a worse case scenario, in which half of the biomass population would be lost from a reactor prior to the addition of new CTS.

Table 3.2 – Acclimation BMP assay bottle compositions

F/M Ratio	Acclimated Inoculum (mL)	Thin Stillage (mL)	Water (mL)	Total Volume (mL)
0.7	50	5.8	44.2	100
1.0	50	8.5	41.5	100
1.5	50	12.5	37.5	100

Both inoculum and thin stillage were characterized prior to the start of the experiment, biogas was monitored daily, VFAs and pH were monitored weekly. Characterization carried out determined TCOD, SCOD, TS, VS, ammonia and alkalinity concentrations of inoculum, substrate and final effluent.

3.2.4 Supplemental Nutrients BMP Assay

It has been previously determined that biogas production can be stimulated by the addition of particular nutrients (Rajeshwari et al., 2000, Agler et al., 2008, Murray and van den Berg, 1981). As described in Chapter 2: Literature Review, the bacteria that carry out anaerobic digestion require micro and macro nutrients such as nitrogen, phosphorus, sulphur, potassium, calcium,

magnesium, iron, nickel, cobalt, zinc, manganese and copper for optimum growth. Despite being required in only very small concentrations, their absence can have a detrimental effect on the performance and growth of the microbial population (Rajeshwari et al., 2000). Research conducted by Agler et al. (2008), employed a full trace element solution described by Zehnder et al., (1980), as well as a few modified versions of the solution, under thermophilic conditions. By eventually reducing the solution to a simple combination of Cobalt ($CoCl_2 \cdot 6H_2O$), EDTA (1g/L) and HCl (1ml/L) it was determined that augmentation of cobalt is required for microbial growth. These results can be further supported by the fact that cobalt is an important factor in enzymatic catalysis of methyl group transfer in methanogenesis (Agler et al., 2008, Murray and van den Berg, 1981) and is also present in enzymes, such as carbon monoxide dehydrogenase (CODH) which plays an important role in acidogenic activity (Kayhanian and Rich, 1995).

In order to determine on the effect of providing supplemental trace elements on mesophilic anaerobic digestion of thin stillage, a BMP assay was carried out using the trace element stock solution in Table 3.3, as described by Schaefer (2006), which the authors also adapted from Zehnder et al., (1980), as well as a BMP assay which made use of the modified Cobalt Solution in Table 3.4, as described by Agler et al., (2008). Both stock solution include EDTA and HCl, which act as chelation agents.

Table 3.3 - Trace element stock solution (Zehnder et al., 1980)

Chemical	Concentration (mg/L)
$FeCl_3 \cdot 4H_2O$	10,000
$CoCl_2 \cdot 6H_2O$	2,000
EDTA	1,000
$MnCl_2 \cdot 4H_2O$	500
Resazurin	200
$NiCl_2 \cdot 6H_2O$	142
Na_2SO_3	123
$AlCl_3 \cdot 6H_2O$	90
H_3BO_3	50
$ZnCl_2$	50
$(NH_4)_6Mo_7O_{24} \cdot 6H_2O$	50
$CaCl_2 \cdot 2H_2O$	38
HCl, (ml/L)	1.0

Table 3.4 - Modified cobalt trace element stock solution (Agler et al., 2008)

Chemical	Concentration (mg/L)
$CoCl_2 \cdot 6H_2O$	2,000
EDTA	1,000
HCl, (ml/L)	1.0

The BMP assays were carried out at F/M ratios of 0.7, 1.0 and 1.5 and bottles were prepared in duplicate as previously described. One set of assay bottles of each F/M ratio was augmented with the trace element or mineral mix solution (denoted MM), while the other set was provided with the modified cobalt solution (denoted Co). Trace element solutions were added to BMP assay bottles in a proportion of 1 milliliter per 15 grams of total COD based on previous research conducted by Agler et al., 2008 and Schaefer, 2006. Both the inoculum and thin stillage were characterized prior to the start of the experiment, biogas was monitored daily, VFAs and pH were monitored weekly. A final characterization was carried out at the conclusion of the assay, which quantified final TCOD, SCOD, TS, VS, ammonia and alkalinity concentrations.

3.2.5 Co-digestion BMP Assays: With and Without Biomass Acclimation

In order to determine if there were means other than directly providing trace elements required for anaerobic digestion via a prepared supplemental mineral mix, co-digestion batch tests with thickened waste activated sludge (TWAS) and kitchen food waste (FW) were conducted. Co-digestion studies were carried out at F/M ratios of 0.7, 1.0 and 1.5, with an equivalent of 25% of the total VS (on a mass basis) provided by either FW or TWAS. Bottles were prepared as described in the previous BMP tests and with compositions outlined in Table 3.5.

Table 3.5 - Co-digestion BMP Assay Bottle Compositions

F/M Ratio	Inoculum (mL)	TWAS (mL)	FW (mL)	Thin Stillage (mL)	Distilled Water (mL)	Total Volume (mL)
0.7	50	0	2.9	4.4	42.4	100
	50	3.2	0	4.4	42.1	100
1.0	50	0	4.1	6.3	39.6	100
	50	4.6	0	6.3	39.1	100
1.5	50	0	6.15	9.4	34.45	100
	50	6.8	0	9.4	33.8	100

Experiment bottles were again monitored for biogas production, VFAs, pH and biogas composition throughout the experiment and fully characterized at the end of the experiment. Acclimation studies were carried out in the same manner as described in section 3.2.3. A well-mixed 50mL sample of the liquid from the BMP was carried over as inoculum, and new thin stillage, alkalinity and either FW or TWAS was added to each bottle. BMP assay bottles were monitored for pH and VFA concentrations weekly until the final characterization of TCOD, SCOD, TS, VS, ammonia and alkalinity.

3.2.6 Effluent Recycle BMP Assay

This BMP experiment was designed in order to facilitate the reuse of digested effluent three times (1st recycle, 2nd recycle and 3rd recycle BMP assays). A high loading rate was chosen in order to observe any build up of VFAs or recalcitrant COD, which assumingly would occur more quickly than would be observed under lower loading conditions. Initially, 32 identical BMP bottles were set up with an F/M ratio of 2.0, using un-acclimated biomass as inoculum in the same manner as described for the initial suitability BMP assay.

Table 3.6 – Recycled Effluent BMP Assay Bottle Compositions

F/M Ratio	Inoculum (mL)	Thin Stillage (mL)	Water (mL)	Total Volume (mL)
2.0	50	17	33	100

The biogas production of all 32 bottles was monitored over 48 days, at which point a random sample of bottles was selected and used to determine the characteristics of the end point of the experiment. Assay bottles were well mixed and then characterized; the final characterization of this portion of the study is presented as the F/M ratio of 2.0 in the initial suitability studies.

After the final analysis of the selected experiment bottles, all 24 remaining bottles were well mixed and half of the liquid was decanted leaving 50mL of acclimated biomass in each bottle. The bottles were prepared in this manner in order to model the worst-case scenario (loss of biomass), as was done in the previous acclimation BMP assays. The decanted mixed liquor was then used as the dilution water for the new thin stillage that was added to the corresponding bottles, any remaining decanted liquid was discarded after all experiment bottles were prepared. All four sets of assay bottles were prepared with the same amount of new thin stillage as was initially provided to achieve an F/M ratio of 2.0, except this time the dilution water for each set of six bottles was comprised of a certain percentage of the treated BMP effluent instead of all clean water. The percentage of effluent refers to the proportion of the effluent ('recycled water') that is used to make up the total volume of dilution water required. The following table summarizes the recycle conditions for each set of six bottles:

Table 3.7 - 1st Recycle BMP Assay Bottle Compositions

% Effluent in Dilution Water	Thin Stillage (mL)	Clean Water (mL)	Digested Effluent (mL)	Acclimated Biomass (mL)
0%	17	33	0	50
25%	17	24.75	8.25	50
50%	17	16.5	16.5	50
100%	17	0	33	50

In summary 6 assay bottles were provided with thin stillage that was diluted with all clean water (0%), 6 bottles were provided with CTS diluted with 25% effluent, 6 bottles were provided with CTS diluted with 50% effluent and 6 bottles had CTS that was diluted entirely (100%) with effluent. Supplementary alkalinity was added and the bottles were then sparged with nitrogen gas, capped and placed into the New Brunswick Scientific incubator shaker and agitated at 100rpm. Volatile fatty acid concentrations and pH were monitored weekly and biogas production was monitored until it approached zero. Two bottles from each of the four sets (0%, 24%, 50% and

100%) were used for final characterization of the assay. This was considered the 1st recycle of effluent BMP assay.

For the second recycle BMP assay, the remaining 16 experiment bottles were allowed to settle until the biomass has visibly accumulated in the bottom of the bottles. Experiment bottles were allowed to settle in order to preserve a greater amount of biomass, in hopes that maximum retention of biomass (best case scenario) may further increase biogas production and rate, COD and VS removals. Once the biomass had visibly settled, 50mL of the supernatant was decanted and 50mL was left in the bottles.

For each set (0%, 25%, 50% and 100% effluent), the decanted liquid was characterized in order to determine the COD and solids concentrations present in the recycle water. Effluent decanted from assay bottles was used to dilute the new CTS for the assay bottles with the same percentage effluent as dilution water for the 2nd recycle of effluent BMP assay (i.e. effluent decanted from assay bottles run with 25% effluent as dilution water in the 1st recycle of effluent BMP was used to diluted the CTS for the 25% effluent assay bottles of the 2nd recycle of effluent BMP, and so on). Assay bottles were prepared with the same proportions and supplemental alkalinity as described for the 1st recycle BMP assay, except that the amount of thin stillage used was adjusted based on the previous recycle BMP assay results. Assay bottles were monitored weekly for pH and VFA concentrations, and the final characterization was carried out when the biogas production rate approached zero.

Table 3.8 - 2nd and 3rd Recycle BMP Assay Bottle Compositions

% Effluent in Dilution Water	Thin Stillage (mL)	Clean Water (mL)	Digested Effluent (mL)	Acclimated Biomass (mL)
0%	13	37	0	50
25%	13	27.75	9.25	50
50%	13	18.5	18.5	50
100%	13	0	37	50

Following the conclusion of the 2nd recycle BMP assay, the eight remaining bottles (two of each recycle condition: 0%, 25%, 50% and 100%) were allowed to settle again, in order to facilitate biomass preservation in the experiment bottles for the 3rd recycle BMP assay. The assay bottles

were prepared in an identical fashion as described for the 2nd recycle BMP assay. This was the final set of experiment bottles for this portion of research. Volatile fatty acids and pH were monitored weekly and when the biogas production rate approached zero, the final recycled BMP assay was ended and end point characterization was carried out.

3.3 Continuous Reactor Studies

In order to accomplish the objectives specific to the DSFF as outlined in Chapter 1, a 12-month laboratory study was conducted. Two DSFF reactors were acclimated and operated at varying OLRs and HRTs over the 12-month period.

3.3.1 Apparatus

Two identical laboratory-scale 28L (empty bed volume) down flow stationary fixed film (DSFF) reactors were used to establish the potential for anaerobic digestion of the corn ethanol thin stillage in a semi-continuous system. Fixed film reactors were chosen due to their ability to facilitate the preservation of biomass by conserving acclimated biomass on support media, thereby reducing washout.

The reactors employed in this experiment consisted of a vertical tank (18.0 x 18.0 x 98.0 cm ID) constructed of 1.25cm thick Plexiglass. Both reactors were covered with 2cm thick removable Plexiglass lids, sealed to the reactors with rubber gaskets and silicone grease. Seal integrity was maintained by pressure applied by 12 bolts running through the cover plate and anchored to a 3cm wide flange attached to the top of the reactor. Three holes in the cover plate provided ports that were fitted with Masterflex Norprene 9.5mm (3/8") ID tubing, of which one was used for feed, one for mixed liquor recycle and one which facilitated biogas production measurement. Biogas production was monitored with a wet test gas meter, which was vented into laboratory ductwork. A glass tee with a built-in gas sampling port equipped with a septum was placed in line between the reactor and the wet test meter to facilitate biogas sampling for determination of composition. The base of the DSFF was tapered at a 45-degree angle to minimize accumulation of solids at the bottom of the reactor (van den Berg and Kennedy, 1981). Figure 3.1 below is a photograph of the continuous reactor laboratory set up, and Figure 3.2 on the following page is a schematic of the set up of an individual reactor.

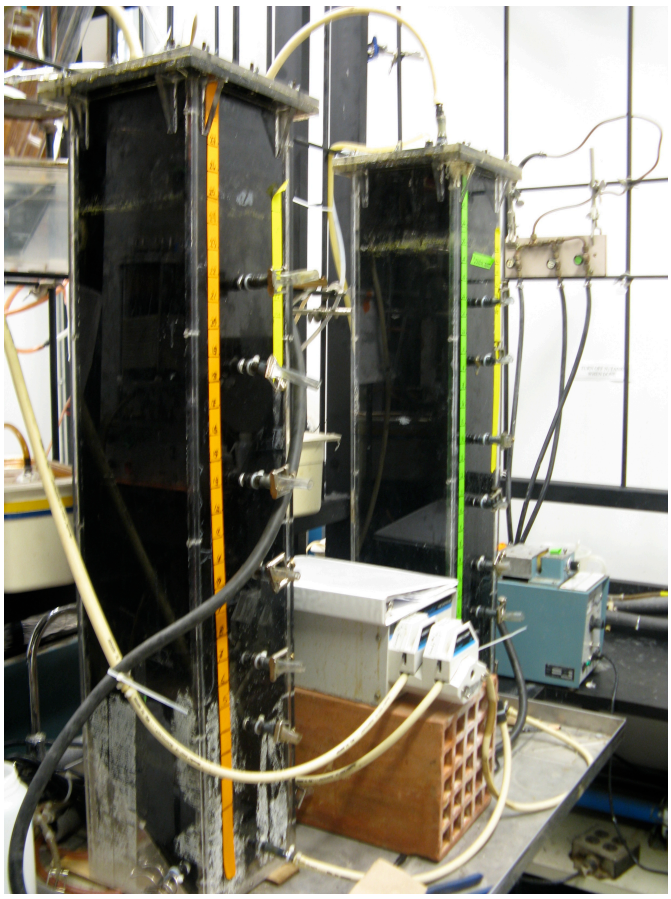


Figure 3.1 – Laboratory continuous reactor set-up

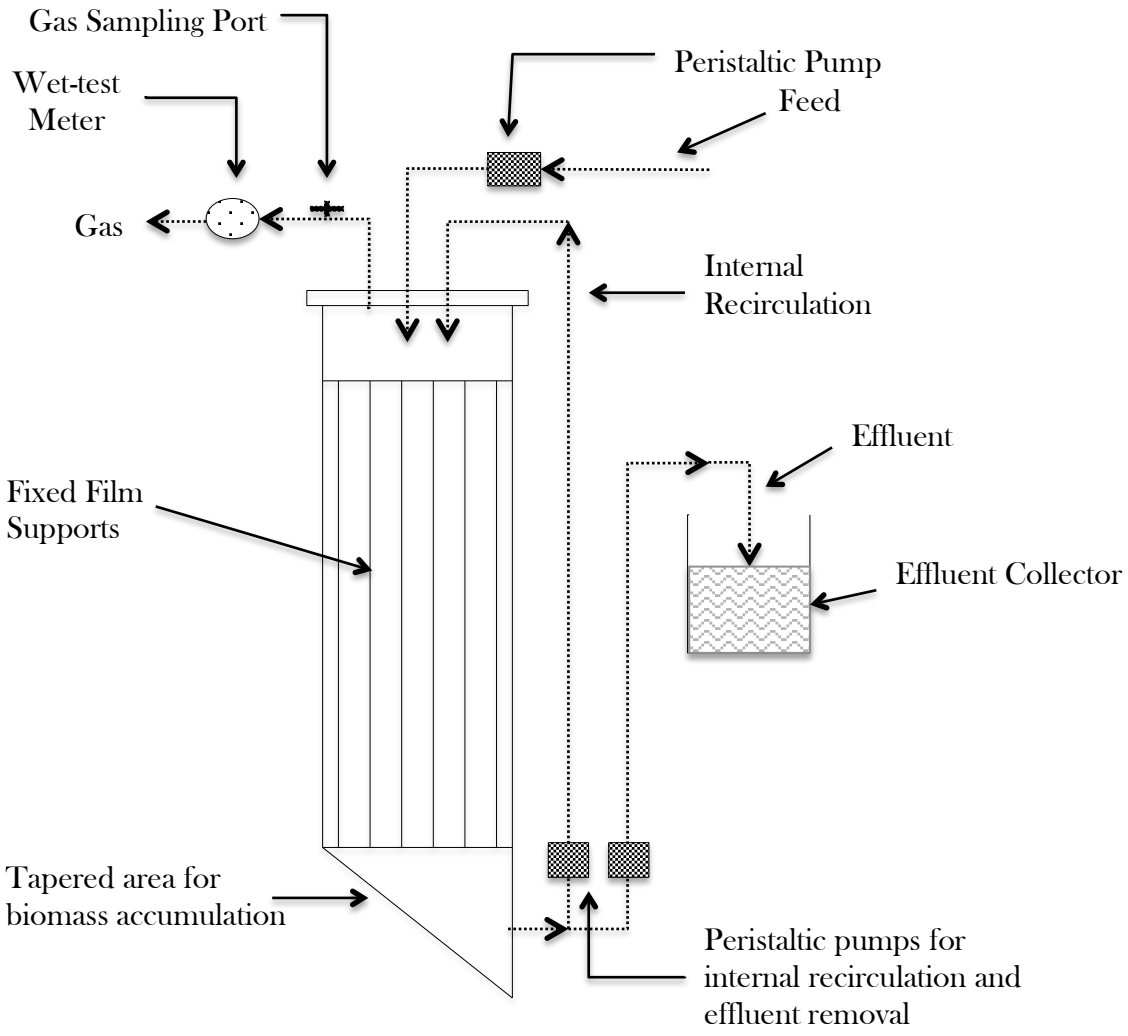


Figure 3.2 – Schematic of a down-flow stationary fixed film reactor

Two Masterflex L/S Model 7524-50 peristaltic pumps each fitted with two Easy Load Model 7518-10 pump heads controlled feed addition and removal of effluent. One pump controlled feed addition for both reactors while the other controlled effluent removal. Effluent was pumped into waste buckets through a 1.3 cm ID hole located 20.5 cm from the bottom of each reactor, just above where the base of the reactors taper. Another Masterflex L/S Model 7553-60 peristaltic pump was fitted with two Easy Load Model 7518-10 pump heads, which maintained a down-flow internal recirculation for both reactors. Recirculation was continuous, except for periods of feeding and wasting, during which it was stopped in order to reduce the possibility of feed loss with the effluent. Fisher Scientific Traceable controllers were used to turn the pumps on and off in order to coordinate feeding, effluent removal and recirculation. The mixed liquor in each reactor

was recycled from a port at the base of the reactor and returned through the recycle port in the lid in order to facilitate mixing in the reactor and feed distribution.

Reactors were run under semi-continuous conditions and were fed and wasted simultaneously once per day (over a 60 minute period) for longer HRTs and twice per day (two 90-minute periods) for shorter HRTs. Feed was placed in 15L buckets next to the reactors each day, immediately prior to feeding times. Due to the high solids content of the thin stillage, feed buckets were placed on Thermix stir plates model 120MR set at speed #5. Stir bars (6 cm) were used to keep the feed homogenous. Effluent samples were collected for analysis from the waste lines leading to effluent collection buckets at the start of feeding/wasting periods.

Each reactor was fitted with vertically oriented stainless steel wire support frames covered with a biomass support material. The needle punched polyester support material was sewn onto the frames to facilitate the development of a biofilm and immobilization of the biomass within the reactor. The supports and support material used were 61 cm in length, propped up 17.8 cm from the bottom of the reactor on a 1cm wide ledge, forming 2.5 by 2.5 cm square and 61 cm long vertical cross sectional channels. 6 large support frames were used (3 on each side) per reactor, with 6 smaller support frames in the center. Figure 3.3(a) shows a reactor support prior to support material being sewn onto it, and Figure 3.3(b) shows the reactor support material.

(a) (b)

Figure 3.3 - Down-flow stationary fixed film reactor support (a) and NPP support material (b)

The support material was a 100% post consumer content non-woven needle punch polyester (NPP) green geotextile (GN 200) provided by Geosynthetic Systems, in Ottawa ON. The NPP was selected due to its ease of use, operation and the effectiveness exhibited in previous research by DSFF reactors utilizing this type of support media (Droste and Kennedy (1985), Kennedy et al. (1988), Kennedy and van den Berg (1982), van den Berg & Kennedy (1981)). Table 2.1 summarizes the physical characteristics of the NPP used.

Table 3.9 - Properties and characteristics of the NPP biofilm support material

Needle Punched Polyester	
Colour	Green
Specific Weight	1.38g/cm^3
Weight	271g/m^2
Thickness	3mm
Tensile Strength	480N
Tear Propagation	275N
Pore Size	30-150µm

The surface area-to-volume ratio of the support material was 75m^2/m^3 (based on empty reactor volume) and the total volume of the reactor was 28L, operated with a 25L working volume and 3L of headspace. The reactors were located in a temperature-controlled room maintained at 35 ± 2°C.

3.3.2 Reactor Start Up

Reactors were inoculated with 12.5L of mesophilic seed with an activity of 0.17gVS/gVS$_{inoc}$/d obtained from ROPEC in Ottawa, Ontario and 12.5L distilled water for a total working volume of 25L. Establishment and acclimation of a healthy biofilm biomass inventory was accomplished over a period of 5 months by adding approximately 50mL of full strength CTS daily (pH adjusted to 6.9) corresponding to an OLR of 0.3g TCOD/L/d. Internal recycle of the reactor liquid was maintained at a rate of 5 reactor volumes per day (125L/d). This was done in order to facilitate biomass attachment to the support media prior to the start of experimentation. At the conclusion of the acclimation period, a measurement of the liquid volume displaced by the biomass that had accumulated was made in order to estimate the volume of biomass present in each reactor.

Research conducted by Kennedy and Droste (1985) using down flow fixed film reactors established a start-up range of 75 - 96 days, with a maximum nominal biofilm thickness observed of 1.8mm.

Immediately following the 5-month acclimation period, both DSFF reactors were operated at an HRT of 20 days fed with buffered thin stillage (pH 6.5 – 7.0). Due to the exceptionally high total COD concentration of thin stillage one DSFF reactor was run with stillage diluted 1 part CTS to 5 parts water (1:6) and the other with thin stillage diluted 1:4, corresponding to OLRs of 1.24 and 1.89 g TCOD/L/d respectively. The different organic loading rates for each DSFF were achieved by varying the dilution of CTS feed for each DSFF reactor. Initial dilutions of 1:6 and 1:4 were gradually changed in order to increase OLRs. As a result of the differing feed dilutions DSFF Reactor #1 (R1) was operated at steady state OLRs of: 1.24, 1.73, 2.85, 4.75, 7.42, and 8.73 g TCOD/L/d, while DSFF Reactor #2 (R2) was operated at slightly higher organic loading rates of: 1.89, 2.60, 4.30, 5.93, 9.90 and 11.6 g TCOD/L/d, corresponding to HRTs of 20, 14.2, 8.7, 6.25, 5 and 4.25 days respectively. Reactors were run at each OLR for a minimum of two hydraulic retention times. The HRT, thin stillage dilution ratios and corresponding OLRs for each of the reactors are summarized in Table 3.10.

Table 3.10 - Reactor hydraulic retention times and related organic loading rates

Hydraulic Retention Time	Reactor #1		Reactor #2	
	Thin Stillage Dilution Factor	OLR (g TCOD/L/d)	Thin Stillage Dilution Factor	OLR (g TCOD/L/d)
20 Days	1 in 6	1.24	1 in 4	1.89
14.3 Days	1 in 6	1.73	1 in 4	2.60
8.7 Days	1 in 6	2.85	1 in 4	4.3
6.25 Days	1 in 5	4.75	1 in 4	5.93
5 Days	1 in 4	7.42	1 in 3	9.90
4.2 Days	1 in 4	9.90	1 in 3	11.6

Gas production (monitored daily) and biogas composition were determined using a wet-test gas meter and gas chromatography machine, respectively. Analyses of effluent total and soluble COD,

pH, ammonia, alkalinity, VFA concentrations, total and volatile solids were conducted in duplicate every other day for both R1 and R2.

3.4 Analytical Methods

3.4.1 pH

The pH of all samples, substrates and inoculum was determined using a Fisher Accumet model XL25 dual channel pH/ion meter equipped with a glass electrode (serial number 498442). The pH electrode was stored in a pH 7 buffer solution and was removed, rinsed with distilled water, and dried with Kimwipes task wipers before sample pH was measured. The electrode was returned to the buffer solution, rinsed again with distilled water and dried between each pH measurement.

Sample pH was typically measured at room temperature, with the exception of continuous reactor effluent samples. The pH of continuous reactor samples was measured immediately after being removed from the reactors in order to reduce the extent of pH change due to the escape of carbon dioxide from solution. It is important to know the pH of the continuous reactors at the operating temperature, which was maintained at 35 ± 2°C, as it gives a more accurate representation of reactor performance. Therefore pH was measured while the continuous reactor samples were still warm, as the pH at room temperature would be less representative of the actual reactor operating conditions.

3.4.2 Alkalinity

Alkalinity determination was carried out according to Standard Method 2320B. Samples were centrifuged in a Thermoscientific Sorvall Legend T+ model centrifuge at 10,000 rpm (relative centrifugal force (RCF) of 11,292) for between 45 and 80 minutes depending on the type of sample. Following centrifugation, samples were poured onto and filtered through 47mm diameter sterile 0.45μm filters by applying a vacuum using a Fisher Scientific pump.

A known volume of filtered sample was then poured into a Pyrex beaker along with a magnetic stirring rod and was placed on a Thermix stirrer model 120MR, set at speed 5. Sample pH was then measured with the Fisher Accumet XL25 dual channel pH/ion meter. Titration of the

sample was carried out using 0.1N sulfuric acid dispensed through a 25 mL Kimax burette, and the volume of acid required to reach a pH of 4.6 was recorded. The pH electrode was thoroughly rinsed with distilled water and dried with Kimwipes between each sample. Alkalinity in terms of mg/L was the determined based on the results of the titration with the following equation:

$$Alkalinity,\ mg\ CaCO_3 / L = \frac{A \times N \times 50{,}000}{mL\ \ sample\ \ titrated} \qquad Eqn.\ 3.1$$

Where:
A = mL standard acid used
N = normality of standard acid

3.4.3 Ammonia

Standard method 4500D was used to determine the dissolved ammonia (NH_3 and NH_4^+) concentration present in sample supernatant. Samples were centrifuged with an RCF of 11,292 for between 45 and 80 minutes in a Thermoscientific Sorvall Legend T+ model centrifuge, equipped with a Heraeus rotor #3334. Following centrifugation, samples were poured onto and filtered through 47 mm diameter sterile 0.45µm filters by applying a vacuum using a Fisher Scientific pump. A Fisher Accumet pH/ion meter model 750 equipped with an Accumet ammonia ion selective electrode was used for sample measurement. The electrode was stored in a 1000mg NH_3-N/L solution when not in use, and was placed in a 10mg NH_3-N/L solution in between sample measurements.

A three-point calibration curve was prepared each time the probe was used. One milliliter of 10N NaOH solution was added to each standard solution (10, 100 and 1000mg NH_3-N/L) to ensure a pH of greater than 11 before ammonia measurement was carried out. Likewise, a drop of 10N NaOH was also added to sample supernatant used for analysis, to ensure a pH greater than 11. Samples were then poured into Pyrex beakers with magnetic stir rods, and placed on a Thermix stirrer 120MR set at speed 5. The ammonia electrode was thoroughly rinsed with distilled water and dried with Kimwipes between each sample. When possible, measurements were carried out in duplicate. Measurements were taken in ± millivolts (mV), and converted to mg NH_3-N/L with the use of the calibration curve equation.

3.4.4 Total and Soluble Chemical Oxygen Demand

Total and soluble chemical oxygen demand (COD) was carried out according to the closed reflux, colorimetric Standard Method 5220D. Procedures for preparation of the digestion solution and catalyst required for COD determination can be found in the Standard Methods.

An eight-point calibration curve was prepared using an 850mg/L stock solution of potassium hydrogen phthalate, which has a theoretical COD of 1000mg O_2/L. Standard tubes were prepared by diluting the stock solution to achieve the following chemical oxygen demand concentrations: 0mg/L, 100mg/L, 200mg/L, 300mg/L, 400mg/L, 500mg/L, 600mg/L and 700mg/L. Once the appropriate dilutions had been made, 10mL of each standard was dispensed along with 6mL of COD digestion reagent and 14mL of the sulfuric acid catalyst to prepare each standard tube. All standard tubes were prepared in duplicate, with the exception of the 0mg/L and 500mg/L standards, which were prepared in triplicate. Tubes were then capped and vortexed for four seconds using a Fisher vortex Genie 2™ and placed in a Precision mechanical convection oven set at 150 degrees Celsius for three hours (plus or minus 15 minutes). After three hours, the tubes were removed from the oven and allowed to cool in a dark place overnight. The next day, the outside of the tubes were cleaned with distilled water and dried with Kimwipes prior to absorbance readings. The absorbance of the standard tubes was measured at a 600nm wavelength with a Coleman Spectrophotometer model 295. Corresponding absorbances were recorded and used to generate a standard curve to determine the COD concentration (in mg O_2/L) of prepared experiment samples.

