EVALUATION OF MULTIPLE FEEDSTOCKS FOR CODIGESTION

by

Tracy Marie Chouinard

A dissertation submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in
Infrastructure and Environmental Systems

Charlotte

2014

Approved by:

_____

Dr. Helene Hilger

_____

Dr. Sandra Clinton

_____

Dr. Jay Wu

_____

Dr. Richard Tsang

_____

Dr. Mona Azarbayjani

ABSTRACT

TRACY MARIE CHOUINARD. Evaluation of multiple feedstocks for codigestion
(Under the direction of DR. HELENE HILGER)

In the U.S., a move toward the use of anaerobic digestion (AD) technologies for solid waste management has been slow. However, recently, a number of factors have coalesced to renew interest in AD because of its potential to produce renewable energy from the wastes. It is well known among AD practitioners that every waste material is unique, and each must be evaluated on a case-by-case basis for a particular digester project. A review of existing literature revealed that the guidance provided by it was not of a quality that would promote and assist with rapid U.S. AD development and advances. The aim of this research was to address some of these deficits and provide some new information to inform feedstock testing reliability and reproducibility.

A database of codigestion articles published between 2000 and 2014 was compiled to examine the nature and quality of existing literature and also new researchers to filter research articles based on a variety of criteria that include feedstocks, operational parameters, and the types of information reported. The database is expandable and available for hosting on a website. Database analysis revealed that 32% of the authors measured biogas production but not methane (CH_4) output specifically. Only 27% of the studies used food to microorganism (F:M) ratio as an operational parameter, and 8% reported both F:M and the nutrients expressed as carbon-to-nitrogen (C:N) ratio.

The batch codigestion experiments revealed that even in the absence of acclimated sludge or alkalinity supplementation, poultry litter (PL) and DAF in equal weight percent loadings proved to be stable cosubstrates. Further, mixes in ratios of PL60:FW15 (poultry litter: food waste) with the remainder brown grease (BG) or DAF were also successful despite the fact that FW and DAF failed in the biochemical methane potential (BMP) and anaerobic toxicity assays (ATA) tests due to acidification. The BG was only mildly inhibitory in ATA testing and performed well in the BMP test with alkalinity supplementation. All batch, BMP and ATA experiments showed glycerin (GLY) and/or canola seed hull cake inhibited CH_4 production. Although acidification was implicated in the canola ATA, it was not the cause of failure in the BMP test or in either GLY test; propionate accumulation or toxins may have been responsible. Methane yields in the BMP tests showed BG to be the most productive (371 ± 76 mL CH_4/gVS), and paper, PL, and cattle manure (CM) had yields in the 120–150 mL CH_4/gVS range. PL and CM were stimulatory in the ATAs, with all other feedstocks showing varying degrees of inhibition. This finding is interesting in light of the fact that feedstock cell counts showed that PL (as well as CM and DAF) contain more live cells per gram volatile solids than the seed used as inoculum. The semi-continuous reactors demonstrated that a mix with up to eight feedstocks could be managed in a stable digestion; however, mixes with lipid-laden feedstocks, high organic loading rates or short solids retention time led to foaming and fouling of two of the reactors.

DAF exposed to thermal pretreatment produced 170 ± 22 mL CH_4/gVS, while untreated DAF yielded no CH_4. Similarly pretreated CM showed a two-fold increase in CH_4 yield, but the same was not true for sewage sludge, where pretreatment inhibited

CH$_4$ production. Thermal pretreatment of PL had no effect on CH$_4$ production, but, along with CM and DAF, it had a positive net energy balance. The energy analysis based on pretreatment studies reported in the literature revealed that chemical and biological pretreatment were the only methods that reliably yielded a net energy gain. Mechanical, thermal, and thermochemical pretreatment were less successful at yielding net positive energy values.

Taken together, this body of work offers a roadmap for codigestion research. Ready access to recent literature is provided along with guidance about the important procedural, operational and reporting features required for sound study. A reproducible batch protocol is described that includes attention to nutrient and inoculant balance, to gas collection and analysis, and to the use of controls. If replicated, it will allow for better comparisons among laboratories. The importance of replication of semi-continuous or continuous flow studies is highlighted, due to the inherent variability between reactors. A discussion of the relevance and application of the BMP and ATA tests is offered, and data from some novel feedstocks is reported. Feedstock cell counts suggest that F:M ratios for some substrates need to be adjusted to account for live cells entering with the feed.

ACKNOWLEDGEMENTS

Completing this dissertation is the culmination of hours of work in the lab and writing. I would not have been able to complete it without the help of so many people. I would like to thank Dr. Helene Hilger, my advisor, mentor, and lifelong friend. Her guidance, influence, and critique have been impactful and life changing. Words cannot express my gratitude for her years of dedication to the pursuit of my doctorate. I would like to thank my committee members: Drs. Sandra Clinton, Jay Wu, Richard Tsang, and Mona Azarbayjani for their time and advice in creating this project. Thank you to Catawba County, the funding authority, especially Barry Edwards, for their innovation and forward thinking without which this project would not have happened.

This dissertation would not have been possible without the many people that worked in the laboratory conducting research. I would like to thank Nathalia Queen, Hye Jin, Seon-Yi Han, Bhargavi Golluru, Bradford Garrigues, Cindy Vang, Kyle Baarson, Maggie Stevens, and the countless others who volunteered their time when I needed it.

DEDICATION

The heart of my journey was greatly influenced by my friends and family. I have been blessed with two families; my friends in North Carolina and my family in Massachusetts. During my travels, they have all taught me many different things that have benefited me in life's journey. To Mom, Dad, Ron, and Nate for always believing in me and encouraging me to pursue my goals. To my Mémé Perrier, who though you have left this world, your love and belief in me has kept me striving for my next adventure. I would like to give special thanks to Vicki, Richard, Angie, Heidi, and Robin for always encouraging me to follow my dreams and for their moral support. They have also been wonderful sounding boards. Next, I would like to give special thanks to Dr. Shubha Oza for her friendship, help in the lab, overall support, and the fabulous dinners. Last but certainly not least, thanks to my two furry little valiums, Allie and Emmitt, for keeping me sane and providing countless hours of laughter.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

AD	anaerobic digestion
ATA	anaerobic toxicity assays
AW	abattoir wastewater
BG	brown grease
BMP	biochemical methane potential
BTA	Biotechnische Abfallverwertung GmBH & CO.
C:N	carbon to nitrogen
$CH_3CH_2COO^-$	propionate
CH_3OH	methanol
CH_4	methane
CHP	combined heat and power
CM	cattle manure
CNG	compressed natural gas
CS	canola seed hull
CO	carbon monoxide
CO_2	carbon dioxide
CTC	5-cyano-2,3-ditoly tetrazolium chloride
CUPs	compostable cups
DAF	dissolved air floatation
DAPI	4'6-diamidion-2-phrnylindole
EIBI	European Industrial Bioenergy Initiative

E_I/E_O	energy input to output ratio
EPS	extracellular polymers
EU	European Union
F:M	food to microorganism
FCs	fuel cells
FM	feed meal
FOG	fats, oils, and grease
FVW	fruit and vegetable waste
FW	food waste
GIW	grease interceptor waste
GLY	glycerin
H^+	hydrogen ions
H_2	hydrogen
H_2O_v	water vapor
H_2S	hydrogen sulfide
HRT	hydraulic retention times
HW	hatchery waste
IC	internal combustion
IEA	International Energy Agency
LCFA	long chain fatty acids
LHW	liquid hot water
MFC	molten-carbonate fuel cells
MSW	municipal solid waste

MW	microwave
Na^+	sodium
NaOH	sodium hydroxide
NH_3	ammonia
NH_4HCO_3	ammonium bicarbonate
O_2	oxygen
OLR	organic loading rate
PACF	phosphoric acid fuels cells
PEM	proton exchange membrane fuel cells
PL	poultry litter
PS	primary sludge
SMR	steam methane reforming
SOFC	solid oxide electrolyte fuel cells
SRT	solids retention time
SS	sewage sludge
SuOC	sunflower oil cake
TA	total alkalinity
TS	total solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VS	volatile solids
WAS	waste activated sludge
WWTP	wastewater treatment plant

CHAPTER 1: INTRODUCTION

Anaerobic digestion (AD) technology was first documented in the 10th century B.C., in Assyria, what is now the Middle East, for bath water heating (Monnet 2003, Pullen 2004, Appels et al. 2008). The popularity of AD systems has tended to rise and fall depending on the competing options for energy production and waste management. For many years, Europe (Monnet 2003, Appels et al. 2008, IEA 2010) and the UK (AD-Community 2008, Appels et al. 2008) employed small, single feedstock, on-farm AD systems (De Baere 2000, AD-Community 2008, IEA 2010), but more recently, numerous centralized and/or codigestion systems with multiple feedstocks are being used (De Baere 2000, IEA 2005, Held et al. 2008, IEA 2010, Weiland 2010). In addition to agricultural wastes, other value-added wastes come from municipal (community, commercial, and light industry), and industrial sources (Steffen et al. 1998). The typical processes required for a modern commercial or municipal system include: (1) pre-treatment of the feedstock (optional), (2) digestion, (3) gas upgrading (optional, depending on use), and (4) digestate treatment (Monnet 2003).

The advancement of AD systems in the European Union (EU) has been aided by legislation that focuses on increasing alternative fuel production (IEA 2010). For example, the European Industrial Bioenergy Initiative (EIBI) aims to advance the most promising technologies for efficient production of biofuels from biomass (IEA 2010). Likewise, Canada has initiated a feed-in tariff program that offers guaranteed pricing for

renewable energy production (IEA 2010). By 2000, the EU had 53 AD systems operating with at least 10% feed from the organic fraction of municipal solid waste (De Baere 2000). In the 2010 country reports to the International Energy Agency (IEA) Bioenergy Task 37, each country noted an increase in AD systems since 2000 and commitments to construct more. For instance, Norway plans a 30% increase in biogas production from livestock manure codigested with food waste by 2020, and Denmark proposes to construct four large codigestion facilities per year until 2020. This will increase the fraction of animal manures and slurries codigested in Denmark from 3–6% to 50% (IEA 2010). Canada reported 17 AD codigestion systems and expects that number to increase with the feed-in tariff incentives.

In the U.S., a move toward AD technologies has been slow. The majority of digestion facilities are at wastewater treatment plants (WWTP), with a smaller collection of digesters located at commercial livestock farms. In 2010, there were 160 agricultural AD facilities registered with AgStar (2013). By 2011, the U.S. had 45 codigestion and/or centralized AD facilities, and Ohio had committed funding for 11 more codigestion facilities (BioCycle 2011). An AgStar inventory in 2013 documented 192 farm digesters, 93% of which were treating manure and harvesting energy. The inventory also reported that of the 1238 WWTP digesters counted, only 69% of them harvested energy from biogas (AgStar 2013). These findings are not entirely surprising, as sewage sludge (SS) is not a high-energy substrate, and economic analyses of energy capture are often unfavorable at small-scale facilities.

In recent years, a number of factors have coalesced to renew U.S. interest in AD technologies as renewable energy sources. First among these is the rising cost of

conventional energy. Another influential factor has been the recognition that excess capacity in WWTP digesters can be converted to tipping fee revenue if additional feedstocks are accepted. A third driver is increased emphasis nationwide on food waste diversion from landfills; AD can accept such waste as a value-added feedstock that can generate energy revenue. Such diversion also extends the capacity of landfills (which has cost benefits that can be calculated).

As codigestion on farms and at municipal WWTPs increases, industries are emboldened to examine AD as a new treatment option. Thus, it can be expected that the feedstock mixes codigested will be more diverse as more candidates for digestion are considered and as more mixes are conceived to optimize energy production. Because each new codigestion project is capital intensive but presents a unique set of feedstock opportunities and circumstances, it is critical that proper testing be conducted. Further, testing should occur at both the laboratory and pilot scale before any commitment to full-scale operation is made because each of these testing stages carries increasing levels of predictive power. Such tests can reveal synergies and maybe more importantly, inhibitory responses between substrates. They can also identify possible mix ratios for full-scale digestion. As codigestion for energy production receives increasing research attention, numerous reports of feed compatibility testing are emerging. However, there is a lack of rigor in many of these reports, and not all known factors of influence are included in each set of tests. Many reports are from studies where the predominant feedstock is manure from farm operations, while much of the new interest in AD is coming from municipal and commercial operations.

When AD studies are performed, they are fundamental examinations of microbial systems. The digestion process is accomplished by a consortium of anaerobic microorganisms that ultimately converts organic material to methane (CH_4) and carbon dioxide (CO_2). Some of the important factors that are investigated in codigestion assessment studies include: the carbon to nitrogen ratio (C:N) available to microbes; the balance of nutrients beyond C and N (especially certain trace nutrients); the ability of one feedstock to enhance or inhibit biogas production from other feedstocks; the inoculant used; and the ratio of feedstock (food) to microorganisms in the inoculant (F:M ratio). In batch experiments, it is the ratio of the mass of volatile solids (VS) in the mix from substrate divided by the mass of VS derived from microorganisms in the inoculant (g $VS_{substrate}$/g $VS_{inoculum}$). In continuous flow, the ratio requires that the feedstock flow rate and the reactor volume be included in the calculation.

While it may not always be possible to keep all of these factors at the same level when various conditions are compared, it should be possible to standardize which ones are varied and how best to control the effects of such variation. Further, the way in which the F:M ratio is calculated needs to be examined more closely.

There are a few researchers who have explored and reported on F:M ratios for different feedstocks including: cellulosic feedstocks (Chynoweth et al. 1993), tannery wastewater (Sri Bala Kameswari et al. 2011), sunflower cake oil (Raposo et al. 2009), food waste and vegetable oil (Maya-Altamira et al. 2008), and synthetic wastewater (Prashanth et al. 2006). However, the feedstocks are wide-ranging, and some of these studies lack good controls or replicates. Further, even when good protocols are followed, reporting practices for F:M ratio do not take into account any additional microbial

biomass that may be introduced with a substrate. It is presumed that only the inoculant provides microbes. Thus, when a substrate itself introduces a significant concentration of microorganisms into a mix, there is no way to account for the additional microbe activity. Substrates such as manures or grease interceptor wastes will introduce large acclimated microbe populations; in fact, some studies use them as the source of inoculant. When they are tested against feedstocks that do not have inherent microbe populations, such as food waste, cellulosic agricultural wastes, tannery, or paper waste, the results may be confounded if the additional microbial loading is not taken into account.

As new waste materials are being assessed for AD, the potential to pretreat those that are more resistant to degradation is being investigated. Pretreatment processes can include thermal (Pinnekamp 1989, Yang et al. 2010), chemical (Carballa et al. 2009, Carrère et al. 2010), and mechanical (Nah et al. 2000, Muller et al. 2009) treatment as well as steam explosion (Dereix et al. 2006), microwaves (Eskicioglu et al. 2008), or a combination of these options (Tanaka et al. 1997, Kim et al. 2003b, Bougrier et al. 2006, Liu et al. 2009c). Such methods can particularly enhance energy yields from individual feedstocks or feedstock mixes high in particulates or with high lignocellulose content. However, because pretreatment methods will add handling steps and may require energy inputs for mechanical equipment and transport, it is important to gauge their net benefit beyond the additional energy gain from pretreatment before digestion. There must be a net energy gain from the overall two-step process for such pretreatment to be worthwhile and cost effective.

The purpose of the proposed research is to address some of the developing issues with feedstock testing. Those issues include (i) the existence of many studies in this

domain but great diversity in their attention to the many important process variables and reporting practices needed to inform future research; (ii) the need for a more standardized guideline that will operationalize codigestion feasibility studies; (iii) the practice of comparing microbe-rich and micro-lean feedstocks without controlling for the uneven microbe loadings; and (iv) the measure of pretreatment success in harvesting additional energy from feedstocks without attention to the added energy inputs required for the pretreatment.

1.1. Objectives

The specific objectives of this research are to:

Objective 1:   Create a codigestion literature database that can organize existing reports according to the factors included in protocols

Specifically, articles will be reviewed and catalogued with information about the inclusion of F:M, C:N, CH_4 reporting units, replication, and VS loading. The result will be a database of existing studies that new investigators can quickly access by feedstock(s) of interest. More importantly, the database will allow users to readily scan for important protocol elements in the studies and make more authentic comparisons between new results and reported data. The database should also serve to emphasize the importance of uniform protocols among research teams.

Objective 2:   Develop a guideline to operationalize codigestion feasibility studies

Through examination of the literature and laboratory study, a guideline will be developed and carefully described, which will inform future AD feedstock feasibility studies and allow them to be compared. The guideline will include a suite of science-based factors of influence that should be assessed each time the performance of codigestion feed candidates are evaluated. Feedstocks available from a local municipality

that is assessing them for AD will be used along with anaerobic toxicity assays (ATA), and biochemical methane potential (BMP) tests to derive final guideline recommendations. If the guideline is widely adopted, it will reduce both the time and cost of feasibility studies and increase the quality and comparability of new data that results from more uniform testing protocols.

Objective 3: Assess the relevance of feedstock: inoculum (food to microorganism) ratios on codigestion mixes containing significant microbial biomass

Many studies reporting on batch testing systems do not report a food to microorganism (F:M) ratio for the test mixes, although such reporting is critical for replication or test standardization among laboratories. Among the studies that report the ratio used, many lack proper controls and/or fail to use replicates and statistical analysis. There are no reports where the inherent microbial load introduced with a feedstock is taken into account. Thus, even if an F:M ratio is set and observed in a guideline reported, results from trials comparing microbe-rich manure and waste cooking oil (a substrate with relatively few microbes) may be confounded. A standard BMP protocol will be used to compare CH_4 yields of poultry litter, DAF, and paper at a F:M of 1 and with the feedstock alone. All reaction volumes will be the same. The microbial load of the feedstocks will be compared using CTC (5-cyano-2,3-ditoly tetrazolium chloride) and DAPI (4'6-diamidion-2-phrnylindole) staining methods. If the microbes introduced with the feedstocks are influential in the digestion, CH_4 production will occur with the feedstock only bottles.

Objective 4: Assess the net energy balances associated with several pretreatment options

The success of pretreatment protocols for improving energy yields from feedstock(s) digestion is important, because it allows more value to be created from former waste materials. However, when such gains are considered independently of the energy inputs required to execute pretreatment, a false impression can be created. Thermal pretreatment requires energy input, as does size reduction, chemical metering pumps, mixing, and movement of materials. This research objective seeks to put pretreatment in the context of overall net energy gains. The post-2000 pretreatment literature for agricultural, municipal, and industrial wastes will be reviewed, and three studies from each of three pretreatment categories will be examined. The substrates will include SS, food waste, organic fraction municipal solid waste, manure, and highly cellulosic materials. Both the thermal and electrical energy consumption and production will be evaluated. The findings will be made available to an audience very interested in seeing a focused analysis of the energy trade-offs associated with pretreatment systems for AD. The environmental advocacy community in the U.S. is showing strong opposition to the use of waste-to-energy technologies and favoring the more "natural" rate of waste degradation offered by AD processes. Thus, the net energy gain of AD is of particular interest in the context of this political tension as new potential users evaluate each technology option.

CHAPTER 2: LITERATURE REVIEW

2.1.   Introduction

AD is accomplished by a consortium of microorganisms that converts organic material to CH_4 and CO_2. The process is valuable for two main reasons: (1) it is a means to stabilize former organic wastes and make a usable (and saleable) product; and (2) it yields energy-rich CH_4 gas (Monnet 2003). Only with the achievement of these two goals can AD systems be a viable economic option for waste treatment (Monnet 2003); however, the priority of these two objectives can vary among users. The typical processes required for a complete commercial or municipal system include: (1) pretreatment of the feedstock (optional); (2) digestion; (3) gas upgrading (optional, depending on use); and (4) digestate treatment (Monnet 2003). Pretreatment processes can include thermal (Pinnekamp 1989, Yang et al. 2010), chemical (Carballa et al. 2009, Carrère et al. 2010), mechanical (Nah et al. 2000, Muller et al. 2009), and biological (Park et al. 2005, Yi et al. 2013, Merrylin et al. 2014) treatment or some combination of these options (Tanaka et al. 1997, Kim et al. 2003b, Bougrier et al. 2006, Liu et al. 2009c).

The digestion occurs in gas-tight containments, and there are a variety of configurations that have been designed to optimize feedstock loading, mixing, monitoring, and allowing the reactions to proceed. Through a series of biochemical reactions, the organic feed material is converted to CH_4-rich biogas, stable undigestable solids, and nutrient-rich digestate.

2.2.   Microbiology and Biochemistry of Digestion

The microbial population that develops in a digester is biomimetic of anaerobic systems found in nature. Though scientists have known about this system for centuries, a deeper understanding of the microbial communities involved was not achieved until the 1950s, when microbiologists began to understand rumen microbes (Hobson & Wheatley 1993), the populations living in the specialized stomachs of herbivore cattle and sheep. Microbiologists were first able to identify some of members of anaerobic communities by culturing them (Hobson & Wheatley 1993, Chouari et al. 2005), but it is well-known that only a small percentage of organisms are culturable. In recent years, some powerful noncultivation-dependent molecular techniques have been applied. They have markedly advanced our understanding of complex microbial communities present in natural anaerobic systems and in digesters (Godon et al. 1997, Zumstein et al. 2000, Daims et al. 2006, Sanz & Kochling 2007, Steinberg & Regan 2008, Steinberg & Regan 2009, Steinberg & Regan 2011). Further, as understanding of the dynamic relationships between populations in digesters becomes more sophisticated, it may be possible to create "designer" mineral mixes that can sustain more stable, robust, and resilient microbial mixes.

Clearly, anaerobic digesters contain facultative and obligate anaerobes because no oxygen (O_2) is present. Digester microorganisms include many organisms from the domain *Bacteria*. For example, one study of sewage sludge (SS) found a core group of organisms that included *Chloroflexi Betaproteobavteria, Bacteroidetes*, and *Synergistetes* (Riviere et al. 2009). Others have identified Firmicute, *Proteobacterial, Bacteriodete*, and *Spirochaetes* (Ramsay & Pullammanappallil 2001, Chouari et al. 2005, Cardinali-

Rezende et al. 2009, Liu et al. 2009a, Garcia-Peña et al.) However, AD microbial consortia also include microbes from another domain, *Archaea*, which largely includes the methanogens responsible for CH_4 production.

Organisms from the two domains are intimately related in the biodegradation of feedstocks, which typically begins with the solubilization of particulate matter. This first step, called hydrolysis, is accomplished by hydrolytic bacteria that excrete exoenzymes (such as glucosidases, proteases, and lipases) (Gerardi 2003). These enzymes degrade and solubilize substrates into simpler compounds, such as glucose, amino acids, fatty acids, and glycerol (Gerardi 2003). Once soluble, the simple compounds can enter the cells of acidogenic (acid-producing) bacteria, which use endoenzymes (internally released enzymes) to ferment the simpler compounds to CO_2, hydrogen (H^+), and volatile fatty acids (VFA) end-products, including acetic acid, butyric acid, and propionic acid (McInerney et al. 1981, Zumstein et al. 2000, Monnet 2003, Appels et al. 2008, Liu et al. 2009a). Acetogens (acetic acid formers) also use endoenzymes to convert VFAs, butyric acid, and propionic acid to acetic acid. (McInerney et al. 1981, Zumstein et al. 2000, Ramsay & Pullammanappallil 2001, Monnet 2003, Appels et al. 2008, Liu et al. 2009a)

There are three principal groups (biochemical pathways) of methanogens that mediate the conversion of these products to CH_4, and each follows a different pathway (Gerardi 2003, Mata-Alvarez 2003, Monnet 2003, Appels et al. 2008, Rapport et al. 2008). Acetotrophs (*Methanosaeta* genus) split acetate into CH_4 via aceticlastic cleavage (Eq. 2.1), and they typically produce 70% of the CH_4 in AD (Riviere et al. 2009). They are sensitive to H^+ accumulation and reproduce more slowly than hydrogenotrophic methanogens, which use CO_2 and four H^+ to produce CH_4 (Eq. 2.2). Hydrogenotrophic

methanogens (M*ethanomicrobiales*) typically comprise 1–29% of digester methanogens and are responsible for less than 30% of the CH_4 produced (Riviere et al. 2009). Methylotophic methanogens use methanol (CH_3OH) and H^+ to produce CH_4 (**Eq. 2.3**) and are responsible for the smallest amount of CH_4 production in digesters (Gerardi 2003, Rapport et al. 2008).

1. Acetotrophic methanogenesis

$$4\,CH_3COOH \rightarrow 4\,CO_2 + 4\,CH_4 \qquad\qquad \text{Eq. 2.1}$$

2. Hydrogenotrophic methanogenesis

$$CO_2 + 8\,H^+ \rightarrow CH_4 + 2\,H_2O \qquad\qquad \text{Eq. 2.2}$$

3. Methylotophic methanogenesis

$$3\,CH_3OH + 6\,H^+ \rightarrow 3CH_4 + 3\,H_2O \qquad\qquad \text{Eq. 2.3}$$

For the purpose of tracking microbial activity during AD, it is helpful to categorize the organic feedstocks as carbohydrates, proteins, and lipids (FIGURE 2.1). Carbohydrates are polymers containing multiple monomers of sugars that are too large to enter a cell. Saccrolytic bacteria hydrolyze polysaccharides into monosaccharides, which are then able to enter bacteria for further degradation. Two of the most common genera that degrade monosaccharides are *Clostidium* and *Propionibacterium*, which degrade monosaccharides into butanol, butyric acid, and isopropanol and into acetic and propionic acid, respectively (Gerardi 2003).
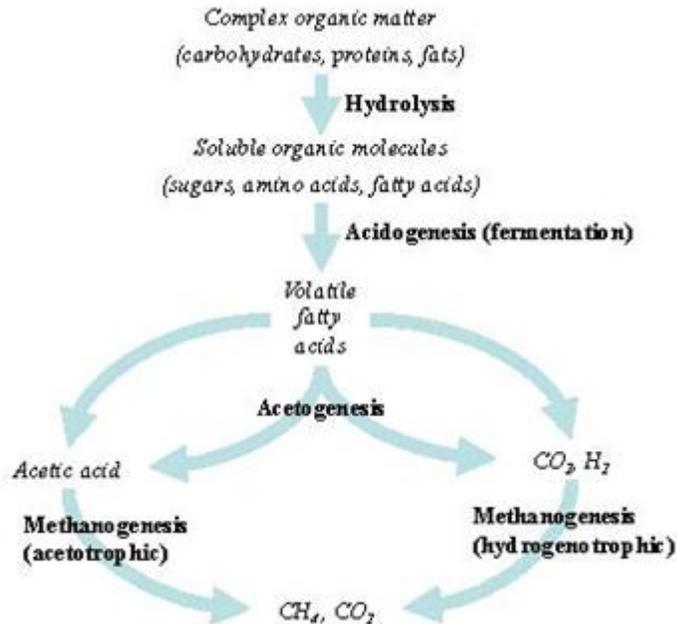
FIGURE 2.1: An overview diagram of the anaerobic digestion process
(Rapport et al. 2008)

Proteins, which are chains of amino acids linked by peptide bonds, cannot enter

bacteria cells until the peptide bonds are broken (Gerardi 2003). Therefore, protease or

peptidase bacterial enzyme excretions are essential if amino acids are to enter bacterial

cells and be further degraded into VFAs. The bacteria that mediate such reactions are

typically *Clostridium propionicium* (which ferment the amino acid alanine), *Clostridium*

tetanomorphium (glutamate), *Peptostreptococcus* (glycine), *Clostridium sticklandii*

(lysine), and *Clostridium* spp., (arginine) (McInerney 1988, Gerardi 2003). Such

fermentations follow one of two pathways: (1) coupled oxidation reduction fermentation

(called the Stickland reaction) and (2) single amino acid fermentation in the presence of

hydrogen-utilizing bacteria (Ramsay & Pullammanappallil 2001). The Stickland reaction

is the preferred method of amino acid degradation, because amino acids will act as

electron acceptors (Nagase & Matsuo 1982, Ramsay & Pullammanappallil 2001). Basically, the Stickland reaction is a coupled oxidation-reduction reaction involving the degradation of amino acids (Nisman 1954). For the reaction to occur, an electron donor amino acid is oxidized, while an electron acceptor amino acid is reduced, as shown in Eq. 2.4, where alanine ($C_3H_7NO_2$) is oxidized and glycine ($C_2H_5NO_2$) is reduced (Nisman 1954):

$$C_3H_7NO_2 + 2C_2H_5NO_2 + 2H_2O \rightarrow 3CH_3CO_2^- + 3NH_4^+ + CO_2 \qquad \text{Eq. 2.4}$$

(Nagase & Matsuo 1982) showed that single amino acid fermentation would only occur if there were no other amino acids available to act as an electron acceptor. Amino acid degradation did not rely on methanogens to act as the electron acceptor, thereby reinforcing the theory that the Stickland reaction is the preferred method (Nagase & Matsuo 1982).

During hydrolysis, lipids are degraded by lipase exoenzymes that separate the long chain fatty acid (LCFA) from the glycerol backbone of the lipid molecules (McInerney et al. 1981, McInerney 1988, Sousa et al. 2007). The glycerol is degraded via acidogenesis, while LCFA degradation proceeds via β-oxidation, which is a syntrophic acetogenesis that is predominately performed by two families: *Syntrophobacteraceae* and *Syntrophomonadaceae* (McInerney et al. 1981, Pereira 2003, Sousa et al. 2007). Because LCFAs vary in chain length and degree of saturation, unsaturated LCFAs need to become saturated (hydrogenated) prior to entering the β-oxidation cycle.

During β-oxidation, the saturated LFCA is first activated by coenzyme A (Figure 2.2). Then, two hydrogen molecules (H_2) are removed (dehydrogenation) and a double bond is formed between the second and third carbon where a hydroxyl group is fixed

$$RCH_2CH_2COOH \xrightarrow{\text{CoA}} RCH_2CH_2CO\text{-}CoA \xrightarrow{-2H} RCH=CHCO\text{-}CoA$$

$$\xrightarrow[\text{H}_2\text{O}]{} RCHOHCH_2CO\text{-}CoA \xrightarrow{-2H} RCOCH_2CO\text{-}CoA$$

$$\downarrow \text{CoA}$$

$$CH_3CO\text{-}CoA \ + \ RCO\text{-}CoA$$

$$\downarrow -\text{CoA}$$

$$CH_3COOH$$
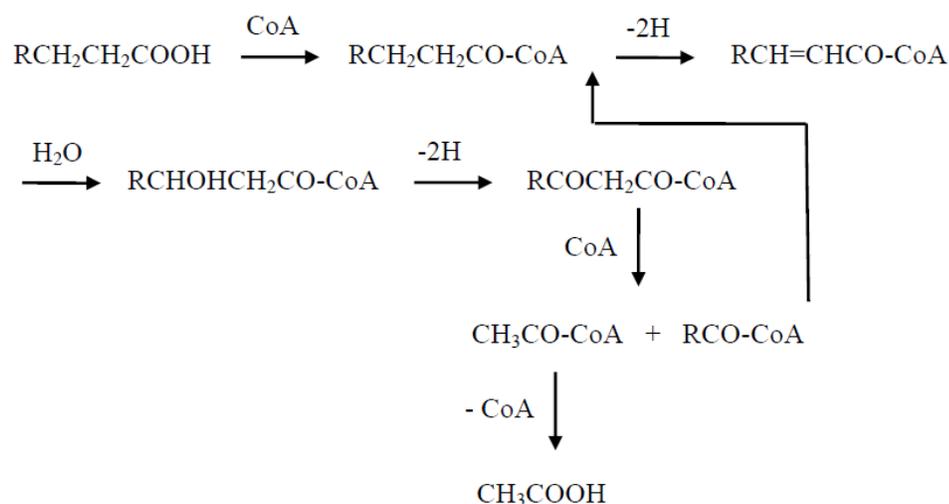
FIGURE 2.2: β-oxidation degradation of the LCFA (Pereira 2003)

(Pereira 2003). The molecule is then dehydrogenated again at the third carbon, and a ketone group is formed. Finally, another coenzyme A splits the chain into an acetic acid and an LCFA that is two carbons shorter. This cycle repeats until the LCFA is fully degraded into acetic acid for even-numbered chains and propionic acid for odd-numbered chains (Pereira 2003). This process can only be accomplished in a synotrophic relationship with hydrogen-utilizing methanogens. Because this reaction has a positive Gibbs free energy value, it requires low H^+ partial pressure for the utilization of H^+ by methanogens (negative Gibbs free energy value).

The organic acids from carbohydrates, proteins, and lipids are processed by acetogens, which are in the same phyla as acidogens. Acetogens have a slow generation time (greater than three days) and are obligate H^+ producers; they can survive only at low H^+ concentrations (Gerardi 2003). Therefore, acetogens have a syntropic relationship with methanogens. When acetate is produced, H_2 is also produced. If there is a significant

amount of H$^+$, then acetogenic activity stops. Methanogens present in the microbial mix consume the H$^+$ (Eq. 2.2); therefore, H$^+$ concentrations do not increase (McInerney et al. 1981, Gerardi 2003, Mata-Alvarez 2003, Speece 2008). This syntrophic relationship between acetogens and methanogens is evident in the thermodynamic relationships of acetate and CH$_4$ formation. Acetate formation is not thermodynamically favored (ΔG = 76.1 kJ, Eq. 2.5) (McInerney et al. 1981), but when it is coupled with CH$_4$ formation, the combined reaction is favorable.

The degradation of propionate (CH$_3$CH$_2$COO$^-$)$CH_3CH_2COO^-$) to CH$_4$ (ΔG =- 275.3kJ) and H$^+$ has similar interdependencies, as shown in Eq. 2.5 through Eq. 2.8 (McInerney 1988, Mucha et al. 1988, Mata-Alvarez 2003). The degradation of propionate to acetate (CH$_3$COO$^-$) is inhibited by the accumulation of H$^+$, requiring the partial pressure of H$^+$ to be below 10^{-6}–10^{-4} atm (Fukuzaki et al. 1990). It has also been observed that the rate of propionate disappearance decreases with increasing propionate and/or acetate concentrations, even at pH levels between 6.0–6.4. This indicates that the inhibition is due to undissociated acids species (propionic acid and/or acetic acid) (Fukuzaki et al. 1990) (Mawson et al. 1991). The undissociated acids may cause accelerated entry into cells and decreased intracellular pH due to exclusion of anions. In order for the cells to maintain a balance under these conditions, the protons would need to be expelled, which requires the hydrolysis of adenosine triphosphate (ATP) therefore there is less ATP available for metabolism and growth (Fukuzaki et al. 1990).

$$CH_3 CH_2 COO^- + 3H_2O \rightarrow CH_3 COO^- + HCO_3^- + H^+ + 3H_2$$
$$\Delta G = 76.1 \; kJ \qquad \text{Eq. 2.5}$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \qquad \Delta G = -135.6 \; kJ \qquad \text{Eq. 2.6}$$

$$CH_3 COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad \Delta G = -31.0 \; kJ \qquad \text{Eq. 2.7}$$

$$4CH_3 CH_2 COO^- + 7H_2O \rightarrow 7CH_4 + 5HCO_3^- + H^+$$
$$\Delta G = -275.3 \; kJ \qquad \text{Eq. 2.8}$$
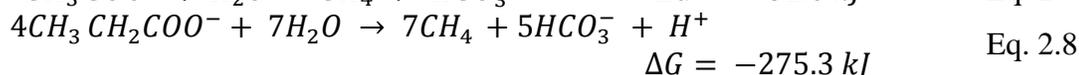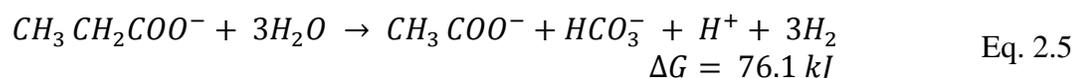
Methanogens in the *Archaea* domain are a diverse and varied group with respect to shape, growth pattern, and size. Some of their unique features are: (1) their cell walls are not rigid and lack muramic acid; (2) their cell membranes do not contain an ether lipid as a major constituent; (3) they can produce CH_4; (4) they possess coenzyme M that allows them to reduce CO_2 to CH_4; and (5) they possess F420 and F430 nickel-containing coenzymes that are H^+ carriers (Gerardi 2003). All are slow growers, with generation times of about three days at 35°C to 50 days at 10°C (Gerardi 2003, Appels et al. 2008).

In addition to the groups of organisms identified as key to the reactions described here, many others are present in digester systems, depending on the microbial loads carried by new feedstocks introduced. Their populations will rise or fall depending upon whether or not there are substrates available in the mix to support them. For example, SS digesters contain high levels of *Escherichia coli,* which are found in human intestines. It was shown that if SS digestate was used to seed a pig waste digester, the *E. coli* population diminished and *Streptococci* flourished because *Streptococci* are dominant in pig waste (Hobson & Wheatley 1993).

2.2.1. Inhibitions

Performance problems that occur in AD can be attributed to inhibitory substances (Chen et al. 2008). These substances can be grouped into two classes: end-products of normal microbial reactions and substances, organic or inorganic, that may be present when these reactions occur (Hobson & Wheatley 1993). Some examples of end-product inhibitory substances include NH_3, sulfide, CO_2, and VFAs, whereas light and heavy metals, LCFAs, and lignin are examples of substances that can be problematic if introduced with the feedstock. There are varieties of mechanisms by which inhibitory

substances exert their influence, and in some instances, the mode of action is poorly understood. Nevertheless, three outcomes tend to predominate when inhibitory substances are present: continued running of the digester but at suboptimal levels, gradual performance decline until failure, and rapid and complete failure (Hobson & Wheatley 1993).

Ammonia is produced when nitrogenous material, such as protein and urea are degraded, which is present as NH_3 or ammonium ion (NH_4^+) (Hobson & Wheatley 1993, Steffen et al. 1998, Chen et al. 2008). Ammonia is a beneficial component of the digester mix at concentrations less than 200 mg/L (Chen et al. 2008), because nitrogen is an essential nutrient for microorganism growth and function (Gerardi 2003). However, higher ammonia concentrations can be inhibitory especially in the free NH_3 form. The level at which inhibition is reported to occur varies, and this has been attributed to differences in substrates and inocula, environmental conditions, and acclimation periods (Chen et al. 2008). Estimates of the concentration at which CH_4 production activity declines 50% range from 1.7 to 14 g/L (Jarrell et al. 1987, Koster & Lettinga 1988, Wittmann C 1995, Steffen et al. 1998, Sung S 2003, Speece 2008, Buendia et al. 2009, Singh et al. 2010, Procházka et al. 2012). The proposed mechanisms of inhibition are related to the hydrophobicity of the NH_3 molecule, which can easily penetrate a cell wall and change the intracellular pH or inhibition of specific enzymatic reactions (Wittmann C 1995). When high NH_3 levels (4051–5736 mg/L) in sludge reduced methanogen activity by 56.5%, acidogen activity was not affected, suggesting that the latter was not as vulnerable to NH_3 inhibition as methanogens (Koster & Lettinga 1988).

One effective approach for NH_3 control is to monitor and adjust pH to create a "regulatory cycle". By raising the pH, the concentration of NH_3 relative to NH_4^+ increases, which results in increased inhibitory effects. However, as pH decreases, there is an accompanying increase in VFA concentrations due to the NH_3, CO_2, and VFA equilibrium (Chen et al. 2008, Procházka et al. 2012). The VFA increase causes the pH to fall, which reduces the NH_3 concentration. This is called an "inhibited steady state" condition (Angelidaki et al. 2003).

Cation addition is another method used to reduce the effects of NH_3 inhibition. The addition of two substances inhibitory to methanogens can cause an antagonistic relationship between the two inhibitors that reduces their inhibition of CH_4 production (Chen et al. 2008). For instance, a digester containing 0.15 M NH_3 reduced CH_4 production from acetic acid by 20%. However, when 0.002–0.05 M Na^+ (sodium) was also added, CH_4 production increased 5% (Koster & Lettinga 1988). Finally, the microbial consortia can adapt to higher concentrations of NH_3 over a period of time. One study showed that digesters failed when NH_3 reached concentrations of 1900–2000 mg N/L (Koster & Lettinga 1988). However, after the inocula adapted to higher NH_3 concentrations, these digesters continued to perform at NH_3 concentrations of 11,000 mg N/L.

Another known end-product inhibition occurs when there is CO_2 in the headspace (Hobson & Wheatley 1993), which affects VFA inhibition through the imbalance of the synergistic relationship between acetogens and methanogens (described previously). Carbon dioxide has been known to inhibit the primary methanogenesis pathway (acetotrophic methanogenesis); however, it will not inhibit the hydrogenotrophic

methanogenesis pathway (Hansson 1981). This phenomenon was investigated in a study, which used a heterogeneous microbial culture from a SS digester to digest glucose under different CO_2 partial pressures (Hansson 1981). It was determined that the degradation of glucose to organic acid occurred at a faster rate than the destruction of organic acids, and CH_4 production was three times greater in a nitrogen rich atmosphere. This was believed to be due to the synergistic relationship between acetogens and methanogens.

The primary VFAs produced were acetic and propionic acid, with lower amounts of butyric acid. If the propionic acid concentration was equal or higher to the acetic acid level, then there would not be any H^+ left for CH_4 production via the hydrogenotrophic pathway. Methane production would rely solely on the acetotrophic pathway. Additionally, the disruption of the synergistic relationship is evident with lower rates of propionic and butyric acid utilization by methanogens at higher pCO_2. This was caused by unfavorable thermodynamics. The production of CH_4 from H^+ is needed to make this more thermodynamically favorable (ΔG = -135.6 kJ) (McInerney et al. 1981, McInerney 1988).