Total COD (TCOD) determination made use of a well mixed sample, while soluble COD (SCOD) required the use of filtered sample supernatant, and as such, SCOD samples were prepared in the same manner as previously described for alkalinity determination. Once filtration of all supernatant samples was complete, dilution of both fully mixed samples (for TCOD) and filtered supernatant (for SCOD) were carried out to ensure sample absorbance would fall within the range of the calibration curve. Dilution was conducted with the use of 5 and 10mL serological pipettes, as well as a 10mL Pyrex graduated cylinders. Volumes were weighed to ensure accurate measurements. Samples were diluted in a 100mL volumetric flask for TCOD, and in a 10mL volumetric flask for SCOD. Once the appropriate dilutions had been made, 10mL of sample (for both total and soluble COD) was measured and dispensed into Kimax tubes, which were then

prepared and digested as described above for the standard curve. All experiment samples were analyzed for TCOD and SCOD in duplicate.

Once the sample tubes were digested, cooled overnight and cleaned, the absorbance of the solution in the tubes were measured following the calibration of the spectrophotometer with the prepared standard curve. Sample absorbance was recorded and subsequently used in the calibration curve equation to determine the corresponding concentration of COD (total or soluble) in mg O_2/L.

3.4.5 Total and Volatile Solids

Total and volatile solids were analyzed according to Standard Method 2540G and all samples were analyzed in duplicate. Prior to solids determination, porcelain evaporating dishes were prepared by scrubbing with soap and water, followed by soaking in a 10% (v/v) sulfuric acid solution over night. The dishes were removed from the sulfuric acid solution the following morning, rinsed with distilled water and subsequently placed in a Thermolyne 62700 muffle furnace model F62730 at 550 degrees Celsius, to ensure no organic residues remained. After 60 minutes the evaporating dishes were removed from the muffle furnace and allowed to cool for 15 minutes in a Precision mechanical convection oven model 23 maintained at 105 degrees Celsius. The dishes were then transferred to a desiccator to cool completely for another 60-minute period.

Following desiccation, each dish was placed on a Mettler Toledo Classic Plus Model AB204 – s/fact analytical balance and the weight (W) was recorded. A well-mixed sample was then transferred into the dish and the weight of the dish with the sample (X) was recorded. The dish containing the sample was subsequently placed in the Precision mechanical convection oven, set at 105°C, until all the water in the sample had evaporated. Samples were typically left in the oven overnight, requiring a minimum of 12 hours for full evaporation. Once oven dried, the dishes were transferred to a desiccator for 60 minutes, and then weighed on the analytical balance and the new mass (Y) was recorded. The oven-dried dishes were then placed in the muffle furnace set at 550°C and were ignited for 60 minutes. The dishes were again transferred to the 105°C oven to cool for approximately 15 minutes and subsequently placed in a desiccator for another 60 minutes. The final weight of the dishes after desiccation (Z) was noted, and the percentage total and volatile solids were determined using the recorded weights (W, X, Y, Z) and the following equations:

$$\%\,Total\,Solids\ (TS) = \frac{(Y - W)}{(X - W)} \times 100\% \qquad Eqn.\,3.2$$

$$\%\,Volatile\,Solids\ (VS) = \frac{(Y - Z)}{(X - W)} \times 100\% \qquad Eqn.\,3.3$$

3.4.6 Volatile Fatty Acids

Measurement of volatile fatty acids (VFAs) was accomplished with the use of an Agilent 6890 Series Gas Chromatography (GC) system equipped with a flame ionization detector (FID), an Agilent 7683 Series auto-sampler and injector, and an Innowax splitless column (30m x 0.25mm ID capillary column, coated with 0.5μm film thickness). HP ChemStation (Rev. 06.03 [509]) chromatographic software was used for sample component separation and identification. The oven temperature was programmed to ramp up to a final temperature of 250°C according to the following sequence: 80°C for 0.2 minutes, followed by an increase from 80 to 120°C at 20°C per minute and held at 120°C for 1 minute, an increase from 120 to 250°C at 25°C per minute and then a final temperature of 250°C maintained for 0.10 minutes. The inlet and detector temperatures were set at 250°C and 300°C respectively. The carrier gas was helium, flowing at a rate of 1.7 mL per minute, and a linear velocity of 43cm/s. Hydrogen and air, at 30 and 400ml/min correspondingly, were used in the GC/FID with N_2 flowing at 25ml/min, as the make-up gas.

A VFA standard mixture was prepared containing 2000mg/L of each acetic, butyric and propionic acid, and an internal standard was prepared with a concentration of 2000mg/L isobutyric acid. Calibration of the GC was accomplished by adding 0.5mL of the standard mixture and 0.5mL of the internal standard to an auto-sampler vial, vortexing followed by placing the vial into the auto-sampler, which injected 0.1μL of sample into the GC for analysis. Injection and re-calibration was iterated until integration resulted in a measured concentration of 2000 ± 50mg/L for acetic, propionic and butyric acids.

Experiment samples were prepared for VFA analysis by centrifugation in 1.5 mL eppendorf tubes in a Brinkmann Eppendorf centrifuge model 5415 at 14,000 rpm for 10 – 15 minutes (RCF of

8984). Centrifuged samples were filtered through an Acrodisc® LC 13 mm diameter 0.2 μm PVDF membrane syringe filter, and 0.5 mL was dispensed into auto-sampler vials, along with 0.5 mL of the internal standard solution. Vials were then vortexed on a Fisher vortex Genie 2™ and placed in the auto-sampling tray and analyzed by the GC. Results were reported after integration by the software in mg/L of acetic propionic and butyric acids.

3.4.7 Biogas Measurement

3.4.7.1 Batch Experiments

Biogas production was measured with the use of a manometer water displacement apparatus for batch bottles. Assay bottles were removed from the incubator and allowed to cool to room temperature prior to biogas volume measurement. A BD 21G1½ needle connected to a U-tube manometer was inserted through the rubber stopper sealing each batch bottle, and were allowed to equilibrate until the pressure in the assay bottle reached atmospheric pressure. The volume of biogas produced was determined by noting the starting and ending point of the water in the burette connected to the manometer. The difference between these two values provided the volume of biogas produced in milliliters. Biogas measurements were standardized to standard temperature and pressure (STP; 0°C and 1 atmosphere).

3.4.7.2 Continuous Reactors

A wet test tip meter was used for measurement of DSFF continuous reactors biogas production. The wet test meters were first calibrated, such that the volume of gas that resulted in one tip was known. Each time that volume of gas passed through the wet test meter the apparatus 'tipped' and a 'count' was recorded on an automatic counting device. The reactor gas line was connected to the wet-test tip meter, and the number of tips counted was recorded daily. The known volume per tip was then multiplied by the recorded tip volume values to determine the volume of biogas produced each day. Biogas measurements were collected at 35 ± 2 °C and corrected to STP.

3.4.8 Biogas Composition

Biogas composition (consisting of methane and carbon dioxide) was determined with a Hewlett Packard 5712A GC equipped with a metal packed column (Chromatographic Specialties Inc. Brockville, ON, Canada, Porapak T, packing mesh size: 50/80, column length x OD: 304.8 x

0.635cm) a 5705A thermal conductivity detector (oven, inlet and outlet temperatures: 70, 100 and 150°C, respectively) and a 3380A model integrator. Carrier gas was maintained at 25mL of helium per minute. Samples for composition analysis were collected by inserting a BD 21G1½ needle tip attached to a 1mL syringe through the stopper of the batch bottles or the septum of the gas sampling port on the continuous reactors. Gas samples were collected and discharged with the syringe two to three times before a sample was collected for analysis, in order to ensure a representative sample. A 1mL of biogas was taken for analysis, of which 0.5mL was wasted into the air and the remaining 0.5mL was injected manually into the GC.

National Instruments™ LabVIEW version 6.0 was installed on the computer connected to the GC. Biogas composition was evaluated by the computer software and presented in percentages of nitrogen, methane and carbon dioxide.

3.5 Sample Preservation

If analysis could not be completed within an acceptable time period, samples were preserved according to the following instructions summarized in Table 3.11, for analysis at another time.

Table 3.11 - Sample preservation techniques

Analyses	Sample Type	Preservation	Bottle Type	Maximum storage time
pH	Sludge	Refrigerated, 4°C	Plastic or glass	14 days
Alkalinity	Filtered (0.45µm) supernatant	Refrigerated, 4°C	Plastic or glass	14 days
Ammonia	Filtered (0.45µm) supernatant	pH to less than 2, with concentrated H_2SO_4 Refrigerated, 4°C	Plastic or glass	28 days
VFAs	Filtered (0.2µm) supernatant	Frozen, -18°C	Plastic microcentrifuge tube	
TS/VS	Sludge	Refrigerated, 4°C	Plastic or glass	7 Days
TCOD	Sludge	pH to less than 2, with H_2SO_4 Refrigerated, 4°C	Amber glass	28 days
SCOD	Filtered (0.45µm) supernatant	pH to less than 2, with H_2SO_4 Refrigerated,4°C	Amber glass	28 days

Chapter 4

Results and Discussion

4.1 Characterization Studies

Initial characterization of substrates and inoculum used for the following research was completed prior to the start of each experiment. Corn thin stillage (CTS), anaerobic sludge (mesophilic inoculum), food waste (FW) and thickened waste activated sludge (TWAS) were all characterized. The anaerobic inoculum used for all studies characterized in Table 4.1 had a specific activity of 0.17gVS/gVS/d. The values of the parameters measured are presented in Table 4.1; all values are in mg/L and are the arithmetic means of replicates and the associated standard devitations.

Table 4.1 – Characterization results of inoculum and experimental substrates

	Substrate			
Parameter	**Mesophilic Inoculum**	**Thin Stillage**	**Food Waste**	**TWAS**
pH	7.36 - 7.45	3.4 – 3.8	3.8 - 4.0	6.6 – 7.0
Total COD	11,090 ± 1249	159,838 ± 15,981	91895 ± 4936.8	59714 ± 5 524
Soluble COD	551 ± 191	74,725 ± 4700	40857 ± 2595	3411 ± 255
Total Solids	21,533 ± 5173	94,264 ± 3145	55,430 ± 5481	53,059 ± 6090
Volatile Solids	11,818 ± 3734	84,937 ± 3882	39,626 ± 2305	37,490 ± 4337
NH_3-N	832 ± 418	Not detected	121.6	808.5
Alkalinity	4583 ± 236	0	0	3,250

Corn thin stillage, obtained from Greenfield Ethanol in Tiverton, Ontario Canada, was further analyzed for total kjeldahl nitrogen (TKN), total phosphorus (P_{total}), nutrient and metal concentrations, which are summarized Table 4.2. Sample analysis results indicate a C:N:P (COD:TKN:P_{total}) ratio of approximately 101:1.7:1.0. Likewise, the C:N ratio was found to be approximately 60, which is well above the optimum range of 20-30 for aerobic digestion and slightly above the recommended ratio of 40 for anaerobic digestion (Horan, 2003). The high COD to TKN ratio indicates that AD performance may be affected due to nitrogen limitation.

However, this could be overcome by co-digestion or supplementing CTS with another source of nitrogen.

Cobalt (Co), nickel (Ni) and molybdenum (Mo), which are all considered important for anaerobic digestion metabolism, were also found at low concentrations. Cobalt levels were below detectable limits, while copper, nickel and molybdenum were found at concentrations of 0.013 and 0.017 mg/L. Research conducted by Murray and van den Berg (1981) using fixed film digesters found that final concentrations of cobalt (50nM, 0.003mg/L) or nickel (100nM, 0.0059mg/L) individually or in combination, stimulated methanogenesis appreciably. A final concentration of 50nM (0.0048mg/L) of Molybdenum was found to only slightly improve methanogenic activity, but only when added in combination with both nickel and cobalt. Murray and van den Berg (1981) speculated this was as a result of required molybdenum concentrations being met by trace amounts present in the effluent or substrate being used for their research. Total gas and methane production was reported to improve by 42% when all three trace elements were provided. Based on the results of Murray and van den Berg's studies, it seems that CTS has sufficient quantities of Ni and Mo available for AD. However, the low concentrations of Co present in the CTS could have an acute or chronic impact on AD performance. The absence of alkalinity and cobalt suggests that co-digestion of CTS may be an alternative to remedy these both of these issues.

Table 4.2 – Corn thin stillage micro and macro-nutrient concentrations

Parameter	Concentration (mg/L)	Parameter	Concentration (mg/L)
Total Kjeldahl Nitrogen	2680	**Copper**	0.165
Total Phosphorus	1580	**Iron**	6.49
Calcium	36	**Lead***	<0.01
Magnesium	674	**Manganese**	0.51
Potassium	2270	**Molybdenum**	0.017
Sodium	276	**Nickel**	0.013
Aluminum	2.51	**Silicon**	21
Barium	0.05	**Silver***	<0.01
Beryllium*	<0.005	**Strontium**	0.024
Boron	2	**Thallium***	<0.01
Cadmium*	<0.005	**Titanium**	4
Chromium	0.021	**Vanadium***	<0.005
Cobalt*	<0.005	**Zinc**	1.77

*Levels were found to be below detection limits

4.2 Initial Suitability BMP Assay

Due to the high organic content (≈159,800 mg TCOD/L), thin stillage must be diluted to appropriate organic concentrations prior to digestion. Accordingly, an initial suitability BMP assay was conducted in order to establish an appropriate range of COD concentrations for AD of thin stillage. F/M ratios between 0.2 and 2.0 (2,455 – 27,172 mg TCOD/L) were examined for this purpose and assays were terminated when biogas production rates began to approach zero. Assay bottles with F/M ratios of 0.2 and 0.5 were ended after 28 days, while F/M ratios of 0.7, 1.0 and 1.5 were allowed to continue for a total of 44 days. Assay bottles with an initial F/M ratio of 2.0 were terminated after 48 days. At the conclusion of the assay, total COD, soluble COD, total and volatile solids concentrations, volatile fatty acid concentration, pH and methane composition were measured. A malfunctioning ammonia probe prevented the measurement of ammonia concentrations of assay bottles for F/M ratios of 0.7, 1.0, 1.5 and 2.0. Data presented represents the arithmetic mean of replicates and the associated standard deviation. Table 4.3 summarizes the samples analysis results for the initial suitability assay:

Table 4.3 – Initial suitability BMP assay sample analysis results

Parameter	F/M Ratio					
	0.2	0.5	0.7	1.0	1.5	2.0
Initial TCOD (mg/L)	2,455 ± 99	6,138 ± 543	9,448 ± 681	13,357 ± 963	21,177 ± 1468	27,172 ± 1071
Final TCOD (mg/L)	146 ± 0	431 ± 202	1,743 ± 259	2,059 ± 36	3,166 ± 355	5,251 ± 296
TCOD Removal (%)	94 ± 0	93 ± 3	86 ± 5	85 ± 0	85 ± 2	81 ± 1
Initial SCOD (mg/L)	1,074 ± 181	2,686 ± 179	4,935 ± 79	6,978 ± 113	11,063 ± 179	12,713 ± 467
Final SCOD (mg/L)	77 ± 16	134 ± 44	270 ± 7	331 ± 14	628 ± 44	685 ± 36
SCOD Removal (%)	93 ± 2	95 ± 2	95 ± 0	95 ± 0	94 ± 0	95 ± 0
Initial VS (mg/L)	1,362 ± 18	3,405 ± 44	4,836 ± 31	6,837 ± 44	10,839 ± 70	14,440 ± 106
Final VS (mg/L)	115 ± 7	200 ± 57	945 ± 7	1155 ± 21	1910 ± 156	3341 ± 233
VS Removal (%)	92 ± 1	94 ± 2	80 ± 0	83 ± 0	82 ± 1	77 ± 1
Total Biogas (mL)	110 ± 1	223 ± 23	389 ± 2	532 ± 8	794 ± 22	1112 ± 36
%CH_4	48%	63%	62%	64%	67%	62%
L CH_4/g COD_{added}	0.22	0.23	0.26	0.25	0.26	0.25
L CH_4/g $COD_{removed}$	0.29	0.31	0.31	0.30	0.30	0.31
NH_3 - N	261 ± 58	170 ± 27	-	-	-	-

Cumulative biogas production was based on average production of duplicates. Biogas production attributed to the mesophilic inoculum was approximately 10mL, and the data presented in Table 4.1 has been corrected for inoculum contribution. The following figure illustrates the cumulative biogas production observed for all assay bottles at standard temperature and pressure (STP), (0°C and 1 atmosphere) over the course of the experiment as a function of digestion time. Figure 4.1 (a) shows cumulative biogas production and Figure 4.1(b) show cumulative biogas production per gram COD added.

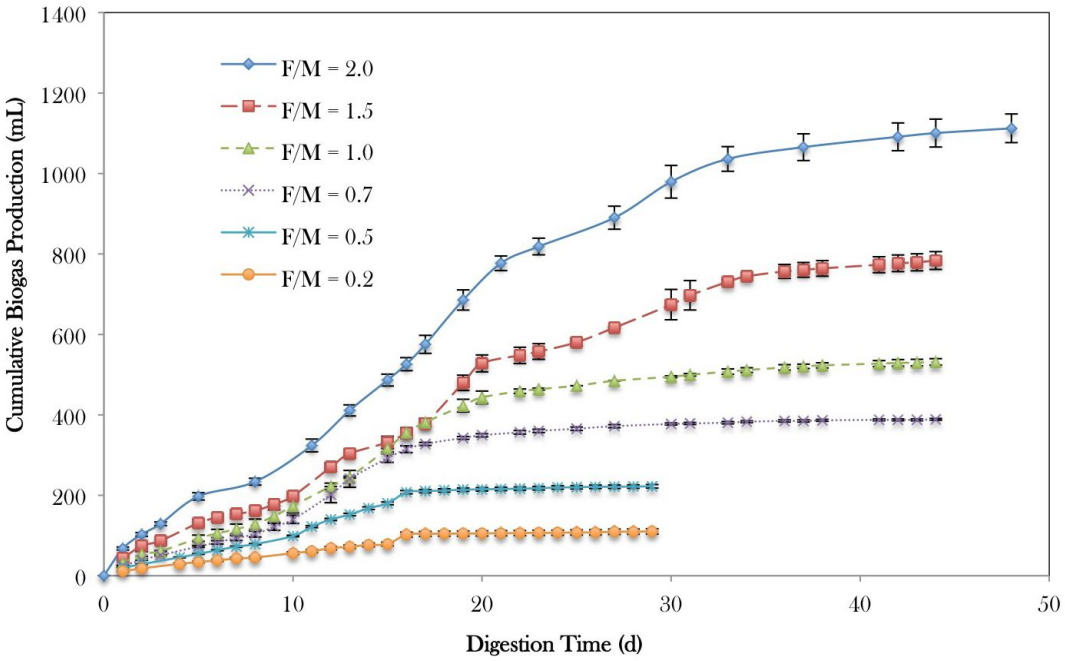


Figure 4.1(a) – Initial suitability assay cumulative biogas production

Thin stillage appears to contain a suitable proportion of easily degradable matter and no inhibition or lag period was observed. Increased organic concentrations (higher F/M values) corresponded to higher cumulative biogas production and maximum biogas production rates. The observed increases in the initial biogas production (day 0 – 9) with increasing F/M ratio suggests that initially the system follows first order kinetics. The normalized values (mL biogas per gram of COD added) seen in Figure 4.1(b) indicate that the F/M values between 0.2 and 0.7 perform the best and that the higher F/M ratios perform nearly the same. This can be observed in the convergence of cumulative specific biogas production near the end of the assay in Figure 4.1(b) on the following page.

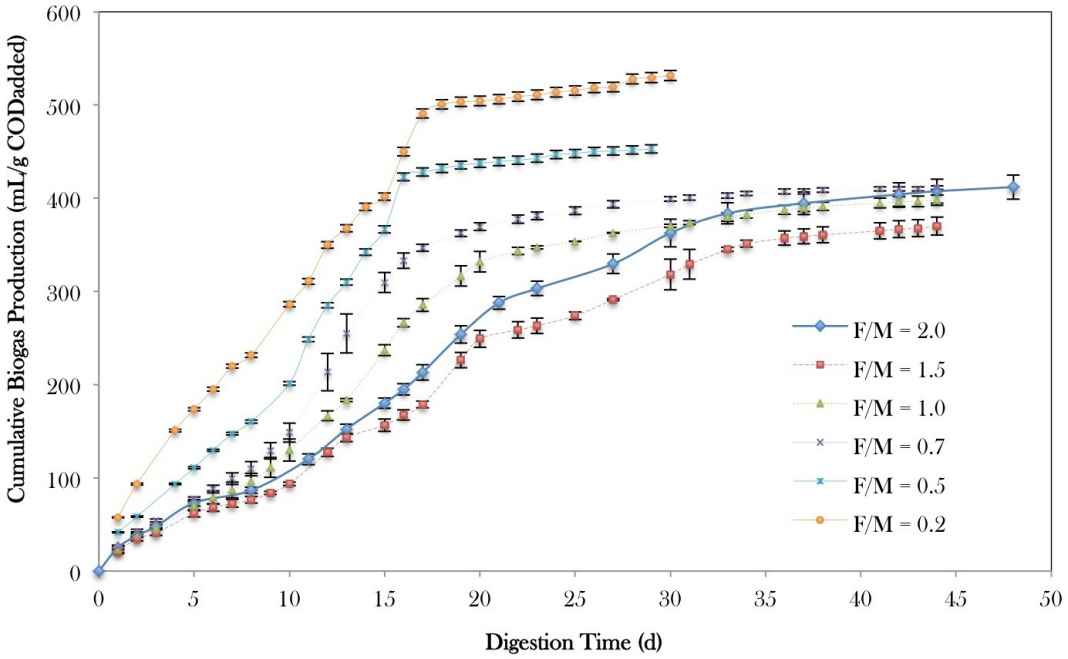


Figure 4.1(b) – Initial Suitability assay specific cumulative biogas production

Assay bottles with F/M ratios of 1.5 and 2.0 required 27 days of digestion in order to achieve 80% of their total biogas production, while F/M = 1.0 required 19 days, and F/M ratios of 0.2, 0.5 and 0.7 required 15, 15 and 16 days respectively. After 25 days of digestion, only F/M ratios of 1.5 and 2.0 continued to produce biogas at a reasonable rate. At this time all other experiment bottles had produced approximately 90% of the total biogas measured, and therefore had likely run out of easily degradable substrate. Table 4.4 summarizes the average biogas production rates that account for the production of 80% of total biogas measured.

Table 4.4 –Initial suitability BMP assay biogas production rates

F/M Ratio	Maximum Rate (L/L/d)	Days of Digestion
0.2	0.06	0 – 15
0.5	0.14	0 – 15
0.7	0.19	0 - 16
1.0	0.22	0 – 19
1.5	0.24	0 – 27
2.0	0.38	0 – 27

Despite the increase in biogas production and production rates, the overall volatile solids, total and soluble COD destruction decreased with higher initial organic concentrations (Table 4.3). At an F/M ratio of 2.0, VS removal decreased below 80% to 77 ± 1%, while the TCOD removal efficiency decreased to just above, at 81 ± 1%. Despite having the lowest VS and TCOD removal efficiencies, assay bottles with an F/M ratio of 2.0 achieved the highest biogas production rates, with a maximum rate of 0.38L biogas/$L_{reactor}$/d. The lowest F/M ratio (0.2), exhibited the poorest biogas production performance overall. The percentage of methane in the biogas of F/M = 0.2 assay bottles reached a maximum of 48% CH_4, with the lowest maximum biogas production rate of 0.06L/L/d.

Assay performance was also evaluated based on production and accumulation of volatile fatty acids. VFA concentrations of assay bottles were measured only once per week, due to the small bottle volume. The maximum concentration of total acids occurred within the first week of digestion for all assay bottles. This rapid accumulation indicates that CTS is composed of readily degradable components. A quick accumulation of VFAs could result in a sudden drop in pH if the system is not sufficiently buffered, leading to an imbalance in acidogenic and methanogenic activity should organic overloading or shock occur. The highest VFA concentrations were observed at an F/M = 2.0, reaching approximatley 6600 mg/L of total acids (approximatley 3440mg/L acetic, 2650mg/L propionic and 514mg/L butyric acid). This rapid accumulation of VFAs occurred after only 6 days of digestion, and a consequent drop in pH (from 7.8 to 7.3) was observed, despite the presence of approximately 8,000mg/L alkalinity as $CaCO_3$. Additional alkalinity was added to F/M = 2.0 bottles at day 6 in order to prevent the pH from dropping further, and a slight plateau in biogas production can be observed in Figure 4.1 (days 5 – 8) as a result.

The temporal changes in VFAs, related to the initial F/M ratio, is an additional indication of maximum CTS utilization. Higher F/M ratios (1.5 and 2.0) experienced greater VFA concentrations and prolonged presence when compared to lower F/M ratios. The concentration of total acids present in the experiment bottles was essentially zero by day 20 for all F/M ratios with the exception of 1.5 and 2.0. It can be observed in Figure 4.2 that as total VFA concentrations decreased to near zero, biogas production rates decreased to the lowest levels observed. This occurred at approximatley day 16 for F/M ratios of 0.2 and 0.5, day 20 for F/M ratios of 0.7 and

1.0, and VFA concentrations approached zero between days 35 and 40 for F/M = 1.5 and 2.0.

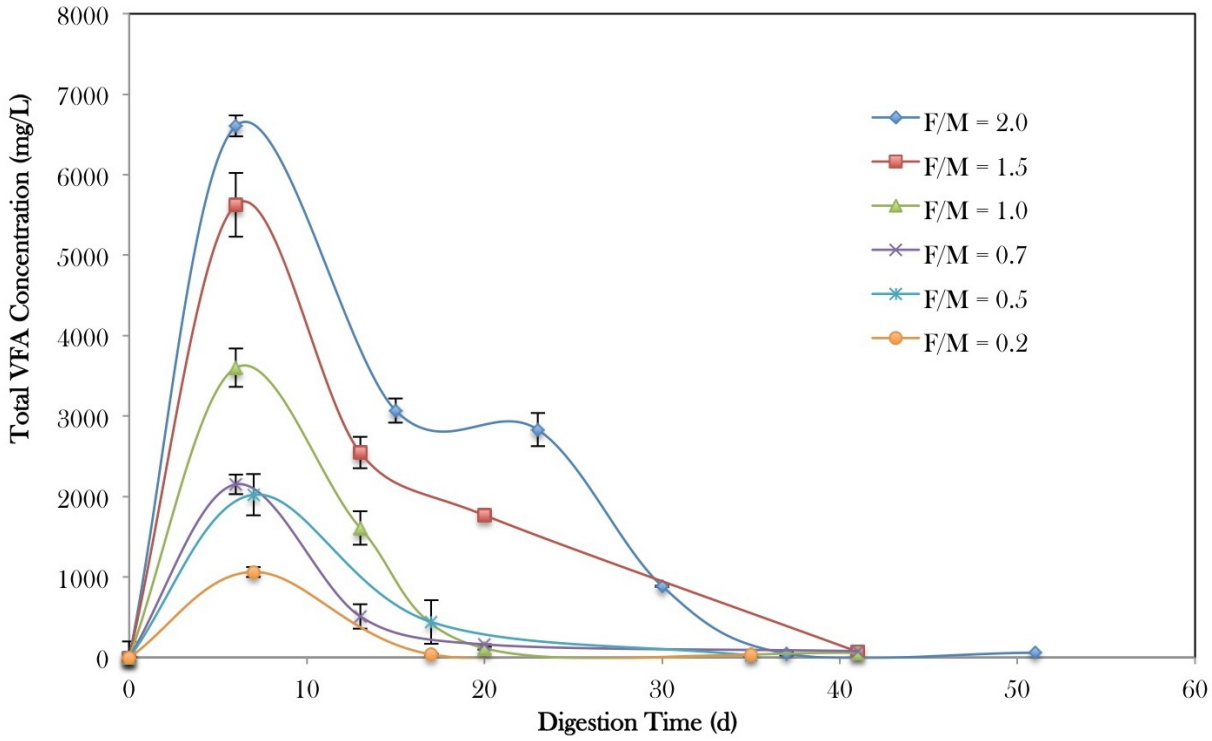


Figure 4.2 – Initial suitability BMP assay volatile fatty acid concentrations

Overall, the highest organic concentration that provided suitable performance was observed for F/M = 1.5 assay bottles. It appears that due to the easily degradable nature of CTS, initial organic concentrations should be near 21,000 mg TCOD/L (F/M = 1.5) due to rapid acidification, and subsequent decreases in pH despite the presence of alkalinity, observed for the higher F/M ratio of 2.0. Although F/M = 1.5 assay bottles accumulated nearly the same concentration of VFAs during the first week of the assay, no drop in pH was observed which indicates that it is a more suitable organic concentration for digestion. Removal efficiencies for TCOD, SCOD and VS remained above 80%, at 85 ± 2%, 94 ± 0% and 82 ± 1%, respectively for assay bottles with an F/M ratio of 1.5. The highest percentage of methane present in the biogas was also achieved at an F/M ratio, of 1.5, reaching 67% methane. Maximum methane yields achieved were similar between all F/M ratios. A yield of 0.30L CH_4/g $TCOD_{removed}$ was achieved at an F/M = 1.5, and is also only slightly less than the maximum yield of 0.31 LCH_4/g $TCOD_{removed}$, which was observed for F/M ratios of 0.5, 0.7, and 2.0.

4.3 Acclimation BMP assay

An acclimation BMP assay was conducted in order to determine if the acclimation of biomass to thin stillage would result in improved biogas yields, production rates or TCOD removal efficiencies. Based on the results of the initial suitability assay, the acclimation BMP assay was carried out at F/M ratios of 0.7, 1.0, 1.5 and 2.0. Initial VS and COD concentrations of the inoculum for this experiment were determined based on the sample results of the effluent of the initial suitability BMP assay. This assay simulated a worst-case scenario, in which half of the acclimated biomass would be lost prior to the addition of new substrate for digestion. Experiments were terminated when the biogas production rate dropped to near zero: F/M ratios of 0.7, 1.0 and 1.5 were all terminated after 35 days, while the F/M = 2.0 experiment bottles were ended after only 20 days of digestion. Table 4.5 summarizes the sample analysis results for the acclimation BMP assay. Data presented represents the arithmetic mean of replicates and the associated standard deviation. Overall, after the digestion of thin stillage with acclimated biomass, moderate improvements in TCOD and SCOD reductions were observed, while VS removal efficiencies remained nearly the same in all cases.

Table 4.5 – Acclimation BMP assay sample analysis results

Parameter	F/M Ratio			
	0.7	1.0	1.5	2.0
Initial TCOD (mg/L)	9,488 ± 681	13,357 ± 963	21,177 ± 1468	27,172 ± 1071
Final TCOD (mg/L)	1,034 ± 251	1,317 ± 415	2704 ± 324	3,485 ± 368
TCOD Removal (%)	89 ± 3	90 ± 3	87 ± 2	88 ± 1
Initial SCOD (mg/L)	4,935 ± 79	6,978 ± 113	11,063 ± 179	12,713 ± 467
Final SCOD(mg/L)	103 ± 15	201 ± 44	483 ± 170	2,028 ± 214
SCOD Removal (%)	98 ± 0	97 ± 1	96 ± 2	84 ± 2
Initial VS (mg/L)	4,836 ± 31	6,837 ± 44	10,839 ± 70	-
Final VS (mg/L)	1,003 ± 117	1,143 ± 11	2,015 ± 28	-
VS Removal (%)	79 ± 2	83 ± 0	81 ± 0	-
Total Biogas (mL)	393 ± 7	560 ± 13	828 ± 15	859 ± 26
%CH_4	60%	60%	63%	63%
L CH_4/g COD_{added}	0.25	0.25	0.25	0.20
L CH_4/g $COD_{removed}$	0.28	0.28	0.28	0.23
NH_3– N (mg/L)	604 ± 60	612 ± 25	587 ± 119	578 ± 15

The highest removal efficiencies for the digestion of CTS with acclimated biomass were achieved at an F/M ratio of 1.0. TCOD, SCOD and VS removal efficiencies were 90 ± 3%, 97 ± 1% and 83 ± 0% respectively. In comparison, the digestion of CTS at an F/M = 1.0 in the initial suitability BMP assay (no acclimation) had slightly lower removal efficiencies of 85 ± 0%, and 95 ± 0% for TCOD and SCOD, although VS reduction was consistent at 83 ± 1%.

Despite increased COD removal efficiencies, methane yields remained similar for all F/M ratios when CTS was digested with acclimated biomass. The highest methane yield achieved was 0.28 L CH_4/g $TCOD_{removed}$ at F/M ratios of 0.7, 1.0 and 1.5. Comparatively, digestion of CTS with non-acclimated biomass achieved similar yields of 0.31, 0.30 and 0.30 L CH_4/g $TCOD_{removed}$ for F/M ratios of 0.7, 1.0 and 1.5 respectively. The lowest methane yield observed for the acclimation assay was 0.23 L CH_4/g $TCOD_{removed}$ for an F/M ratio of 2.0. Assay bottles with an F/M ratio of 2.0 also exhibited the poorest performance in general, and were the only bottles to experience a decrease in SCOD removal efficiency. Soluble COD removal was 95 ± 0% without acclimation, and dropped to 84 ± 2% for CTS digestion with acclimated biomass.

Figure 4.3 shows the cumulative biogas production over the course of the initial suitability BMP assay, followed by the biogas production of CTS digestion with acclimated biomass. Only moderate improvements were observed in total biogas production. The major impact of biomass acclimation appears to be on biogas production rates as well as TCOD removal efficiencies. Biogas production rates for CTS digested with acclimated biomass were observed to increase for all F/M ratios, except F/M = 0.7.

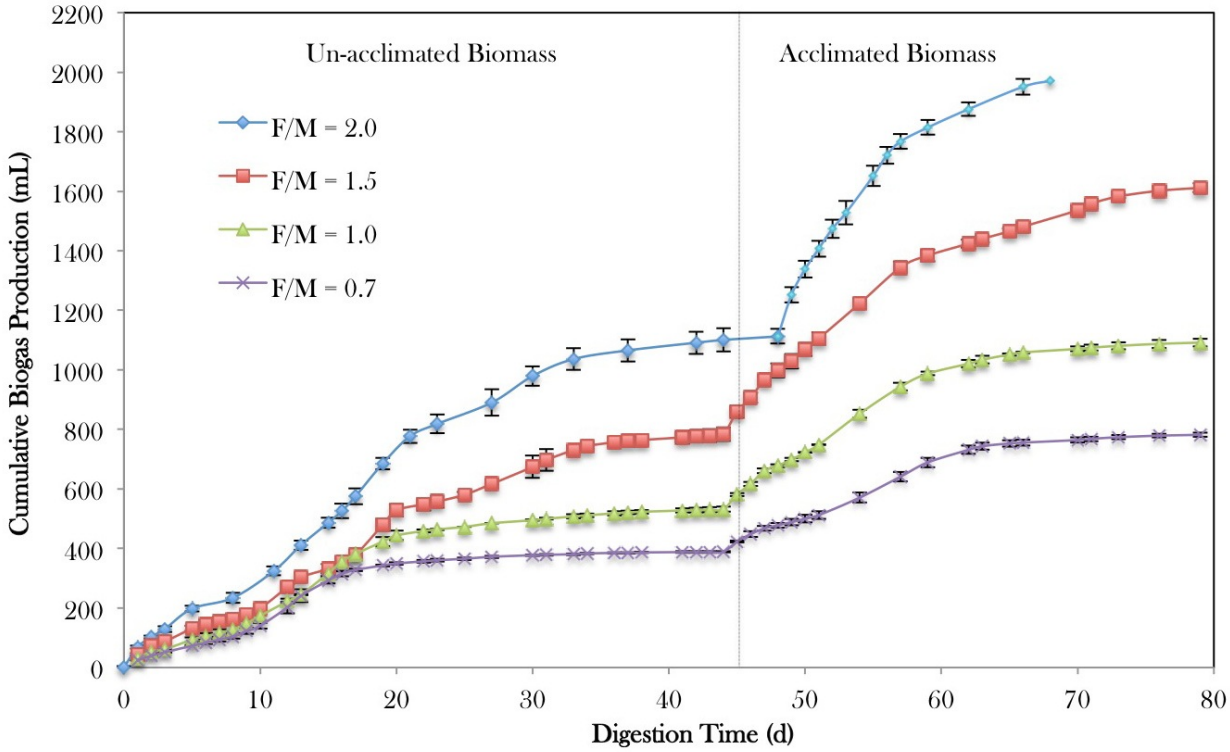


Figure 4.3 – Initial suitability and acclimation BMP assays cumulative biogas production

Without biomass acclimation, 80% of biogas production was achieved at 16, 19, 27 and 27 days, with acclimation 80% of total biogas production was observed by 18, 15, 19 and 11 days for F/M ratios of 0.7, 1.0, 1.5 and 2.0 respectively. Table 4.6 summarizes the maximum biogas production rates achieved by the biomass acclimated to thin stillage. As in section 4.1, these rates account for the production of 80% of total biogas.

Table 4.6 – Acclimation BMP assay biogas production rates

F/M Ratio	Maximum Rate (L/L/d)	Days of Digestion
0.7	0.18	44 – 62
1.0	0.29	44 – 59
1.5	0.33	44 – 63
2.0	0.63	48 – 59

Biogas production for the digestion of CTS with acclimated biomass resumed immediately with no lag period. As previously discussed, this is likely due to the quick degradation of the readily degradable portion of the substrate. Digestion with acclimated biomass resulted in improved

biogas production rates for all F/M ratios with the exception of 0.7, which remained nearly the same to the rate of 0.19L/L/d observed for digestion with non-acclimated biomass, at 0.18L/L/d of digestion with acclimated biomass. As well, the time required for the production of approximately 80% of total biogas decreased in all cases, again with the exception of the F/M ratio of 0.7. The greatest improvement observed was for the F/M = 2.0 experiment bottles which had biogas production rates which improved from 0.38 to 0.63 L/L/d, a 67% increase in the biogas production rate and a 44% reduction in the amount of time required to produce 80% of total biogas.

Maximum VFA accumulation was observed within the first week of digestion, as was the case in the initial suitability BMP assay. Acids decreased in a similar manner as well, approaching zero after approximately 20 - 25 days of digestion for F/M ratios of 0.7 and 1.0. Total acid concentrations appeared to persist for F/M ratios of 1.5 until approximately day 40 - 45. Figure 4.4 illustrates the VFA accumulation by un-acclimated biomass followed by that of the acclimated biomass on thin stillage.

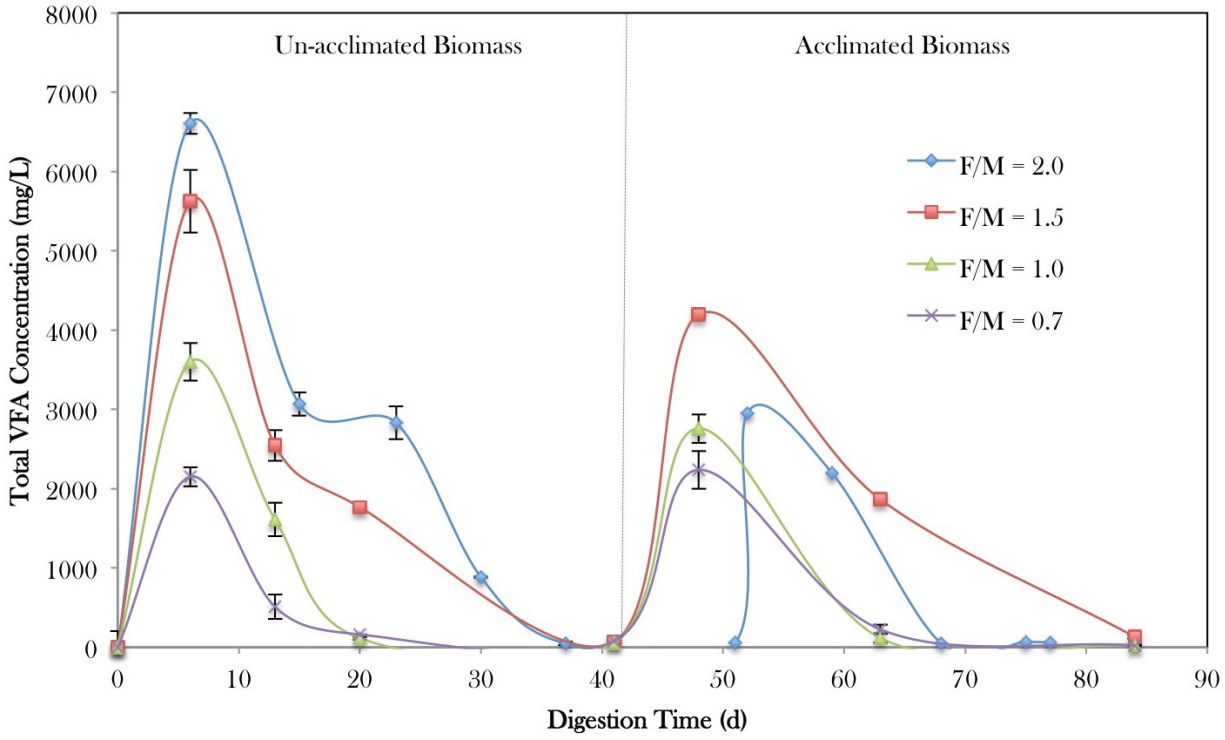


Figure 4.4 – Initial suitability and acclimation BMP assays volatile fatty acid concentrations

Interestingly, peak VFA concentrations at each F/M ratio in the acclimation assay were typically less than those measured in the initial suitability BMP assay. This is a good indication that the microbial population was adapting to the CTS with an improved balance of acidogenic and methanogenic activity in the inoculum. Biogas production rates were improved by between 30 and 65% with the acclimation of biomass, which indicates potential for reductions in digestion time required. Improvements in removal efficiencies were also observed at appropriate F/M ratios. Based on the initial suitability assay, as well as the acclimation assay, F/M ratios in the range of 1.0 and 2.0 appear to be the suitable for CTS digestion.

4.4 Supplemental Nutrients BMP Assay

Due to the low levels of trace elements measured in CTS, a BMP assay to examine the potential for improved digestion by supplementing nutrients was carried out at F/M ratios of 0.7, 1.0 and 1.5. Two sets of assay bottles were prepared at each F/M ratio, one supplemented with the full trace element stock solution (MM) and another with the cobalt stock solution (Co) as previously described in Chapter 3 – Materials and Methods. The assay was carried out using un-acclimated biomass and was terminated after 82 days of digestion.

When compared to the digestion of thin stillage alone (Initial suitability studies – Table 4.1), VS, and TCOD removals are lower in all cases. SCOD removal efficiencies were similar for F/M ratios of 0.7 and 1.0, but decreased slightly for 1.5. Thin stillage digested at an F/M = 1.5 dropped from a SCOD removal efficiency of 94 ± 0% when digested alone (no acclimation) to 87 ± 1% and 89 ± 0% when supplemented with Co and the MM, respectively. Levels of ammonia present at the conclusion of this experiment are quite a bit higher than anticipated. In comparison to CTS digestion with acclimated biomass data, ammonia levels are nearly double for all F/M ratios in this assay but within the acceptable range of concentrations described by Sawayama et al. 2004. The reason for this latter finding is presently unknown, but Agler et al. (2008) also reported increases (30%) in ammonia concentrations in reactor effluent when CTS was augmented with trace elements. Sample analysis results for this assay are presented in Table 4.7. Data presented represents the arithmetic mean of replicates and the associated standard deviation.

Table 4.7 – Supplemental nutrients BMP assay sample analysis results

Parameter	F/M Ratio					
	Cobalt Solution (Co)			Mineral Mix (MM)		
	0.7	1.0	1.5	0.7	1.0	1.5
Initial TCOD (mg/L)	9,448 ± 681	13,357 ± 963	21,177 ± 1468	9,448 ± 681	13,357 ± 963	21,177 ± 1468
Final TCOD (mg/L)	3,138 ± 893	3,177 ± 223	4,418 ± 168	1,721 ± 146	2,596 ± 367	3,689 ± 532
TCOD Removal (%)	67 ± 9	76 ± 2	79 ± 1	82 ± 2	81 ± 3	83 ± 3
Initial SCOD (mg/L)	4,935 ± 79	6,986 ± 113	11,076 ± 179	4,942 ± 79	6,986 ± 113	11,076 ± 179
Final SCOD (mg/L)	301 ± 18	301 ± 18	1,440 ± 70	280 ± 46	268 ± 40	1166 ± 28
SCOD Removal (%)	94 ± 0	96 ± 0	87 ± 1	94 ± 1	96 ± 1	89 ± 0
Initial VS (mg/L)	4,836 ± 31	6,837 ± 44	10,839 ± 70	4,836 ± 31	6,837 ± 44	10,839 ± 70
Final VS (mg/L)	1935 ± 49	2410 ± 28	3680 ± 269	2100 ± 71	2170 ± 198	3375 ± 120
VS Removal (%)	60 ± 1	65 ± 0	66 ± 2	57 ± 2	68 ± 3	69 ± 1
Total Biogas (mL @ STP)	745 ± 20	878 ± 27	1179 ± 70	761 ± 7	916 ± 5	1188 ± 45
$\%CH_4$	66 ± 3	66 ± 1	75 ± 0	69 ± 1	62 ± 1	66 ± 1
$L\ CH_4/g\ COD_{added}$	0.52	0.43	0.42	0.56	0.43	0.28
$L\ CH_4/g\ COD_{removed}$	0.78	0.57	0.53	0.68	0.53	0.49
NH_3 - N	1024 ± 182	1323 ±182	1102 ± 429	865 ± 42	1072 ± 9	1166 ± 117

Biogas production rates cannot accurately be compared to those observed in the previous BMP assays due to the effect of a significant accumulation of VFAs and the corresponding drop in pH had on the biogas production. VFA concentrations of between approximately 5000 and 6500mg/L were observed for F/M ratios of 0.7 and 1.0, while concentrations of between 9000 and 10,000mg/L for assay bottles with an F/M ratio of 1.5 were observed after 8 days of digestion. Assay bottles supplemented with Co and MM at F/M ratios of 0.7 maintained a nearly neutral pH at approximately 6.9. Assay bottles with F/M ratios of 1.0 and 1.5 supplemented with Co dropped notably to 6.7 and 5.9 respectively, while bottles supplemented with the mineral mix solution dropped to 6.9 and 5.9. The rapid drop in pH necessitated the addition of alkalinity in order to maintain a neutral pH. Biogas production rates had decreased significantly at this point and were approaching zero for all F/M ratios between days 10 and 15. The addition of alkalinity returned all bottles to a pH of approximately 7.8, and biogas production was observed to show signs of recovery approximately 10 days later. Experiment bottles with F/M ratios of 1.5 were the slowest to recover, likely due to the fact that they experienced the highest level of VFA accumulation and

greatest drop in pH of all the assays. They returned to a normal biogas production rate after approximately 30 days following the pH adjustment.

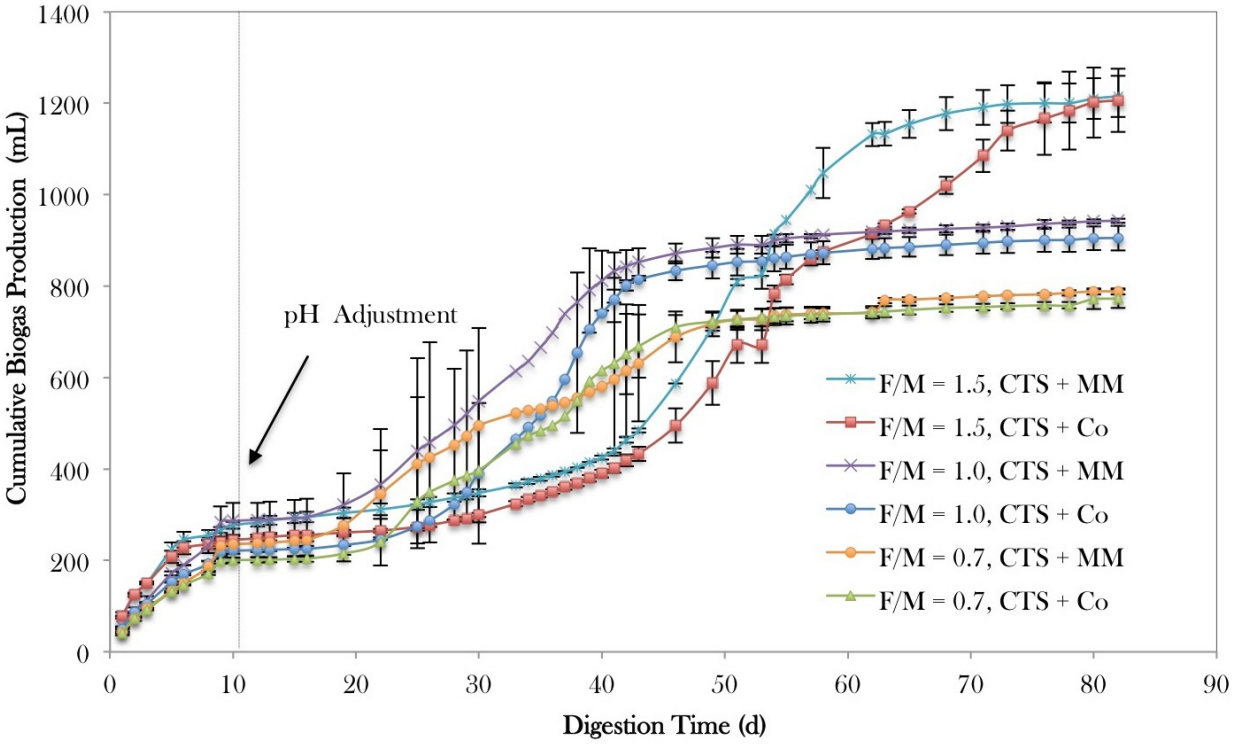


Figure 4.5 – Supplemental nutrients BMP assay cumulative biogas production

Despite the initial drop in pH, the assay bottles recovered fully and high biogas production was observed. As can be noted from Figure 4.5, there is a significant standard deviation associated with the average total biogas production values over the period of recovery, but these values decrease as the systems reached the end of digestion time and final cumulative biogas production values have acceptable standard deviations associated with them.

However, in comparison to the digestion of CTS as the sole carbon source with non-acclimated biomass, total biogas production increased for all F/M ratios in this assay. Comparable values observed between the bottles supplemented with only Cobalt and those supplemented with the mineral mix solution. These findings corroborate the results of Murray and van den Berg (1981) as previously discussed, and more recent results reported by Agler et al. (2008), who concluded that long-term stable digestion of thin stillage required the augmentation of cobalt. In comparison to the biogas production of thin stillage digestion without nutrients (or acclimation), total biogas production increased by 98%, 70% and 54% for thin stillage supplemented with the cobalt solution

and by 100%, 77% and 55% for thin stillage digested with the full mineral mix solution for F/M ratios of 0.7, 1.0 and 1.5 respectively.

Methane yields calculated are well above theoretical values indicating that perhaps a greater amount of thin stillage was added than intended. If the COD concentrations in the assay bottles were actually higher than initially estimated, this would account for the vast improvements in total biogas production, greater than theoretical methane yields, and rapid accumulation of VFAs (Figure 4.6). Due to the high organic and solids content of CTS, adding only a very small amount over the intended volume of thin stillage can drastically change the initial organic concentration.

Acumulation of VFAs was not monitored as closely as other experiments due to the fact that it was doubtful whether or not the assay bottles would recover from the drop in pH enough to warrant conducting a final analysis. The following figure shows the VFA data points collected over the course of the experiment. As can be observed in Figure 4.6 , total VFA concentrations are very high within the first week of digestion, corresponding to the point in time when the biogas production rates began to decrease for all bottles. Once the pH was adjusted, the system began to produce acids again, creating the second peak in the graph. From this point on digestion continued fairly normally, with total biogas production reaching a plateau as total VFA concentrations decreased to zero.

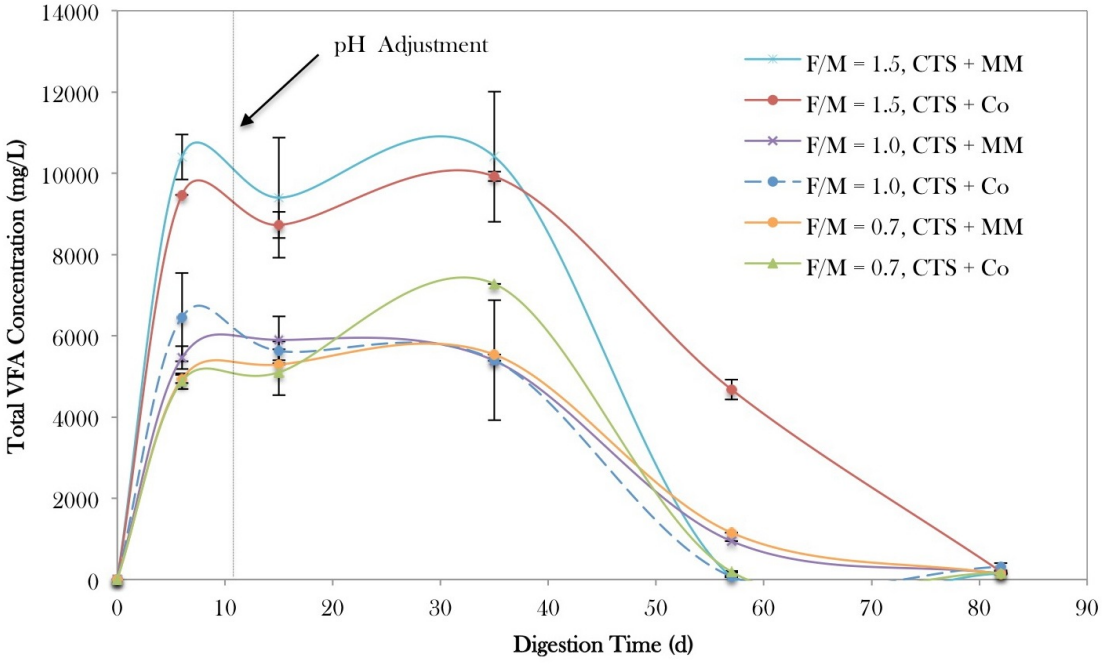


Figure 4.6 – Supplemental nutrients BMP assay volatile fatty acid concentrations

Overall, there appeared to be little difference in the performance of experiment bottles supplmented with the mineral mix solution or with the colbalt only solution at the same F/M ratios. Soluble COD and VS removal efficiencies were very similar, as was total biogas production, while higher TCOD removal efficiencies were observed for assay bottles supplemented with the MM. Total COD removal efficiencies for CTS + MM assay bottles were 82 ± 2%, 81 ± 3%, 83 ± 3%, while assay bottles with cobalt were 67 ± 9%, 76 ± 2%, and 79 ± 1% for F/M ratios of 0.7, 1.0 and 1.5 respectivley. In contrast to the removal efficiencies achieved in the intial suitability study BMP assay, removals for this assay were lower in all cases, with the exception of the reduction in SCOD which was similar for F/M ratios of 0.7 and 1.0 F/M ratios between 94 and 96%. Soluble COD removal efficiencies for F/M ratios of 1.5 decreased from 94 ± 0%, to 87 ± 1% for Co and 89 ± 0% for MM supplemented assay bottles.

4.5 Co-digestion BMP Assay

Co-digestion of CTS offers the potential to address its shortcoming as a substrate for anaerobic digestion. Low concentrations of some essential nutrients, as well as a lack of naturally occurring alkalinity are two characteristics in particular that make co-digestion of CTS an attractive option. Substrates suitable for co-digestion with CTS may address one or both of these deficiencies and potentially improve biogas production and rates, as well as COD and solids removal efficiencies.

Assay bottles were prepared with un-acclimated inoculum at F/M ratios of 0.7, 1.0 and 1.5, with 25% of total VS (on a mass basis) provided by either TWAS or food waste (FW). Data for the F/M = 1.0 supplemented with TWAS is not presented, as the bottles broke early on in the experiment. This experiment was conducted with un-acclimated biomass and was terminated after 44 days of digestion. Results from the final analysis are presented in Table 4.8. Data presented represents the arithmetic mean of replicates and the associated standard deviation.