Finally, CH_4 inhibition can occur due to the addition of oily, greasy and/or fatty feedstocks such as slaughterhouse waste; fats, oils, and grease (FOG); brown grease (BG): oil/fat refineries:, and dairy wastewater. These feedstocks are easily hydrolyzed to LCFAs, which have been known to cause problems with inhibition, flotation, and washouts (Chen et al. 2008). LCFA inhibition is attributed to an acute toxic effect on microorganisms involved in β-oxidation and the different methanogenic pathways (Angelidaki & Ahring 1992, Hwu & Lettinga 1997, Pereira et al. 2003, Shin et al. 2003, Pereira et al. 2004, Pereira 2005). LCFA accumulation with biomass is recognized as

having three mechanisms: precipitation, absorption, and entrapment (Figure 2.3). As noted in Figure 2.3, calcium can aid in LCFA precipitation by the formation of insoluble salts (Angelidaki & Ahring 1992, Chen et al. 2008). Additionally, LCFA will absorb onto cell membranes, which interferes with cell transport, thereby inhibiting cell function (Pereira et al. 2003, Pereira 2003, Pereira et al. 2004, Pereira 2005). A physical barrier forms, hindering transfer of substrates and products, inducing a delay in initial CH_4 production (Pereira 2005). Also, entrapment of LCFAs within the sludge can cause a scum layer to form as well as flotation, which increases accumulation and limits transport. LCFA inhibition does not appear to be sludge type dependent but is dependent on the physical characteristics of the sludge, e.g. surface area and size distribution (Hwu & Lettinga 1997, Chen et al. 2008). Suspended and flocculent sludge have higher instances of inhibition than granular sludge because the former have more surface area than the latter (Hwu & Lettinga 1997). However, LCFA inhibition can be reversible by mineralization (Pereira et al. 2003, Pereira 2003, Pereira et al. 2004, Pereira 2005). This reversal has been noted with biomass-associated LCFA, biomass with LCFA absorbed onto it, which is inhibited by the physical barrier that the LCFA creates. Despite this barrier, H^+ is small enough to be able to pass through the LCFA layer and into the cell, thereby allowing the hydrogenotrophic pathway to proceed (Pereira et al. 2004). This also suggests that biomass-associated LCFA do not become damaged by the LCFA barrier. When given time without high lipid feedstocks, the biomass-associated LCFA will degrade and mineralize the LCFA to CH_4 and reduce the amount of LCFA in the biomass (Pereira et al. 2004, Pereira 2005). Additionally, it was noted that because loose,

suspended sludge absorbs the LCFA faster, it will also mineralize and degrade it at a

faster rate than granular sludge (Pereira et al. 2004).
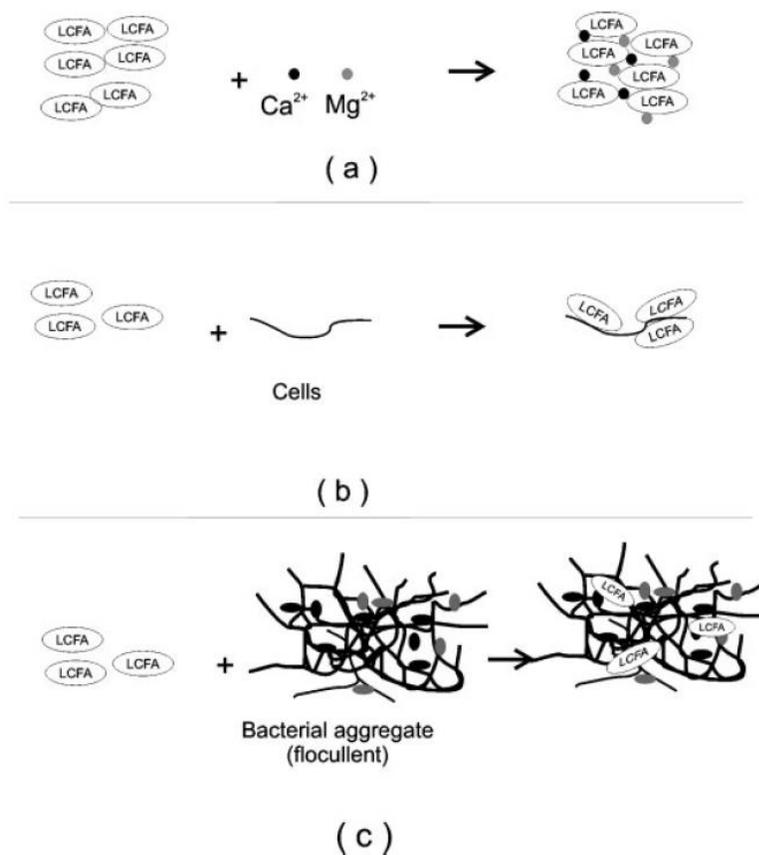
FIGURE 2.3: A schematic of the mechanisms of biomass-associated
LCFA accumulation: (a) precipitation, (b) absorption, and (c)
entrapment (Pereira et al. 2004)

2.3. Digester Systems

Digester configurations have evolved to accommodate factors such as the

feedstock(s) to be processed and their rate of biodegradability; the footprint available;

and even the ambient temperature ranges in which they will operate. A digester system

includes pretreatment, which is optional; digestion (Figure 2.4); and then post-treatment

of the biogas and solids. Each unit operation is described and discussed below.

2.3.1. Digestion

Digestion goals can focus on waste management (volume reduction and stabilization) or product production (energy-rich biogas and nutrient-rich fertilizer), and reactor designs can reflect different optimization aims. There are key design and operating parameters that define AD types (Figure 2.4), and they include operating temperature; mixing regime; solids retention time (SRT); feedstock C:N; the operating F:M; and the pH and concentrations of solids, alkalinity, and VFA concentrations in the digester.

2.4. Digester Configurations

2.4.1. Reactor Number

Reactors can be single or dual stage. In a single stage continuous-flow reactor, all stages of the biochemical conversion of organic matter to CH_4 and CO_2 occur simultaneously in a single tank. In a dual stage design, liquid with a high concentration of VFAs is metered to a second tank where a dense population of methanogens is maintained. This decouples methanogens from pH fluctuations that might occur and inhibit CH_4 production. Azbar and Speece (2001) reviewed several options for staging and operating dual stage systems and noted that the feedstock composition and operating conditions are highly influential factors for obtaining high CH_4 yields. The greater technical complexity of dual stage may explain why higher CH_4 yields are not always realized during full scale operation (Monnet 2003). Nevertheless, their usefulness continues to be explored as a means to simultaneously produce both H^+ and CH_4. Liu et al.(2006) reported a 21% increase in CH_4 yield over single stage digestion when a two stage system was used to digest municipal solid waste at 10% TS, and the CH_4 production was accompanied by production of useable H^+ gas.

2.4.2. Mixing

In liquid-based digestion systems, mixing can be passive or active. Passive mixing is typical in lagoons and plug flow digesters. Lagoons are covered ponds used primarily to treat manure and SS, while plug flow reactors are usually rectangular horizontal-flow tanks where liquid introduced at one-end moves slowly, over a design detention time, to the outlet (Hamilton 2013). Unintended mixing will occur.

Advocates of active mixing argue that the agitation allows more contact between microorganisms and substrates. In these, the mixing minimizes and promotes the release of volatile inhibitory products into the gas phase so that they do not accumulate. It also helps to prevent gradient formation, such as temperature pockets (Grady et al. 1999), or discrete areas where certain microbial populations predominate. Mixing also minimizes the the development of scum sediment layers (dead zones), which is an important operational concern. (Speece 2008).

However, mixing speed and duration must be optimized in order for these benefits to accrue. One known operational problem of waste activated sludge (WAS) fed digesters is the formation of foam. There have been different methods of foam reduction to varying success (Fang et al. 1994, Ghosh et al. 1995, Westlund et al. 1998). When Kim et al. (2002) compared dog food digestion in mesophilic reactors with and without mixing, both reactors had similar volatile solids (VS) reduction; however, the reactor that was not mixed had greater biogas production. The results were repeated in reactors tested at 55°C. Likewise, other studies using substrates such as dog food (Kim & Speece 2002) or municipal solid waste (MSW) codigested with primary sludge (PS) and WAS (Stroot et al. 2001) reported that continuously mixed reactors produce less biogas than once-daily

or minimally mixed reactors. Evidently, reduced mixing aids reactor stability. It has been suggested that mixing should be kept to ". . . zones most likely to gel and solidify, not in the digester volume" (Speece 2008).

2.4.3. Post-treatment

Post-treatment refers to the handling of biogas, digested solids, and liquid digestate. Although biogas is energy-rich, as of 2013, only 5% of U.S. municipal WWTP digesters used the biogas produced (AgStar 2013). Instead, it was flared, because the key objectives of digestion efforts were waste stabilization and volume reduction. With increasing emphasis on renewable energy sources and sustainable resource use, various levels of post-treatment for biogas are being employed to make it suitable for fuel, and options beyond land application are under investigation for the liquid and solid fractions.

2.4.3.1. Gas Upgrading

Biogas typically contains about 60% CH_4 and 38% CO_2. The remaining 2% includes hydrogen sulfide (H_2S), NH_3, hydrogen gas H_2, nitrogen, O_2, carbon monoxide (CO), saturated or halogenated carbohydrates, $H_2O_{(v)}$, dust particles, and siloxanes (Wellinger & Linberg 2000, Wellinger & Linberg 2005). Table 2.1 summarizes gas composition of two biogas samples (one sourced from a landfill and one from an AD) and one sample of natural gas from the North Sea. The final quality of upgraded biogas must match its eventual end-use. Methane in the biogas can be used as fuel in the following systems: for heating water in boilers; to run internal combustion engines in combined heat and power (CHP) systems; as compressed natural gas (CNG) in pressurized tanks; and as feedstock for fuel cells. Biogas used in boilers can be of lower quality than biogas used as CNG, which needs significant upgrading to remove
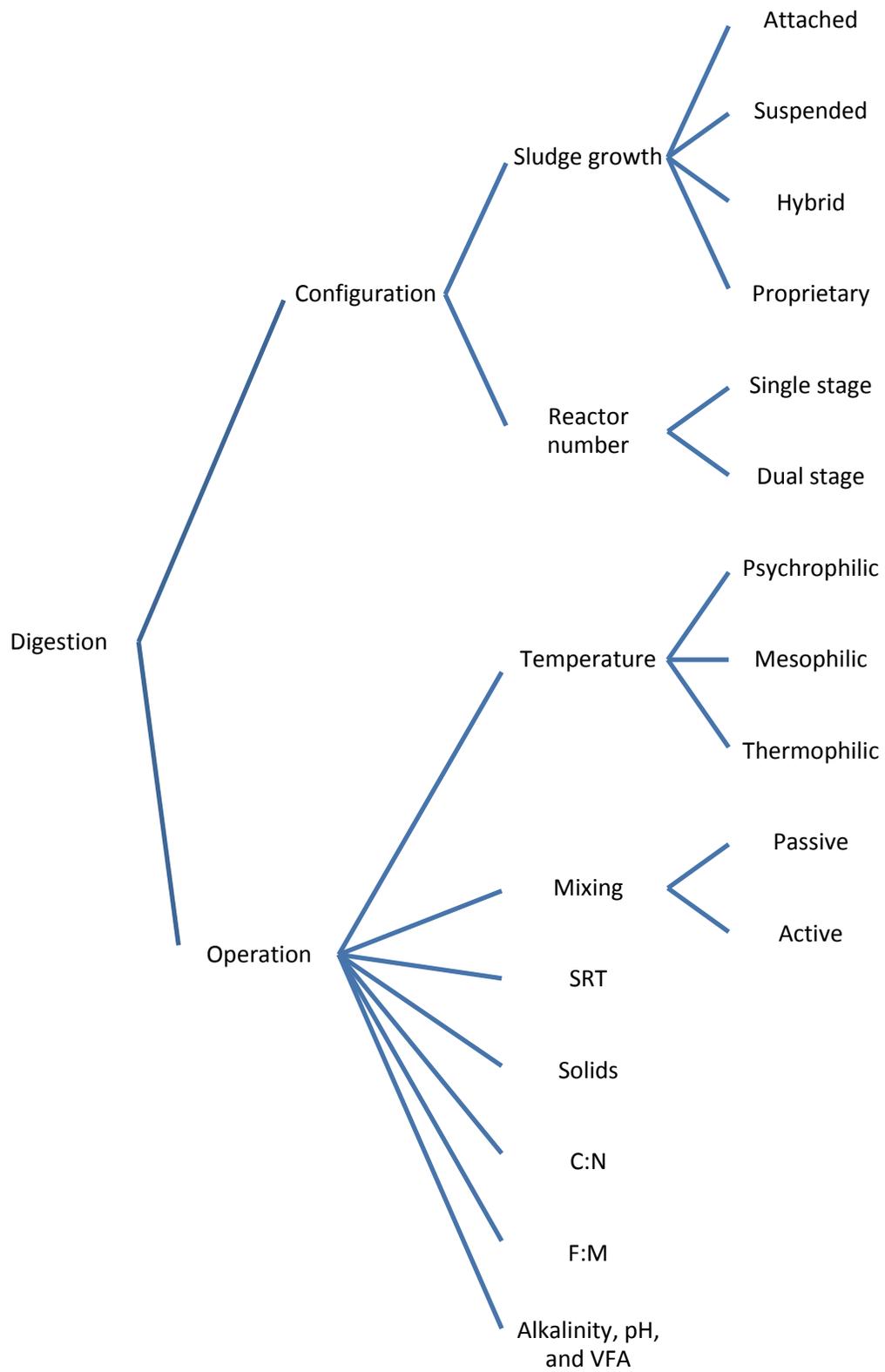
FIGURE 2.4: A schematic of possible digestion configurations and design options

TABLE 2.1: Comparison of biogas characteristics from two infrastructure sources and one natural source (Appels et al. 2008)

Parameter	Unit	Landfill gas	Digestion biogas	North Sea NG
Lower heating value	MJ/Nm^3	16	23	40
	KWh/ Nm^3	4.4	6.5	11
	MJ/kg	12.3	20.2	47
Density	Kg/ Nm^3	1.3	1.2	.84
Methane number		>130	>135	70
Methane (and variation)	Vol%	45 (30–65)	63 (53–70)	87
Higher hydrocarbons	Vol%	0	0	12
Hydrogen	Vol%	0–3	0	0
Carbon monoxide	Vol%	0	0	0
Carbon dioxide	Vol%	40 (15–50)	47 (30–50)	1.2
Nitrogen	Vol%	15 (5–40)	0.2	.3
Oxygen	Vol%	1 (0–5)	0	0
Hydrogen sulfide	Ppm	<100 (0–500)	<1000 (0–10^4)	1.5 (1–2)
Ammonia	Ppm	5	<100	0
Total chlorine (as Cl$^-$)	Mg/ Nm^3	20–200	0–5	0

contaminants prior to use (Wellinger & Linberg 2000, Wellinger & Linberg 2005, Appels et al. 2008, Miltner et al. 2009).

2.4.3.1.1. Heating

Boilers offer the easiest and least costly method of biogas use, and they offer 80% heat production efficiency (EPA 2013a). Upgrading is typically limited to removal of $H_2O_{(v)}$ and H_2S (<1000ppm H_2S) (Holm-Nielsen et al. 2009), and gas pressure adjustment to 8–25 mbar (Wellinger & Linberg 2000).

2.4.3.1.2. Internal Combustion Engines

Use of biogas in internal combustion engines (IC) requires little upgrading of the gas, and such systems are well-proven and reliable. They can be configured as a CHP system, which means that additional energy can be harvested from heat that would
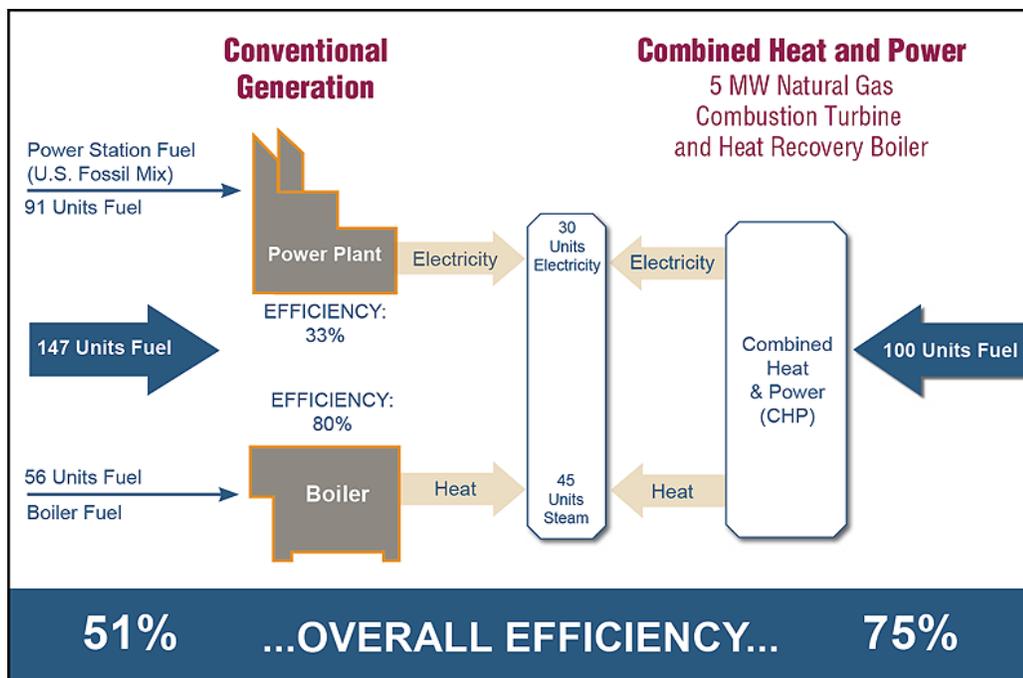
FIGURE 2.5: An example of typical boiler, IC, and CHP systems with their relative efficiencies (EPA 2013a)

otherwise be lost (75% vs. 33% from the engines) (Figure 2.5) (EPA 2013a). Typical engine sizes range from 12 kW$_e$ on small farms to several MW$_e$ at large scale facilities.

2.4.3.1.3. Compressed Natural Gas (CNG)

If biogas is upgraded to natural gas standards, it can be mixed with or substituted for commercial natural gas or sold as CNG fuel for vehicles. Such upgrading requires new technology and infrastructure as well as vehicle conversion and new fueling station accommodations. In 2013, the cost for a U.S. large truck conversion to CNG use was $30,000–40,000; the cost for passenger vehicles and pickup trucks was $9,000–15,000; and the cost to purchase new CNG vehicles was 10–20% more than for conventional vehicles (Voell 2013). Nevertheless, the payback period is expected to be 1–5 years if gas and diesel prices remain at $3.50–4.00 per gal. Emission reductions in volatile organic

carbon, particulate matter, and other air toxins would also accrue, although no dollar estimate has been placed on these benefits. As of 2005, more than 2 million CNG vehicles and about 10,000 biogas fueled cars and buses were operated worldwide (Wellinger & Linberg).

2.4.3.1.4. Fuel Cells

Biogas-powered fuel cells (FCs) generate direct current electricity by combining fuel and O_2 in an electrochemical reaction. Because FCs do not require a step where fuel is converted to mechanical energy and heat, they produce extremely low emissions and have at least 50% efficiency (Wellinger & Linberg 2000). The typical fuel used is H_2, which can be from pure H_2 or from a hydrocarbon, and air is introduced as a source of O_2 (Minnesota 2003). Hydrogen is converted to electrical current, with the resultant H^+ ions combining with the O_2 to form water, which releases two electrons that are made available to power an external circuit (Figure 2.6). In the U.S., most H_2 produced from CH_4 comes from a process known as steam methane reforming (SMR) (DOE 2013).