Table 4.8 - Co-digestion BMP Assay Sample analysis results

Parameter	F/M Ratio				
	Co-digested with FW			Co-digested with TWAS	
	0.7	1.0	1.5	0.7	1.5
Initial TCOD (mg/L)	10,752 ± 345	15,346 ± 493	22,928 ± 736	10,139 ± 328	21,635 ± 701
Final TCOD (mg/L)	2751 ± 0	2751 ± 0	5581 ± 0	2751 ± 0	4867 ± 1010
TCOD Removal (%)	74 ± 0	82 ± 0	76 ± 0	73 ± 0	78 ± 5
Initial SCOD (mg/L)	4,951 ± 25	7,051 ± 37	10,544 ± 55	2,971 ± 61	6,345 ± 131
Final SCOD(mg/L)	343 ± 119	379 ± 0	456 ± 23	393 ± 83	608 ± 124
SCOD Removal (%)	93 ± 2	95 ± 0	96 ± 0	90 ± 2	93 ± 2
Initial VS (mg/L)	4,630 ± 23	6,611 ± 33	9,877 ± 49	4,799 ± 5	10,239 ± 11
Final VS (mg/L)	1195 ± 35	1510 ± 0	1975 ± 7	2105 ± 827	2545± 21
VS Removal (%)	74 ± 1	77 ± 0	80 ± 0	56 ± 17	75 ± 0
Total Biogas (mL @ STP)	488 ± 95	584 ± 11	830 ± 107	352 ± 2	747 ± 60
$\%CH_4$	61%	66%	67%	62%	64%
L CH_4/g COD_{added}	0.28	0.25	0.24	0.22	0.22
L CH_4/g $COD_{removed}$	0.37	0.31	0.32	0.29	0.29
NH_3 - N	928 ± 11	910 ± 5	924 ± 72	1055 ± 91	898 ± 64

Ammonia concentrations are similar to those reported for the digestion of CTS with nutrients, and are almost twice as high as the concentrations observed for the digestion of CTS as the sole carbon source. Again, the reason for these findings is unclear at this time, but concentrations are still not above levels thought to affect methanogeneic activity (Sawayama et al., 2004). Excellent SCOD removal efficiencies are observed, although TCOD removal efficiencies are typically below 80%, with the exception of CTS co-digested with FW at an F/M ratio of 1.0, which achieved 82 ± 0% removal. VS removals are also lower than 80%, for all conditions except CTS + FW at an F/M = 1.5. In general, assay bottles co-digested with FW performed better than those with TWAS: total and soluble COD, and VS removal efficiencies are higher, as are methane yields observed for corresponding F/M ratios.

The following Figure 4.7 illustrates the cumulative biogas production at STP over the course of the experiment. In comparison, the large standard deviations associated with the biogas production in this assay make it difficult to conclude if one co-substrate is more beneficial than the other in terms of total biogas production. The relatively low degradability of TWAS (35-60%) compared with

food waste that is generally readily degraded (70-90%), tends to indicate that FW should produce more biogas, but this cannot be concluded from the data presented.

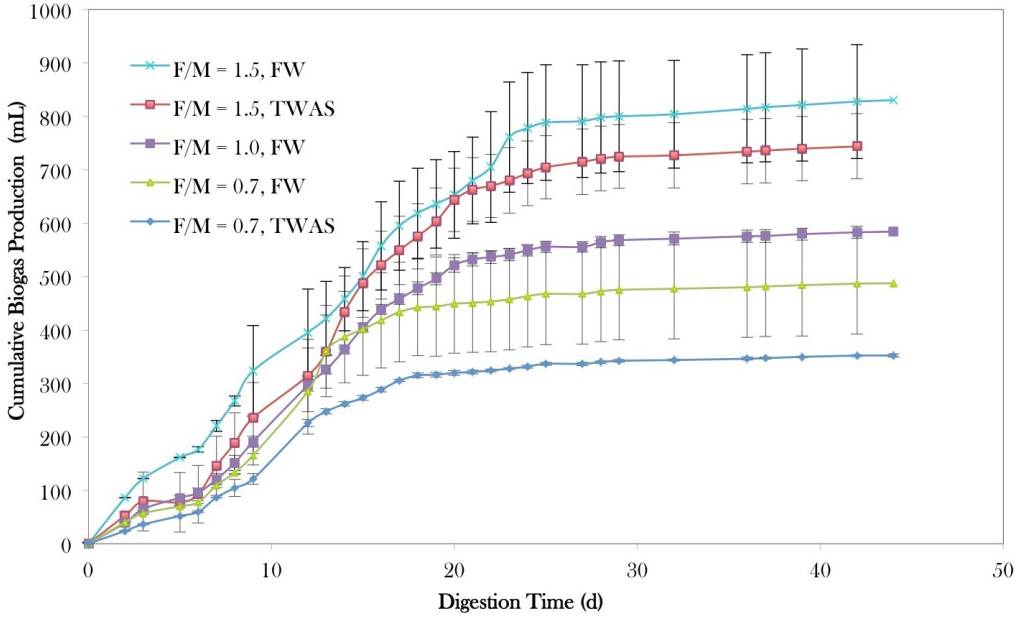


Figure 4.7 – Co-digestion BMP assay cumulative biogas production

The biogas production rates presented in Table 4.9 are similar for corresponding F/M ratios when compared to those observed for CTS digested as the sole carbon source (with acclimation). The exception was F/M = 0.7 assay bottles co-digested with FW, which did have a higher rate. The higher rates observed would appear to imply that a greater amount of readily degradable substrate is available initially, resulting in the increased biogas production rates and ultimately less time required for full digestion. However, the low removal efficiencies indicate that although there may be a portion of easily degradable substrate present in both TWAS and FW that can be quickly consumed, that there is also a portion that is extremely resistant to microbial action.

Table 4.9 – Co-digestion BMP assay biogas production rates

F/M Ratio	Maximum Rate (L/L/d)	Days of Digestion
0.7 + TWAS	0.20	0 – 16
1.5 + TWAS	0.34	0 – 19
0.7 + FW	0.28	0 – 14
1.0 + FW	0.29	0 – 18
1.5 + FW	0.34	0 – 21

When comparing the co-digestion of FW and TWAS, a higher biogas production rate was observed when thin stillage was co-digested with FW at an F/M ratio of 0.7. Identical biogas production rates were noted for F/M ratios of 1.5 for TWAS and FW assay bottles, at 0.34 L/L /d. In the case of F/M ratios of 0.7 and 1.0 (for both TWAS and FW), the time to reach 80% of ultimate biogas production was almost identical to that of thin stillage digested alone, at approximately 15 and 18 days respectively. The time required for the F/M = 1.5 assay bottles was shorter than the 27 days required for the digestion of thin stillage alone, and 80% of total biogas was produced by approximately 19 for TWAS, and 21 days for FW assay bottles. Table 4.9 summarizes the average biogas production rates observed for 80% of total biogas production.

Total VFA accumulation was lower in all cases when compared to the initial suitability BMP assay results. A more gradual accumulation of VFAs for assay bottles co-digested with TWAS, illustrated in Figure 4.8, further solidifies previous assumptions that TWAS is less easily degradable than FW. VFAs for FW co-digested assay bottles quickly accumulated but were nearly zero by day 20 of digestion. Biogas production for assay bottles with F/M ratios of 0.7 and 1.0 (for TWAS and FW) had essentially reached a biogas production rate near zero at this point as well.

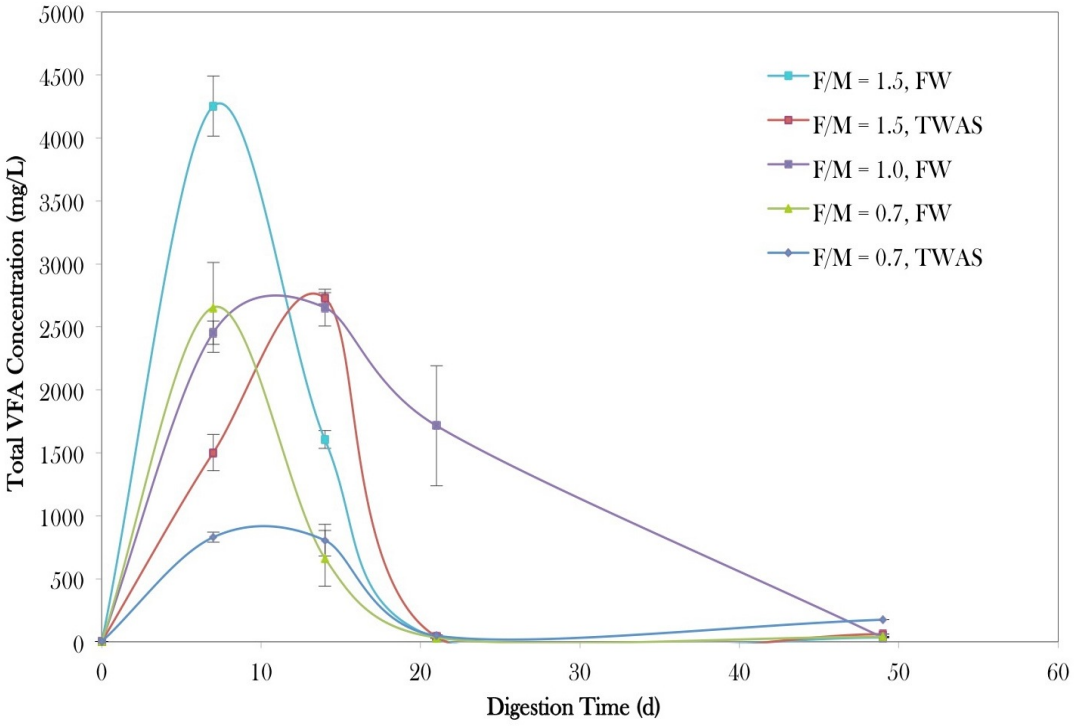


Figure 4.8 – Co-digestion BMP assay volatile fatty acid concentrations

The apparently high concentration of easily degradable substrate in FW is quickly converted to VFAs, and adequate buffer capacity is especially important so that the pH of the system does not drop below acceptable levels. Adequate buffer is also important for co-digestion with TWAS, but not nearly as critical as indicated by the lower maximum concentration of VFAs reached when compared to the co-digestion of CTS with FW.

4.6 Co-digestion BMP Assay with Biomass Acclimation

Once the final characterization for the initial co-digestion study was completed, the remaining mixed liquor was used as inoculum for a new set of co-digestion bottles. Proportions of corn thin stillage, food waste and TWAS added were identical to the initial set of bottles. This portion of the experiment was to observe the effects of acclimation of biomass on biogas production, COD and solids removals simulated under the worst-case scenario, the loss of half of biomass prior to the addition of new thin stillage and co-substrates. Table 4.10 summarizes the sample analysis results. Data presented represents the arithmetic mean of replicates and the associated standard deviation.

Table 4.10 - Co-digestion with biomass acclimation BMP assay sample analysis results

Parameter	F/M Ratio				
	Co-digested with FW			Co-digested with TWAS	
	0.7	1.0	1.5	0.7	1.5
Initial TCOD (mg/L)	10,752 ± 345	15,346 ± 493	22,928 ± 736	10,139 ± 328	21,635 ± 701
Final TCOD (mg/L)	701 ± 0	1415 ± 0	3091 ± 202	1891 ± 421	3386 ± 262
TCOD Removal (%)	93 ± 0	91 ± 0	87 ± 1	81 ± 4	84 ± 1
Initial SCOD (mg/L)	4,951 ± 25	7,051 ± 37	10,544 ± 55	2,971 ± 61	6,345 ± 131
Final SCOD(mg/L)	182 ± 7	475 ± 71	862 ± 67	116 ± 25	545 ± 61
SCOD Removal (%)	96 ± 2	93 ± 1	92 ± 1	96 ± 1	91 ± 1
Initial VS (mg/L)	4,630 ± 23	6,611 ± 33	9,877 ± 49	4,799 ± 5	10,239 ± 11
Final VS (mg/L)	1103 ± 95	1445 ± 7	2103 ± 194	758 ± 92	2388 ± 92
VS Removal (%)	76 ± 2	78 ± 0	79 ± 2	84 ± 2	77 ± 0
Total Biogas (mL @ STP)	645 ± 17	880 ± 49	1402 ± 73	460 ± 166	1144 ± 6
$\%CH_4$	63%	65%	62%	60%	60%
L CH_4/g COD_{added}	0.46	0.38	0.39	0.52	0.36
L CH_4/g $COD_{removed}$	0.49	0.42	0.45	0.64	0.43
NH_3 - N	498 ± 46	575 ± 50	817 ± 51	642 ± 2	962 ± 5

In general, similar VS removal efficiencies for the co-digestion of CTS and FW with and without acclimated biomass were observed. However, improvements in VS and COD removal efficiencies were observed for co-digestion of TWAS with acclimated biomass. VS reductions improved by 50% and COD removal improved by 8% for CTS co-digested with TWAS at an F/M ratio of 1.5. An 11% improvement in COD removal and 3% in VS removals were calculated for the co-digestion of TWAS at an F/M ratio of 0.7. Higher COD removal efficiencies for CTS co-digested with FW were noted as well: F/M ratios of 0.7, 1.0 and 1.5 improved by 26%, 14% and 14% respectively. In general, soluble COD reduction remained similar when compared to co-digestion with non-acclimated biomass, with removals greater than 90% in all cases.

Concentrations of ammonia were all lower than those reported for co-digestion without acclimated biomass, with the exception of F/M = 1.5 (TWAS) which was slightly higher than reported for digestion with un-acclimated biomass. Methane yields were again much higher than theoretical yields. This may be as a result of more CTS present in assay bottles than intended, or potentially the carry over of un-digested substrate with the acclimated biomass used for this experiment. The low removal efficiencies reported in the co-digestion without acclimation study would imply that there is likely still COD present in the biomass and this may partially explain the greater than theoretical methane yields. Improved cumulative biogas production was also observed in this assay (Figure 4.9 and Table 4.10). The potential carry over of un-digested substrate with the acclimated biomass would also explain the improvements in biogas production observed.

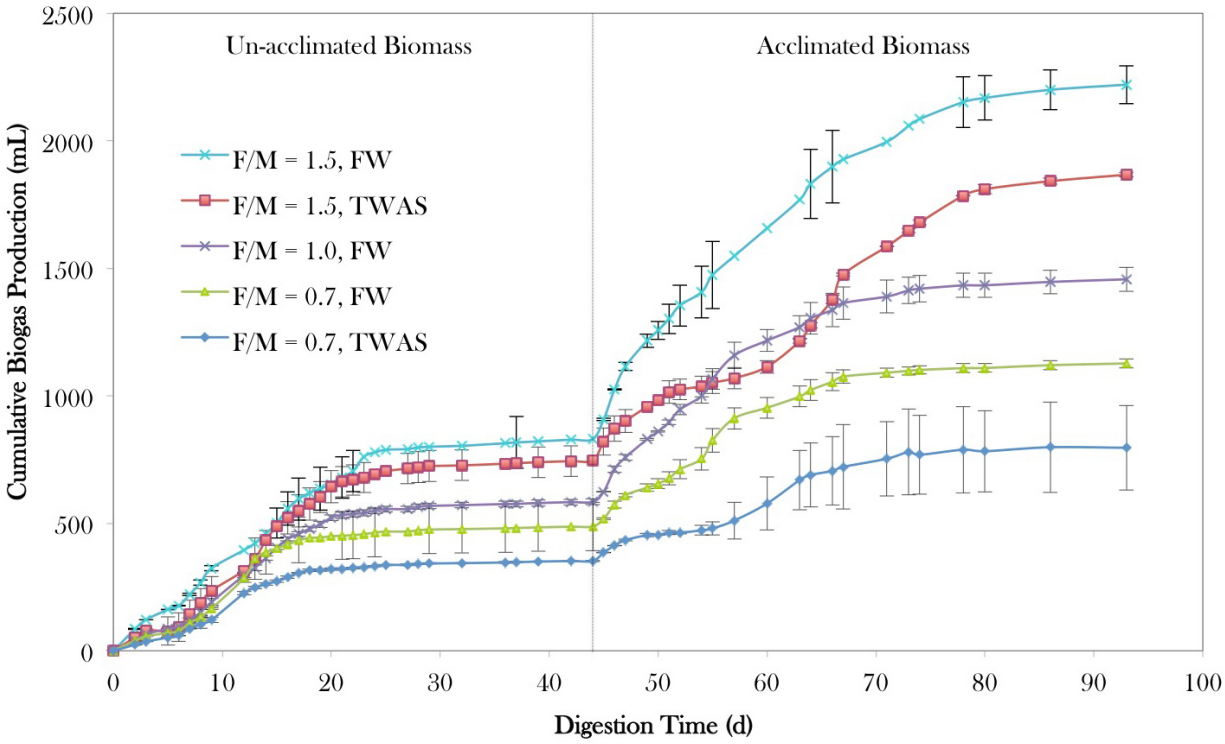


Figure 4.9 – Cumulative biogas production for co-digestion of CTS with and without acclimated biomass

In comparison to co-digestion of CTS with non-acclimated biomass, assay bottles with FW as the co-substrate experienced improved biogas production; by 32%, 51% and 69% for F/M ratios of 0.7, 1.0 and 1.5 respectively. Assay bottles co-digested with TWAS attained 31% and 53% improvements in total biogas production for F/M ratios of 0.7 and 1.5 respectively. However, the amount of time to reach 80% of total biogas production was slightly longer in all cases. Improvements in total biogas production were even greater than those observed for CTS digested (as the sole carbon source) with acclimated biomass. As previously discussed it is unclear if the improved biogas production in this co-digestion and acclimation assay is as a result of biomass acclimation or of the presence of substrate that cannot be accounted for. Initial studies with CTS as the sole carbon source did indicate that acclimation of biomass does offer some improvements for AD. Nevertheless, due to the possibility of carry over of un-digested substrate with the inoculum in this assay, it cannot be concluded that acclimation improves biogas production or methane yield under co-digestion conditions. However, one improvement that we can be certain that acclimation does offer is a reduction in the amount of digestion time required. If un-digested substrate were carried over, in theory we would expect required digestion time to be longer than

digestion without acclimated biomass, due to the higher concentration of COD present in each assay bottle. Table 4.11 summarizes the maximum biogas production rates for 80% of total biogas production observed for co-digestion of CTS with acclimated biomass.

Table 4.11 – Co-digestion with biomass acclimation BMP assay biogas production rates

F/M Ratio	Maximum Rate (L/L/d)	Days of Digestion
0.7 + TWAS	0.15	44 - 66
1.5 + TWAS	0.28	44 – 73
0.7 + FW	0.26	44 – 67
1.0 + FW	0.37	44 – 63
1.5 + FW	0.45	44 – 67

Maximum biogas production rates were higher for CTS co-digested with FW at F/M ratios of 1.0 and 1.5, when compared to the digestion of CTS as the sole carbon source with acclimated biomass at the same F/M ratios. Reduced biogas production rates were observed for co-digestion with TWAS at all F/M ratios and for the F/M = 0.7 + FW assay bottles. For these assay bottles, rates decreased compared to co-digestion without biomass acclimation, and there was an increase in the duration of the rates. Duration increased from 16 to 22 days for F/M = 0.7 + TWAS assay bottles, and from 19 to 29 days for F/M = 1.5 + TWAS assay bottles. The length of time for F/M = 0.7 + FW to produce 80% of the total biogas production also increased from 14 days to 23 days. Slight drops in the pH can explain the decreased rates and prolonged periods of digestion in order to reach 80% of ultimate biogas production for these particular assay bottles, which can be observed at approximately day 57 as slight plateaus in Figure 4.9.

The maximum biogas production rate achieved was for the co-digestion of CTS with FW at an F/M = 1.5 and rate of 0.45 L/L/d. This rate was greater than that achieved by the co-digestion of CTS with FW with non-acclimated biomass (0.34L/L/d) and of CTS digested alone with acclimated biomass (0.33L/L/d) at the same F/M ratio. The time to reach 80% of total biogas for co-digestion of CTS at F/M = 1.5 was similar between un-acclimated and acclimated biomass at 21 and 23 days respectively. Digestion of CTS alone (F/M = 1.5) with acclimated biomass also required a similar length of time (19 days) for 80% of total biogas production. Biogas production rates appear to indicate that FW is a good choice for co-digestion. This may indicate a greater

amount of biodegradable substrate at the same F/M ratio per g VS or COD added available in FW when compared to CTS. In other words, FW has a greater biogas production capacity per g VS added compared to thin stillage.

An interesting observation of this assay was the pronounced increase in VFA accumulation within the first week of digestion with acclimated biomass (Figure 4.10). VFA concentrations of over 8000mg/L for F/M ratios of 1.5 (FW and TWAS), 5000mg/L for F/M = 1.0 + FW and approximately 4000mg/L for F/M = 0.7 (FW and TWAS) were observed, which could potentially be the cause of the decreased biogas production rates observed earlier in Figure 4.9.

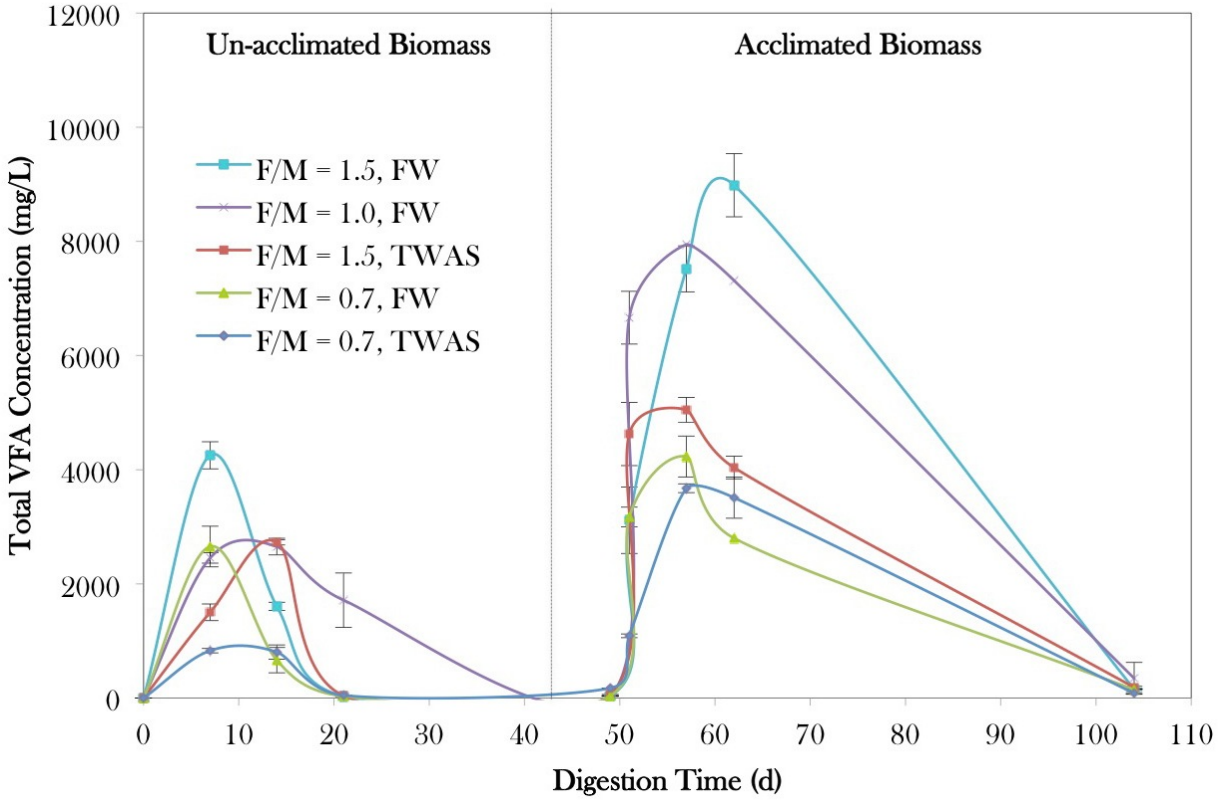


Figure 4.10 - Volatile fatty acid concentrations for co-digestion of CTS with and without acclimated biomass

In a similar fashion to the accumulation of VFAs in the co-digestion of CTS with non-acclimated biomass, assay bottles with FW appear to produce acids more rapidly than those with TWAS. VFA concentrations peak for FW assay bottles within 5 days of digestion, while TWAS assay bottles reach maximum concentrations after approximately 7 – 10 days of digestion. The reason

for the increase in total VFAs is unknown, but may indicate that the microbial population has shifted towards predominately acidogenic bacteria. A shift in microbial population away from methanogenic bacteria would also explain the increased digestion time required to reach 80% of total biogas production. The previous acclimation study indicated that acclimation may offer the benefit of reduced digestion time, however it seems that this advantage depends upon a balanced microbial population.

4.7 Effluent Recycle BMP Assay

Based on the results of previous BMP assays, it has become apparent that the high organic content of thin stillage necessitates dilution in order to provide conditions favourable for anaerobic digestion and to achieve acceptable levels of stabilization in terms of TCOD or VS destruction. This BMP builds upon previous assays to illustrate the effect of the use of treated effluent as a replacement for clean dilution water, as well as the advantages of acclimated biomass.

A portion of this recycle BMP assay is presented as the results for F/M = 2.0, in the initial suitability BMP assay. The initial digestion of thin stillage at an F/M ratio of 2.0 was carried out with un-acclimated biomass and CTS was diluted with clean water. Please see Chapter 3 – Materials and methods for a more detailed description on how this experiment and subsequent 1st, 2nd and 3rd recycle BMP assays were carried out. COD destruction achieved in the initial suitability assay at an F/M ratio of 2.0 was 81% for total COD and 95% for soluble COD, while VS removal was approximately 77%. Cumulative biogas production reached 1112 mL after 44 days of digestion, with a biogas composition of 62% methane and yield of 0.31L CH_4/g $TCOD_{destroyed}$.

Once the initial suitability BMP assay was completed at an F/M ratio of 2.0, experiment bottles for the 1st recycle BMP assay were prepared. Despite initial intentions to carry out all 3 subsequent recycle experiments at an F/M ratio of 2.0, the amount of thin stillage added in the 2nd and 3rd recycle BMP assays was reduced, due to the high accumulation of VFAs observed for the F/M = 2.0 over the course of the initial suitability BMP assay. As a result, assay bottles were provided with thin stillage to achieve an organic load of approximately 21g TCOD/L, corresponding to an F/M ratio of 1.5.

At the start of each recycle assay, 50mL of treated effluent was removed from each assay bottle and replaced with thin stillage diluted to the desired concentration (between 22,000 and 26,000mg TCOD/L) with either: 100% tap water, 25% effluent/75% tap water, 50% effluent/50% tap water or 100% effluent, by volume. It should be noted that for the 100% recycled effluent no clean water was used in any of the recycle BMP assays. Only a small amount of effluent (equal to the volume of the diluted thin stillage to be added) was removed from each bottle in order to prepare for the start of each new recycle BMP assay. Table 4.12 summarizes the sample analysis results for the 1st set of assay bottles in which CTS was diluted with digested BMP effluent. All data presented represents the arithmetic mean of replicates and the associated standard deviation.

Table 4.12 – 1st Recycle BMP assay sample analysis results

1st Recycle of Effluent	F/M = 2.0			
Parameter	**All clean dilution water**	**25% Effluent/75 % Tap water**	**50 % Effluent/50 % Tap water**	**100% Effluent**
Initial TCOD (mg/L)	27,172 ± 1468	27,909 ± 1468	28,646 ± 1468	30,120 ± 1468
Final TCOD (mg/L)	3485 ± 368	2904 ± 179	3173 ± 292	5048 ± 736
TCOD Removal (%)	88 ± 1	90 ± 1	90 ± 1	83 ± 2
Initial SCOD (mg/L)	12,703 ± 179	12,798 ± 179	12,892 ± 179	13,083 ± 179
Final SCOD (mg/L)	2028 ± 214	1546 ± 68	2085 ± 393	2207 ± 0
SCOD Removal (%)	84 ± 2	88 ± 1	84 ± 3	83 ± 0
Total Biogas @ STP (mL)	880 ± 28	873 ± 37	876 ± 32	804 ± 79
$\%CH_4$	62%	62%	62%	62%
L CH_4/g COD_{added}	0.20	0.19	0.19	0.17
L CH_4/g $COD_{removed}$	0.23	0.22	0.21	0.20
NH_4 – N (mg/L)	-	-	-	-

The results of 1st recycle BMP assay showed slightly higher TCOD removals, and slightly lower SCOD removal efficiencies when compared to the initial suitability BMP assay results at an F/M ratio of 2.0. The increases in TCOD removal efficiencies are consistent with the TCOD results of both previous acclimation studies. However, lower soluble COD removals were observed, which could be accounted for by the reduced time of digestion (approximately 33 days) in comparison to the 44 days of digestion of the initial F/M = 2.0 assay bottles. Biogas production was also slightly lower than the initial suitability BMP assay for F/M = 2.0 experiment bottles, which again can likely

be attributed to the shorter digestion time. Initial VS concentrations were also slightly lower than those in the initial suitability assay, which may account for the differences in removals and gas production observed. All methane yields presented in Table 4.12 were lower when compared to the F/M ratio of 2.0 in the initial suitability study. This is consistent with the decreased methane yields also observed when comparing the results of digestion of CTS as the sole carbon source with acclimated biomass, to un-acclimated biomass.

After the first successful use of effluent as dilution water in the 1st recycle BMP assay, the treated effluent from these bottles was used to dilute new thin stillage for the 2nd recycle BMP experiment bottles. BMP results are presented in Table 4.13.

Table 4.13 - 2nd Recycle BMP assay sample analysis results

2nd Recycle of Effluent	F/M = 1.5			
Parameter	**All clean dilution water**	**25% Effluent/75 % Tap water**	**50% Effluent/50 % Tap water**	**100% Effluent**
Initial TCOD (mg/L)	21,177 ± 1468	21,320 ± 1468	21,561 ± 1468	21,993 ± 1468
Final TCOD (mg/L)	5060 ± 1426	6646 ± 872	6839 ± 255	5221 ± 0
TCOD Removal (%)	76 ± 7	69 ± 4	68 ± 1	76 ± 0
Initial SCOD (mg/L)	9,714 ± 179	9,730 ± 179	9,752 ± 179	9.815 ± 179
Final SCOD(mg/L)	356 ± 138	257 ± 68	222 ± 36	579 ± 36
SCOD Removal (%)	96 ± 1	97 ± 1	98 ± 0	94 ± 0
Total Biogas @ STP (mL)	813 ± 32	789 ± 15	811 ± 33	743 ± 3
$\%CH_4$	62%	62%	62%	62%
L CH_4/g COD_{added}	0.24	0.23	0.23	0.27
L CH_4/g $COD_{removed}$	0.31	0.33	0.34	0.27
NH_4 – N (mg/L)	578 ± 15	695 ± 40	729 ± 14	1151 ± 54

As in the 1st recycle assay, 50mL of effluent was removed from each assay bottle and was replaced with CTS diluted with the removed effluent. This was the second time that the effluent was recycled as dilution water, and the third time that it was used for digestion in a BMP assay. The re-use of acclimated biomass was also changed slightly in order to allow the maximum retention of biomass within the experiment bottles. Bottles were allowed to sit undisturbed for 1 day, in order to preserve biomass by settling prior to the effluent decanting. The decanted supernatant was

analyzed for TCOD and SCOD in order to estimate any carry over to the 2nd recycle assay BMP bottles. After the completion of the 2nd recycle BMP assay, sample analysis indicated reduced effluent total COD removal efficiencies, although the percentage soluble COD removal improved in comparison to the 1st recycle BMP assay. After the 1st recycle BMP assay, TCOD removals were 88 ± 1%, 90 ± 1%, 90 ± 1%, and 83 ± 2%, and decreased to 76 ± 7%, 69 ± 4%, 68 ± 1% and 76 ± 0% for the 2nd recycle assay bottles with CTS diluted with 0%, 25%, 50% and 100% effluent respectively. A slight decrease in biogas production of the experiment bottles diluted with all recycled effluent (100% effluent) can be noted, with only a minor improvement in methane yield observed when compared to the 0%, 25% and 50% assay bottles.

Again, assay bottles were settled and in the same manner as for the previous assay, and effluent collected from the 2nd recycle BMP assay bottles was used as dilution water for the 3rd and final recycle BMP assay at this point. This assay is the 3rd time the effluent has been recycled and the 4th time that it has been used for dilution to facilitate the digestion of thin stillage. Table 4.14 summarizes the results of the 3rd recycle assay.

Table 4.14 - 3rd Recycle BMP assay sample analysis results

3rd Recycle of Effluent	**F/M = 1.5**			
Parameter	**All clean dilution water**	**25% Effluent**	**50 % Effluent**	**100% Effluent**
Initial TCOD (mg/L)	21,177 ± 1468	21,495 ± 1468	21,561 ± 1468	22,183 ± 1468
Final TCOD (mg/L)	3867 ± 736	3335 ± 206	4482 ± 449	6249 ± 340
TCOD Removal (%)	82 ± 3	84 ± 1	79 ± 2	72 ± 2
Initial SCOD (mg/L)	9,714 ± 179	9,730 ± 179	9,752 ± 179	9,815 ± 179
Final SCOD(mg/L)	183 ± 34	224 ± 2	357 ± 60	877 ± 92
SCOD Removal (%)	98 ± 0	98 ± 0	96 ± 0	91 ± 1
Total Biogas @ STP (mL)	907 ± 4	960 ± 36	932 ± 38	902 ± 30
$\%CH_4$	60	60	60	66
L CH_4/g COD_{added}	0.26	0.27	0.26	0.27
L CH_4/g $COD_{removed}$	0.31	0.32	0.33	0.37
NH_4 – N (mg/L)	524 ± 10	734 ± 9	877 ± 127	1444 ± 266

After the 3rd and final dilution of thin stillage with digested effluent, final COD removal efficiency was observed to increase in comparison to the 2nd recycle BMP assay, but was still lower than the removal efficiencies in the 1st recycle assay. Soluble COD removal efficiency remained fairly comparable for all assay bottles, with the exception of those diluted with 100% effluent, which dropped 3% from 94 ± 0% in the 2nd assay to 91 ± 1% in the 3rd recycle assay and 50% effluent, which dropped 2%. Figure 4.11 illustrates the trends for TCOD removal efficiency for all three effluent recycle assays.

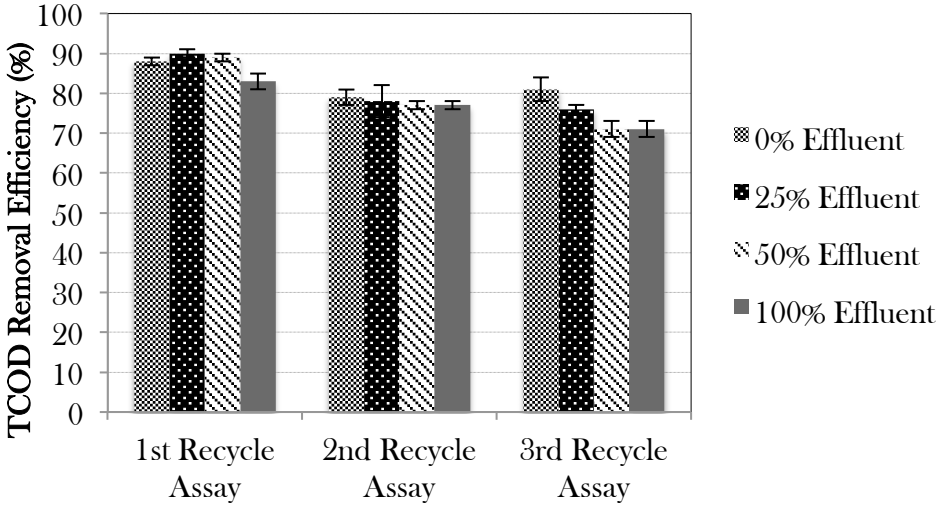


Figure 4.11 – Total COD removal efficiencies for 1st, 2nd and 3rd recycle BMP assays

Despite the observed decrease in TCOD removal efficiencies, an improvement in methane yield was observed in the 2nd and 3rd recycle BMP assays. Improved yields are likely a result of the destruction of degradable COD carried over via the inoculum, which was not included in the initial organic concentration for each assay bottle, and not in fact representative of improved COD digestion. This would also explain the reduced TCOD removal efficiencies observed in Figure 4.11 for assay bottles diluted with 25%, 50% and 100% effluent. Recalcitrant COD would be carried over repeatedly with the effluent used for dilution and build up. This could result in conditions inappropriate for digestion due to accumulation of recalcitrant or toxic substances (such as ammonia) and may affect performance, affecting biogas production.

In the 3rd recycle assay ammonia concentrations were 6%, 20% and 25% higher for assay bottles diluted with 25%, 50% and 100% effluent respectively, when compared to the 2nd recycle BMP assay. Ammonia levels for experiment bottles diluted with all clean water actually decrease by 9% from values measured in the 2nd recycle BMP assay.

Table 4.15 – Methane yields for digestion of CTS diluted with digested effluent

% Effluent for Dilution	Methane yield (L CH_4/g $TCOD_{destroyed}$)		
	1st Recycle BMP	2nd Recycle BMP	3rd Recycle BMP
0%	0.23	0.31	0.31
25%	0.22	0.33	0.32
50%	0.21	0.34	0.33
100%	0.20	0.27	0.37

Figure 4.12 on the following page shows cumulative biogas production versus time for all 3 sequential BMP assay cycles, at the four different dilution conditions. Each section of Figure 4.12 (1, 2, 3 or 4) identifies the biogas production curve for the corresponding BMP assay. Section 1 represents the digestion of thin stillage at an F/M ratio of 2.0 diluted with all clean water (as presented in the initial suitability BMP). Section 2 of the biogas production curve represents the digestion of new CTS diluted with either all clean water, 25%, 50% or 100% effluent with acclimated biomass (under the worst case scenario, removal of half of the biomass prior to the addition of new thin stillage), which is the 1st recycle BMP assay. Section 3 represents the digestion of thin stillage diluted again with either all clean water, 25%, 50% or 100% treated effluent. In the case of section 3 the maximum amount of biomass was retained by settling (best case scenario) in the assay bottles prior to the addition of new thin stillage to observe any differences in performance. The assay bottle conditions for section 4 of the biogas production curve were the same as those described for section 3.

The first curve (series F/M = 2.0, all clean water) is presented from the initial BMP for comparison purposes. It can be noted that there was little difference in biogas production for the first and second cycles using clean water, 25%, 50% or 100% effluent for dilution of CTS. However, in the 3rd cycle the trend observed in Figure 4.12 indicates that the assay bottles with diluted with 100% effluent experience a decrease in both the cumulative biogas production and rate. Even so, total

biogas production (cumulative of 4 assays) at the conclusion of the 3rd assay, was approximately 95% of the maximum biogas produced by assay bottles diluted with all clean water, 25% effluent and 50% effluent.

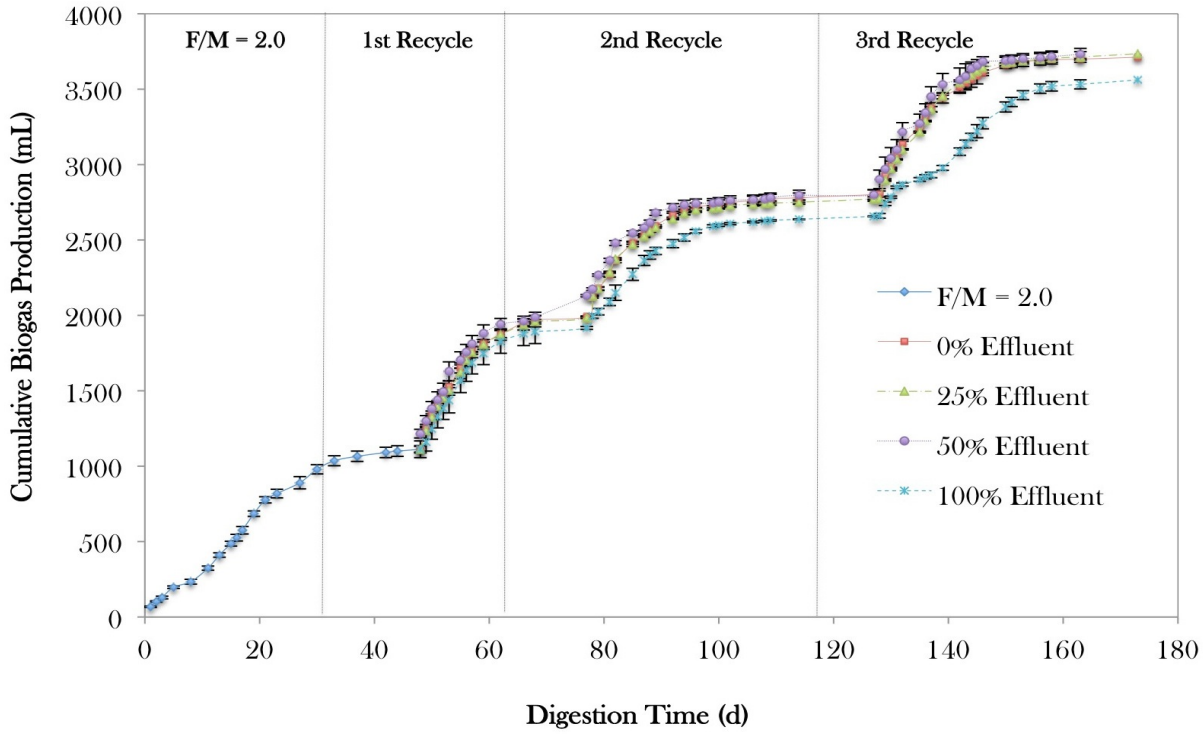


Figure 4.12 – Cumulative biogas production for 1st, 2nd and 3rd recycle BMP assays

When comparing the biogas production rates from each part of the curve, the best rates were typically observed for assay bottles digesting thin stillage diluted with half clean water and half recycled effluent (50% effluent/50% tap water). The rates presented in Table 4.16 represent the biogas production rates at which 80% of total biogas is produced for each of the 3 recycle assays.

Table 4.16 – 1st, 2nd and 3rd Recycle BMP assay Biogas production rates

% Effluent for Dilution	F/M = 2.0		1st Recycle BMP		2nd Recycle BMP		3rd Recycle BMP	
	Days	Biogas Production Rate (L/L/d)	Days	Biogas Production Rate (L/L/d)	Days	Biogas Production Rate (L/L/d)	Days	Biogas Production Rate (L/L/d)
0%	8-21	0.43	48-59	0.63	78-92	0.43	129-142	0.50
25%	-	-	48-59	0.63	78-92	0.42	129-142	0.54
50%	-	-	48-59	0.65	78-92	0.45	129-143	0.49
100%	-	-	49-59	0.61	78-94	0.36	139-150	0.30

As previously discussed, Figure 4.12 seems to suggest that the use of 100% digested effluent for CTS dilution does not significantly impede biogas production, although it does appear to affect production rate. Assay bottles digested with 100% effluent dilution water had the lowest rate of biogas production consistently, and in the 3rd recycle BMP assay production rate had dropped to half of the rate observed in the 1st BMP assay.

Figure 4.13 illustrates VFA accumulation over the course of the four BMP assays, with section numbers corresponding to assay conditions previously described for Figure 4.12. Again, curve 1 represents the digestion of CTS with all clean dilution water at an F/M ratio of 2.0 for comparison purposes. Maximum VFA accumulation remains between 3,400mg/L and 4,400mg/L for assay bottles diluted with 0% effluent, 25% effluent and 50% effluent over the course of all three recycle BMP assays. However, it appears that the assay bottles digested with 100% effluent experience increased maximum VFA accumulation with each subsequent BMP assay. Maximum concentrations reach on average approximately 3,400mg/L during the 1st recycle BMP, 4,900mg/L during the 2nd recycle BMP and 5,100mg/L for the 3rd recycle BMP. Visually, the VFA peaks also become wider with each successive recycle assay, indicating that it is taking a longer period of time for the methanogens to consume the acids that have been produced by the digestion of CTS. This characterizes an imbalance in the microbial population, indicating there has been a shift towards conditions that are more favourable for acidogenic bacteria. This results in decreased methanogenic activity and consequently lower biogas production, as was observed for assay bottles digested with 100% effluent dilution water (Figure 4.12).

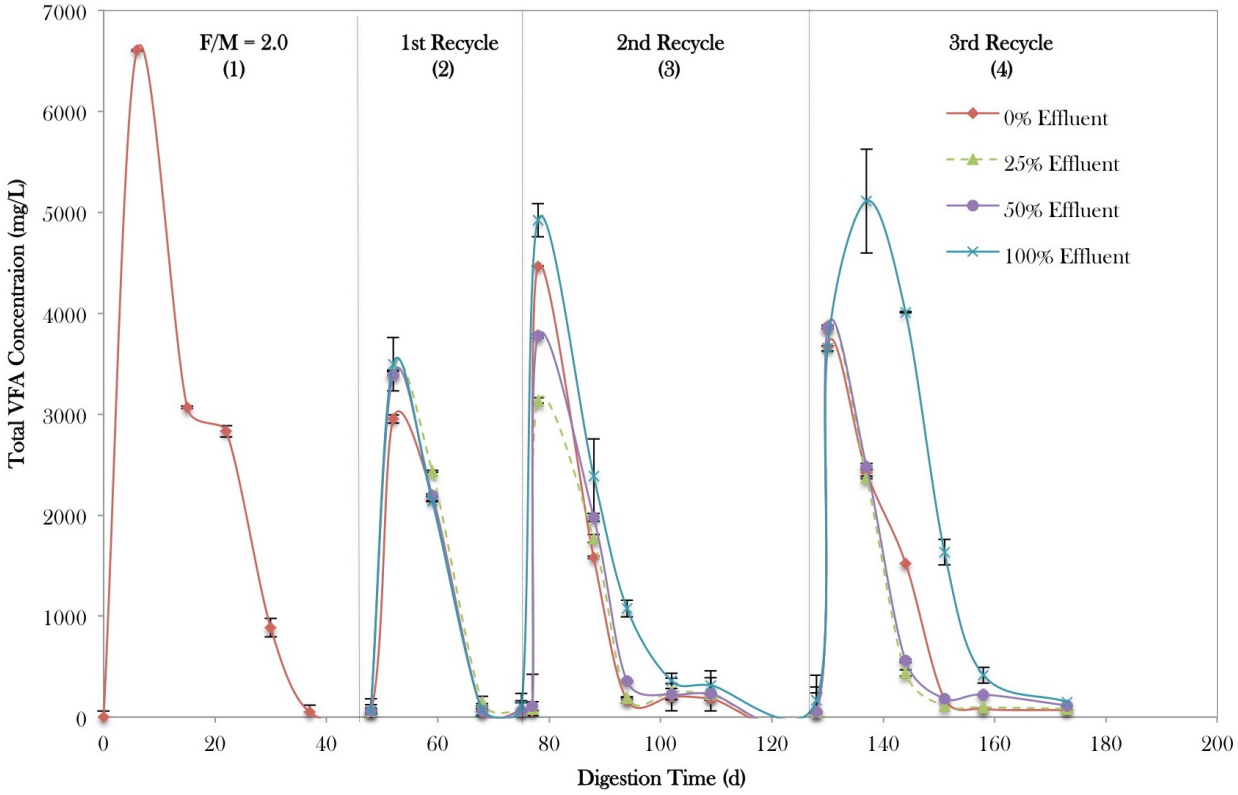


Figure 4.13 – Volatile fatty acid concentrations for recycle BMP assays

These trends appear to suggest that the use of treated effluent as dilution water is a potential approach to reduce fresh waster consumption in the anaerobic digestion of high strength wastewaters, such as CTS. The most favourable results were observed for the digestion of CTS diluted with 25% effluent. Removal efficiencies (SCOD, TCOD, VS) were the second highest with the exception of assay bottles diluted with all clean tap water, which was anticipated. It also exhibited the lowest build up of ammonia and maintained high total biogas production and production rates.

Based on decreased in TCOD removal efficiencies as well as reduced biogas production, it can be speculated that further cycles of CTS digestion diluted with 100% effluent, would result in a further deterioration in performance. However at the present time it would seem that additional cycles can be performed with 25/75, 50/50 or 75/25 clean water/effluent mixtures to ascertain when negative effects on AD performance are likely to occur. While not in the scope of the present

study, future work with continuous AD of CTS could evaluate impact of effluent recycle on process performance.

4.8 Continuous Reactor Studies

High-rate fixed film reactors are thought to provide many advantages over classical continuous reactor configurations. Specifically, the key advantage of fixed film reactors is their ability to retain biomass, addressing biomass washout, which has been identified as one of the most prevalent problems of industrial anaerobic digestion plants (Speece 1996). Low biomass yield, often considered one of the major benefits of anaerobic digestion, is also its major disadvantage when attempting to preserve slow growing methanogens and increase biomass inventory. The ability of fixed film reactors to create and maintain a healthy inventory of biomass on suspended support materials helps to temper the effects of slow growing methanogens and washout on the anaerobic digestion process. Maintaining a fixed biomass inventory not only helps to greatly reduce the chance of washout, but also allows the de-coupling of hydraulic and solid retention times. This ability allows fixed film reactors to handle higher loading rates while still achieving the desired removal efficiencies.

These characteristics make fixed film reactors an excellent candidate for the digestion of a high strength feedstock such as corn ethanol thin stillage. The potential for long-term continuous anaerobic digestion of corn ethanol thin stillage was evaluated with the use of two 28L (empty bed volume) bench-scale, down flow stationary fixed film (DSFF) reactors. Reactors contained vertically oriented needle punched polyester geotextile support media with a surface to volume ratio of $75 m^2/m^3$. Reactors were inoculated with 12.5L of mesophilic seed with an activity of 0.17g VS/gVS_{inoc}/d obtained from ROPEC, and 12.5L distilled water for a total working volume of 25L. Establishment and acclimation of a healthy biofilm biomass inventory was accomplished over a period of 5 months by adding approximately 50mL of full strength CTS daily (corresponding to an OLR of 0.3g TCOD/L/d) and recycling the reactor liquid at a rate of 5 reactor volumes per day (125L/d). This was done in order to facilitate biomass attachment to the support media prior to the start of experimentation. Throughout the period of biofilm establishment and acclimation, accumulation of volatile fatty acids remained negligible.

At the conclusion of the acclimation period, a measurement of the volume displaced by the biomass that had accumulated was made. This measurement indicated that approximately 7L of biomass was present in Reactor #1 and 9L in Reactor #2. Visual inspection of both reactors suggested that initially the biomass was predominantly suspended on the reactor support media, but was also present in the mixed liquor of both reactors.

Following start up, full strength CTS was diluted to two different concentrations (approximately 25g TCOD/L and 37g TCOD/L for Reactor #1 and Reactor #2 respectively) with tap water containing sodium bicarbonate ($NaHCO_3$) and potassium bicarbonate ($KHCO_3$) to achieve an alkalinity of between 4000 and 6000mg/L as $CaCO_3$. Throughout the course of this research, both reactors were operated at 5 different HRTs, and various organic loading rates were achieved by diluting the CTS used as feed for each reactor accordingly. Dilutions ranged from 1-part CTS to 5 parts water, (1:6) at the beginning of experimentation down to dilutions of 1:4 and 1:3 for the final HRTs investigated. A dilution of 1:6 was chosen as the starting point based on the performance in the initial suitability BMP assay. The best performance was observed at an F/M ratio of 1.5, which corresponded to an initial organic concentration of approximately 22g/L. Based on the average total CTS COD determined of 159,800mg/L, a dilution of 1:6 was established to provide feed concentrations in this range.

Reactor #1 was operated at organic loading rates (OLRs) of: 1.24, 1.73, 2.85, 4.75, 7.42, and 8.73 g TCOD/L per day, while reactor #2 was operated at slightly higher organic loading rates of: 1.89, 2.60, 4.30, 5.93, 9.9 and 11.6 g TCOD/L/d corresponding to HRTs of 20, 14.2, 8.7, 6.25, 5 and 4.2 days respectively. Reactors were maintained at each OLR for a minimum of two HRTs. Please see Table 3.10 in Chapter 3 – Materials and Methods, for CTS feed dilution ratios corresponding to each OLR.

Table 4.17 summarizes the organic loading rates, removal efficiencies and methane yields for both reactors. Data presented represents the arithmetic mean of replicates and the associated standard deviation. Total and soluble COD, as well as VS removal efficiencies were maintained above 80% for both reactors up until an organic loading rate of 8.73 g TCOD/L/d (4.2 day HRT) for Reactor #1 and 9.9 g TCOD/L/d (5 day HRT) for Reactor #2. At these OLRs Reactor #1 and Reactor #2 achieved total COD removal efficiencies of 81 ± 2% and 83 ± 2% and VS removal efficiencies of

80 ± 2% and 82 ± 2% respectively. Soluble COD reductions fell to 72 ± 2% for reactor #1 and 78 ± 2% for reactor #2.

Figure 4.14 shows the average SCOD and TCOD removal efficiencies at each loading rate. A general trend can be observed, indicating a decrease in average reactor performance once an organic loading rate of approximately 7.4 g TCOD/L/d is reached. Error bars represent the standard deviation of sample replicates.

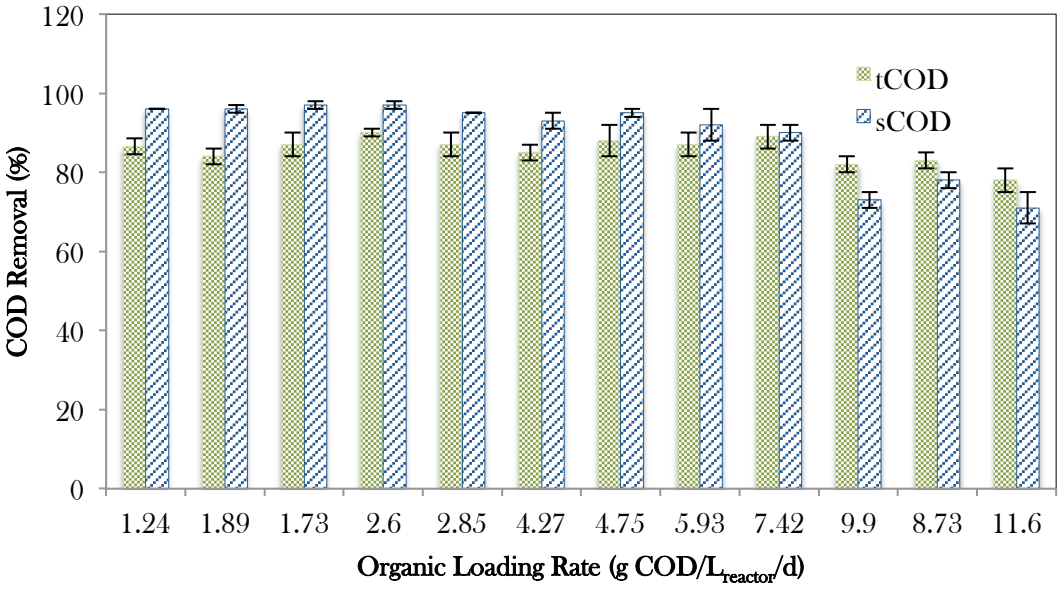


Figure 4.14 (a) – COD removal efficiencies for DSFF Reactors #1 and #2 at corresponding OLRs

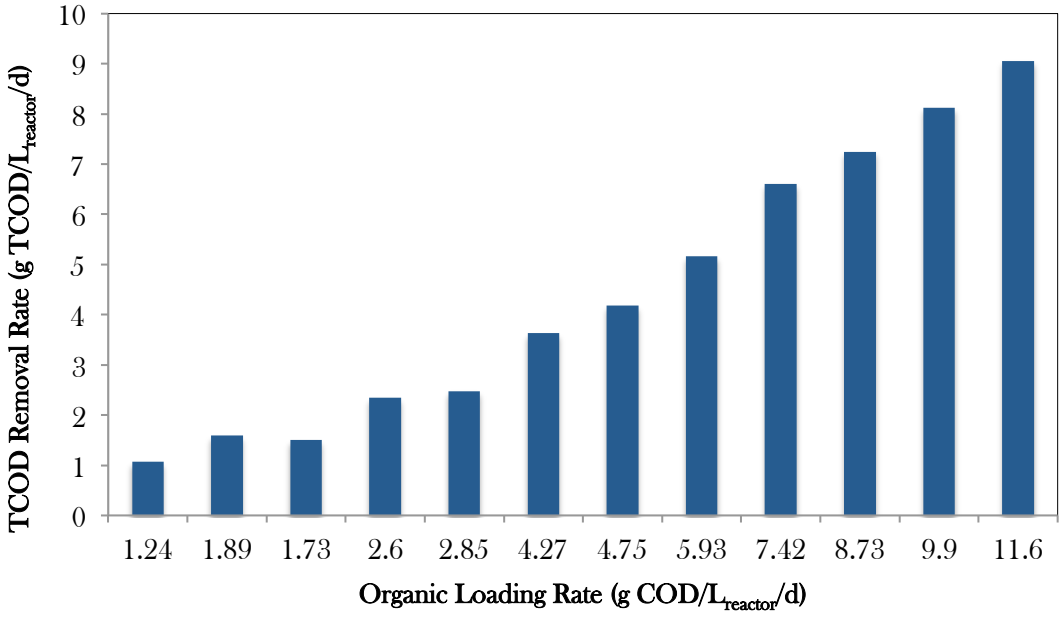


Figure 4.14(b) – COD removal rates for DSFF Reactors #1 and #2 at corresponding OLRs

Soluble COD removal falls from 89% at an OLR of 7.42 g TCOD/L/d to 78% for an OLR of 9.9g TCOD/L/d, while total COD removal drops from 89 ± 3% to 83 ± 2%. Volatile solids removals remained essentially the same, with values within the standard deviations calculated. The first drop in biogas production rates were observed at an OLR of 9.9 g TCOD/L/d. Up until this loading rate, biogas production had been steadily increasing with increased organic loading rates, which can be seen in Figure 4.15. Again, as Reactor #2 is transitioned from a 5.93 g TCOD/L/d to 9.9 g TCOD/L/d OLR, the first drop in biogas production rate occurs. Reactor #1 however, maintains the upward trend in biogas production rate when the OLR is changed from 4.75 to 7.42 g TCOD/L/d. Biogas production rates presented in Figure 4.15 were determined based on the average production rates over the final HRT of the corresponding OLR, as were the average COD removal efficiencies presented in Figure 4.14. Figure 4.15 illustrates the average volumetric biogas production with associated standard deviation over time at the various HRTs and corresponding organic loading rates for Reactors #1 and 2.