The process uses high-temperature steam (700–1000°C) to produce H_2. The CH_4 reacts with the steam to produce H_2, CO, and small amounts of CO_2. Another source of H_2 is AD, where small amounts of H_2 are naturally generated. This has been the focus of recent research (Chang et al. 2002, Fountoulakis & Manios 2009, Dong et al. 2011). Altering the pH and hydraulic retention time (HRT) increases the amount of H_2 produced (Fountoulakis & Manios 2009) while CH_4 is co-generated. The combined production of CH_4 and H_2 increases the overall energy efficiencies from 33.5% (H_2 alone) and 83.2% (CH_4 alone) to 89.0% (H_2 and CH_4) (Dong et al. 2011).
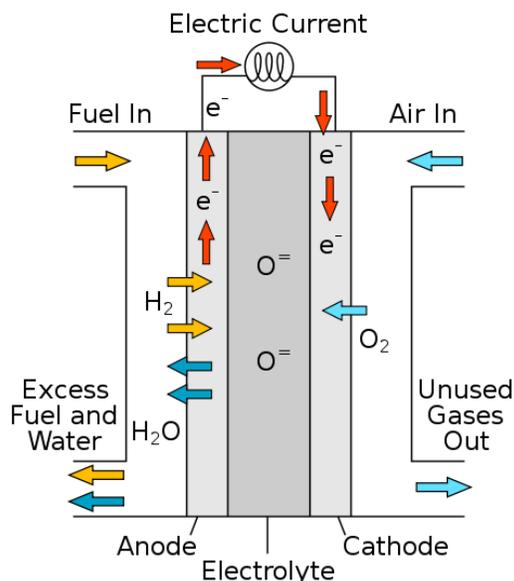
FIGURE 2.6: A schematic of a typical fuel
cell (Fuel 2009)

Several FC types are in operation, and they are distinguished by the material that
allows charges to move between the two sides of a cell. Some of these materials include
phosphoric acid, molten-carbonate, solid oxide, and proton exchange membranes.
Typical FC types and characteristics are shown in Table 2.2.

TABLE 2.2: Fuel cell types and characteristics (Wellinger and Linberg 2000)

Fuel cell and characteristics	PAFC	MFC	SOFC	PEM
Electrolyte	Phosphoric acid (H_3PO_4)	Molten carbonate ($LiKCO_3$)	Solid oxide (Y_2O_3 and ZrO_2)	Membranes
Operating temperatures (°C)	200	650	1000	50–120
System Efficiency (%)	40–45	50–57	45–50	
Module Size	200 kW – 2MW	2 MW	3 – 100 kW	
Fuel type	Natural, coal, or landfill gas			Gases MeOH

2.4.3.2. Digestate

AD digestate is a combination of undigested solids and liquid left after batch digestion or that exit digestion after a fixed hydraulic and/or solids retention time. It contains microbial biomass and undigested (biodegradable and non-biodegradable) material (Frischmann 2012). The solids fraction can represent 60–80% of the original feedstock solids, and will typically retain 60–80% of the feedstock phosphorus, 20–25% of its nitrogen, and 10–15% of its potassium (Holm-Nielsen et al. 2009). As a general rule, the amount of digestate (liquid and solid fraction) created will be 85% (weight basis) of the total feedstock added (Frischmann 2012), and its characteristics are highly dependent on the feedstock (Holm-Nielsen et al. 2009). However, after separation of the two fractions, the solid fraction will contain 25–35% total solids after drying, because of its high water holding capacity.

In the U.S., most digestate is used for on-site agricultural application. The liquid fraction can be applied as fertilizer, while the solids can be composted and used for agriculture or as animal bedding (Alexander 2012). Therefore, great care needs to be taken to ensure the feedstocks do not contain any contaminants (Frischmann 2012). Alternatively, digestate can produce energy via combustion, and the resulting ash can be utilized in building material (Li et al. 2013). While combustion will result in loss of valuable nutrients within the digestate, it does reduce the risk of other contaminants entering the food chain. If contaminants are a concern, combustion would need to be monitored to ensure that contaminants did not enter the environment via air exhaust or via improper ash disposal. In municipal or commercial codigestion facilities, digestate

marketing will likely be part of facility financial plans. Digestate post-processing and characterization may be necessary, and quality control measures may be required.

2.5.   Digester Operation

2.5.1.   Temperature

There are three temperature ranges used for AD designs: psychrophilic (less than 20°C) (Gerardi 2003), mesophilic (20–45°C), and thermophilic (50–65°C) (Monnet 2003, Abbasi et al. 2012). Psychrophilic reactors rely on ambient temperatures and require longer retention times (>12 weeks). As a result, reactor volumes must be larger, and their use is typically limited to small WWTPs and farms, where they may be designed as lagoons (Appels et al. 2008).

Mesophilic and thermophilic reactors encompass the majority of reactors today, with mesophilic reactors predominating (Abbasi et al. 2012). Table 2.3 shows that use of the alternate temperature ranges results in differences in loading rates, solids reduction efficiency, pathogen destruction, and toxicant sensitivity among other distinctions between the operating modes. Many of the factors listed in Table **2.3** are due to differences in the microbe populations that predominate under the two temperature regimes. Mesophilic bacteria can withstand a 2–3°C per day temperature change, while thermophilic bacteria cannot (less than 1°C per day) (Gerardi 2003).

In one study, codigestion of fruit and vegetable waste (FVW) with abattoir wastewater (AW) at two different HRTs (10 d and 20 d) showed that CH_4 yields increased when the temperature was raised from 35ºC to 55ºC at a 20 d HRT. The same was not true for the trial operating at a 10 d HRT, as the reactor failed due to overloading (Bouallagui et al. 2009). The fact that the mesophilic reactors were not affected by the

change in HRT, illustrates an attribute that often makes mesophilic operation a favored design choice; they tend to be more stable and less sensitive to changes. This was well demonstrated in a study comparing reactors treating FOG wastewater (Hwu & Lettinga 1997). Anaerobic sludge was collected from mesophilic and thermophilic reactors, and the FOG substrate (oleate) was digested by each of the inoculants at its source temperature. Each reaction vessel was then dosed with varying levels of oleate to determine its inhibition concentration, and the thermophilic reactors proved to be more susceptible to acute oleate toxicity. Thus, while thermophilic reactors can often produce higher CH_4 yields for a given reactor volume, designers are also cognizant of the costs of maintaining higher temperature levels and the greater sensitivity of the microbial biomass to shifts in temperature, feeding regimes, new substrates, and toxins.

2.5.2. pH, Volatile Fatty Acids (VFA), and Alkalinity

The optimal pH range for AD CH_4 production is about the same as the range for optimal methanogen performance (Grady et al. 1999, Monnet 2003, Appels et al. 2008, Speece 2008). For SS, the optimum pH range for CH_4 production is 6.4–7.2. Municipal

TABLE 2.3: Comparison of mesophilic and thermorphilic reactors (adapted from (Gerardi 2003))

Feature	Mesophilic Digester	Thermophilic digester
Loading rates	Lower	Higher
Pathogen destruction	Lower	Higher
Toxicant sensitivity	Lower	Higher
Operational costs	Lower	Higher
Temperature control	Less difficult	More difficult
Bacteria growth	Higher	Lower
Diversity	Higher	Lower
Solids destruction	Lower	Higher
Solids retention time	Higher	Lower
Methane yields	Lower	Higher

solid waste (MSW) digestion shows a similar optimum range of 6.6–7.0 (Monnet 2003). Methanogens are quite sensitive to pH, with all studies showing clear activity declines below pH of 6.4; however, other microbes, such as the fermentative bacteria that produce VFAs, can tolerate low pH (Appels et al. 2008).

The maintenance of pH levels in a digester mix occurs primarily as a result of the bicarbonate buffering system and the NH_3-NH_4^+ system (Grady et al. 1999, Gerardi 2003, Deublein & Steinhauser 2008). During fermentation of organic material, CO_2 is produced. Also, CO_2 and NH_3 are released during amino acid and protein degradation (Grady et al. 1999, Gerardi 2003). The CO_2 and NH_3 partition themselves between the headspace and liquid fractions of the mix, which adds to the alkalinity of the liquid phase. Dissolved CO_2 can react with water to form carbonic acid. The latter participates in pH-dependent equilibrium reactions that can lead to the presence of bicarbonate or carbonate alkalinity. Dissolved NH_3 exists in equilibrium with NH_4^+, so that this system has buffering capacity as well. Finally, with NH_3 and CO_2 dissolved in water, ammonium bicarbonate (NH_4HCO_3) can form, which further alters the equilibrium chemistry. The relevant equilibrium equations are shown in Eq. 2.9 through Eq. 2.11 (Grady et al. 1999, Gerardi 2003, Deublein & Steinhauser 2008, Speece 2008):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$
$$\leftrightarrow 2H^+ + CO_3^{2-}$$
\hfill Eq. 2.9

$$NH_3 + H^+ \leftrightarrow NH_4^+$$
\hfill Eq. 2.10

$$NH_3 + CO_2 + H_2O \leftrightarrow NH_4HCO_3$$
\hfill Eq. 2.11
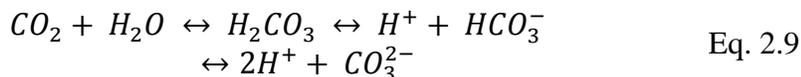
While alkalinity is consumed by VFAs generated during organic degradation, alkalinity is created during the production of CH_4 (Gerardi 2003, Speece 2008). This complementary system helps maintain digester stability. For example, when glucose is degraded to an

organic acid (Eq. 2.12), the acid product consumes some alkalinity (Eq. 2.13). But that alkalinity is recovered when CH_4 is produced from ammonium acetate (Eq. 2.14).

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \qquad \text{Eq. 2.12}$$

$$3CH_3COOH + 3NH_4HCO_3 \\ \rightarrow 3CH_3COONH_4 + 3CO_2 + 3H_2O \qquad \text{Eq. 2.13}$$

$$3CH_3COONH_4 + 3H_2O \rightarrow 3CH_4 + 3NH_4HCO_3 \qquad \text{Eq. 2.14}$$
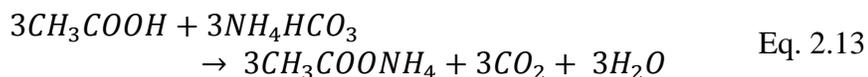
Stability is most robust in the middle of the optimum pH range, because this is where the buffering capacity is greatest and the bacteria are least vulnerable to toxic effects (Gerardi 2003, Deublein & Steinhauser 2008, Speece 2008). If VFA production out-paces downstream reaction rates, buffering capacity can be rapidly depleted and instability can result (Appels et al. 2008). Certain feedstocks tend to promote such instability. Substrates with high lipid content; FVW; high carbohydrates (grass, food, corn, and straw); and high proteins (whey and animal products) have all been shown to trigger instability (Steffen et al. 1998).

There is some evidence that the particular species distribution of VFAs in a digester may be a function of pH (Zoetemeyer et al. 1982, Horiuchi et al. 2002). When digestate microbes processed glucose in a reactor operating at pH 5.0–7.0, the main soluble VFA products were butyric and acetic acid. When the pH was shifted to 8.0, the products shifted to more acetic and propionic acid, with low butyric acid concentrations (Horiuchi et al. 2002). When municipal WWTP secondary sludge microbes processed glucose, the same acid product distribution was evident in the lower pH range, but when the pH was raised to 8.0, the main products shifted to lactic acid, formic acid, and ethanol (Zoetemeyer et al. 1982). Although there are some methodological issues to consider when comparing the two studies (e.g. (Horiuchi et al. 2002) did not use replicates or

statistical analyses). The results reflect the interactive effects of pH on chemical equilibria, buffering, and microbial species selection.

Reactor stability is often monitored in terms of VFA content and bicarbonate alkalinity, which together can be used to determine total alkalinity (TA) (Gerardi 2003). A VFA:TA ratio (Grady et al. 1999, Speece 2008) close to 0.1 is desirable, with greater instability as the ratio approaches 0.5. At a VFA:TA ratio > 0.8, a digester is typically considered unstable and can be expected to "sour". As acid build-up consumes alkalinity, pH levels fall, and methanogenesis is inhibited. This further retards the uptake of CO_2, which leads to further pH decline as more of the gas dissolves and reacts with water to form carbonic acid (Grady et al. 1999).

Alkalinity amendments can be used to increase buffering capacity in digestion, but their use adds to operating costs and must be evaluated on a case-by-case basis; the reactions within a digester are complex, and there is the potential for such additions to lead to toxic effects (Grady et al. 1999). The most common chemical option for pH adjustment is sodium bicarbonate because it is seldom associated with harmful side effects (Grady et al. 1999). Codigestion of feedstocks can be a means to avoid chemical amendments for alkalinity control (Grady et al. 1999, Monnet 2003, Speece 2008, Bouallagui et al. 2009). Because the amount of buffering capacity generated by different feedstocks varies, feedstocks can be paired, such that those likely to create unstable pH conditions are combined with others that modulate those effects. When AW was paired with FVW, the alkalinity contributed by the high nitrogen content of the AW was credited in part for the more successful CH_4 production by the mix than by either feedstock alone (Bouallagui et al. 2009).

2.5.3. Sludge Growth

Efficient microbial activity is at the heart of a digester system, and a wide variety of systems have been devised to support their growth. The earliest systems were suspended growth reactors such as the classic cylindrical tank. The contents are well mixed with no separation of liquid and solid layers, so that the HRT and SRT are the same (Grady et al. 1999). The typical retention time is 15–20 d. The microbes are able to float freely within the digestion liquid, although many attach to particles from the substrate. Such reactors can accept a wide range of wastes; but large reactor volumes are required (Abbasi et al. 2012).

A variation on conventional suspended growth digester design is the upflow anaerobic sludge blanket (UASB). In this system, inflow to the reactor enters at the bottom and passes upward through a blanket of sludge "granules" that form and are dense with microbes. Settleable solids fall by gravity to the bottom of the containment. The composition of the granules and the layering of key microbial groups within them can change depending on the substrates being digested (Fang et al. 1994). The collective mass of granules creates a blanket that is suspended by the upflow of the wastewater. (Grady et al. 1999). UASBs constitute a more compact and vertically oriented reactor design, and they permit operation with differences between HRT and SRT. These systems require excellent separation of the gas, liquid, and solid phases, and their success depends on the development and maintenance of dense, settleable solids (Grady et al. 1999). Show et al. (2004) demonstrated the efficacy of polymer addition to enhance start-up and granule formation in UASBs, which is a critical element for their successful operation. Recently, UASBs have considered for H_2 production because of their ability to

maintain high levels of biomass. However, they also require a long startup time to cultivate H_2 producing bacteria (Jung et al. 2013). Use of a settling tank with high rate recirculation to cultivate H_2 producers reduced startup time from a few months to 10 d (Jung et al. 2013). Fixed film digester designs were developed to further exploit the propensity of microbial biomass to attach. Some ideal medium properties reviewed by (Agamuthu 1999) included porosity, high surface area, surface properties that promote adherence, low weight, and low cost. Media such as wood chips or small plastic rings are common materials used to create packed columns through which low-solids (1–5% TS) substrate laden liquid passes (Abbasi et al. 2012) (Figure 2.7). Other materials showing good promise include coconut coir and sisal fiber waste (Acharya et al. 2008).

Flow within a fixed film system can be upward or down. The main consequence of the differing flow patterns is the retention of suspended material (Grady et al. 1999). In an upflow reactor, biomass that detaches and becomes suspended is still retained; in a downflow reactor, it passes through the media and is less available for substrate digestion. Because fixed film systems can provide a very high density of microbes in the reactor, retention times can be much shorter (0.5–4 d) (Grady et al. 1999), which means that digester sizes can be smaller. The effluent is usually recycled to help maintain a constant flow (Abbasi et al. 2012).
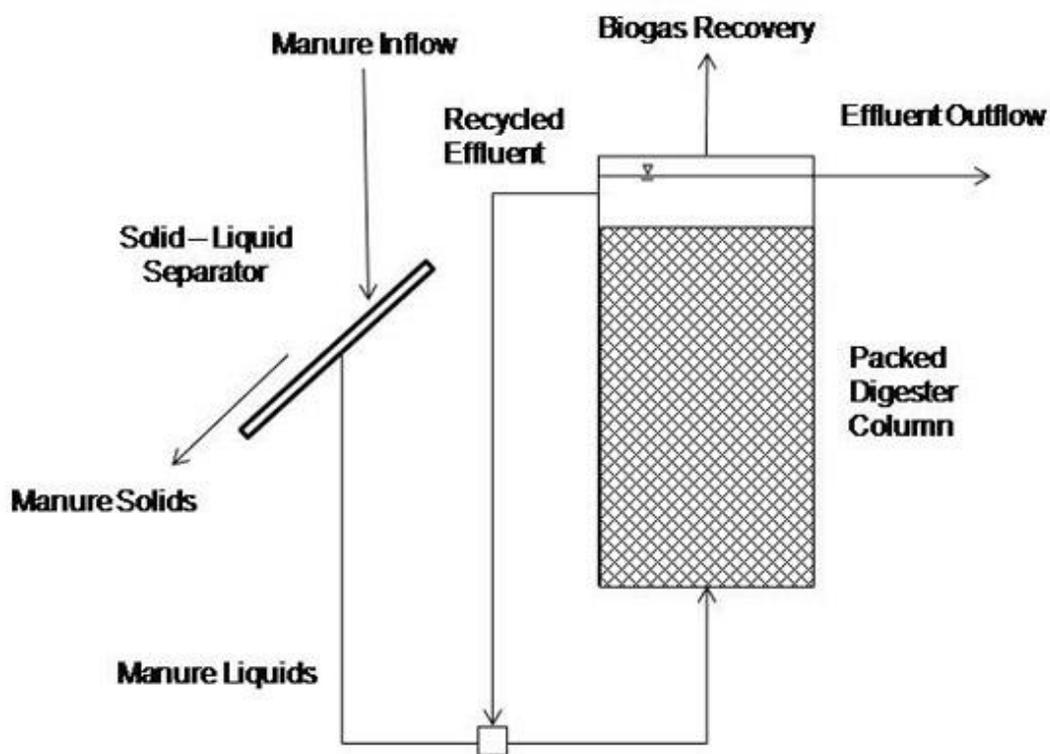
FIGURE 2.7: An upflow fixed film reactor for manure digestion (AgSTAR 2012)

A variety of hybrid and propriety systems have been developed to exploit certain strengths of these various systems for particular applications. A fixed film-UASB hybrid passes influent upward through a granular sludge layer (suspended growth) and then into a fixed film column (attached growth) (Grady et al. 1999). Finally, there are several proprietary digesters, such as BTA, DRANCO, and Biogas-GW. These systems are designed and marketed by companies to treat certain wastes. BTA is a German-based company (Biotechnische Abfallverwertung GmBH & CO.) that targets FW, biowaste, commercial waste, and MSW. It utilizes a hydropulper with grit removal and separation of light and heavy fractions before wet digestion (Haines 2008). DRANCO is a Belgium company (Organic Waste Systems) that uses thermophilic dry digestion for MSW,

biowaste, and SS (OWS 2013). Biogas-GW is a US-based company (Eisenmann), targeting grease, food, and yard waste. Their system is thermophilic, and feedstock moves along a long horizontal tube during digestion (Eisenmann 2013).

2.5.4.   Carbon to Nitrogen Ratio (C:N)

Neglecting water, a typical bacterial cell is about 50% carbon and 12% nitrogen (Gerardi 2003, Tchobanoglous et al. 2003). Thus, the C:N ratio in a reactor is an important parameter of digester stability (Backus et al. 1988, Yen & Brune 2007, Liu et al. 2008, Rughoonundun et al. 2012), because bacteria and other microbes in the reactor mix require these nutrients for cellular function and replication. Also, the ratio has been shown to influence the distribution and variation of VFAs present (Liu et al. 2008, Rughoonundun et al. 2012) as well as the amount of biogas produced and its CH_4 content (Backus et al. 1988). The optimal C:N ratio for stable growth varies from organism to organism (Tchobanoglous et al. 2003). In a digester's anaerobic environment, a C:N level below 10 can lead to high total NH_3 release, which can inhibit methanogens. While a C:N level above 30 can restrict cell growth due to lack of nitrogen (Monnet 2003, Deublein & Steinhauser 2008, Liu et al. 2008).

There are numerous examples of variations in C:N ranges optima under different operating conditions. In a mesophilic batch study, SS was digested in seed sludge from an UASB digester (Liu et al. 2008). The C:N was altered by adding maize starch and soybean protein, and the optimal C:N ratio range was found to be 10–30. In a thermophilic batch study, bagasse sugarcane was mixed with primary and secondary SS (Rughoonundun et al. 2012). The mix was digested in digestate from a WWTP, and the results showed an optimal C:N range of 13–25. A similar C:N range (12–23) was

observed when leafy biomass waste (sisal pulp) and fish waste were digested in sisal wastewater sludge at varying C:N ratios (Mshandete et al. 2004). This batch study was conducted at ambient temperature (27°C). In a semi-continuous mesophilic study, algal sludge and paper waste were tested at differing percentages and C:N ratios (Yen & Brune 2007). The optimal C:N range was 20–25. Finally, in another mesophilic semi-continuous study, cheese whey was digested in effluent from a whey treatment facility (Backus et al. 1988), with the whey mixed with lactose and ammonium hydroxide to vary the C:N level. The optimal C:N range for cheese whey digestion was 22–28. Together the examples provided ranges of 10–30 (Liu et al. 2008), 13–25 (Rughoonundun et al. 2012), 12–23 (Mshandete et al. 2004), 20–25 (Yen & Brune 2007), and 22–28 (Backus et al. 1988), which although different, share a central tendency around the ratio of 20:1. It should also be noted that at their best, such ranges are "only an indication, because nitrogen can also be bound in lignin structures" (Deublein & Steinhauser 2008).