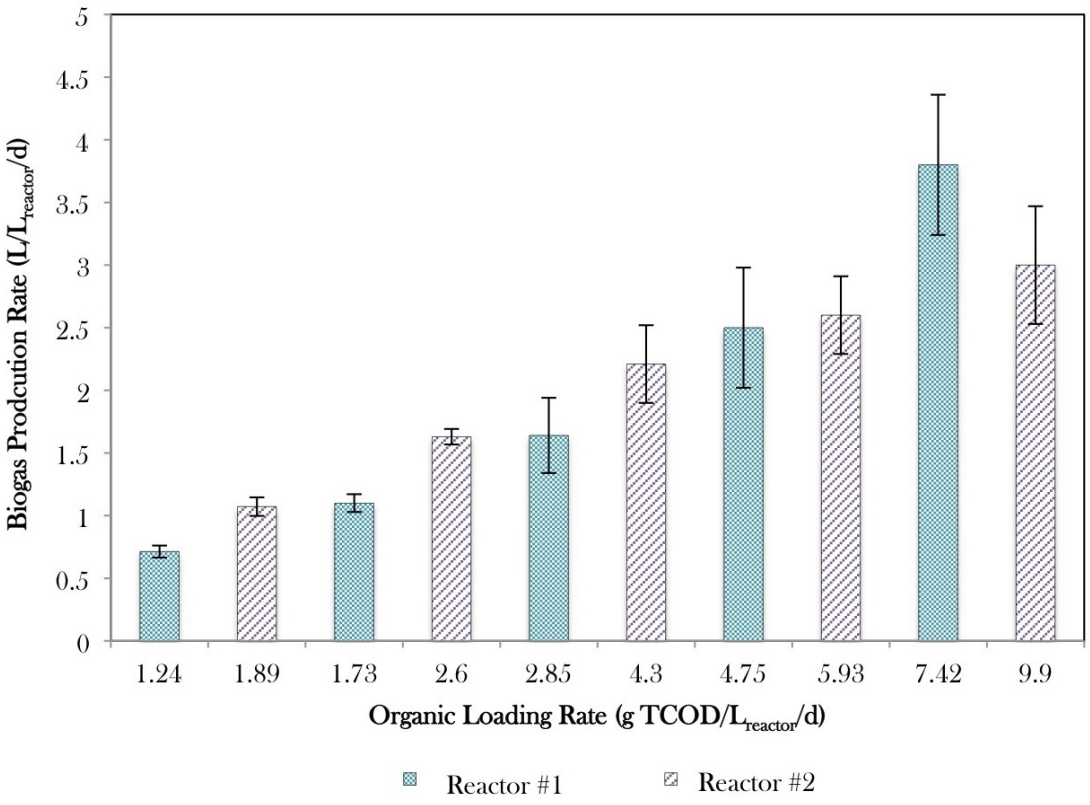


Figure 4.15 – Biogas production rates at corresponding OLRs for DSFF reactors #1 and #2

Table 4.17 – Continuous reactor sample analysis results summary; all parameters are in mg/L unless otherwise specified

Parameter	Hydraulic Retention Time (d)											
	20		14.3		8.7		6.25		5		4.2	
Reactor	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
Organic Loading Rate (g TCOD/L/d)	1.24	1.89	1.73	2.6	2.85	4.3	4.75	5.93	7.42	9.90	8.73	11.6
Surface Loading Rate (g TCOD/m²/d)	16.5	25.2	23.1	34.7	38	57.3	63.3	79.1	98.9	132.0	116.4	154.7
Influent VS	14300 ± 222	21400 ± 333	14300 ± 222	21400 ± 333	14300 ± 222	21400 ± 333	17100 ± 267	21400 ± 333	21400 ± 333	28500 ± 444	21400 ± 333	28500 ± 444
Effluent VS	2182 ± 242	3126 ± 243	-	3157 ± 404	2714 ± 244	3737 ± 539	2646 ± 615	3419 ± 643	3303 ± 551	5116 ± 557	4720 ± 421	6061 ± 845
VS Removal (%)	85 ± 2	85 ± 1	-	85 ± 1	81 ± 2	83 ± 3	85 ± 4	84 ± 3	85 ± 3	82 ± 2	80 ± 2	79 ± 3
Influent TCOD	24744 ± 462	37116 ± 693	24744 ± 462	37116 ± 693	24744 ± 462	37116 ± 693	29693 ± 554	37116 ± 693	37116 ± 693	49489 ± 923	37116 ± 693	49489 ± 923
Effluent TCOD	2665 ± 455	4571 ± 529	3219 ± 764	3729 ± 428	3574 ± 731	5459 ± 777	3410 ± 1126	4283 ± 1163	4,234 ± 1063	8,510 ± 1,077	6,957 ± 878	10,816 ± 1,310
TCOD Removal (%)	89 ± 2	88 ± 1	87 ± 3	90 ± 1	86 ± 3	85 ± 2	89 ± 4	88 ± 3	89 ± 3	83 ± 2	81 ± 2	78 ± 3
Influent SCOD	11387 ± 509	17081 ± 764	11387 ± 509	17081 ± 764	11387 ± 509	17081 ± 764	13664 ± 611	17081 ± 764	17081 ± 764	22774 ± 1018	17081 ± 764	22774 ± 1018
Effluent SCOD	415 ± 38	600 ± 85	329 ± 61	523 ± 90	602 ± 32	1189 ± 270	810 ± 183	1289 ± 664	1674 ± 310	5106 ± 462	4741 ± 301	6768 ± 928
SCOD Removal (%)	96 ± 1	96 ± 1	97 ± 1	97 ± 1	95 ± 0	93 ± 2	94 ± 1	92 ± 4	89 ± 2	78 ± 2	72 ± 2	70 ± 4
% CH_4	60	61	58	59	58	59	59	59	54	50	61	57
Biogas Production (L/L/d)	0.71 ± 0.05	1.07 ± 0.07	1.10 ± 0.07	1.63 ± 0.06	1.64 ± 0.30	2.21 ± 0.31	2.50 ± 0.48	2.60 ± 0.31	3.8 ± 0.56	3.0 ± 0.47	-	-
Methane Yield (L CH_4/L/d)	0.43 ± 0.03	0.64 ± 0.04	0.64 ± 0.04	0.96 ± 0.04	0.95 ± 0.17	1.3 ± 0.18	1.48 ± 0.28	1.53 ± 0.18	2.05 ± 0.32	1.5 ± 0.24	-	-
Effluent NH_3-N	303 ± 39	326 ± 51	-	-	110 ± 9	122 ± 22	128 ± 24	215 ± 26	239 ± 18	288 ± 35	337 ± 25	311 ± 20

Daily biogas production rates were observed to remaine relatively stable for HRTs of 20 and 14.3 days. However, near the beginning of the 8.7-day HRT (approximately day 80), corresponding to OLRs of 2.85 and 4.3 g TCOD/L/d for Reactor #1 and Reactor #2 respectively, daily biogas production becomes more inconsistent.

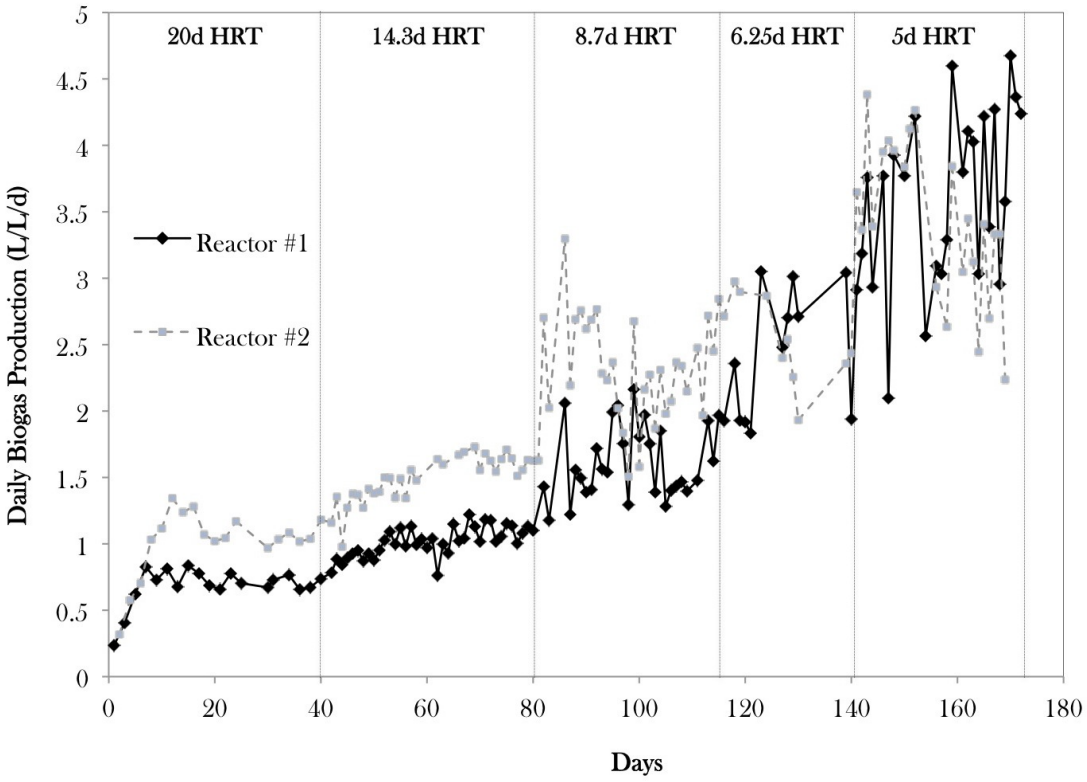


Figure 4.16 – Daily biogas production from DSFF reactors #1 and #2

Despite the increasingly variable daily biogas production rates, both DSFF reactors continued to operate without problem and average VS, SCOD and TCOD removal efficiencies remained above 80% until an HRT of 4.2 days for Reactor #1 and an HRT of 5 days for Reactor #2. No large accumulation of ammonia was observed at any OLR or HRT. Although ammonia concentrations did increase slightly with increased OLRs, they were still well below concentrations considered to affect methanogenic activity. A slight accumulation of foam was observed in the headspace of both reactors for the first time during the 8.7 day HRT period, but it did not cause any operational issues at this point. Daily biogas production for Reactor #2 was always greater than Reactor #1 for HRTs of 20, 14.3 and 8.7 days respectively. Based on the increased loading of Reactor #2 (due to

higher influent CTS concentrations resulting from lower dilution ratios) this is not unexpected during stable operation and is indicative of similar COD removal efficiencies.

It should be noted that at operational HRTs of 20, 14 and possibly 8.7 days, reactor performance could be the result of suspended microbial activity, biofilm activity or combination of both. However, at shorter HRTs where washout of methanogenic cultures will occur, greater reliance will be placed on the biofilm for ultimate DSFF performance.

In general, higher biogas production was also seen for R2 at HRT of 6.25 days indicating that the specific biofilm was not yet exceeded and that both reactors were achieving similar COD stabilization efficiencies. At OLRs of 8.73 g TCOD/L/d (4.2 day HRT) for R1 and 9.9 g TCOD/L/d (5 day HRT) for Reactor #2, removal efficiencies remained above 80% at 81 ± 2% and 83 ± 2% for COD and 80 ± 2% and 82 ± 2% for VS, respectively. However, soluble COD reductions fell from 89 ± 2% to 72 ± 2% for R1 and from 92 ± 4% to 78 ± 2% for R2 when compared to the previous organic loading rates (in the same reactor). Additionally it can be noted from the temporal biogas production at these loading rates, that both R1 and R2 were similar in the range of about 3.5 L/L/d at these. This convergence of the biogas productions suggests that Reactor #1 and definitely Reactor #2 were approaching their maximum specific biofilm loading and that for the given concentrations tested the maximum DSFF loading is in the range of 8.7-10 g TCOD/L/d (120-130 g TCOD/m^2/d). Assuming that the biofilm is mature and that the optimum surface to reactor volume ratio is being used significant improvements in the volumetric loadings with stable COD stabilization (>80%) are unlikely.

Figure 4.17 on the following page shows the corresponding VFA accumulation with time for Reactors #1 and #2. In Figure 4.17, total VFA concentration accumulated to approximately 2000mg/L for reactor #2 at approximately day 152 – 153 (pH remained stable near 7), which correlated to a decrease in biogas production rate from a maximum of 4.2L/L/d, down to approximately 3.0L/L/d. This drop occurs approximately over days 152 – 165 of digestion. For Reactor #1, VFA concentrations of 2000mg/L were not observed to accumulate until approximately day 171 of digestion, and likewise a drop in biogas production rates were observed at this point.

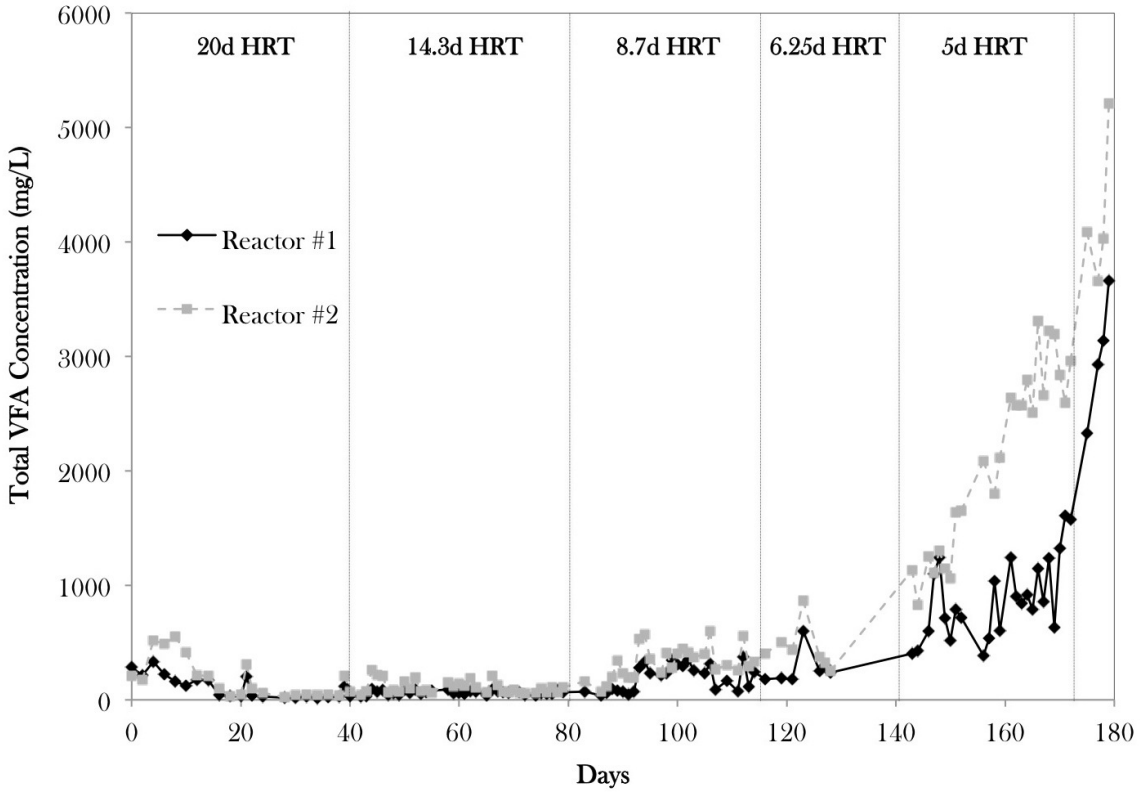


Figure 4.17 – Volatile fatty acid accumulation for DSFF reactors #1 and #2

The accumulation of total VFAs has been associated with the inhibition of methane production due to microbial stress. Organic or hydraulic overloading may be the cause of increased VFA concentrations in a digester, although at a neutral pH, levels of acetic and butyric acids up to 10,000mg/L are reported not to be inhibitory (Eskicioglu et al., 2011). Adverse effects have only been shown for propionate concentrations greater than 1000mg/L. Throughout the operation of the reactors, acetic acid production dominated accounting for on average, more than 60% of the total acids in both reactors. Propionate levels measured reached approximately 1000mg/L or greater towards the end of the 5 day HRT (~ day 167) for reactor #2, while reactor #1 reached levels of propionate greater than 1000mg/L within the first few days of digestion at the 4.2 day HRT (days 171 - 172). The accumulation of propionate indicated that the hydrogenotrophic methanogens are being inhibited. In both cases, the increase in propionate concentrations to near 1000mg/L corresponded to a drop in reactor efficiency.

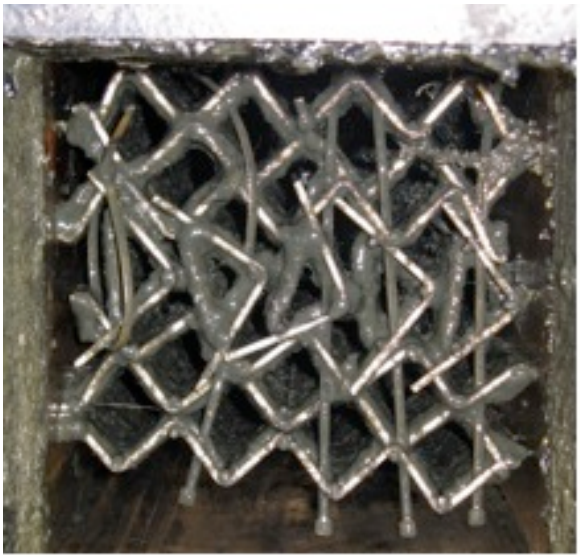
At day 172 both reactors were shut down. Accumulation of VFAs was occurring much faster than was observed for previous OLRs (although pH remained above 7) and biogas

production rates were decreasing, which was an indication that the reactors were reaching their operational limits. Throughout continuous reactor experiments no clogging of influent, effluent and recycle tubing was experienced, despite rather high total solids present in CTS. However, foaming which was initially observed during the 8.7 day HRT (approximately day 85 of digestion) began to accumulate in the headspace of both reactors and became excessive at the final HRT of 4.2 days. Wet-test tip meters used throughout the experiment to measure biogas produced became clogged with foam and accurate measurements could no longer be made at this point.

At the conclusion of this study the volume of biomass in each reactors was measured. Average biofilm thickness was measured, with an average thickness of 5mm (between 4 - 6mm) in Reactor #1 and 6mm (5 - 7mm) in Reactor #2. The thickness of the biofilm support media itself was 3mm prior to the development of the biofilm. The volume of biomass on the supports was measured and determined to be 4.7L of Reactor #1 and 5.6L for Reactor #2. A slight accumulation of biomass (sludge) was also observed in the bottom of each reactor. The accumulated sludge settled below the bottom of the biofilm support in the tapered portion of the reactors. Reactor #1 had an accumulation of approximately 1.8L of sludge, while Reactor #2 had slightly less build up with a volume of 1.7L. This corresponds to a total estimated biomass inventory of approximately 6.5L for Reactor #1 and 7.3L for Reactor #2. The slightly larger amount of biomass in Reactor #2 can be explained by the extra substrate that was accessible to its microbial population for growth, in comparison to Reactor #1.

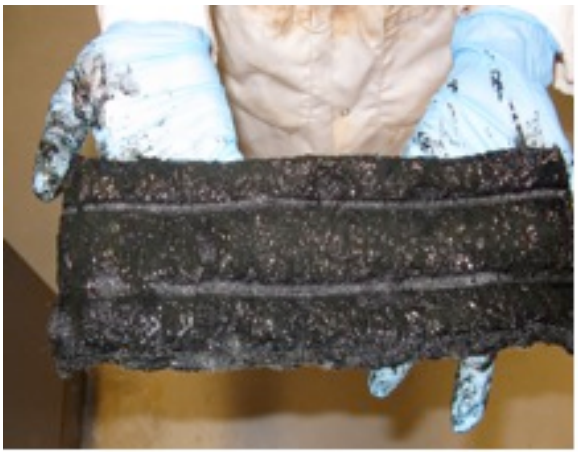
A visual inspection of reactor biofilms found that both exhibited a denser biomass population in the upper half of the reactors. Total and volatile solids analysis of the biofilms of Reactors #1 and #2 summarized in Table 4.18, indicate greater concentrations (for both TS and VS) on the upper half of the reactor than the lower half. Similar results were found by Murray and van den Berg (1981), and may be as a result of increased exposure to substrate, since feed enters DSFF Reactors from the top. Table 4.18 summarizes the total and volatile solids on the top and bottom halves of each reactor, as well as the sludge that accumulated in the bottom of each. Figures 4.18 (a), (b), (c) and (d)

show the biomass on the reactor supports within the DSFF reactors, on the supports and the thickness.

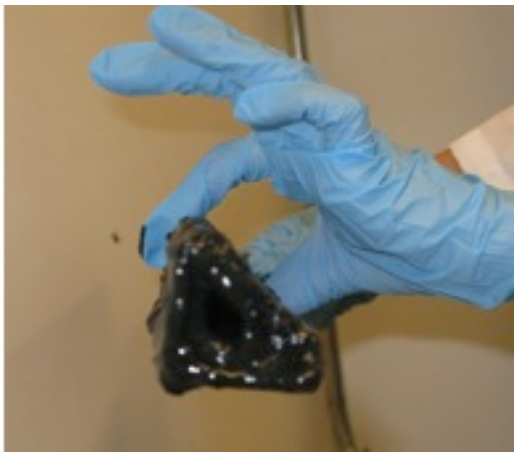


(a) Supports in the DSFF reactor

(b) Support with established biofilm



(c) NPP with established biofilm



(d) Side view of support with established biofilm

Figure 4.18 – DSFF reactor supports with biofilm established on NPP

Table 4.18 – Solids analysis for biofilm and accumulated sludge of DSFF reactors #1 and #2

Parameter	Reactor #1			Reactor #2		
	Top	Bottom	Sludge	Top	Bottom	Sludge
Total Solids (%w/w)	5.39 ± 0.42	5.29 ± 0.13	5.91 ± 0.02	7.33 ± 0.14	6.58 ± 0.05	5.56 ± 0.06
Volatile Solids (%w/w)	3.73 ± 0.15	2.46 ± 0.12	3.15 ± 0.01	3.92 ± 0.02	2.82 ± 0.06	2.69 ± 0
VS/TS ratio	0.70	0.50	0.53	0.50	0.43	0.48

When the reactor supports were examined, a crystalline clear/white substance was observed to have accumulated in both the underflow sludge as well as on the biomass support material. Previous studies by Agler et al., (2008) on anaerobic digestion of CTS also noted a white precipitate accumulation in the bottom of their reactors. Based on results of X-ray powder diffraction the authors concluded that the substance was likely struvite. It seems probable that this is also the substance observed in the DSFF reactors used in this research. Agler et al. concluded based on a nitrogen balance that magnesium was the limiting species for struvite formation, which was present at a concentration of 370mg/L in the thin stillage used for their research and 674 mg/L for the CTS used in this research. Although it is possible that struvite may result in operational problems in some reactor configurations as suggested by Agler et al. (2008), it did not appear to negatively impact the performance of the DSFF reactors. Despite the potentially negative impact of struvite, there is also potential to recover it for use as a slow-release fertilizer (Agler et al., 2008).

The total reactor biomass volumes calculated are in agreement with mixed liquor volume measurements taken from each reactor at the conclusion of the continuous reactor studies. The accumulation of biomass consequently alters the active mixed liquor volume of the reactors, and instead of a 25L active reactor volume, Reactor #1 actually has a mixed liquor volume of 18.5L and Reactor #2 has a volume of 17.7L. This in turn has an effect on the actual loading rates and HRTs of the reactors, which up until this point have been based on a 25L liquid volume in each reactor. Table 4.19 shows the adjusted HRTs and OLRs, which result from the accumulation of biomass and reduced volume of mixed liquor in

each reactor. Based on the volume of biofilm in the reactor as well as the VS/TS ratio and VS concentration of the biofilm material, R1 and R2 contained a biofilm biomass inventory of approximately 8.2 and 10.9g VS/L. At an HRT of 5 days the corresponding specific loading rate could be estimated as 0.9 and 0.93g COD/gVSS/d for R1 and R2 respectively. It would seem based on biogas production decrease and rapid accumulation of VFAs that the maximum specific loading rate for CTS is roughly 0.9 g COD/gVSS/d. This specific loading rate is approximately in agreement with the batch assay F/M ratios (between 0.7 and 1.5g COD/g VSS) determined to be the best for thin stillage digestion. The specific loading rates determined here are in agreement with the published literature values, which range from 0.9 - 1.2g COD/gVSS/d, for fixed film reactors (Najafpour et al., (2006), Kennedy and Droste, 1986)

Table 4.19 – Adjusted HRTs and OLRs for DSFF Reactors #1 and #2

Empty Bed HRT (d)	DSFF Reactor #1			DSFF Reactor #2		
	Biofilm Adjusted HRT (d)	Empty Bed OLR (gCOD/L/d)	Biofilm Adjusted OLR (gCOD/L/d)	Biofilm Adjusted HRT (d)	Empty Bed OLR (gCOD/L/d)	Biofilm Adjusted OLR (gCOD/L/d)
20	14.8	1.2	1.7	14.2	1.9	2.7
14.3	10.6	1.7	2.3	10.1	2.6	3.7
8.7	6.4	2.9	3.9	6.2	4.3	6.1
6.25	4.6	4.8	6.4	4.4	5.9	8.4
5	3.7	7.4	10.0	3.5	9.9	14.0
4.2	3.1	8.7	11.8	3.0	11.6	16.4

Upon the adjustments of hydraulic retention times, it appears that both reactors are likely able to handle higher organic loading rates that initially estimated. The best performance for Reactor #1 was observed at the theoretical HRT of 5 days corresponding to an OLR of 7.42 g TCOD/L/d, which upon adjustment indicates that the reactor can potentially truly handle an HRT of closer to 3.7 days and a corresponding adjusted OLR of 10.03 g TCOD/L/d. Reactor #2 performed the best at the theoretical HRT of 6.25 days, which is equal to an adjusted 4.4 day HRT, and an organic loading rate of 8.38 g TCOD/L/d.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Anaerobic digestion of corn ethanol thin stillage offers the potential to improve the net energy balance of corn grain ethanol. The use of thin stillage as a substrate for anaerobic digestion will reduce drying costs associated with the production of DDGS and produce methane, which can potentially be used to supplement ethanol plant power requirements. Anaerobic digestion of thin stillage as the sole carbon source was carried out successfully at a variety of organic concentrations, with and without acclimated biomass. Successful co-digestion with food waste and TWAS was also achieved, with and without acclimated biomass. However, when CTS was supplemented with additional nutrients and minerals, achieving stable and efficient digestion proved problematic.

A general conclusion that can be drawn from the initial suitability BMP assays carried out is the necessity of thin stillage dilution in order to facilitate anaerobic digestion. The high strength nature of thin stillage appears to limit the concentrations at which digestion can be effectively carried out. AD of thin stillage at a concentration of approximately 27,000 mg TCOD/L (F/M = 2.0) resulted in rapid accumulation of VFAs and a drop in pH despite adequate alkalinity (6000mg/L as $CaCO_3$), which was not observed at lower F/M ratios. This seems to imply the upper limit of organic concentrations suitable for anaerobic digestion of thin stillage. With an average full strength thin stillage concentration of 159,800 mg/L, a concentration of 27,000mg/L corresponds to a required dilution ratio of approximately 1 to 6.

The best performance observed for initial suitability studies was at an F/M ratio of 1.5, which corresponds to an organic concentration of 21,177 mg TCOD/L. At an F/M ratio of 1.5, TCOD, SCOD and VS removal efficiencies achieved were 85 ± 2%, 94 ± 0% and 82 ± 1%, respectively, with a methane yield of 0.30 L CH_4/g $COD_{destroyed}$. VFA production was

similar to that observed for the F/M ratio of 2.0, however no problematic changes in pH were observed.

Results obtained in the acclimation BMP assays appear to indicate that acclimation is advantageous of CTS as the sole carbon source for two reasons. Total COD removal efficiencies were improved compared to digestion with non-acclimated biomass by between 2 and 9%. As well, improvements of between 30 and 65% in biogas production rates (accounting for 80% of total biogas production) were observed. The observed increased biogas production rates are particularly beneficial, as this subsequently decreases the amount of digestion time required.

Assays investigating the use of digested effluent for substrate dilution presented in this thesis indicate promise for reductions in water consumption resulting from anaerobic digestion. Of assays conducted with 25%, 50% or 100% digested effluent used to supplement substrate dilution water; only assay bottles diluted with 100% effluent appeared to be negatively effected. Total COD removal efficiencies decreased from 83% in the first recycle BMP assay, to 76% and 72% in the 2nd and 3rd recycle assays. Assay bottles with CTS diluted with 100% digested effluent were also the only ones to exhibit a decrease in biogas production rates and total biogas production over the course of the three sequential recycle assays. Biogas production rates of all other effluent recycle assay bottles either improved or remained nearly the same. After the 1st recycle BMP assay, the biogas production rate was 60.7 mL/d for 100% effluent assay bottles, which decreased to 36.4 and 29.9 mL/d, in the 2nd and 3rd recycle assays respectively. An increase in ammonia was observed for the digestion of CTS with recycled effluent, with the maximum increase observed in the assay bottles diluted with 100% effluent. Concentrations were observed to increase between the 2nd and 3rd recycle assays by 6%, 20% and 25% for CTS diluted with 25%, 50% and 100% effluent respectively.

Volatile fatty acid analysis during the recycle assays also appeared to indicate reduced performance in the 100% effluent assay bottles. While all other effluent recycle assay bottles produced and consumed VFAs in essentially the same manner from one recycle assay to another, bottles with 100% effluent produced more acids, and required a greater amount of time to show reduced concentrations. This would appear to indicate that the

microbial populations in the 100% effluent assay bottles had shifted towards acid generating populations.

Continuous studies with down-flow fixed film reactors under mesophilic conditions provided excellent results under a variety of organic loading rates and hydraulic retention times. High organic loading rates at low HRTs were achieved, with removal efficiencies greater than 80%. Soluble COD removal efficiencies were maintained above 90%, and total COD and VS removal efficiencies were maintained above 85% until reactors were transitioned to organic loading rates of 8.7 g TCOD/L/d for DSFF reactor #1, corresponding to an HRT of 4.25 days and 9.9 g TCOD/L/d for DSFF reactor #2, corresponding to an HRT of 5 days. The maximum biogas yield was realized at an OLR of 7.4 g TCOD/L/d by DSFF reactor #1 at an HRT of 5 days, at 2.8 L/L/d. This corresponds to a methane yield of 2.05 L of CH_4/L/d. Continuous reactor results further solidify the conclusion that corn thin stillage requires dilution in order to achieve acceptable reactor performance drawn from BMP assay results. The lowest dilution factor that resulted in acceptable digestion performance was 1-part CTS to 3 parts water or a 1:4 dilution, at a maximum OLR of 7.4 g TCOD/L/d and an HRT of 5 days in DSFF reactor #1.

5.2 Recommendations for future research

The following recommendations are intended to build upon the research presented in this thesis, in order determine the full potential for the digestion of corn ethanol thin stillage:

- Examine the potential for co-digestion of thin stillage in a continuous system
- Determine the extent for the possible re-use of digested effluent as dilution water by conducting additional batch experiments that investigate the number of cycles that effluent can be used for dilution without affecting digestion performance.
- Examine the re-use of digested effluent as dilution water in a continuous system.
- Examine the potential for the application of a 2-phase anaerobic digestion system of reactors for AD of thin stillage, due to its tendency to produce acids rapidly when digested at high concentrations.

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APPENDIX A – BMP DATA

TABLE A.1 – BMP Ammonia data and standard curves

Initial Suitability BMP Assay				
F/M Ratio	**Replicate**	**Dilution**	**[mV]**	**NH_3-N (mg/L)**
Standard Curve			-25 -88 -145	10mg/L 100mg/L 1000mg/L
0.2	1 2 3	n/a n/a n/a	-105 -111 -116	207.23 260.89 316.06
0.5	1 2 3	n/a n/a n/a	-96 -99 -104	146.71 164.61 199.43
Standard Curve			ND	10mg/L 100mg/L 1000mg/L
0.7	1 2	n/a n/a	10.6 10.6	ND ND
1.0	1 2	n/a n/a	11 11.7	ND ND
1.5	1 2	n/a n/a	12.8 13.7	ND ND
2.0	1 2 3 4 5 6 7 8	n/a n/a n/a n/a n/a n/a n/a n/a	ND	ND
Acclimation BMP Assay				
F/M Ratio	**Replicate**	**Dilution**	**[mV]**	**NH_3-N (mg/L)**
Standard Curve			63 3.9 -50.7	10mg/L 100mg/L 1000mg/L
0.7	1 2	n/a n/a	-40.6 -37.1	646.94 561.43
1.0	1 2	n/a n/a	-39.9 -38.5	628.86 594.19

TABLE A.1 – BMP Ammonia data and standard curves (continued)

F/M Ratio	Replicate	Dilution	[mV]	NH_3-N (mg/L)
1.5	1	n/a	-34.4	503.28
	2	n/a	-41.5	670.96
2.0	1	n/a	ND	ND
	2	n/a	ND	ND
Co-digestion BMP Assay, without acclimation				
Standard Curve			63.5	10mg/L
			4.2	100mg/L
			-52.4	1000mg/L
0.7 + FW	1	n/a	-48.8	920.76
	2	n/a	-49.2	935.86
1.0 + FW	1	n/a	-48.6	913.31
	2	n/a	-48.4	905.91
1.5 + FW	1	n/a	-47.5	873.37
	2	n/a	-50.2	974.68
0.7 + TWAS	1	n/a	-50.6	990.66
	2	n/a	-53.6	1119.15
1.5 + TWAS	1	n/a	-46.9	852. 32
	2	n/a	-49.4	943.50
Co-digestion BMP Assay, with acclimation				
Standard Curve			63.5	10mg/L
			4.2	100mg/L
			-52.5	1000mg/L
0.7 + FW	1	n/a	-36.5	531.07
	2	n/a	-33.2	465.65
1.0 + FW	1	n/a	-36.9	539.60
	2	n/a	-40	610.54
1.5 + FW	1	n/a	-48.4	853.21
	2	n/a	-46.2	781.61
0.7 + TWAS	1	n/a	-41.2	640.44
	2	n/a	-41.3	642.99
1.5 + TWAS	1	n/a	-51.3	957.71
	2	n/a	-51.5	965.39
Supplemental Nutrients BMP Assay				
Standard Curve			63	10mg/L
			3.9	100mg/L
			-50.7	1000mg/L
0.7 + Co	1	n/a	-50.8	998.75
	2	n/a	-52.0	1048.68
1.0 + Co	1	n/a	-60.0	1451.70
	2	n/a	-55.2	1194.36
1.5 + Co	1	n/a	-45.3	798.65
	2	n/a	-59.2	1405.25

TABLE A.1 – BMP Ammonia data and standard curves (continued)

% Effluent	Bottle No.	Dilution	[mV]	NH_3-N (mg/L)
0.7 + MM	1	n/a	-46.4	835.18
	2	n/a	-48.1	894.93
1.0 + MM	1	n/a	-52.4	1065.87
	2	n/a	-52.7	1078.95
1.5 + MM	1	n/a	-52.8	1083.34
	2	n/a	-56.3	1248.98
1st Recycle BMP Assay				
Standard Curve			ND	10mg/L 100mg/L 1000mg/L
0%	27	n/a	ND	ND
	28	n/a		
25%	19	n/a	ND	ND
	20	n/a		
50%	11	n/a	ND	ND
	12	n/a		
100%	3	n/a	ND	ND
	4	n/a		
2nd Recycle BMP Assay				
Standard Curve			36.5 -10.2 -64.1	10mg/L 100mg/L 1000mg/L
0%	29(1)	n/a	-50.5	566.04
	29(2)	n/a	-50.6	568.64
	30(1)	n/a	-51	579.15
	30(2)	n/a	-51.7	598.00
25%	21(1)	n/a	-54.6	682.88
	21(2)	n/a	-56.7	751.77
	22(1)	n/a	-53.8	658.33
	22(2)	n/a	-54.7	686.01
50%	13(1)	n/a	-55.7	718.13
	13(2)	n/a	-56.6	748.33
	14(1)	n/a	-56.0	728.06
	14(2)	n/a	-55.8	721.43
100%	5(1)	n/a	-65.1	1104.19
	5(2)	n/a	ND	ND
	6(1)	n/a	-65.8	1140.13
	6(2)	n/a	-67.1	1210.03

TABLE A.1 – BMP Ammonia data and standard curves (continued)

3rd Recycle BMP Assay				
% Recycled Effluent	Bottle No.	Dilution	[mV]	NH_3-N (mg/L)
Standard Curve			38.9 -16.6 -66.9	10mg/L 100mg/L 1000mg/L
0%	31 32	1:2 1:2	-36.9 -36.3	531.26 517.52
25%	23 24	1:2 1:2	-44.5 -44.1	740.35 727.53
50%	15 16	1:2 1:2	-50.6 -45.9	996.32 787.02
100%	7 8	1:2 1:2	-62.6 -56.6	1631.92 1255.77

TABLE A.2 – BMP Final Solids Data

Initial Suitability BMP Assay							
F/M Ratio	Bottle No.	Dish (g)	Dish +Sample (g)	DW_{105}	DW_{550}	TS(%)	VS(%)
0.2	1	14.0449	20.0447	14.0966	14.0772	0.862	0.323
	2	13.0516	18.4777	13.0869	13.0755	0.651	0.210
	3	12.7561	17.7886	12.7885	12.778	0.644	0.209
0.5	1	14.0417	21.391	14.096	14.0786	0.739	0.237
	2	13.2853	19.8689	13.3399	13.3201	0.829	0.301
	3	12.9825	20.7256	13.0345	13.0168	0.672	0.229
0.7	*1*	14.0414	19.2406	14.0836	14.0676	*0.812*	0.308
	2	14.0134	18.1832	14.0469	14.0341	*0.803*	0.307
1.0	*1*	14.0643	19.3608	14.1104	14.0929	*0.870*	0.330
	2	14.1658	20.152	14.2187	14.1991	*0.884*	0.327
1.5	*1*	13.168	18.1593	13.2198	13.2002	*1.038*	0.393
	2	13.6698	14.9947	13.6841	13.6786	*1.079*	0.415
2.0	1(1)	10.2862	16.2173	10.3997	10.3663	1.914	0.563
	1(2)	10.842	18.3364	10.9874	10.9452	1.940	0.563

TABLE A.2 – BMP Final Solids Data (continued)

F/M Ratio	Bottle No.	Dish (g)	Dish +Sample (g)	DW_{105}	DW_{550}	TS(%)	VS(%)
2.0	2(1)	13.3361	18.6201	13.4361	13.4081	1.893	0.530
	2(2)	16.7253	28.667	16.9523	16.8896	1.901	0.525
	9(1)	13.5455	19.1153	13.6552	13.623	1.970	0.578
	9(2)	14.8224	20.9539	14.9533	14.9185	2.135	0.568
	10(1)	13.7198	20.2503	13.8464	13.8109	1.939	0.544
	10(2)	15.634	22.5788	15.7687	15.7309	1.940	0.544
	17(1)	13.9247	21.3032	14.0684	14.0262	1.948	0.572
	17(2)	14.0532	20.0251	14.169	14.1369	1.939	0.538
	18(1)	14.2688	20.2251	14.3843	14.3513	1.939	0.554
	18(2)	13.8479	20.4354	13.9749	13.9393	1.928	0.540
	25(1)	13.5997	19.8535	13.7201	13.6865	1.925	0.537
	25(2)	13.7076	20.5651	13.8405	13.8028	1.938	0.550
	26(1)	13.2018	19.4523	13.3237	13.2905	1.950	0.531
	26(2)	14.2886	19.9525	14.3994	14.3702	1.956	0.516
Acclimation BMP Assay							
0.7	*1*	10.9121	15.7822	10.9537	10.9417	**0.854**	0.246
	2	10.5123	16.0121	10.559	10.5446	**0.849**	0.262
1.0	*1*	10.3993	16.6027	10.4575	10.4401	**0.938**	0.280
	2	10.6399	16.4568	10.6945	10.6784	**0.939**	0.277
1.5	*1*	10.8454	17.0775	10.9182	10.8933	**1.168**	0.400
	2	10.6712	16.254	10.7346	10.7119	**1.136**	0.407
2.0	*1*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	2	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
Co-digestion BMP Assay, without acclimation							
0.7 + FW	1	12.085	16.0513	12.1719	12.1399	2.19	0.807
	2	13.7191	17.1848	13.7944	13.7666	2.17	0.802
1.0 + FW	1	14.2928	18.2056	14.3804	14.3477	2.24	0.836
	2	13.337	18.0408	ND	ND	ND	ND
1.5 +FW	1	12.0511	16.8559	12.1636	12.1212	2.34	0.882
	2	14.1827	18.3376	14.2795	14.2428	2.33	0.883
0.7 + TWAS	1	14.8375	19.0286	14.9301	14.8901	2.21	0.954
	2	14.1347	18.4701	14.2306	14.1943	2.21	0.837
1.5 + TWAS	1	14.0735	18.7305	14.188	14.1443	2.46	0.938
	2	13.5483	17.5017	13.6454	13.6082	2.46	0.941

TABLE A.2 – BMP Final Solids Data (continued)

Co-digestion BMP Assay, with acclimation							
F/M Ratio	**Bottle No.**	**Dish (g)**	**Dish +Sample (g)**	**DW_{105}**	**DW_{550}**	**TS(%)**	**VS(%)**
0.7 + FW	1	13.82	19.281	13.9295	13.9018	2.01	0.507
	2	13.6035	18.9734	13.7101	13.6823	1.99	0.518
1.0 + FW	1	14.4978	19.8337	14.612	14.582	2.14	0.562
	2	13.7162	19.5642	13.8429	13.81	2.17	0.563
1.5 +FW	1	13.8848	19.9186	14.0293	13.9892	2.39	0.665
	2	12.0413	18.44	12.1886	12.1478	2.30	0.638
0.7 + TWAS	1	10.5114	17.0078	10.6377	10.6041	1.94	0.517
	2	13.9294	19.6084	14.0407	14.0106	1.96	0.530
1.5 + TWAS	1	10.2595	16.1805	10.4054	10.3634	2.46	0.709
	2	10.3602	17.281	10.5285	10.4795	2.43	0.708
Supplemental Nutrients BMP Assay							
0.7 + Co	1	12.0847	18.1534	12.1745	12.1446	1.48	0.493
	2	13.8809	20.2812	13.9784	13.9473	1.52	0.486
1.0 + Co	1	10.1479	15.4912	10.2492	10.2206	1.90	0.535
	2	14.2722	19.7228	14.384	14.3546	2.05	0.539
1.5 + Co	1	13.0948	18.9262	13.2417	13.2041	2.52	0.645
	2	14.2927	20.8522	14.4628	14.418	2.59	0.683
0.7 + MM	1	14.073	19.473	14.1584	14.1308	1.58	0.511
	2	14.5087	20.2618	14.599	14.5702	1.57	0.501
1.0 + MM	1	13.7171	19.6456	13.8208	13.7912	1.75	0.499
	2	14.8367	21.3685	14.9694	14.935	2.03	0.527
1.5 + MM	1	14.1431	20.3557	14.2992	14.2604	2.51	0.625
	2	13.7099	19.9364	13.8663	13.8263	2.51	0.642
2nd Recycle BMP Assay							
0%	29(1)	14.5148	18.5598	14.5882	14.5649	1.8146	0.576
	29(2)	14.1325	18.6595	14.2153	14.1895	1.8290	0.570
	30(1)	14.1855	18.2944	14.2629	14.2374	1.8837	0.621
	30(2)	13.7335	17.7686	13.8076	13.7848	1.8364	0.565
	S(1)	14.1893	19.1342	14.2467	14.2372	1.1608	0.192
	S(2)	13.2561	18.4113	13.3157	13.3062	1.1561	0.184
25%	21(1)	14.7037	18.2114	14.7756	14.7532	2.0498	0.639
	21(2)	15.4374	19.3028	15.5169	15.4919	2.0567	0.647
	22(1)	14.1578	18.8725	14.2512	14.2223	1.9810	0.613
	22(2)	13.2816	16.4726	13.3459	13.3249	2.0150	0.658

TABLE A.2 – BMP solids data (continued)

% Recycled Effluent	Bottle No.	Dish (g)	Dish +Sample (g)	DW_{105}	DW_{550}	TS(%)	VS(%)
25%	S(1)	13.7011	18.8537	13.7673	13.758	1.2848	0.181
	S(2)	10.3904	15.4182	10.4554	10.4458	1.2928	0.191
50%	13(1)	14.1811	18.5906	14.2693	14.242	2.0002	0.619
	13(2)	12.0745	16.4474	12.1618	12.1354	1.9964	0.604
	14(1)	15.6152	19.2787	15.6907	15.6672	2.0609	0.642
	14(2)	14.1067	18.3353	14.1943	14.1665	2.0716	0.657
	S(1)	13.711	17.7775	13.7649	13.7554	1.3255	0.234
	S(2)	12.8605	18.3116	12.9312	12.92	1.2970	0.206
100%	5(1)	13.3306	17.3441	13.4238	13.3973	2.3222	0.660
	5(2)	13.3818	16.8951	13.4646	13.4419	2.3568	0.646
	6(1)	13.816	18.0685	13.9148	13.8891	2.3233	0.604
	6(2)	14.0103	17.5649	14.0946	14.0715	2.3716	0.650
	S(1)	12.6403	15.0957	12.6839	12.6761	1.7757	0.312
	S(2)	10.8992	13.944	10.9529	10.9443	1.7637	0.282
3rd Recycle BMP Assay							
0%	31(1)	13.5923	20.731	13.7258	13.684	1.8701	0.586
	31(2)	13.4551	19.8336	13.5735	13.5378	1.8562	0.560
	32(1)	10.6585	17.9437	10.7986	10.7563	1.9231	0.581
	32(2)	14.0486	20.1717	14.1658	14.1265	1.9141	0.642
25%	23(1)	13.2344	19.2907	13.3602	13.3232	2.0772	0.616
	23(2)	13.7107	19.5809	13.8309	13.7945	2.0476	0.620
	24(1)	11.9586	18.6606	12.0972	12.0561	2.0680	0.613
	24(2)	13.6603	20.1988	13.796	13.7556	2.0754	0.618
50%	15(1)	14.3958	21.2214	14.5455	14.5013	2.1932	0.648
	15(2)	13.9199	20.0404	14.0552	14.0144	2.2106	0.667
	16(1)	13.0851	19.5587	13.2272	13.1848	2.1951	0.655
	16(2)	13.7277	19.3804	13.8517	13.8139	2.1936	0.669
100%	(1)	14.2325	20.211	14.3998	14.3573	2.7984	0.711
	5(2)	13.5443	20.3618	13.7354	13.6871	2.8031	0.709
	6(1)	10.7983	17.8039	10.9927	10.9439	2.7749	0.709
	6(2)	13.2493	19.504	13.4236	13.3796	2.7867	0.697

TABLE A.3 – BMP total and soluble COD data

Initial Suitability BMP Assay – Slope = 0.0007, b = 0.0169							
F/M Ratio	No.	Soluble COD			Total COD		
		Dilution	Abs. @ 600nm	COD (mg/L)	Dilution	Abs @ 600nm	COD (mg/L)
0.2	1	2	0.12	344.0	10	0.28	3934.3
	1	2	ND	ND	10	ND	ND
	2	2	0.12	344.0	10	0.20	2862.9
	2	2	ND	ND	10	ND	ND
	3	2	0.13	372.6	10	0.25	3577.1
	3	2	ND	ND	10	ND	ND
0.5	1	2	0.14	401.1	10	0.23	3291.4
	1	2	ND	ND	10	ND	ND
	2	2	0.13	372.6	10	0.29	4148.6
	2	2	ND	ND	10	ND	ND
	3	2	0.16	458.3	10	0.21	3005.7
	3	2	ND	ND	10	ND	ND
0.7	1(1)	2	0.16	408.9	10	0.36	4901.4
	1(2)	2	0.16	408.9	10	0.37	5044.3
	2(1)	2	0.16	408.9	10	0.35	4758.6
	2(2)	2	0.17	437.4	10	0.32	4330.0
1.0	1(1)	2	0.19	494.6	10	0.35	4758.6
	1(2)	2	0.18	466.0	10	0.35	4758.6
	2(1)	2	0.18	466.0	10	0.35	4758.6
	2(2)	2	0.18	466.0	10	0.36	4830.0
1.5	1(1)	2	ND	ND	10	0.40	5472.9
	1(2)	2	0.27	723.1	10	0.45	6187.1
	1(3)	ND	ND	ND	10	0.45	6187.1
	2(1)	2	0.29	780.3	20	0.23	6088.6
	2(2)	2	0.30	808.9	20	0.23	6088.6
2.0	1(1)	5	0.13	931.4	20	0.27	7725.7
	1(2)	10	0.06	862.9	20	0.27	7725.7
	2(1)	5	0.11	788.6	20	0.28	8011.4
	2(2)	5	0.12	860.0	20	0.28	8011.4
	9(1)	5	0.14	1002.9	20	0.29	8297.1
	9(2)	5	0.13	931.4	20	0.29	8297.1
	10(1)	5	0.14	1002.9	25	0.24	8585.7
	10(2)	5	0.15	1074.3	25	0.22	7871.4
	17(1)	5	0.15	1074.3	25	0.22	7871.4
	17(2)	5	0.14	1002.9	25	0.23	8228.6
	18(1)	5	0.14	1002.9	25	0.22	7871.4
	18(2)	5	0.15	1074.3	25	0.23	8228.6
	25(1)	5	0.12	860.0	25	0.22	7871.4
	25(2)	5	0.12	860.0	25	0.21	7514.3
	26(1)	5	0.14	1002.9	25	0.22	7871.4

TABLE A.3 – BMP total and soluble COD data (continued)

Acclimation BMP Assay – Slope = 0.0007, b = 0.0169							
F/M Ratio	No.	Soluble COD			Total COD		
		Dilution	Abs. @ 600nm	COD (mg/L)	Dilution	Abs @ 600nm	COD (mg/L)
0.7	1(1)	2	0.12	294.57	10	0.26	3472.9
	1(2)	2	0.13	308.9	10	0.22	2901.4
	2(a)	2	0.13	308.9	10	0.25	3330.0
	2(b)	2	0.13	323.1	10	0.22	2901.4
1.0	1(a)	2	0.16	408.9	10	0.27	3615.7
	1(b)	2	0.19	494.6	10	0.31	4187.1
	2(a)	2	0.17	437.4	10	0.24	3187.1
	2(b)	2	0.16	408.9	10	0.29	3830.0
1.5	1(a)	2	0.33	894.6	10	0.43	5901.4
	1(b)	2	0.34	923.1	10	0.41	5615.7
	2(a)	3.33	0.17	704.5	20	0.22	5802.9
	2(b)	3.33	0.18	775.9	20	0.20	5231.4
	2(c)	ND	ND	ND	20	0.21	5517.1
	2(d)	ND	ND	ND	20	0.22	5802.9
2.0	1(a)	10	0.17	2434.3	25	0.2	7157.14
	1(b)	10	0.16	2291.4	25	0.2	7157.14
	2(a)	10	0.19	2720	25	0.21	7692.9
	2(b)	10	0.19	2720	25	0.22	7871.4
Co-digestion BMP Assay, without acclimation							
0.7 + FW	1(a)	3.33	0.17	884.4	100	0.1	12041.3
	1(b)	3.33	0.12	646.5	100	0.1	12041.3
	2(a)	3.33	0.12	646.5	100	0.1	12041.3
	2(b)	3.33	0.12	646.5	100	0.1	12041.3
1.0 + FW	1(a)	3.33	0.14	741.6	100	0.1	12041.3
	1(b)	3.33	0.14	741.6	100	0.1	12041.3
	2(a)	3.33	0.14	741.6	100	0.1	12041.3
	2(b)	3.33	0.14	741.6	100	0.1	12041.3
1.5 + FW	1(a)	3.33	0.15	789.2	100	0.09	10585.7
	1(b)	3.33	0.16	813.0	100	0.09	10585.7
	2(a)	3.33	0.16	836.8	100	0.1	12041.3
	2(b)	3.33	0.16	836.8	100	0.12	14871.4
0.7 + TWAS	1(a)	5	0.1	827.9	100	0.1	12041.3
	1(b)	5	0.1	827.9	100	0.1	12041.3
	2(a)	5	0.08	685.0	100	0.1	12041.3
	2(b)	5	0.08	685.0	100	0.1	12041.3

TABLE A.3 – BMP total and soluble COD data (continued)

F/M Ratio	No.	Soluble COD			Total COD		
		Dilution	Abs. @ 600nm	COD (mg/L)	Dilution	Abs @ 600nm	COD (mg/L)
TWAS	1(b)	5	0.11	899.3	100	0.1	12041.3
	2(a)	5	0.14	1113.6	100	0.12	14871.4
	2(b)	5	0.11	899.3	100	0.11	13442.9
Co-digestion BMP Assay, with acclimation							
0.7 + FW	1(1)	2	0.17	426.0	50	0.11	6721.4
	1(2)	2	0.17	440.3	50	0.10	6007.1
	2(a)	2	0.17	440.3	50	0.10	6007.1
	2(b)	2	0.17	440.3	50	0.11	6721.4
1.0 + FW	1(a)	3.33	0.19	828.2	50	0.12	7435.7
	1(b)	3.33	0.20	875.8	50	0.12	7435.7
	2(a)	3.33	0.21	923.4	50	0.12	7435.7
	2(b)	3.33	0.18	756.9	50	0.12	7435.7
1.5 + FW	1(a)	3.33	0.27	1208.8	50	0.13	8150.0
	1(b)	3.33	0.27	1208.8	50	0.14	8507.1
	2(a)	3.33	0.28	1256.4	50	0.15	9221.4
	2(b)	3.33	0.30	1351.5	50	0.14	8507.1
0.7 + TWAS	1(a)	3.33	0.13	554.6	50	0.12	7435.7
	1(b)	2	0.21	542.8	50	0.13	8150.0
	2(a)	2	0.20	526	50	0.11	6721.4
	2(b)	2	0.19	497.4	50	0.13	8150.0
1.5 + TWAS	1(a)	3.33	0.24	1066.1	50	0.15	9221.4
	1(b)	3.33	0.23	1018.5	50	0.14	8507.1
	2(a)	3.33	0.21	923.4	50	0.16	10,292.9
	2(b)	3.33	0.22	970.9	50	0.16	9935.7
Supplemental Nutrients BMP assay							
0.7 + Co	1(a)	5	0.1	588.5	50	0.11	6614.0
	1(b)	5	0.1	588.5	50	0.13	7707.5
	2(a)	5	0.1	588.5	50	0.14	8436.5
	2(b)	5	0.11	625.0	50	0.11	6614.0
1.0 + Co	1(a)	5	0.1	588.5	50	0.13	7707.5
	1(b)	5	0.1	588.5	50	0.12	7343.0
	2(a)	5	0.1	588.5	20	0.27	7238.3
	2(b)	5	0.11	625.0	20	0.27	7238.3

TABLE A.3 – BMP total and soluble COD data (continued)

F/M Ratio	No.	Soluble COD			Total COD		
		Dilution	Abs. @ 600nm	COD (mg/L)	Dilution	Abs. @ 600nm	COD (mg/L)
1.5 + Co	1(a)	5	0.27	1827.8	20	0.32	8769.2
	1(b)	5	0.26	1754.9	20	0.32	8769.2
	2(a)	5	0.25	1682.0	20	0.31	8477.6
	2(b)	5	0.25	1682.0	20	0.31	8477.6
0.7 + MM	1(a)	3.33	0.13	537.6	20	0.23	6144.8
	1(b)	3.33	0.15	634.7	20	0.22	5853.2
	2(a)	3.33	0.15	634.7	20	0.22	5853.2
	2(b)	3.33	0.14	586.1	20	0.22	5853.2
1.0 + MM	1(a)	3.33	0.14	586.1	20	0.24	6436.4
	1(b)	3.33	0.13	537.6	20	0.25	6728.0
	2(a)	3.33	0.14	586.1	20	0.27	7311.2
	2(b)	3.33	0.15	634.7	20	0.25	6728.0
1.5 + MM	1(a)	3.33	0.32	1460.1	20	0.27	7311.2
	1(b)	3.33	0.33	1508.6	20	0.28	7602.8
	2(a)	3.33	0.33	1508.6	20	0.31	8477.6
	2(b)	3.33	0.32	1460.1	20	0.30	8186.0
1st Recycle BMP Assay							
0%	27(a)	10	0.17	2434.3	25	0.2	7157.1
	27(b)	10	0.16	2291.4	25	0.20	7157.1
	28(a)	10	0.19	2720.0	25	0.22	7871.4
	28(b)	10	0.19	2720.0	25	0.22	7871.4
25%	19(a)	10	0.14	2005.7	25	0.19	6800.0
	19(b)	10	0.15	2148.6	25	0.19	6800.0
	20(a)	10	0.14	2005.7	25	0.19	6800.0
	20(b)	10	0.15	2148.6	25	0.20	7157.1
50%	11(a)	10	0.21	3005.7	25	0.20	7157.1
	11(b)	10	0.20	2862.9	25	0.21	7514.3
	12(a)	10	0.16	2291.4	25	0.20	7157.1
	12(b)	10	0.16	2291.4	25	0.19	6800.0
100%	3(a)	10	0.19	2720.0	25	0.27	9657.1
	3(b)	10	0.19	2720.0	25	0.27	9657.1
	4(a)	10	0.19	2720.0	25	0.23	8228.6
	4(b)	10	0.19	2720.0	25	0.24	8585.7