When a single feedstock type is digested, there is little operator control over C:N ratio, but codigestion of two or more feedstocks offers the opportunity for adjustment and modulation of the ratio into an optimum range (Monnet 2003, Deublein & Steinhauser 2008, Zhi & Zhou 2011). Paper, yard waste, and woody material have high C:N ratios, while manure, poultry litter, and high-protein waste tend to have low C:N ratios (Steffen et al. 1998, Vandevivere et al. 2003, Bouallagui et al. 2009, Jihen et al. 2010, Singh et al. 2010).

2.5.5. Solids

The solids content of a feedstock is often used as a basis for determining feedstock loading rates into a digester. A measure of total solids (TS) is taken after all the

water has been driven from a sample by heating it at 105°C until only dried solids remain. Subsequent combustion at high heat (550°C) can be used to distinguish between volatile solids (VS, those that are volatilized during combustion) and fixed solids (FS, those that remain). Because organic biodegradable molecules composed of carbon and nitrogen tend to volatilize at high temperature, the VS fraction is often used to represent the organic fraction of a substances like feedstocks or microbial mass (Monnet 2003). Of course, use of VS to estimate organic content is imprecise, because short chain VFAs are not solids suspended in the liquid. They may volatilize during drying of the substrate when determining TS (Sommer et al. 2013). Additionally, the VS measurement includes slow or non-degradable fraction of the substrate. Other measures of organic content that are used include chemical oxygen demand (COD) and total organic carbon (TOC). COD is a measure of the equivalent oxygen of the organic matter than can be oxidized, using a strong chemical oxidizing agent in an acidic medium (Tchobanoglous et al. 2003). It is a quick method, about 2 h; however, there are limitations when working with solid and semi-solid materials. These substrates reduce the reproducibility, reliably, and accuracy in replicates, producing high standard deviations. This has prompted researchers to use VS measurements instead when dealing with high solids materials (Moody et al. 2011a). TOC is the measure of the organic carbon in a sample. (Total carbon is a measurement of all the carbon in a sample, inorganic and organic.) It is measured by injecting a known quantity into a high-temperature furnace where the organic carbon can be oxidized to CO_2 in the presence of a catalyst (Tchobanoglous et al. 2003). The CO_2 produced is then measured. While this is another quick organic content measurement, not all organic compounds are oxidized. Some resistant organic compounds may not oxidize thereby

reporting a reduced TOC content. Also, leaks associated with the gas flow system can cause false positive TOC results and drying or pretreating the sample may remove volatile organic compounds and loss of other organic compounds (Schumacher 2002).

Most biological material contains 45–60% carbon, so that the mass of carbon in a sample is about 55% of the mass of organic matter in a sample (Richard 1996). Digester volumes are often designed based on VS loadings or organic loading rates (OLR), where the organic loading is described in terms of the daily mass of VS applied per unit of reactor volume (Monnet 2003, Appels et al. 2008). As the organic material is degraded and converted into biogas, the amount of VS remaining within the digester declines to some steady-state value, providing a reliable indicator of stabilization. The percent VS reduction can be monitored to detect problems with reactor operations (Appels et al. 2008).

There are three ranges of solids concentration levels typically maintained in digesters. Wet digestions (low TS) have no more than 10% TS, semi-dry digestion (medium solids) have 15–20% TS, and dry digestion (high solids) have 20–40% TS. The concentrations are measured as weight per volume or wet weight per weight. Most AD systems are operated as either low or high solids systems. Wet digestion corresponds to the earliest suspended growth systems, and they can be operated as continuous stirred tank reactors (CSTRs), plug flow systems, or even in batch. A wet digestion system may require the addition of water, especially with high solid feedstocks, and this water may be wastewater from another operation. Dry digestion systems allow for simpler pretreatment of the waste. They can handle particle sizes up to 40 mm, so that only large impurities require removal. The feedstock mix is highly viscous, and plug flow reactors are

generally used. High solids digestion has the advantage of not requiring mixing devices and the associated energy costs, although mechanical devices are often included to press the feed through a vertical or horizontal cylindrical containment. Also, adequate mixing of the feedstocks with digestate prior to feeding is required to seed the mix. Plug flow dry digesters are smaller and require less heating, pumping, and dewatering because there is less water content (Monnet 2003). However, the time required for complete digestion is often longer in such systems, because the feedstock is compacted and susceptible to degradation by only a localized population of microbes.

2.5.6. Food to Microorganism Ratio (F:M)

The F:M ratio (g VS $_{substrate}$/g VS $_{inoculum}$) reflects the amount of organic substrate provided per unit mass of inoculum in the system. While the inoculum is typically SS or animal manure, the "food" can be very diverse. It might be watery grease interceptor wastes, highly dewatered grease interceptor wastes, solid highly biodegradable food waste with 70% water content, or dry and very heterogeneous poultry waste and litter that is only partially biodegradable. The F:M ratio is a particularly important parameter for biogas optimization and biodegradability because it provides a balance between the amount of food and organisms (Kayhanian 1995, Grady et al. 1999, Prashanth et al. 2006, Sri Bala Kameswari et al. 2011). For instance, if there is too much food (high F:M), then there would be more food left over, less VS reduction. In contrast, if there isn't enough food (low F:M), then the microorganisms must compete for any available food, which would lead to microorganism in starvation mode and reduced CH_4 production. Finding and maintaining the correct F:M ratio is a balancing act to provide the microorganisms

with just enough food to make them efficient without having any leftovers (Grady et al. 1999).

When mesophilic biochemical methane potential (BMP) tests were conducted on cellulosic feedstocks (cellulose, napiergrass, and energycane) using active digester sludge inoculum at F:M ratios of 1.1, 0.67, and 0.53 g VS $_{substrate}$/g VS $_{inoculum}$ (Chynoweth et al. 1993), the the optimal F:M ratio was 0.5 g VS $_{substrate}$/g VS $_{inoculum}$ (Chynoweth et al. 1993). In another BMP study, fleshing, a solid tannery processing waste was codigested with primary and secondary sludge from a tannery wastewater treatment facility (Sri Bala Kameswari et al. 2011). The inoculum was waste activated sludge (WAS) from a wastewater treatment facility and the optimal F:M range was 0.43–1.0 g VS $_{substrate}$/g VS $_{inoculum}$ (Sri Bala Kameswari et al. 2011). There was no incubation temperature reported, but the C:N ratio was 6. The most noticeable CH_4 production increase was observed when the F:M was decreased from 1.5 to 1.0 g VS $_{substrate}$/g VS $_{inoculum}$; there was no significant difference in yields when the F:M was changed from 0.43–1.0 g VS $_{substrate}$/g VS $_{inoculum}$. While reactors were tested in duplicate, no statistical analysis was reported to support the authors' claim of non-significance. Yet, their F:M range was in line with findings from other researchers (Chynoweth et al. 1993, Tanaka et al. 1997, Raposo et al. 2009).

When sunflower oil cake (SuCO), a high lipid feedstock, was digested, the optimal F:M range was 0.33–1.25 g VS $_{substrate}$/g VS $_{inoculum}$ (Raposo et al. 2009). Seven mesophilic (35°C) reactors were used to analyze different F:M treatments; however, no replication was indicated. The SuCO was digested using granular anaerobic digestion sludge from a brewery treatment facility. Conversely, another BMP study of food waste

and vegetable oil in food-processing industrial wastewater showed a negative relationship between CH_4 yield and F:M ratio (Maya-Altamira et al. 2008). Under mesophilic conditions, the optimal F:M ratio range was 0.25–0.50 g VS $_{substrate}$/g VS $_{inoculum}$. Similar negative relationships between F:M and CH_4 yields have been reported by others for synthetic wastewater samples inoculated with wastewater digestate, where the optimal F:M ratio range was 0.40–0.48 g VS $_{substrate}$/g VS $_{inoculum}$ (Prashanth et al. 2006).

These studies varied widely in their level of detail and the types of materials studied. Optimal F:M ratio appears to depend on the type of substrate analyzed. All of the ranges tested reported good CH_4 production at F:M of 0.50 g VS $_{substrate}$/g VS $_{inoculum}$ is captured among all of the ranges. Therefore, it may be a good starting F:M for codigestion.

2.5.7. Solids Retention Time (SRT)

SRT (θ_c) is an important parameter in AD because it controls the type and number of microorganisms grown within the digester (Grady et al. 1999). The microbial mass (X) and volume (V_r) of the reactor versus the microbial mass (X_w) and flow rate (Q_w) leaving the reactor determines SRT (Eq. 2.15).

$$\theta_c = \frac{V_r X}{Q_w X_w}$$
Eq. 2.15

When a reactor is completely mixed then the equation can be simplified because the biomass wasted and biomass in the reactor are assumed to be equal.

Due to the long generation times of methanogens, a retention time greater than 10 d is required to prevent them from washing out of the system (Gerardi 2003). While SRT is not affected by the nature of the feedstock, it will influence CH_4 production and

digester design (Gerardi 2003). Increasing the SRT, increases the contact time available for microbes to completely degrade a feedstock and yield CH_4. For a given feedstock flow to be retained longer in a reactor, the reactor volume must increase, so digesters with high design SRTs must be sized larger. Higher SRTs may also provide a buffer against possible toxic compounds and shock loadings because the microorganisms have time to acclimate to the changes in the system (Gerardi 2003).

2.6.   Feedstock

AD feedstocks are typically grouped into three categories by source: agricultural, municipal (community, commercial, and light industry), and industrial (Table 2.4) (Steffen et al. 1998). Because degradation success is largely a function of the physical and biochemical properties of a substrate, each feedstock tends to have its own processing and digestion characteristics. For instance, FVW is known to be readily biodegradable. However, it can cause a rapid initial decline in pH because it is so quickly converted to VFAs before much buffering capacity becomes available (Steffen et al. 1998, Velmurugan et al. 2010). Velmurugan et al. (2010) determined that the best way to counter the effects of FVW was to codigest it with SS because the latter was a source of alkalinity for the system. Likewise, poultry litter (PL) has distinct characteristics: 1) its bedding composition can vary by location, 2) it has very high NH_3 content, and 3) it can be very heterogeneous and may include carcasses, feathers, straw, manure, and waste feed (Singh et al. 2010). As new feedstocks become available through waste diversion efforts, new crop development, or industry startups or relocations, each material must be assessed for its anaerobic degradability alone or codigested.

TABLE 2.4: A list of various feedstocks for AD (Steffen et al. 1998)

Category	Feedstock type
Agriculture	Animal manure
	Energy crops
	Algal biomass
	Harvest remains
Community	OFMSW
	MSW
	Sewage sludge
	Grass clippings/garden waste
	Food remains
Industry	Food/beverage processing
	Dairy
	Starch industry
	Sugar industry
	Pulp and paper
	Slaughterhouse/rendering plant

There are a variety of measures in the literature that are used to describe feedstock anaerobic biodegradability or its BMP. A typical test would include feedstock mixed with a microbe-rich inoculum incubated for a period of time until CH_4 production ceased. Nutrients and alkalinity supplementation are sometimes included. Some authors report their data as biogas volume produced, while others assume that a percentage of any measured biogas is CH_4 and report it as such. Others measure the biogas volume and CH_4 concentrations in the gas mix to report the volume of CH_4 measured. Further, some studies will report the volume of gas produced per volume of TS added or digested, while others will normalize gas production to volume of VS added or digested. The percent reduction in VS is another parameter often reported, although it can be confounded by VS contributed by biomass growth in the mix as substrate VS diminishes. Studies that fail to include blanks (inoculum but no substrate) can over-report CH_4 production, as some of the product may stem from activity introduced with the inoculum. Data from

such studies can be useful within a laboratory to compare various substrates, even if conditions are not comparable for comparison with results from another laboratory. Data from individual substrates may not predict success or failure of a feedstock when it is paired in codigestion with another feedstock.

2.6.1. Agriculture

Small farms have been digesting agricultural wastes for centuries. Along with SS, these are the most well-studied substrates (Carlsson et al. 2012), and predominant among them are harvest residue, cattle manure (CM), and PL. Agricultural wastes can include crop remnants (stalks, leafs, and grains), spoiled or low-quality fruits and vegetables, silo leachate, straw, and energy crops that as a category, are the most resistant to digestion (Steffen et al. 1998). Their organic structure is largely cellulose, hemicellulose, and lignin (Taherzadeh & Karimi 2008, Hendriks & Zeeman 2009), all of which are challenging polymers to degrade relative to simple soluble compounds.

Cellulose is a crystalline polymer comprised of linear polysaccharide molecules packed together in microfibril units (Ha et al. 1998). Each unit is a combination of fibrils bound together by hemicelluloses and covered in lignin (Delmer & Amor 1995, Hendriks & Zeeman 2009). Lignin is particularly resistant to most microbial enzymes, and the unit structure of the microfibrils makes it difficult for microbial enzymes to access the more biodegradable hemicellulose and cellulose. Various pretreatment strategies have been applied to overcome these challenges, but they add to the cost of treatment and energy production. Harvest residuals can also introduce physical and chemical contaminants, such as pesticides or entrained sand and grit, that can interfere with digestion (Steffen et al. 1998).

It is estimated that there are over 30 million digesters worldwide processing animal manure (Chen et al. 2010, Rao et al. 2010). In the U.S., digestion of dairy manure has been used with some success, although the U.S. Environmental Protection Agency estimates that there is the potential for far more energy generation from manure sources than is currently occurring (AgStar 2013). Some analysts suggest that AD, especially for energy production, requires large herds (200–500 cows) to be economically feasible (Mehta 2002, AgStar 2013). The rise of industrial scale farms with confined animal feeding operations (CAFOs) does provide more opportunities for concentrated quantities of manure feedstock to be available, and their use of AD can avoid many of the risks associated with managing dense manure accumulations. These include risk avoidance of environmental pollution that occurs when natural hazard events or management failures occur (Sánchez et al. 2000, Nayyeri et al. 2009); avoidance of problems linked to manure storage (high air emissions of NH_3, nitrous oxide (N_2O), CH_4, and odors (Amon et al. 2006a)); and problems related to land applications of manure slurries (Table 2.5). Further, CAFOs tend to be complex operations with multiple waste streams, so that additional sources of waste-related feedstocks are generated. For instance, in CAFOs and poultry processing plants, additions like hatchery waste (HW) or dissolved air floatation (DAF) wastes from wastewater pretreatment operations can generate additional waste streams.

TABLE 2.5: High solids manure slurry problems (Amon et al. 2006a)

	Problems
Storage	Crust formation and sedimentation of solids
	High energy consumption for pumping and mixing
	Emissions of N_2O, CH_4, and odor
Spreading	NH_3 losses
	High technical effort for even and low emission application
	Suffering of plants due to scorching by slurry
Fertilization	Less effective than mineral fertilizer
	Effect less predictable than from mineral fertilizer
	N immobilization in the soil
	Denitrification and subsequent N_2O emissions

Swine manure digestion has not been developed as fully as that for dairy manure (Liu et al. 2006). The U.S. EPA estimated in a 2010 report that there were 159 operating dairy farm digesters but only 23 swine farm digesters (AgStar 2013). Swine manure is more challenging to digest as a single substrate or even in codigestion with dairy manure because it has higher NH_3 content (Fang et al. 1994). Without more restrictive regulations, there has been little incentive to move toward AD-based swine manure management systems. Such systems could produce energy and other beneficial products. North Carolina, which has about 2500 swine farm operations, recently provided grant funds to incentivize energy production from CH_4 captured over covered swine lagoons. Six farms are installing these systems (Agamuthu 1999).

Poultry manure or manure combined with bedding, feathers and feed is distinct in that it can be so variable from site to site, depending on its source (Singh et al. 2010). It also tends to be much drier in its "as delivered" state than cow manure or swine manure, which are typically collected as slurries (Arora 2011). Interestingly, poultry house operational practices have changed in recent years, such that less external bedding

material is provided for the animals (which makes it better for the animals' feet), so that the distribution of major constituents has changed. Litter now contains less woody material and more manure than previously, which makes it a more valuable digestion feedstock (Arora 2011). Like swine manure, however, it has a high NH_3 concentration, which requires special management for successful AD outcomes (Singh et al. 2010). When PL was digested in mesophilic batch reactors at solids concentrations ranging from 1–10% TS, the maximum CH_4 production (0.41–0.44 m^3/kg VS) occurred at 4–6% TS (Webb & Hawkes 1985). At higher solids concentrations, the higher NH_3 levels present inhibited CH_4 production.

All of the manure feedstocks bring fermentative microbes to the digestion mix as well as organic substrates for biodegradation. Studies of cattle rumen function that were performed in the 1950s informed the microbiology and biochemistry of AD (Hobson & Wheatley 1993). In many instances, CM is codigested with other feedstocks because it is being used as an inoculant as well as a feedstock. This is true for all manures, but among them, CM microbes are the most studied.

2.6.2. Municipal

Food waste (FW) has been investigated as an AD feedstock with the advent of major campaigns to divert more wastes from landfills. With over 35 million tons of readily biodegradable FW generated annually (EPA 2013b), there is the potential to move FW CH_4 production from the landfill to a digester, where the process can be optimized, and landfill space can be reserved for better uses (Heo et al. 2011, Li et al. 2013). FW can be characterized by its carbohydrate, protein, lipid, and fiber composition, which varies widely from mix to mix (Chen et al. 2010). It includes both solid and liquid waste

fractions (Heo et al. 2011). A review of literature reporting on FW digestion reveals that substrates can range from single-source origins, such as fish wastes or beet wastes; or include only FVW; or only meat products (AW); or include a heterogeneous mix of wastes from cafeteria food. Sometimes FW substrates are tested as grab samples from a waste stream, and sometimes composites are created to better represent the natural composition variation that occurs in a waste stream flow.

Because foods vary in their chemical composition and biodegradability, CH_4 generation can be source-dependent, as evidenced by one study conducted by (Chen et al. 2010) on four different FW sources (soup processing, cafeteria, commercial kitchen, and fish farm). Each source sample was batch digested either mesophilically or thermophilically at an OLR of 3 g VS/L (0.27% TS) with an F:M ratio of 0.5 and 1.0. The mesophilic inoculum was digestate from a WWTP, and the thermophilic inoculum was thermophilic digestate from a WWTP. The temperature variations did not affect the trials that tested soup, cafeteria, or commercial kitchen waste, which all demonstrated similar CH_4 yields ranging from 0.25–0.45 L/g VS. However, the mesophilic fish farm waste digested at F:M 1.0 yielded more CH_4 than when digested thermophilically (0.92 vs. 0.38 L CH_4/g VS). The fish farm waste was higher in carbohydrate, protein, and lipid content but lower in fiber (wet weight basis) than the other FW sources investigated.

When these authors blended the various FW sources together with grease trap waste (it is not clear whether this was solids and grease only or whether it included the liquid fraction) and digested the mix mesophilically in continuous flow reactors, the digestions were challenged by rapid acidification. The mix was comprised of 0.6% fish, 14% soup, 20% grease, 24% kitchen, and 42% cafeteria waste at a loading rate of 0.5 g

VS/L-d using an HRT of 20 d. To remedy the rapid pH decline, 0.2 g NaOH/g VS was fed to provide additional alkalinity. With the supplement, the loading rate was eventually doubled, but the shift decreased CH_4 yields from 0.24 to 0.18 g VS/L-d (the authors do not indicate whether the difference was statistically significant). Others report using NH_4HCO_3 to counteract rapid acidification during FW digestion (Bodkhe & Vaidya 2012).

(Bouallagui et al. 2009) summarizes a number of FVW digestion studies and notes that some are successful without alkalinity addition, but others fail due to acidification that inhibits methanogenesis. Trials at less than 5% TS tend to succeed, while those at 8% or higher fail. This observation is echoed by Nagao et al. (2012) who pointed out that although successful FW digestion may be achieved at 1–4 g/L-d loading rates, it is insufficient for economical full-scale digester performance. Such concerns have led to the proposal of a variety of new reactor configurations that include two-stage systems and semi-dry reactors (Bolzonella et al. 2005, Bolzonella et al. 2006, Nagao et al. 2012). Other causes for problematic FW digestion include high lipid content in the waste, a C:N ratio that is sub-optimum, and an imbalance of certain cationic elements required for microbial growth (Zhang et al. 2013).