TABLE A.3 – BMP total and soluble COD data (continued)

2nd Recycle BMP Assay							
% Effluent	No.	Soluble COD			Total COD		
		Dilution	Abs. @ 600nm	COD (mg/L)	Dilution	Abs. @ 600nm	COD (mg/L)
0%	29(a)	5	0.11	788.6	25	0.14	5014.3
	29(b)	5	0.15	1074.3	25	0.17	8106.2
	30(a)	5	0.12	824.3	33.3	0.18	8581.9
	30(b)	5	0.11	788.6	33.3	0.17	8106.2
25%	21(a)	5	0.12	860.0	33.3	0.2	9533.3
	21(b)	5	0.11	788.6	33.3	0.2	9533.3
	22(a)	5	0.10	717.1	40	0.14	5014.3
	22(b)	5	0.10	717.1	40	0.14	5014.3
50%	13(a)	5	0.10	717.1	33.3	0.19	9057.6
	13(b)	5	0.11	788.6	33.3	0.19	9057.6
	14(a)	5	0.10	717.1	40	0.16	9165.7
	14(b)	5	0.10	717.1	40	0.15	8594.3
100%	5(a)	5	0.15	1074.3	40	0.16	9165.7
	5(b)	5	0.15	1074.3	40	0.16	9165.7
	6(a)	5	0.15	1074.3	40	0.16	9165.7
	6(b)	5	0.16	1145.7	40	0.15	8594.3
3rd Recycle BMP Assay							
0%	31(a)	3.33	0.16	736.1	25	0.24	8585.7
	31(b)	3.33	0.15	715.5	25	0.24	8585.7
	32(a)	3.33	0.14	667.9	25	0.20	7157.1
	32(b)	3.33	0.14	667.9	25	0.21	7514.3
25%	23(a)	3.33	0.16	763.1	25	0.25	8942.9
	23(b)	3.33	0.16	763.1	25	0.26	9300.0
	24(a)	3.33	0.16	763.1	25	0.25	8942.9
	24(b)	3.33	0.16	763.1	25	0.26	9300.0
50%	15(a)	3.33	0.20	953.3	25	0.30	10,728
	15(b)	3.33	0.18	858.2	25	0.27	9657.1
	16(a)	3.33	0.18	858.2	25	0.29	10,371.4
	16(b)	3.33	0.17	810.6	25	0.29	10,371.4
100%	7(a)	2	0.51	1458.3	25	0.29	10,371.4
	7(b)	2	0.51	1458.3	25	0.28	10,041.3
	8(a)	3.33	0.29	1381.5	25	0.27	9657.1
	8(b)	3.33	0.27	1262.6	25	0.29	10,371.4

TABLE A.4 – BMP Volatile Fatty Acid data

Initial Suitability BMP Assay						
F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
0.2	Day 7	1	946.90	175.38	11.23	1133.51
		2	826.62	205.47	10.84	1042.93
		3	799.44	202.30	9.65	1011.39
	Day 17	1	21.04	5.22	4.90	31.16
		2	43.53	4.16	3.27	50.96
		3	28.21	3.34	3.05	34.60
	Day 35	1	22.64	5.47	5.88	33.99
		2	22.66	3.91	4.68	31.25
		3	17.87	3.34	4.05	25.26
0.5	Day 7	1	1465.66	583.33	55.55	2104.54
		2	1561.86	608.83	56.38	2227.07
		3	1143.84	563.95	27.21	1735.00
	Day 17	1	389.36	13.33	2.90	405.59
		2	181.41	5.04	2.51	188.96
		3	642.89	81.91	3.53	728.33
	Day 35	1	20.40	2.35	3.44	26.19
		2	16.19	2.04	3.16	21.39
		3	17.49	1.60	2.86	21.95
0.7	Day 6	1	1022.60	992.86	50.35	2065.81
		2	1124.98	1092.12	20.84	2237.94
	Day 13	1	117.89	251.36	32.77	402.02
		2	107.61	487.95	24.61	620.17
	Day 20	1	87.29	54.75	19.94	161.98
1.0	Day 6	1	1746.58	1597.55	88.16	3432.29
		2	2010.62	1681.93	76.01	3768.56
	Day 13	1	276.48	1439.08	43.02	1758.58
		2	262.92	1170.61	29.56	1463.09
	Day 20	1	72.41	38.57	16.42	127.40
		2	63.84	24.72	12.86	101.42
	Day 41	1	18.42	41.22	3.33	62.97
		2	17.65	32.00	3.27	52.92
1.5	Day 6	1	3340.89	2444.90	120.40	5906.19
		2	2858.55	2295.00	192.05	5345.60
	Day 13	1	94.48	2523.33	66.06	2683.87
		2	110.51	2285.50	13.03	2409.04
	Day 20	1	67.46	1690.90	9.89	1768.25
	Day 41	1	37.33	25.22	3.21	65.76
		2	52.48	22.18	2.60	77.26
2.0	Day 6	1	3245.12	2385.05	533.00	6163.17

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
2.0	Day 6	2	3423.14	2668.91	371.69	6463.73
		9	3653.76	2661.49	457.61	6772.86
		10	3543.02	2687.34	489.17	6719.52
		17	3562.28	2703.80	463.87	6729.95
		18	3401.78	2655.82	592.66	6650.26
		25	3336.30	2722.16	650.15	6708.61
		26	3332.31	2746.11	558.81	6637.23
	Day 15	9	335.51	2587.26	53.37	2976.15
		10	416.39	2726.04	17.94	3160.37
	Day 23	1	30.94	2478.50	3.17	2512.61
		2	44.21	2887.14	4.06	2935.41
		9	34.97	2683.73	3.35	2722.04
		10	28.47	2826.43	3.42	2858.33
		17	30.01	2846.04	3.26	2879.31
		18	27.65	2808.57	3.30	2839.51
		25	37.27	2913.16	3.66	2954.09
		26	31.16	2912.41	3.75	2947.32
	Day 30	1	281.34	275.11	79.20	635.66
		2	65.21	1784.76	24.75	1874.73
		9	43.19	72.35	17.88	133.42
		10	136.34	567.53	15.97	719.84
		17	232.42	771.67	11.05	1015.14
		18	182.09	873.80	11.36	1067.25
		25	209.81	218.16	5.87	433.85
		26	205.77	995.06	10.48	1211.30
	Day 37	1	49.99	2.48	2.75	55.22
		2	25.51	1.90	2.69	30.10
		9	41.47	1.87	2.78	46.12
		10	38.59	2.92	2.77	44.28
		17	43.01	1.69	2.65	47.36
		18	49.05	2.31	2.73	54.09
		25	51.44	2.33	2.70	56.47
		26	53.99	1.86	2.54	58.39
	Day 51	1(a)	43.1	10.2	17.7	71.08
		1(b)	41.8	2.96	8.4	53.08
		1(c)	38.4	2.47	6.5	47.34
		2(a)	28.3	2.20	5.97	36.43

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
2.0	Day 51	2(b)	27.0	2.1	5.69	34.9
		2(c)	26.9	1.87	3.28	28. 8
		9(a)	43.1	4.19	6.98	54.30
		9(b)	44.2	2.26	3.11	49.6
		9(c)	44.2	2.05	5.10	51.4
		10(a)	76.4	5.92	7.61	90.0
		10(b)	78.4	2.34	5.55	86.3
		10(c)	79.3	2.41	2.68	84.4
		17(a)	34.2	2.02	2.80	39.0
		17(b)	30.6	2.02	2.48	35.1
		17(c)	36.4	12.2	9.4	58.0
		18(a)	53.7	2.2	2.4	58.3
		18(b)	53.2	6.1	6.8	66.1
		18(c)	53.7	2.2	5.3	61.2
		25(a)	86.4	2.1	4.9	93.4
		25(b)	86.2	6.4	7.9	100.5
		25(c)	85.9	6.9	8.3	101.0
		26(a)	33.4	1.8	2.2	37.3
		26(b)	32.9	6.7	7.5	47.1
		16(c)	32.6	1.3	2.0	35.9
Acclimation BMP Assay						
0.7	Day 7	1	1152.06	852.50	64.59	2069.15
		2	1395.98	956.21	53.20	2405.39
	Day 22	1	15.89	5.09	4.76	25.74
		2	15.42	4.12	4.37	23.91
	Day 43	1(a)	197.83	68.14	87.08	353.05
		1(b)	159.39	76.07	49.95	285.41
		2(a)	137.61	47.00	34.29	218.90
		2(b)	112.49	36.47	23.85	172.81
1.0	Day 7	1	1395.44	1379.09	110.99	2885.52
		2	1277.71	1231.90	121.60	2631.21
	Day 22	1	13.43	15.55	4.18	33.16
		2	14.40	4.15	3.84	22.39
	Day 43	1(a)	96.93	26.35	17.60	140.88
		1(b)	87.06	20.27	14.32	121.65
		2(a)	76.64	14.92	11.25	102.81
		2(b)	79.91	12.36	9.71	101.98
1.5	Day 7	1	1558.50	2264.84	386.13	4209.47
		2	1558.09	2235.93	391.27	4185.29
	Day 22	1	284.69	1531.14	5.72	1821.55
		2	246.24	1657.86	5.49	1909.59

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
1.5	Day 43	1(a)	130.80	10.58	8.58	149.96
		1(b)	133.60	11.02	8.00	152.62
		2(a)	98.84	10.74	6.62	116.20
		2(b)	97.54	10.17	5.82	113.53
2.0	Day 6	27	1049.87	1810.80	92.90	2953.57
		28	1247.64	1573.80	195.68	3017.12
	Day 15	27	418.84	1738.50	40.44	2197.78
		28	598.83	1546.20	20.87	2165.90
	Day 22	27	44.12	3.61	4.35	52.08
		28	87.30	4.04	9.90	101.23
	Day 30	27	51.69	3.80	12.64	68.12
		28	64.49	3.85	5.33	73.67
	Day 37	27	40.8	13.78	25.21	79.80
		28	35.0	7.13	18.56	60.71
Co-digestion BMP Assay, without acclimation						
0.7 + FW	Day 7	1	2110.8	717.1	78.6	2906.4
		2	1687.4	652.4	62.7	2402.4
	Day 14	1	62.1	439.5	4.6	506.3
		2	73.8	742.4	4.3	820.4
	Day 21	1	27.6	1.6	3.7	32.9
		2	29.3	1.1	3.7	34.1
	Day 49	1	35.5	15.1	11.8	62.4
		2	32.0	8.6	10.1	50.7
	Day 51	1	29.7	4.8	4.7	39.3
1.0 + FW	Day 7	1	3218.9	1101.7	99.8	4420.2
		2	2981.0	981.9	121.1	4084.0
	Day 14	1	312.6	1328.3	14.1	1655.0
		2	295.1	1244.7	15.4	1555.2
	Day 21	1	23.5	2.0	3.33	28.9
		2	22.7	3.7	0	26.5
	Day 49	1	28.7	5.2	8.6	42.5
		2	23.8	5.8	7.1	36.7
	Day 51	1	27.1	3.2	3.6	33.8
1.5 + FW	Day 7	1	77.1	2116.7	326.4	2520.2
		2	118.6	2056.9	213.3	2388.8
	Day 14	1	217.3	2524.5	15.3	2757.1
		2	95.9	2437.5	16.4	2549.8
	Day 21	1	62.1	1311.6	4.1	1377.8
		2	50.9	1997.2	4.3	2052.4
	Day 49	1	39.6	5.2	6.4	51.2

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
1.5 + FW	Day 49	2	36.4	5.5	5.4	47.3
	Day 51	1	25.8	3.5	3.2	32.5
0.7 + TWAS	Day 7	1	450.1	399.7	8.3	858.1
		2	422.5	375.5	3.6	801.6
	Day 14	1	55.7	829.6	8.3	893.7
		2	44.7	665.6	6.5	716.8
	Day 21	1	36.5	10.2	4.8	51.5
		2	31.8	8.7	4.3	44.8
	Day 49	1	101.0	70.1	49.7	220.8
		2	70.7	42.7	30.1	143.6
	Day 51	1	100.3	44.6	29.9	174.8
1.5 + TWAS	Day 7	1	25.6	1297.6	76.0	1399.2
		2	23.7	1540.4	40.3	1604.4
	Day 14	1	491. 9	2179.3	24.2	2695.3
		2	513.4	2209.6	35.9	2758.9
	Day 21	1	35.0	1.0	4.1	40.1
		2	32.3	4.0	3.9	40.1
	Day 49	1	55.7	28.4	20.7	104.8
		2	46.1	19.2	14.2	79.6
	Day 51	1	43.6	1.3	7.8	60.5
Co-digestion BMP Assay, with acclimation						
0.7 + FW	Day 2	1	2018.3	795.5	236.4	3050.2
		2	2430.1	654.2	210.3	3294.6
	Day 8	1	2499.0	1891.6	94.2	4484.8
		2	2307.4	1557.9	111.9	3977.3
	Day 13	1	1157.0	2078.5	30.7	3266.2
		2	702.4	1615.8	17.3	2335.4
	Day 52	1	38.0	95.9	6.1	140.1
		2	35.7	109.4	9.5	154.6
1.0 + FW	Day 2	1	2713.0	909.4	590.8	4213.2
		2	3253.7	1039.5	744.8	5038.1
	Day 8	1	2113.1	2917.2	302.8	5333.1
		2	1914.4	2686.1	161.7	4762.2
	Day 13	1	1324.6	3061.0	43.8	4429.4
		2	864.4	2765.0	16.8	3646.2
	Day 52	1	74.4	105.3	7.2	186.9
		2	105.4	76.6	5.1	187.2
1.5 + FW	Day 2	1	4164. 4	1777.5	1048.7	6990.6
		2	4293.5	523.5	1519.5	6336.5
	Day 8	1	3965.1	4262.5	964.3	9191.9

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

F/M Ratio	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
1.5 + FW	Day 8	2	3309	2758.8	630.0	6697.8
	Day 13	1	4673.7	3877.0	245.9	8796.6
		2	2892.7	2823.5	116.8	5833.0
	Day 52	1	455.3	82.4	6.6	544.3
		2	85.0	59.5	4.2	148.6
0.7 + TWAS	Day 2	1	741.5	200.1	124.7	1066.4
		2	770.8	258.0	84.4	1113.2
	Day 8	1	2282.1	1162.9	174.7	3619.6
		2	2398.9	1280.9	48.8	3728.6
	Day 13	1	1676.1	1404.5	174.8	3255.4
		2	2330.1	1292.8	142.9	3756.7
	Day 52	1	1.6	68.4	4.7	74.7
		2	47.7	25.7	18.3	91.8
1.5 + TWAS	Day 2	1	1622.7	701.4	399.4	2723.4
		2	1950.9	809.6	745.5	3506.0
	Day 8	1	3694.4	3235.9	743.9	7674.2
		2	3954.9	2934.0	474.6	7363.5
	Day 13	1	5118.7	3375.0	349.0	8842.7
		2	4822.1	3798.7	504.3	9125.1
	Day 52	1	19.1	141.7	7.6	168.5
		2	33.7	103.3	4.1	141.2
Supplemental Nutrients BMP Assay						
0.7 + Co	Day 6	1	2562.1	2228.4	222.7	5013.2
		2	2628.1	2035.5	78.6	4742.2
	Day 15	1	1952.3	2543.2	208.4	4703.9
		2	2468.1	2914.5	115.6	5498.2
	Day 35	1	4213.3	3064.4	190.8	7468.5
		2	ND	ND	ND	ND
	Day 57	1	37.4	164.5	2.8	204.7
		2	93.5	78.0	2.9	174.4
	Day 82	1(a)	139.4	20.3	4.5	164.2
		1(b)	128.5	19.5	4.6	152.6
		2(a)	141.8	12.8	4.3	158.9
		2(b)	154.1	17.4	4.4	175.9
1.0 + Co	Day 6	1	2792.7	2378.2	519.1	5690.1
		2	3647.8	3247.5	329.5	7224.8
	Day 15	1	2287.6	2673.5	504.4	5465.5
		2	2750.0	2307.0	336.0	5789.0
	Day 35	1	2830.5	1521.6	3.6	4355.7

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
1.0 + Co	Day 35	2	3905.4	2458.5	81.9	6445.8
	Day 57	1	78.4	6.4	2.5	87.3
		2	51	5.1	2.7	58.8
	Day 82	1(a)	297.3	130.9	11.3	439.5
		1(b)	225.7	88.1	7.95	321.75
		2(a)	194.7	60	7.1	261.8
		2(b)	176.4	46.6	6.5	229.5
1.5 + Co	Day 6	1	4889.5	3877.6	701.7	9468.8
		2	ND	ND	ND	ND
	Day 15	1	4471.0	3695.6	792.6	8959.2
		2	4237.7	3496.1	730.0	8499.8
	Day 35	1	5859.0	3627.7	355.1	9841.8
		2	6121.8	3587.8	297.4	10006.7
	Day 57	1	294.1	4202.9	6.1	4503.1
		2	331.3	4516.5	4.5	4852.3
	Day 82	1(a)	157.9	36.2	6.03	200.13
		1(b)	147.5	28.15	5.4	181.05
		2(a)	173.8	26.4	5.3	205.5
		2(b)	159.2	23.2	4.9	187.3
0.7 + MM	Day 6	1	2830.3	2070.0	112.6	5012.9
		2	2429.3	2376.5	60.8	4866.5
	Day 15	1	2370.3	2780.2	143.5	5294.0
		2	1040.9	2458.5	78.5	3577.9
	Day 35	1	3606.5	1891.4	39.1	5537.0
		2	ND	ND	ND	ND
	Day 57	1	37.6	19.2	2.5	59.4
		2	1136.9	1100.4	8.7	2246.0
	Day 82	1(a)	120.9	0.5	3.2	124.6
		1(b)	114.3	8.3	3.2	125.8
		2(a)	130.6	8.8	3.4	142.8
		2(b)	126.6	8.5	3.4	138.5
1.0 + MM	Day 6	1	2809.4	2520.1	328.3	5657.8
		2	2630.2	2275.1	359.0	5264.3
	Day 15	1	1914.1	3314.8	264.1	5493
		2	2953.3	2930.4	421.8	6305.5
	Day 35	1	2488.5	2852.8	45.6	5386.9
		2	ND	ND	ND	ND
	Day 57	1	646.4	294.1	4.6	945.1
		2	ND	ND	ND	ND

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
1.0 + MM	Day 82	1(a)	128.8	14.7	4.7	148.2
		1(b)	131.4	14.3	4.4	150.1
		2(a)	126.4	11.3	3.9	141.6
		2(b)	132.7	11.2	3.6	147.5
1.5 + MM	Day 6	1	5775.3	4284.8	739.2	10799.3
		2	5277.5	4010.9	724.1	10012.5
	Day 15	1	4127.6	3460.9	767.7	8356.2
		2	5671.3	4035.7	739.9	10446.9
	Day 35	1	7010.5	4214.7	320.0	11545.2
		2	5449.3	3582.4	243.3	9275.0
	Day 57	1	35.2	30.1	2.6	67.9
		2	24.5	30.1	2.6	57.2
	Day 82	1(a)	124.1	12.5	3.4	140
		1(b)	128.1	11.5	3.8	143.4
		2(a)	126.5	10.1	3.5	140.1
		2(b)	128.7	10.2	3.4	142.3
1st Recycle BMP Assay						
0%	Day 4	27	1049.87	1810.80	92.90	2953.57
		28	1247.64	1573.80	195.68	3017.12
	Day 11	27	418.84	1738.50	40.44	2197.78
		28	598.83	1546.20	20.87	2165.90
	Day 20	27	44.12	3.61	4.35	52.08
		28	87.30	4.04	9.90	101.23
	Day 27	27	51.69	3.80	12.64	68.12
		28	64.49	3.85	5.33	73.67
	Day 29	27(a)	40.81	13.78	25.20	79.80
		27(b)	35.03	7.13	18.55	60.71
		28(a)	36.02	7.13	18.37	61.52
		28(b)	35.10	3.83598	6.55	45.49
25%	Day 4	19	1119.29	1974.17	336.39	3429.85
		20	1168.68	1741.89	210.35	3120.92
	Day 11	19	252.09	2166.49	15.42	2434.00
		20	338.55	1714.51	60.35	2113.41
	Day 20	19	127.11	8.58	10.82	146.51
		20	49.07	3.35	7.67	60.09
	Day 27	19	47.04	7.96	15.13	70.13
		20	50.37	13.75	16.57	80.69
	Day 29	19(a)	46.85	9.12	24.88353	80.85
		19(b)	46.54	5.53	19.66	71.73

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
25%	Day 29	20(a)	58.14	13.35	27.07	98.56
		20(b)	54.6	4.75	18.57	78.01
50%	Day 4	11	1334.19	1842.50	211.47	3388.16
		12	1261.27	1811.50	149.09	3221.86
	Day 11	11	449.08	1589.12	151.63	2189.83
		12	345.98	1869.29	50.07	2265.34
	Day 20	11	53.33	3.77	4.18	61.27
		12	60.90	3.99	8.31	73.20
	Day 27	11	50.92	4.61	5.77	61.30
		12	45.29	4.42	11.23	60.95
	Day 29	11(a)	65.93	18.4	48.4	132.72
		11(b)	66.16	15.1	40.4	121.60
		12(a)	49.74	11.99	34.6	96.36
		12(b)	47.88	6.8723	24.9	79.69
100%	Day 4	3	1691.57	1585.05	218.89	3495.51
		4	1147.32	2046.04	128.79	3322.15
	Day 11	3	359.36	1694.70	85.35	2139.41
		4	355.32	2033.26	126.93	2515.51
	Day 20	3	58.86	4.20	5.28	68.34
		4	58.19	3.87	11.47	73.53
	Day 27	3	88.37	10.56	8.44	107.37
		4	72.46	6.07	11.25	89.78
	Day 29	3(a)	281.74	219.31	264.1	765.2
		3(b)	99.60	62.68	100.2	262.5
		4(a)	68.38	29.97	57.5	155.9
		4(b)	58.82	18.97	41.9	119.6
2nd Recycle BMP Assay						
0%	Day 1	29	2279.81	1612.35	575.32	4467.48
		30	2433.4	1653.72	612.62	4699.74
	Day 11	29	179.8	1364.4	19.06	1563.26
		30	179.8	1410.1	8.68	1598.58
	Day 17	29	91.62	9.81	66.01	167.44
		30	118.04	5.37	77.52	200.93
	Day 25	29	99.21	4.49	100.68	204.38
		30	86.31	4.85	129.6	220.76
	Day 32	29	59.66	3.31	119.42	182.39
		30	59.51	1.52	84.44	145.47
	Day 51	29(a)	39.86	4.66	8.57	53.09
		29(b)	40.73	2.2	2.47	45.4

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
0%	Day 51	30(a)	42.85	2.3	2.64	47.79
		30(b)	43.76	5.06	8.11	56.93
25%	Day 1	21	1310.11	935.07	890.29	3135.47
		22	1351.16	880.17	983.75	3215.08
	Day 11	21	300.07	1384.1	20.55	1704.72
		22	238.65	1574.2	21.44	1834.29
	Day 17	21	108.01	12.39	68.66	189.06
		22	170.71	103.09	10.89	284.69
	Day 25	21	96.16	5.5	150.17	251.83
		22	90.16	4.65	118.47	213.28
	Day 32	21	42.02	3.93	179.26	225.21
		22	41.83	3.3	122.51	167.64
	Day 51	21(a)	36.26	2.3	3.33	41.89
		21(b)	35.02	2.27	7.0	44.29
		22(a)	43.1	10.58	14.94	68.62
		22(b)	33.87	5.74	9.22	48.83
50%	Day 1	13	1674.49	1218.29	881.27	3774.05
		14	1546.76	1178.12	984.85	3709.73
	Day 11	13	389.2	1628.1	19.91	2037.21
		14	266.44	1631.9	22.24	1920.58
	Day 17	13	202.29	102.45	50.51	355.25
		14	137.32	82.38	72.69	292.39
	Day 25	13	77.01	6.74	140.57	224.32
		14	77.12	6.48	164.51	248.11
	Day 32	13	69.96	5.34	155.24	230.54
		14	65.37	4.24	217.65	287.26
	Day 51	13(a)	41.92	4.43	15.22	61.57
		13(b)	38.7	3.57	5.1	47.37
		14(a)	40.68	5.71	12.64	59.03
		14(b)	40.51	2.8	3.52	46.83
100%	Day 1	5	2414.47	1624.63	883.45	4922.55
		6	2382.51	1575.62	725.72	4683.85
	Day 11	5	375.17	1870.8	24.84	2270.81
		6	552.77	1928	21.61	2502.38
	Day 17	5	308.09	729.49	37.73	1075.31
		6	418.47	1141.48	40.56	1600.51
	Day 25	5	129.25	31.83	197.39	358.47
		6	90.32	7.75	143.49	241.56
	Day 32	5	126.6	29.17	158.24	314.01

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
100%	Day 32	6	97.91	5.67	100.93	204.51
	Day 51	5(a)	173.39	91.61	118.64	383.64
		5(b)	9-2.24	24.36	43.55	67.91
		6(a)	78.93	14.79	30.44	124.16
		6(b)	76	6.17	13.01	95.18
3rd Recycle BMP Assay						
0%	Day 1	31	2081.1	491.45	1098.69	3671.24
		32	1858.08	494.38	1015.36	3367.82
	Day 7	31	760.57	1640.03	25.79	2426.39
		32	604.81	1511.27	51.1	2167.18
	Day 15	31	130.53	1342.9	50.5	1523.93
		32	170.21	490.39	40.73	701.33
	Day 22	31	115.57	7.48	58.6	181.65
		32	48.95	4.33	106.17	159.45
	Day 29	31	1.8	44.57	80.01	1.8
		32	1.63	85.42	121.6	1.63
	Day 44	31(a)	64.01	7.15	20.02	91.18
		31(b)	63.85	7.25	19.95	91.05
		32(a)	30.85	3.6	5.64	40.09
		32(b)	30.79	5.73	16.45	52.97
25%	Day 1	23	2076.61	389.9	1411.52	3878.03
		24	2174.21	410.15	1405.48	3989.84
	Day 7	23	1221.58	1115.59	35.96	2373.13
		24	1380.97	1193.9	31.04	2605.91
	Day 15	23	231.02	198.06	7.34	436.42
		24	184.27	757.06	6.2	947.53
	Day 22	23	37.69	5.77	67.55	111.01
		24	66.19	4.49	45.11	115.79
	Day 29	23	19.43	2.53	75.6	97.56
		24	74.88	1.37	37.2	113.45
	Day 44	23(a)	55.65	32.93	38.57	127.15
		23(b)	27.7	12.05	32.03	71.78
		24(a)	26.39	10.82	27.42	64.63
		24(b)	25.54	10.15	28.91	64.6
50%	Day 1	15	2335.95	292.97	1232.88	3861.8
		16	2503.98	358.34	1229.27	4091.59
	Day 7	15	1266.03	1158.84	56.02	2480.89
		16	1225.2	1233	32.64	2490.84
	Day 15	15	298.96	252.86	5.87	557.69

TABLE A.4 – BMP Volatile Fatty Acid data (continued)

% Effluent	Day No.	Bottle No.	Acetic Acid (mg/L)	Propionic Acid (mg/L)	Butyric Acid (mg/L)	Total Acids (mg/L)
50%	Day 15	16	187.61	623.46	9.49	820.56
	Day 22	15	100.5	28.89	52.92	182.31
		16	69.85	8.21	81.82	159.88
	Day 29	15	104.88	7.07	110.27	222.22
		16	93.02	3.54	83.07	179.63
	Day 44	15(a)	86.43	14.93	16.44	117.8
		15(b)	80.57	6.09	4.17	90.83
		16(a)	72.14	14.35	34.45	120.94
		16(b)	68.46	12.64	47.3	128.4
100%	Day 1	7	2397.34	638.97	616.53	3652.84
		8	2193.5	581.06	694.54	3469.1
	Day 7	7	3104.33	1899.75	107.57	5111.65
		8	3193.02	1901.96	53.52	5148.5
	Day 15	7	2526.92	1634.11	28.93	4189.96
		8	2213.46	1606.71	11.37	3831.54
	Day 22	7	628.16	878.11	127.04	1633.31
		8	584.95	995.54	43.8	1624.29
	Day 29	7	195.21	51.79	168.14	415.14
		8	131.62	12.61	93.27	237.5
	Day 44	7(a)	136.22	47.83	82.85	266.9
		7(b)	101.18	10.2	7.39	118.77
		8(a)	65.79	18.02	19.76	103.57
		8(b)	65.58	16.29	24.32	106.19