Blending FW with other feedstocks, such as SS or grease trap wastes is another strategy to overcome some of the problems inherent in using FW as a digestion substrate. When (Marañón et al. 2012) compared different ratios of SS, FW, and CM (70:20:10% and 70:10:20%) in continuous flow mesophilic digestions, the trial with higher FW content outperformed the trial with more SS. The trials were conducted at about 4% TS and with a low organic loading rate (1.2–1.5 g/L-d). The trials had no replicates, so no

statistical analyses were performed. There was also no discussion of pH, and presumably at the low loadings used for testing, excessive acidification was not encountered. (Kim et al. 2003a) sought to study FW and sludge codigestion using a BMP test protocol. Digested SS was tested alone or with FW (collected from a cafeteria) at low TS and VS loadings (2 g VS/L), with trial vessels amended with nutrients and alkalinity. The samples were incubated either thermophilically or mesophilically. Thermophilic trials produced higher CH_4 yields than mesophilic trials, and CH_4 yield per gram VS increased with increasing FW content (Kim et al. 2003a). Similarly, 25%, 50%, and 75% FVW mixes (no meat or bread) were combined with a balance of primary SS (based on VS) and tested in semi-continuous reactors. A low loading rate of 1 g VS/L-d was used, and the samples were incubated at $37^{o}C$ with an inoculum pre-acclimated to FW. The largest biogas volume was collected from the sample with the most FVW waste (Velmurugan et al. 2010). These authors reported their data as averages but showed no statistics, so it is not clear that the differences between trials were statistically significant.

Grease interceptor waste (GIW) (wastes from devices that are plumbed after the dishwasher, food disposal, and sink at food service establishments) is not a municipal waste by strict definition. Most municipalities follow or supplement state guidelines that direct how these wastes are to be managed, and they are typically handled by independent haulers. The wastes are mostly water but contain putrescible food and problematic FOG. The grease and entrained solids fraction together is often referred to as "brown grease" (BG). A 2011 study in Mecklenburg County, NC reported a per capita GIW generation rate (water, solids and FOG) of 3.62 gal (26.5 lb). Of this, the FOG content on average constituted 1.87% of the waste. However, there was high variability in the samples, with

many containing negligible FOG and others containing up to 10% FOG (Hilger et al. 2011). Disposal options for BG include land application, landfilling, incineration, composting, biodiesel production, or AD (Wiltsee 1998). Its high lipid content and even the food solids offer substrate for significant CH_4 production (Kabouris et al. 2008b, Kabouris et al. 2009, Noutsopoulos et al. 2013). However, high lipid levels can be problematic in AD because they can include LCFAs that can inhibit CH_4 production; and they have been linked to digester foaming, sludge flotation, and washout (Long et al. 2012).

Codigestion of GIW with other feedstocks could mitigate some problems with digesting it alone (Acharya & Kurian 2006), and in recent years, the notion of adding it to existing WWTP digesters has been a popular topic of investigation. For instance, Davidsson et al. (2008) observed in 37 d incubations that while GIW (thickened) digested alone in batch reactors produced higher amounts of CH_4 than when codigested with SS (PS and WAS), stable operation of GIW alone in a pilot scale reactor could not be achieved.

In a similar study, GIW (thickened with lime or polymer) was digested alone or with SS (PS and thickened WAS) for 120 d using inoculum from a WWTP (mix liquor) that had been predigested for 90 d (Kabouris et al. 2007). Kabouris et al. (2007) also determined that GIW alone produces more CH_4 than when codigested with SS in batch reactors. However, this study observed less CH_4 production (14% GIW had 410 mL/gVS and 20% GIW had 406 mL/gVS) than Davidsson et al. (2008) for their batch reactors (10% GIW had 425 mL/gVS and 25% GIW had 472 mL/gVS) even though they had similar F:M ratios. The CH_4 yields may appear to be similar; however, (Kabouris et al.

2007) achieved their yields after 120 d compared to only 37 d for (Davidsson et al. 2008). The differences could be attributed to variations in experimental design. (Kabouris et al. 2007) had a higher VS loading of 3.48 gVS/L (14% GIW) and 3.75 gVS/L (20% GIW), while (Davidsson et al. 2008) had a VS loading of 2 gVS/L. The higher loading rate may have been more than the system could handle even with nutrient addition. Also, (Kabouris et al. 2007) manually shook each bottle daily. This could have resulted in improper mixing and reduction of gas release.

Both Davidsson (2008) and (Wang et al. 2013) digested GIW in semi-continuous flow reactors. Davidsson et al. (2008) codigested GIW (30% GIW, VS basis) with thicked WAS and SS, using digestate from a mesophilic WWTP digester as inoculum at a VS loading of 2.4 gVS/L-d and a SRT of 13 d. (Wang et al. 2013) also codigested the GIW with thickened WAS and used digestate from a mesophilic WWTP digester as inoculum. They created a synthetic GIW made from separated portions of GIW collected from a local hauler (10% FOG, 40% food particles; and 50% water by volume) and operated the reactor at an SRT of 20 d. Their VS loading was low (1.6 gVS/L-d), but their synthetic GIW composed 46% of the VS of their inflow. A comparison of some key features of the two studies is shown in Table 2.6. Wang et al. (2013) observed higher CH_4 production. The longer SRT could have contributed to the higher CH_4 production, but Wang et al. (2013) possibly had lower lipid content in their feed because their GIW was not thickened and contained only 10% FOG.

SS is a good co-substrate, especially with substrates that have low buffering capacity (Velmurugan et al. 2010); however, SS has a low biogas yield (250–350 m^3/ton VS) (Braun & Wellinger 2012). It contains a high loading of microorganisms, which can

participate by metabolizing organic materials in the mix or by being particulate substrate for degradation (Dereix et al. 2006, Speece 2008, Beszédes et al. 2011). At WWTPs located in the U.S., 8.3% digest SS (Biogasdata.org 2012). There is a large potential for energy production from SS digestion, with over 8 million dry tons of SS produced annually (EPA 2010).

TABLE 2.6: A summary of the two the continuous and semi-continuous reactor studies

Parameter	Davidsson et al. (2008)	Wang et al. (2013)
VS Loading (g VS/L-d)	2.4	1.6
Percent GIW (% VS basis)	30	46
SRT (d)	13	20
Methane production (L CH_4/gVS)	0.34	0.50

2.6.3. Commercial Poultry Processing Wastes and Biodiesel Wastes

Commercial poultry processing generates two categories of waste that are candidates for AD. The first is hatchery waste (HW), which consists of shells, dead chicks, and unhatched eggs (Glatz et al. 2011). Traditionally, hatchery waste has been treated by composting, rendering, and burning, and there is little information reporting on its behavior as a digestion feedstock (Glatz et al. 2011). The second waste stream is DAF skimmings that stem from treating the poultry processing wastewater. DAF units are suitable for a variety of industrial wastewater streams. DAF (20%) from a yogurt facility was digested in batch with 80% CM. The DAF addition led to increased CH_4 production relative to a manure-only control (Callaghan et al. 1999).

A final feedstock included in this review is canola seed hull (CS), a waxy pellet obtained when canola seeds are pressed to extract oil, a popular feedstock for making biodiesel. While the anaerobic biodegradability of CS has not been studied, digestion of

sunflower oil cake (SuOC) was investigated across different particle sizes (De la Rubia et al. 2011). Samples of three different particle size fractions were digested at 37°C in batch experiments conducted with an F:M of 0.5 (7.5 g:15 g VS) using inoculum from a municipal WWTP (De la Rubia et al. 2011). The highest CH_4 yields negatively correlated with particle size, with a statistically significant difference between the largest (0.213 L/g VS) and smallest (0.180 L/gVS) size fractions. The authors attributed the fractional differences to the solubility of carbon compounds in each fraction and to the VFA profiles they generated. A subsequent study revealed that ultrasonic pretreatment was not an effective means to improve CH_4 production from SuOC (Fernández-Cegrí et al. 2012).

2.6.4. Pretreatment

Over the past 30 years, as AD has shifted emphasis from a technology of waste management to one of energy production, researchers have been exploring ways to optimize CH_4 production and VS reduction. This has led to more investigation of feedstock pretreatment, because the initial microbial interactions that occur with an organic feedstock typically determine how much energy will ultimately be harvested (Carlsson et al. 2012). In AD, hydrolytic bacteria initiate a biodegradation sequence that makes organic material available for other microorganisms in the mix. When a substrate is resistant to hydrolysis, the rate at which all subsequent reactions occur is limited. Therefore, a variety of pretreatment methods have been used as a means to change the properties of resistant feedstocks to make them more bioavailable during digestion (Taherzadeh & Karimi 2008).

In resistant substrates, the desirable organic material tends to be locked behind a barrier. This barrier can be structural, as is the case with the lignin in lignocellulosic
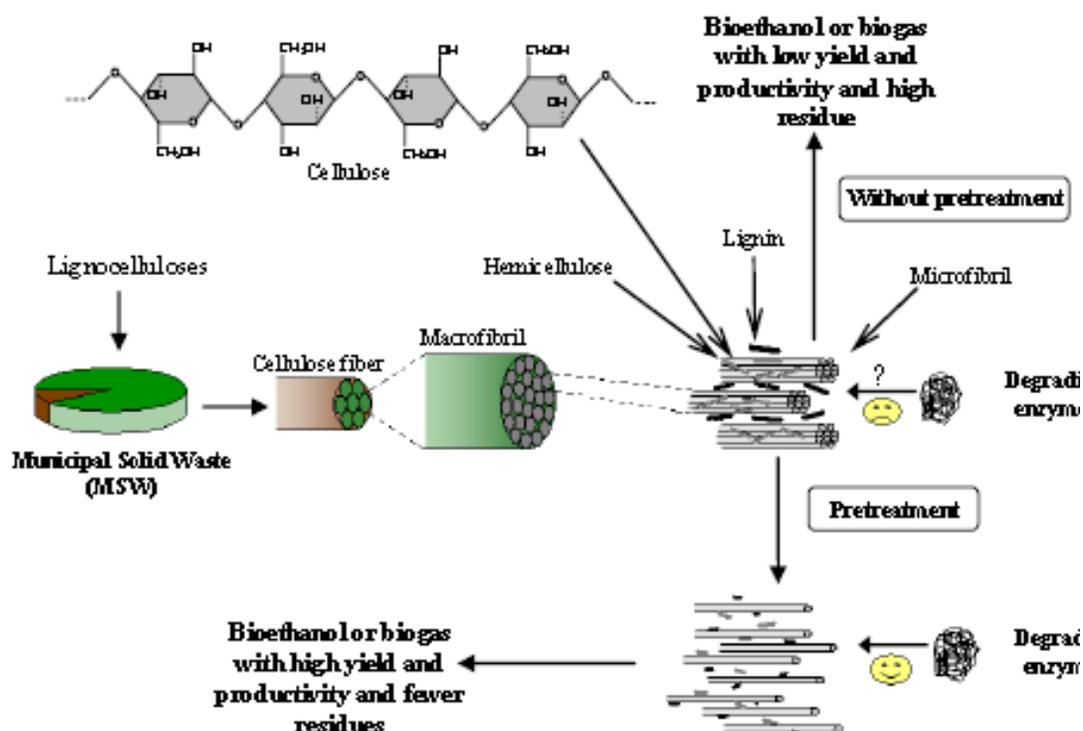
FIGURE 2.8: A depiction of the structure and degradation of cellulosic materials (Taherzadeh and Karimi 2008)

materials; or physical, such as the cell walls and cell membranes of microorganisms in WAS and some components of FW. Lignocellulosic materials include manures, some components of household FW, and many fibrous energy crops. The cellulose, hemicellulose, and lignin are intimately related structurally, so that although they differ in their susceptibility to degradation (Figure 2.8), even the easily digestible fractions may remain unmetabolized, (Taherzadeh & Karimi 2008, Hendriks & Zeeman 2009), because they can be shielded by lignin, which is a three dimensional structure comprised of complex molecules of phenylpropane units that are hydrophobic (Fernandes et al. 2009).

While FW is easily degradable, it benefits from pretreatment that makes lipids, proteins, and lignocellulosic materials more accessible (Stabnikova et al. 2008, Marin et

al. 2010). Pretreatment for substrates like WAS, which contains microorganisms and extracellular polymers (EPS) (Dereix et al. 2006, Speece 2008, Beszédes et al. 2011) aids in breaking through cell walls, which contain glycan strands crossed-linked by peptide chains (Speece 2008). Cell-associated EPS that can surround a cell membrane or be loose in WAS may add another layer of resistance to degradation (Beszédes et al. 2011) that pretreatment can disperse.

In addition to feedstock barrier problems, feedstocks can also be resistant to degradation based on the nature of the products released upon their hydrolysis. For instance, slaughterhouse wastes are lipid rich. When these wastes are hydrolyzed in the digester by extracellular lipases, LCFAs and glycerol are produced. Glycerol is directly degraded into VFAs (Battimelli et al. 2009), but LCFAs are not water soluble. They are adsorbed onto a microbial surface, then transferred through the cell membrane where they are degraded to VFAs by β-oxidation (Li et al. 2002) (See Sec. 2.2). Lipid insolubility makes degradation a slower process. It has been stated that ". . . the limiting step in this process [methanization of pure fats] is assumed to be the physical mass transfer from solid to liquid phase and/or the biological step of LCFA degradation" (Battimelli et al. 2009).

To increase the biodegradation of feedstocks, strategies such as delignification; increasing the surface area of a feedstock available to microbes; cell disruption; and saponification (where a strong base is used on high lipid substrates to hydrolyze triglycerides) can be employed. For lignocellulosic materials, delignification removes the ligneous outer layer (Taherzadeh & Karimi 2008), making it easier for microbes to access the hemicellulose and cellulose fractions. Also, cellulose degradation is aided by

increasing the surface area available to microorganisms. FW biodegradation also benefits from increasing its surface area and disrupting cell walls (Speece 2008, Stabnikova et al. 2008). Finally, saponification can be useful for slaughterhouse wastes or other oily feedstocks to increase their biodegradability (Battimelli et al. 2009). Each of these methods can be achieved by employing different (thermal, chemical, mechanical, and biological) pretreatment options.

2.6.4.1.   Thermal Pretreatment

Thermal pretreatment is conducted over a range of temperatures (150–320°C) and pressures (150–3200 psi) (Speece 2008), and the methods can be described as conventional, hydrothermal, microwave (MW), and freeze/thaw. In conventional thermal pretreatment, the substrate is placed in a sealed container and heated via circulating water or oil (Sheng et al. 2011). In hydrothermal pretreatments, liquid hot-water (LHW) is added to the substrate. MW heats the substrate using irradiation, thereby reducing the reaction time and energy requirement (Speece 2008). Heating takes place because the chemical bonds (charged particles) within the substrate interact with the electromagnetic field (Speece 2008, Qiao et al. 2010). Finally, in freeze/thaw pretreatment, the substrate is frozen then thawed to disrupt the cellular structure (Stabnikova et al. 2008).

In thermal pretreatment, the bonds in lignocellulosic materials are destroyed, making the cellulose available for microorganisms at temperatures ranging from 150–180°C (Menardo et al. 2012). Hemicellulose starts to degrade at 150°C, followed by lignin dissolution in water at 180°C (Hendriks & Zeeman 2009). However, toxic products can form with this method (Carlsson et al. 2012); for example, toxic phenol compounds appear at 160°C (Hendriks & Zeeman 2009). When milled sunflower stalks were

digested under mesophilic conditions after pretreatment at 30, 55, 80, and 170°C (Monlau et al. 2012), there was no significant CH_4 production from samples subjected to thermal pretreatment at temperatures below 100°C; there was a small increase in CH_4 production from samples pretreated at 170°C relative to those that were not pretreated. During trials at less than 100°C, the lignin and hemicellulose were likely intact. This same phenomenon is not seen in manure that contains lignocellulosic material. Because manure contains less lignocellulosic material than energy crops, increased CH_4 yields have been observed at temperatures less than 100°C (González-Fernández et al. 2008, Rafique et al. 2010).

Digestion of thermally pretreated pig manure was investigated at seven pretreatment temperatures, ranging from 25–150°C, using SS digestate as the seed (Rafique et al. 2010). The highest biogas yield was observed in samples pretreated at 100°C. At temperatures above 100°C, there was a negative correlation between pretreatment temperature and CH_4 production, suggesting that inhibitory compounds were forming. This pretreatment temperature was also the optimum in a similar study in which pig manure was pretreated at temperatures that ranged from 32 to170°C and then digested with SS digestate (González-Fernández et al. 2008).

In hydrothermal pretreatment, LHW penetrates the substrate, hydrates cellulose, and removes hemicellulose and some of the lignin (Taherzadeh & Karimi 2008). With this treatment, neither size reduction nor additional chemicals are required. LHW can also make cellulose more accessible to hydrolytic enzymes (Taherzadeh & Karimi 2008). Cow and pig manure were hydrothermally pretreated at 170°C for 60 min before digestion with seed from a pilot digester (Qiao et al. 2011). In CM samples, total biogas

production increased with pretreatment, but CH_4 yield fell by 6.9%. In pig manure samples, the pretreatment enhanced both biogas and CH_4 production (7.8% and 14.6%, respectively) relative to controls (no pretreatment).

In a study investigating MW pretreatment of wheat straw, cut wheat straw was microwaved at a power range of 400–1600 W over a temperature range of 100–180°C. Treated and untreated samples were subjected to BMP tests at mesophilic temperatures, using SS and pig manure for inoculum (Jackowiak et al. 2011). The wheat straw heated to 150°C had the maximum CH_4 yield, which was 28% higher than observed in the untreated samples.

Thermal pretreatment of WAS and FW aims to degrade the cellular structure and release the organic material and linked water (Sheng et al. 2011). This increases the solubility and dewaterability of WAS (Carlsson et al. 2012). The pretreatment conditions recommended for optimal WAS digestion are 160–180°C for 30-60 min (Speece 2008, Sheng et al. 2011). In a study investigating hydrothermal pretreatment (170°C for 60 min) of municipal SS, FVW, and FW, Qiao et al. (2011) found that the biogas produced by pretreated samples was 67.8% and 18.5% higher for sludge and FVW, respectively, than that produced by untreated controls. Similar trials with FW showed that biogas production decreased 3.5% in samples that were hydrothermally pretreated, relative to untreated controls.

(Qiao et al. 2010) used MW pretreatment on SS, heating it to 120–170°C for 5 and 10 min prior to digestion. Biogas production increased after pretreatment for all temperatures tested. The authors noted that MW pretreatment times are short (5 min instead of 30 min). Also, the increased dissolution of organics (increased hydrolysis)
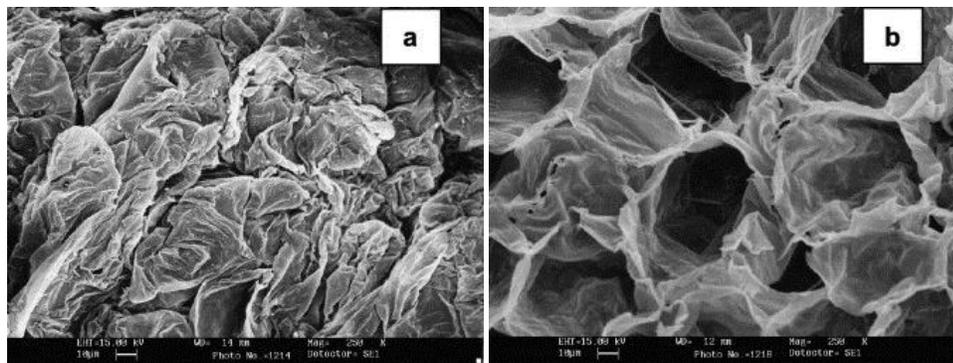
FIGURE 2.9: Scanning electron microscopy image of food waste before (a) and after (b) freeze/thaw pretreatment (Stabnikova et al. 2008)

allowed for a decrease in SRT. In one study, it was demonstrated that a freeze/thaw (-80°C) pretreatment method applied to FW was comparable to thermal pretreatment at 150°C for 1 h, and it required three times less energy (Stabnikova et al. 2008). The freeze/thaw cycle resulted in a looser feedstock structure and higher dissolved organic concentrations (Figure 2.9). The digestion was accomplished in a hybrid anaerobic solid–liquid (HASL) system. A modified two-phase AD with an acidogenic column reactor was used treated solid FW, while an UASB methanogenic reactor treated liquid leachate.

2.6.4.2. Chemical Pretreatment

Chemical pretreatment at ambient or elevated temperatures can enhance solubilization of cells for hydrolysis (Speece 2008). There are four main categories of chemical pretreatment: acid, alkaline, oxidative, and thermo-chemical. One advantage cited for alkaline pretreatment is that the base is neutralized by CO_2, helping to maintain a neutral pH (Speece 2008). Oxidative pretreatment is an efficient but complicated process. Finally, thermo-chemical pretreatment uses acid, alkaline, or oxidative chemicals for pretreatment at higher temperatures and pressures (Speece 2008).

Chemical pretreatment is the most researched option for substrates containing lignocellulose (Carlsson et al. 2012). However, the formation of refractory compounds or loss of organic material has been reported with all types of chemical pretreatment (Carlsson et al. 2012). In acid pretreatment, the goal is to hydrolyze hemicellulose and lignin before digestion to make cellulose readily available in the digestion mix (Hendriks & Zeeman 2009). However, there is a risk that solubilized lignin will condense and precipitate, which would be counterproductive (Hendriks & Zeeman 2009).

In alkaline pretreatment, the alkaline chemical causes the lignocellulosic material to swell, making the material more accessible for degradation (Hendriks & Zeeman 2009). However, there is a loss of hemicellulose to degradation and inhibitory effects from the solubilization of lignin. One study chemically pretreated ground corn straw with 8% sodium hydroxide (NaOH), 5% NH_3, and 4% urea (wt%) for 20 d at 15°C (Zhong et al. 2011b). When the pretreated corn straw was digested with activated sludge from an anaerobic digester at a local WWTP at 35°C, samples pretreated with NaOH yielded the most CH_4, but showed the highest loss of lignin, cellulose, and hemicellulose.

Chemicals can enhance thermal pretreatment (Hendriks & Zeeman 2009). Monlau et al.(2012) pretreated milled sunflower stalks in 4% hydrogen peroxide (H_2O_2), NaOH, or calcium hydroxide ($Ca(OH)_2$) for 24 h at 30, 55, and 80°C and 10% $FeCl_3$ and 4% HCl for 1 h at 170°C. Then the samples were digested in mesophilic anaerobic sludge. There was a 33% and 30% increase in CH_4 potential when then the sunflower stalks were treated with 4% H_2O_2 and 10% $FeCl_3$, respectively. However, the largest increase in CH_4 potential occurred when the sunflower stalks were pretreated with 4% NaOH at 55°C.

Chemical pretreatment of pig manure was conducted with a flocculent agent, a strong acid (HCl), and an alkali (NaOH) (González-Fernández et al. 2008). The samples were digested in SS at 32°C. An increase in CH_4 production was observed for each chemical pretreatment, with the largest effect (13%) observed when NaOH was used. However, when chemical treatment was combined with heat, the combined treatment increased CH_4 production 41% more than heat or chemical treatment alone (Rafique et al. 2010). The treatments included $Ca(OH)_2$ exposure at temperatures ranging from 25–150°C. There was a 7% increase in CH_4 production relative to untreated controls when $Ca(OH)_2$ pretreatment was applied at room temperature but an 88% increase when the chemical was applied at 70°C.

2.6.4.3. Mechanical Pretreatment

Mechanical pretreatment is the physical manipulation of a feedstock material, and it includes practices such as screening, milling, blending, shearing, cutting, grinding, and exposure to ultrasounds. These methods increase the surface area of the material and disrupt cells (Speece 2008, Fernández-Cegrí et al. 2012). Only rarely are they tested for their efficacy alone; rather, they are typically used as routine preparatory steps prior to other pretreatments or digestion. For instance, in several of the studies cited above, the lignocellulosic material was milled (Monlau et al. 2012) and chopped (Jackowiak et al. 2011, Zhong et al. 2011b). Likewise, a study investigating the effects of ultrasonic pretreatment of SuCO shredded the feedstock prior to ultrasonication, but the shredding was not isolated as an additional pretreatment factor (Fernández-Cegrí et al. 2012). These actions are not typically considered to be part of a formal pretreatment process.

During ultrasonic pretreatment, cyclical sound pressure is applied in high frequencies (Speece 2008, Apul & Sanin 2010). Microbubbles that form in the liquid lead to collapse and destruction of cellular material. Sonication causes intense heat and high pressure at the liquid-gas interface as well as shearing in the liquid phase and formation of radicals (OH*, HO_2*, H*) (Bougrier et al. 2006, Speece 2008). The latter is an undesirable by-product of sonication because the radicals degrade volatile compounds via certain pyrolysis processes that occur in the microbubbles (Speece 2008, Fernández-Cegrí et al. 2012). This phenomenon is observed more often at higher frequencies and lower specific energies (Speece 2008, Apul & Sanin 2010, Fernández-Cegrí et al. 2012). Fernández-Cegrí et al. (2012) used ultrasonic pretreatment on SuOC, varying the specific energies from 24,000–597,600 kJ/kg TS. When the mixes were digested at 35°C with granular brewery sludge inoculant, the highest COD solubilization and greatest CH_4 yield were obtained from samples subjected to the lowest specific energy. Hog manure followed similar trends. After sonication at specific energies from 250–30,000 kJ/kg TS, samples were digested in AD sludge at 37°C. The best specific energy for COD solubilization and CH_4 yield was in the lower range (500 kJ/kg TS)(Elbeshbishy et al. 2011). WAS sonication has yielded up to 40% increases in COD solubilization, and it has also been noted that digesters utilizing ultrasonic pretreatment tend to have better buffering (Speece 2008).

2.6.4.4. Biological Pretreatment

Biological pretreatment typically refers to the addition of bacterial cultures or enzymes to the feedstock followed by a timed incubation before the material is fed into a digester. The bacteria and enzymes degrade the feedstocks in much the same manner as

hydrolysis within the digester. Corn straw was pretreated with *Pleurotus florida* for 30 and 60 d before samples were digested in anaerobic digestate at 35°C with a 50 g/L loading rate (Zhong et al. 2011a). The highest biogas production (12.3 L) was observed for samples exposed to the longer incubation (60 d) samples. For the 30 d sample, 11.24 L biogas was produced. The long incubation time required for biological pretreatment is one of the drawbacks to its use. Bruni et al.(2010) tested the use of biological and thermal pretreatment by subjected biofibers (that had been separated from digested manure) to biological pretreatment with a commercially available enzyme product with and without steam. The samples were digested using thermophilic digestate from a biogas facility that treated CM. Enzymatic pretreatment improved CH_4 yield only when steam was added.

CHAPTER 3: MATERIAL AND METHODS

3.1.   Codigestion Literature Review Analysis

The literature review was limited to articles published after 2000. Various keywords and keyword combinations including codigestion, co-digestion, co-digest*, co*digestion, and anaerobic digestion were used, and 66 articles were identified. The studies were catalogued in a MS Access 2007 (Microsoft, Inc) database with the capacity to sort by feedstock and other appropriate terms, such as batch or continuous flow and other parameters (e.g. C:N ratio, F:M ratio, VS loading, VS reduction, and gas production and yield). A form was created within Access for cataloging data, and for each study, a unique entry was created with the pertinent information. Figure 3.1 is a sample of the form used to input article information. Additional information about the other samples with the same feedstock combination was mentioned in the "additional information by the Author(s)" section. Additionally, comments from the author and reader were added regarding information such as statistical analysis performed, if applicable, and other positive and negative aspects of the research

3.2.   Feedstock and Inoculum Collection and Storage

A variety of feedstocks under consideration for a Catawba County AD facility were used to develop a standardized guideline. These included FW, PL, CM, the solids from grease interceptor waste BG, GLY, DAF skimmings, SS, hatchery waste (HW), paper,   and   canola   seed   hulls   (CS).   For   the   FW,   because   it   is

FIGURE 3.1: A sample of the form used for the Access database

heterogeneous and its composition can change from day-to-day, a synthetic FW mix was developed that could be replicated from trial to trial and experiment-to-experiment. The formula was based on data from a sort study of FW from 350 commercial kitchens in San Francisco, CA (Chen et al. 2010), which provided the carbohydrate, protein, fat, and fiber ratio of the mix (Table 3.1). A review of FW characterization studies revealed that this study offered the most representative data available. Preliminary trials show that these ratios could be achieved with a mixture of raw collards, cooked potatoes, lard, and raw boneless, skinless chicken breast.

All other feedstocks were provided by Catawba County, NC. The PL consisted of bedding material, feathers, and manure, and it was cut into 1 cm pieces for analysis. CM was obtained from a NC dairy farm, which included fecal material, urine, and stormwater. It was sieved to concentrate the solid content to at least 10% TS. GLY and CS residue were provided from Catawba County's biodiesel production facility. The CS was ground before use.

Paper and compostable cups (CUPs) were tested to explore the potential for fast food restaurant FW collection. Catawba County hopes that at a future date, coordination with local restaurants will allow collection of FW, paper products used for wrapping, and compostable cups and utensils for AD. Paper wetted with food residue is not suitable for

TABLE 3.1: Typical commercial kitchen food waste characteristics

Characteristic	Grams	Percent
Carbohydrates	164.8	70.6
Protein	31.9	13.7
Crude Fat	15.5	6.6
Fiber	21.3	9.1

Source: (Chen et al. 2010).

recycling but could be an asset in AD mixes. Because no such collection is currently offered in Catawba County, the paper was represented by 100% recycled content napkins and compostable tableware by Greenware brand cups. Greenware was selected based on interviews from a small subset of Charlotte restaurants that could be identified as using such products for takeout food. The paper and CUPs were cut into 1 cm by 1 cm squares for testing.

The BG was from a composite sample from several food service establishments in the Charlotte area. A local grease interceptor company pumped these establishments, and then dewatered the grease waste at their Charlotte facility. A dewatered composite sample was collected and used for all of the experiments.

Finally, SS (60% PS and 40% WAS v/v), digestate, and filter cake were obtained from Mallard Creek Water Reclamation Facility (MCWRF). The filter cake or digestate were used as inocula as needed. All feedstocks were stored at 5°C except for the FW and PL, which were frozen. All feedstocks provided by Catawba County were analyzed for TS and VS according to Standard Methods (APHA et al. 1998). Briefly, crucibles were heated in a 550°C furnace for three hours, weighed, filled with a sample, and re-weighed. The crucibles with samples were placed in a 105°C oven overnight (6-8 h). They were re-weighed after cooling to calculate %TS and then ignited in a 550°C furnace for six hours to calculate %VS. Also, all substrates were analyzed for total carbon and total nitrogen.

3.3.   Feedstock Testing

The feedstocks provided by Catawba County (and the FW composite designed to represent future FW that might be included in a county digester) were used to develop standardized batch and semi-continuous flow digestion protocols that could be adopted

by other laboratories conducting similar testing. The aim was to offer and describe procedures that do not suffer from some of the flaws identified in the literature review and database. Some of the variables that were examined included C:N ratio, F:M ratio, and SRT, as appropriate, in batch and semi-continuous flow reactors respectively; and recommending best practice for measuring gas evolution and gas concentrations to report CH_4 yields.

Two other tests were used and assessed for substrate and mix evaluations. They included the BMP and ATA assays. The rationale for each of these tests and their protocols is described below.

3.4. Biochemical Methane Potential (BMP)

The BMPs are a simple and inexpensive procedure, measuring substrate biodegradability, which can supply researchers with a wealth of information such as CH_4 production, potential toxicity, and solids reduction (Owen et al. 1979, Speece 2008). They were first proposed by Owen et al.(1979) as an alternative method to Warburg respirometry, which was the prevailing method at the time. The Warburg respirometer did measure biodegradability and possible toxicities; however, it had several limitations including sample size, cost, operator skill level, and duration (Owen et al. 1979). Subsequently, Speece (2008) described the BMP general procedure; however, it differs from (Owen et al. 1979) in that they measured biogas only, whereas, Speece also measured CH_4 content. Since its premier in 1979, BMP have been modified by researchers until no single protocol is utilized for the test, although there have been attempts to standardize it and identify the important factors that will allow test results from one laboratory to be compared with those from another (Angelidaki et al. 2009,

Moody et al. 2011a). There is a general consensus that BMP results should be reported as CH_4 yield (L CH_4/g VS added), although this is not uniformly observed.

Feedstocks provided by Catawba County were subjected to BMP analysis; however, tests were conducted at 10% TS, because this is the designed waste strength that Catawba County plans to use. This solids concentration is at the high end for liquid digestions. It is higher than WWTP digesters (which operate at 2–3% TS), but it is not too high to preclude adequate mixing and is often used as the strength that represents cost-effective design (Vandevivere et al. 2003).

Trials were conducted in triplicate at 10% TS (w/w) in bottled spring water using an F:M of 1 gVS_{feed}/gVS_{seed}. Substrates, inoculum, and nutrients (Moody et al. 2011a) (Table 3.2) were combined in 250 mL Corning glass bottles with gas-tight caps modified with 1/8-inch National Pipe Thread (NPT) Swagelok fittings to accommodate a septum for gas sampling. The bottles were incubated at 35±2ºC and continuously shaken at 100

TABLE 3.2: BMP nutrient medium[1] composition

Stock Solution 1	Stock Solution 2
Dissolve the following and add de-ionized water to make 2 L of solution	Dissolve the following and add de-ionized water to make 1 L of solution
60 g NH_4Cl	0.75 g $MnCL_2 \cdot 4H_2O$
63.3 g $MgCl_6$	0.75 g NH_4VO_3
18 g $CaCl_2 \cdot 2H_2O$	0.75 g $CuCl_2 \cdot 2H_2O$
60 g KCl	0.75 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$
12 g $(NH_4)_2HPO_4$	0.75 g $AlCl_3 \cdot 6H_2O$
8.25 g $FeCl_3 \cdot 6H_2O$	0.75 g $NaMoO_4 \cdot 2H_2O$
1.5 g $CoCl_2 \cdot 6H_2O$	0.75 g H_3BO_3
1.5 g KI	0.75 g $NiCl_2 \cdot 6H_2O$
	0.75 g $NaWO_4 \cdot 2H_2O$
	0.75 g Na_2SeO_3

Note: [1]Nutrient medium: Combine 151g sodium bicarbonate, 200 mL of stock solution 1, and 10 mL of stock solution 2. Dilute with tap water (that has been out overnight in an open container to reduce chlorine levels) to 15 L.

rpms. If required, initial pH was adjusted to >7 using 1M NaOH. Gas analyses were conducted daily for the first seven days and then twice weekly for the remainder of the experiment. The total cumulative CH_4 and the CH_4 yield were calculated. Methane addition to the headspace was measured as the product of (i) the volume of excess gas produced and (ii) the concentration of CH_4 in the headspace gas relative to the concentration of CO_2. Typical trial durations were about 60 d.

3.5.   Anaerobic Toxicity Assays (ATA)

The ATA were first proposed by (Owen et al. 1979) as a simple and cost effective analysis to isolate a substrate and determine its effect on the inoculum organisms. A series of bottles are prepared with an increasing amount of the test substrate (% inclusion rate) added to a standard amount of inoculum. The inoculum is also provided with a readily biodegradable feedstock (glucose) and nutrients (TABLE 3.3) (Moody et al. 2011b). The ATA test gauges whether the test substrate enhances, inhibits, or leaves unchanged the inoculum's propensity for metabolizing the standard feedstock provided.

Every sample receives an equal volume of supplement and microbial inoculant, but different strengths of test feedstock-in-water and is incubated at $35^{o}C$ (Table 3.4). Control samples received no feedstock. The premise of the test is that over a short incubation (3–5 d), the microorganisms will grow mainly on the glucose and supplements provided (rather than the feedstock), while the control bottles will show this baseline

TABLE 3.3: ATA supplement mixture (Moody et al. 2011b)

Nutrient broth (g)	**5**
Yeast extract (g)	5
D-glucose (g)	5
DI water (mL)	50

performance. If a particular feedstock is inhibitory, then CH_4 production will perform more poorly than controls and inhibitory effects will be more pronounced with increasing concentration. The degree of inhibition was reported in terms of "I," calculated according to Eq. 3.1:

$$I = \left(1 - \frac{V_{CH_4 Toxicant}}{V_{CH_4 Control}}\right) \times 100 \qquad \text{Eq. 3.1}$$

where $V_{CH4Toxicant}$ is the amount of CH_4 produced when the test substrate is present, and $V_{CH4Control}$ is the amount of CH_4 produced from the control.

A negative value indicates no inhibition, while a positive value indicates inhibition.

TABLE 3.4: Typical ATA proportions of nutrient substrate, inoculum, and feedstock

	Control	1	2	3	4	5	6	7
% Inclusion	0%	1%	3%	5%	6%	7%	9%	10%
Inoculum (mL)	50	50	50	50	50	50	50	50
Substrate (mL)	0	1	3	5	6	7	9	10
DI water (mL)	50	49	47	45	44	43	41	40
Supplement (mL)	2	2	2	2	2	2	2	2
Total Vol (mL)	102	102	102	102	102	102	102	102

3.6.   Batch Reactors

Batch digestions were conducted in 1L Corning glass bottles with gas-tight caps modified with 1/8-inch NPT Swagelok fittings to accommodate a septum for gas sampling (Figure 3.2). After capping, the headspace of the bottle was sparged with nitrogen gas. Preliminary trials showed that a 400 mL mix volume at 10% TS (w/w) could be accommodated in these bottles along with inoculum. Because no single seed source is acclimated to the variety of feedstocks to be tested, bottles were step-fed over the first four days to allow the inoculum to adjust to the feed and prevent rapid acidification (after preliminary trials showed that this step-feeding was necessary). On

Day 0, a mix representing 25% of the total feed (100mL) was added to each bottle in combination with the inoculum. The headspace was sparged with nitrogen gas, and incubation in a Precision incubator at 35 ± 2 °C on a shaker at 110 rpm began (Figure 3.2). After 24 h, each bottle was opened and Day 1 pH measurements were taken before and after an additional 25% of the total feed was added. This process was repeated for two more days until the total mix volume was established in each bottle. All the bottles were then sparged for a final time with nitrogen gas and incubated undisturbed for the duration of the incubation period (typically around 60 d), while being monitored for headspace gas concentration and volume. Control blanks contained spring water and inoculum only. Gas measurements and yield were calculated as described above for BMP tests, and details of the gas concentration analysis are described in Analytical Methods below. All trials were conducted in triplicate.

3.7.   Semi-continuous Reactors

In addition to guideline development, the BMP, ATA, and batch feedstock studies

FIGURE 3.2: A photograph of the modified 1L glass bottles on a shaker

were used to develop promising mix combinations for success in a Catawba County AD system. Two final mixture designs were chosen based on the codigestion batch experiments along with results from subsequent ATA and BMP tests (Table 3.5). A third mixture was proposed by Catawba County and represented proportions of feedstock constituents that reflected their respective availabilities so that no accumulation would occur. Reactors were created from 2 L glass Corning bottles with GL 45 threaded caps. Each cap was fitted with a 3/8" brass ball valve for feeding and wasting, and a 1/8 in NPT Swagelok fitting with a septum for gas sampling. The reactors were mechanically stirred with magnetic mixers, and incubation was at 35±2°C in a temperature controlled room. Trials were conducted in triplicate for at least 2–3 SRTs. The reactors were fed and wasted (the same amount based on volume) daily to maintain an F:M ratio of 0.5. Gas analyses and calculations were conducted as described previously.

TABLE 3.5: A summary of the three mixtures used in the semi-continuous reactors

	Poultry Litter	Food Waste	Sewage Sludge	DAF (W)	DAF (H)	Paper	Hatchery Waste	Leaf Matter	Feed Meal	Brown Grease
Mix	Fraction of TS (w/w)									
1	0.05	0.10	0.05		0.05	0.05	0.35	0.05	0.30	
2	0.25	0.05			0.35					0.35
3	0.02	0.00	0.08	0.77	0.09	0.00	0.03	0.00	0.01	

3.8. Cell Counts

3.8.1. Batch Studies

BMP assays were conducted on PL, DAF (W), and paper as described for single substrate BMPs. Additionally, the substrates were digested without seed to determine if CH_4 would occur from the bacteria introduced by the sample. After the feed and seed were added to the bottles, the volume within the bottle was increased by the addition of

10 mL nutrient water (Moody et al. 2011a) and spring water to a final volume of 150 mL. A blank, seed and water only, was evaluated to determine the amount of CH_4 produced from the seed. All gas-tight bottles were purged with nitrogen gas and incubated at 35 ± 2^oC on a shaker at 100 rpm for 45 days. Gas analyses were conducted thrice weekly when the gas production was high, then twice weekly as gas production decreased.

3.8.2. CTC-DAPI Staining

To enumerate the total and viable bacterial biomass of each feestock CTC-DAPI (5-cyano-2,3-ditoly tetrazolium chloride and 4'6-diamidion-2-phrnylindole) counterstaining was conducted according to a modified technique of Rodriguez et al. (1992). CTC is a ditetrazolium redox dye that, when reduced biologically or chemically in a bacteria, produces fluorescent formazans, indicating cellular activity (Rodriguez et al. 1992). While DAPI binds to the DNA and RNA of cells, staining both living and fixed bacteria for a total bacteria count (Chivu 2010). Environmental samples are often counter stained with CTC-DAPI to accurately determine the microbial bacterial count (Cappelier et al. 1997, Besnard et al. 2000, Coello et al. 2010). CTC-DAPI has the advantage over traditional direct plate counts because it is not hindered by bacteria that are viable but non-culturable (VBNC) or difficult to culture (Besnard et al. 2000), with DAPI presenting 2–3 times the bacteria (Besnard et al. 2000, Chivu 2010). Additionally, CTC has been proven to be a reliable measure of waste samples (Griebe et al. 1997, Coello et al. 2010) with less staining of background organic material compared to other redox stains, providing greater sensitivity (Rodriguez et al. 1992). Each feedstock was diluted with sterilized DI water (10–100 fold dilution). The dilution was gently mixed for 2 minutes. The purchased CTC (Polysciences, Inc. Warrington, PA) was used to prepare a

4 mM solution with autoclaved DI water. The sample and CTC were added to a 2 mL tube in a 1:1 ratio (500 µL each) in a darken room. Then, the tubes were covered and placed in a drawer overnight (for at least 8 hours), which allowed the VBNC bacteria time to reduce the CTC. Next, the cells were counterstained with 50 µL of a prepared solution of 1µg/mL DAPI and incubated for 30 min in the dark. After incubation, the cells were filtered using 0.2µm black filters (Isopore Membrane Filters, Millipore). Finally, the filters were fixed onto a glass slide using immersion oil. The slides were stored at 5°C until viewed under a fluorescent microscope.

3.9.   Pretreatment Analyses

As the economics and technology of renewable energy production change, waste materials considered infeasible a decade ago are now being reconsidered. There is a growing body of work assessing the potential for various pretreatment methods to yield greater energy production from such feedstocks. Some pretreatment-feedstock combinations show promising results. However, such results must be evaluated in the context of the required energy input. An analysis was conducted of some literature reports of feedstock pretreatment results combined with estimates of the required energy inputs to achieve those results to confirm that there was a net energy gain when pretreatment steps were performed in advance of AD.

3.9.1. Pretreatment Literature Data Collection

A review of research reports was used to conduct energy balances for biogas production on four categories of feedstocks (Table 3.6): WAS, FW and the organic fraction of municipal solid waste (OFMSW), lignocellulosic wastes, and manures. Each balance considered both pretreated and untreated feedstocks. For each feedstock

category, the energy balances were calculated for thermal, chemical, thermochemical, and mechanical pretreatment. In addition, lignocellulosic and manure feedstocks were assessed for biological pretreatment. Two-stage reactors were not considered as a form of biological pretreatment in this analysis, although some have argued that they serve this function. Data were obtained from 34 articles, resulting in 53 energy balances (Table 3.6). For evaluation purposes, the pretreated energy balances were assigned a dummy variable based on whether E was positive (1) or negative (0).

3.9.2. Energy Balance Calculations

Net energy (E) from an AD system is calculated as the difference between the system's energy output (E_{out}) and the required energy input (E_{in}). Typically, E_{in} is derived from engines, heaters, feedstock conveyance, biogas upgrading equipment, and mixers, and pretreatment − if applicable. Energy out was determined by the CH_4 production reported. For the purpose of this study, the amount of energy added to the system was derived solely from pretreatment steps (equipment operation or the provision of heat). Because the AD systems assessed were the same with and without pretreatment, all other inputs were assumed to be equal and therefore neglected in the analysis. Figure 3.3 illustrates the defined system boundaries for the energy balance calculations.

TABLE 3.6: A list the articles used for energy analysis

	Pretreatment	Author(s)
Food waste and organic fraction of municipal solid waste	Chemical	(López Torres & Espinosa Lloréns 2008)
		Shahriari et al. (2012)
		Wang et al. (2009)
	Mechanical	(Luste et al. 2009)
		(Elbeshbishy & Nakhla 2011)
		(Izumi et al. 2010)
	Thermal	(Marin et al. 2010)
		(Beszédes et al. 2011)
		(Liu et al. 2012)
		(Liu et al. 2012)
		Wang et al. (2009)
	Thermochemical	Shahriari et al. (2012)
		Wang et al. (2009)
Waste activated sludge	Chemical	Kim et al. (2003)
		(Dhar et al. 2011)
		(Devlin et al. 2011)
	Mechanical	Kim et al. (2003)
		Dhar et al. (2011)
		(Apul & Sanin 2010)
	Thermal	(Kuglarz et al. 2013)
		(Eskicioglu et al. 2006)
		Kim et al. (2003)
	Thermochemical	Kim et al. (2003)
		(Bougrier et al. 2006)
		(Valo et al. 2004)
	Biological	(Frigon et al. 2012)
		(Frigon et al. 2012)
		(Romano et al. 2009)
Lignocellulosic feedstocks (grass and straw)	Chemical	(Frigon et al. 2012)
		(Taherdanak & Zilouei 2014)
		(Chandra et al. 2012)
	Mechanical	(Fernández-Cegrí et al. 2012)
		(Frigon et al. 2012)
		(Menardo et al. 2012)
	Thermal	Jackpwiak_2011
		(Antonopoulou et al. 2010)
		Menardo et al. (2012)
	Thermochemical	(Fernandes et al. 2009)
		Fernandes et al. (2009)
		(Xie et al. 2011)
Manure	Chemical	(González-Fernández et al. 2008)
		(Rafique et al. 2010)
		(Carrère et al. 2009)
	Mechanical	González-Fernández et al. (2008)
		(Elbeshbishy et al. 2011)
		(Castrillón et al. 2011)
	Thermal	(Qiao et al. 2010)
		González-Fernández et al. (2008)
		(Bonmati et al. 2001)
	Thermochemical	(Costa et al. 2012)
		Rafique et al. (2010)
		Carrère et al. (2009)
	Biological	Costa et al. (2012)
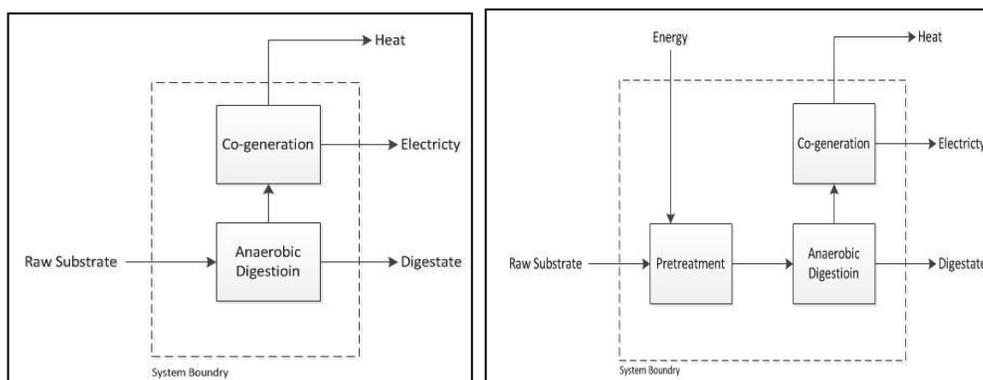
FIGURE 3.3: System boundaries without (left) and with (right) pretreatment

For heating feedstock slurries (thermal, microwave, or thermochemical pretreatment), the thermal energy (Q) required was calculated using the specific heat capacity (C_p) of water (Eq. 3.2), and the energy required to heat the dry feedstock solids was assumed to be negligible.

$$Q = m\,Cp\,\Delta t$$

<div align="right">Eq. 3.2</div>

where, Q is thermal energy in calorie, m is the mass of water in grams, Cp is the heat capacity of water (1 calorie/g°C), and Δt is the change in temperature (°C).

For instance, the calculated required energy input for FW to heat the water in the food was 124 kJ, while the energy required to heat the food solids was 18 kJ (Beszédes et al. 2011). For uniform comparison, thermal energy requirements were expressed as electrical energy using the conversion factor 4.187 J/calorie. The energy requirement for ultrasonic pretreatment (E_{US} as kJ) was calculated based on the specific energy specifications (SE as kJ/kg TS) of the equipment and the dry solids content (TS) of the sample (kg TS) (Eq. 3.3).

$$E_{US} = (SE) * (TS)$$

<div align="right">Eq. 3.3</div>

For pretreatment methods that utilized equipment requiring electrical energy, the following equation was used:

$$E_{el} = (Pt)/1000$$

where, E_{el} is electrical energy (kWh), P is power (Watts), and t is time (h/d).

The E_{out} will be determined based on the volume of biogas or CH_4 reported. To simplify this calculation, it was assumed that the biogas is burned in an internal combustion engine that has a 38% efficiency rating (Menardo et al. 2012). The amount of thermal energy (E_{th}) contained in the gas was calculated (Eq. 3.5) and then converted to electrical energy based on the 38% efficiency rating (Eq. 3.6); lost heat was not be considered.

$$E_{th} = h_m * S_{VS,i} * M$$

Eq. 3.5

where, E_{th} is thermal energy from CH_4 production; h_m is specific energy constant (22 KJ/L for biogas and 35.8 KJ/L for CH_4); $S_{VS,i}$ is VS influent (gVS); and M is CH_4 or biogas yield rate (L CH_4/gVS or L biogas/gVS).

$$E_{out} = E_{th} * \eta_{el}$$

Eq. 3.6

where, E_{out} is the electrical energy from biogas (kJ), and η_{el} is the efficiency of a biogas generator (assumed to be 38%). E was determined using Eq. 3.7.

$$E_{out} - E_{in} = E$$

Eq. 3.7

In addition to E calculations, another method to evaluate positive energy balances or the energy efficiency of a system was the energy input to output ratio (E_I/E_O) (Eq. 3.8). This analysis was used to evaluate how efficiently the energy is used. The lower the

E_I/E_O ratio the greater the efficiency of the system with a negative E value related to E_I/E_O ratio as it approaches or surpasses unity (1) (Pöschl et al. 2010).

$$\frac{\sum E_{in}}{\sum E_{out}} = \frac{E_I}{E_o}$$

Eq. 3.8

3.9.3. BMP Tests and Thermal Pretreatment

To evaluate thermal pretreatment of some feedstocks under consideration for Catawba County, BMPs were conducted in duplicate with the CM, PL, SS, and DAF (H) feedstocks. Samples were heated to 70˚C for 30 min before testing as described in Section 3.4. The samples were heated as received (undiluted). They there then adjusted to a 10% TS after thermal pretreatment. Non-pretreated controls and inoculum-only blanks were incubated as well. The feedstocks were tested at 10% TS, at an F:M of 1, and filter cake from MCWRF was used as seed. The BMPs were conducted for 66 d. The volumes and yields of CH_4 were normalized for the amount of CH_4 produced by the inoculum-only blanks.

3.10. Analytical Methods

Concentrations of O_2 (negligible), CO_2, nitrogen, and CH_4 were measured using a SRI GC fitted with a CTR-1 column with sequential flow through a TCD and then an FID detector. Helium carrier gas was used at a flow rate of 60 ml/min. Gas volume was measured either by liquid displacement or a continuous flow meter. The liquid displacement method required a gas-tight connection be made between the headspace of the sample and a graduated pipet prefilled with an acid-brine solution (APHA et al. 1998). The pressure differential resulted in headspace gas displacing some of the acid-brine solution until the headspace reaches atmospheric pressure (Figure 3.4). Another

flow device utilized was a continuous flow digital flow meter (Agilent ADM 2000, California). It was placed in-line (using gas-tight connections) with tubing that was valved to the headspace of a test vessel. When the valve was opened, if there was overpressure in the vessel headspace, gas would flow through the flow meter, and a computer program was used to record meter readings in two second intervals until flow was negligible. The flow meter measures flow independent of gas type. The gas volume was calculated as the area under the curve of flow vs. time.

The CO_2:CH_4 ratio of the gas mixture (measured by GC) was used to calculate the CH_4 produced and the CH_4 production rate. TS and VS was measured using Standard Methods as previously described (APHA et al. 1998). Also, all substrates were analyzed for total carbon and total nitrogen via a third party laboratory.
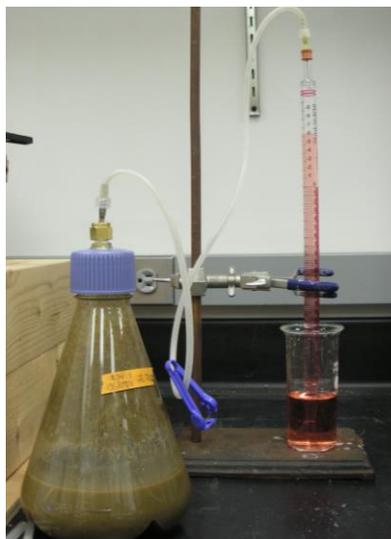
FIGURE 3.4: A photograph of the acid-brine gas displacement apparatus for measuring gas volume

3.11.   Statistical Analysis

Data collected during this research was statistically analyzed using JMP software (v10.0.0, SAS Institute, Inc). One-Way ANOVA with Tukey's post-hoc analysis was used to determine statistical significance. All tests for significance were conducted at a 0.05 significance level. In addition, Dixon's Q test for small samples sizes (n=3) was used to probe the data for possible outliers (Rorabacher 1991).

CHAPTER 4: RESULTS

4.1.   Literature Database

The purpose of the database was two-fold. First, for research purposes, it was assembled to demonstrate the nature and quality of studies available for reference to those interested in codigestion research or practice. Second, it was compiled to expedite others' investigations of existing literature and study findings. The collection contains 66 articles that span the years 2000 to 2014. The database can be sorted by a variety of search terms, and if an agency wanted to host it, maintain it, or expand it, it is in a ready state to be acquired. Table 4.1 and Table 4.2 are examples of query outputs that users can make so that the data can be quickly filtered and explored. The queries can reference first author, digestion mode (e.g. batch, semi-continuous flow, thermophilic, mesophilic), temperature, substrates, mix ratios, OLR, OLR units, gas measured, units of gas measurement, F:M ratio, units of F:M ratio, and C:N ratio.

Database queries reveal that six different units are used among various research groups to report biogas and CH_4 production. These included volume of CH_4 and yield units that were based on gram VS added, gram VS removed, gram wet weight, gram COD, or gram TS. One article reported CH_4 production as grams of CH_4. The database revealed a lack of standard gas reporting, with 32% reporting only biogas and not CH_4 production, which makes comparisons among studies difficult. Also, many of the studies did not normalize the data to adjust cumulative CH_4 observed to reflect the amount of

substrate that was fed to generate it. Only 35% of the articles normalized data by expressing it as a yield.

Additionally, the type of information presented in the articles varies. For example, not all researchers report the F:M or C:N ratios, initial and final pH values, and OLR. Of the 66 articles reviewed, 27% reported F:M in units of either gVS/gVS or gCOD/gVS (Table 4.2); 21% measured and reported C:N ratios; and only 8% reported both F:M and C:N (Table 4.1). From these queries, we were able to determine that all of the articles that reported F:M and C:M or just F:M were from engineering journals, while of those that reported only the C:N, 79% were from the engineering literature, 14% from biology discipline journals, and 7% other, which includes student theses.

TABLE 4.1: Sample of output from database query for articles that reported both F:M and C:N

First Author's Last Name	Type of Digestion	Temp (°C)	Sub-strates	Substrate mix ratio (FVW:CM:WAS :: 15:15:70)	OLR	OLR units	Biogas	Biogas units	F:M ratio	F:M ratio units	C:N ratio
Mshandete, Anthony	Batch	27	FO, SP	FO:SP:: 33:67 (wet weight basis)			0.62	m^3 CH_4/kgVS	1	gVS/gVS	16
Wang, Xiaojiao	Batch, Mesophilic	35	CM, ChickM, Straw	CM:Chick:Straw ::18.4:55.3:26.3 (VS basis)			243.2	mL CH_4/gVS	0.5	gVS/gVS	29
Sosnowski, P.	Semi-continuous, Thermophilic, UASB	56	WAS, OFMSW	WAS:OFMSW :: 75:25 (%vol) (2:1 TS basis)	1.5	$gVSS/dm^3$-d	0.42	dm^3 biogas/gVSS	0.1	gVS/gVS	14.2
Hosseini Koupaie, E.	Batch, Mesophilic	35	WAS, ScCake, MuCake	0.3g ScCake, 4g WAS, 0.5g MuCake (ww)			458.7	mL biogas/gVS	1.65	gVS/gVS	12
Li, Yeqing	Batch, Mesophilic	37	CM, CornSil	CM:Corn :: 1:3 (gVS)	3	gVS/L	298.2	mL CH_4/gVS	0.5	gVS/gVS	27.3

TABLE 4.2: A sample output from database query for articles that reported F:M values

First Author's Last Name	Type of Digestion	Biogas yield	Biogas yield units	F:M ratio	F:M ratio units
Davidsson, A.	Batch, Mesophilic	681	NmL CH$_4$/ gVS	0.6	gVS/gVS
Flor, A.	Batch, Mesophilic	0.34	L CH$_4$/ kgVS	4.5	gVS/gVS
Buendia, I. M.	Batch, Mesophilic	352	L CH$_4$/ kgVS	1.2	gVS/gVS
Luostarinen, S.	Batch, Mesophilic	788	m^3 CH$_4$/tVS	1	gVS/gVS
Martinez, E. J.	Batch, Mesophilic			1	gVS/gVS
Mshandete, Anthony	Batch	0.62	m^3/kgVS	1	gVS/gVS
Astals, S.	Batch, Mesophilic	215	mL CH$_4$/gCOD	0.75	gCOD/gVSS
Li, Chenxi	Batch, Mesophilic	418	mL CH$_4$/ gVS	0.46	gVS/gVS
Wang, Xiaojiao	Batch, Mesophilic	243.2	mL CH$_4$/ gVS	0.5	gVS/gVS
Wang, Li-Hong	Batch, Mesophilic	159.7	mL CH$_4$/ gTS	0.45	gVS/gVS
Wall, David M.	Batch, Mesophilic	345	L CH$_4$/ kgVS	0.5	gVS/gVS
Sosnowski, P.	Semi-continuous, Thermophilic, UASB	0.42	dm^3 biogas/gVSS	0.1	gVS/gVS
Silvestre, G.	Semi-continuous, Mesophilic	0.54	Nm^3/m^3-d	1	gCOD/gVSS
Amon, Th	Batch, Mesophilic	617	L CH$_4$/ kgVS	7	gVS/gVS
Hosseini Koupaie, E.	Batch, Mesophilic	458.7	mL biogas/gVS	1.65	gVS/gVS
Ferrer, Pablo	Batch, Mesophilic	337.96	mL CH$_4$/gVS	1.4	gVS/gVS
Li, Yeqing	Batch, Mesophilic	298.2	mL CH$_4$/gVS	0.5	gVS/gVS
Lisboa, Maria Sol	Batch, Mesophilic	452	mL CH$_4$/gVS	3.4	gVS/gVS
Davidsson, A.	Batch, Mesophilic	681	NmL CH$_4$/ gVS	0.6	gVS/gVS

4.2.   Codigestion Batch Studies

4.2.1. Experiment 1

Experiment 1 consisted of a set of preliminary trials to evaluate basic testing protocols and collect some initial data on some of the substrate materials. It was used to assess the CH_4 production potentials of two PL-based mixtures that were each tested at two different mixture ratios with FW or DAF (Table 4.3). The feedstocks varied in moisture content, but all were high in organic matter, as evidenced by the high percent VS concentrations (Table 4.4).

TABLE 4.3: Experiment 1 trial mixtures

Trial	Food Waste	Poultry Litter	DAF
	Percent Total Solids (w/w)		
1	50	50	0
2	25	75	0
3	0	50	50
4	0	75	25

Methane produced was normalized in two ways. First, the average cumulative CH_4 produced by the blanks was subtracted from the mean cumulative CH_4 produced by each treatment. Second, the resulting cumulative CH_4 was then divided by the grams of feedstock VS introduced and expressed as yield, mL CH_4/gVS. The trials with DAF

TABLE 4.4: Experiment 1 Feedstock characteristics.

Feedstock	Total Solids (%)	Volatile Solids (% of TS)
Food waste [a]	14.8	92.1
Poultry litter	37.6	85.1
DAF	95.5[c]	93.3

[a] Tested as individual feedstocks (chicken, lard, potato, and cabbage) and calculated based on their weight contribution to the food waste mix

yielded more CH_4 than the blanks (seed with spring water only) and the trials with FW (Figure 4.1), and a one-way ANOVA with Tukey's post hoc test confirmed that the differences were statistically significant ($p<0.05$). However, there was no significant difference between the CH_4 yields of the two trials containing DAF; Trials
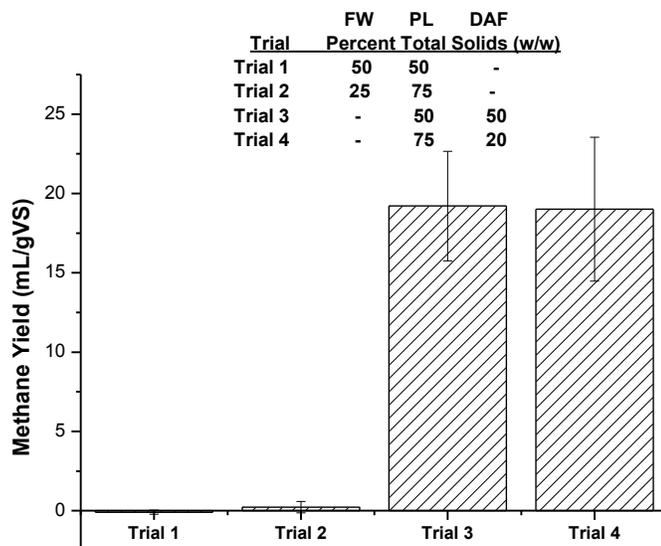
FIGURE 4.1: Experiment 1 specific methane yields after 64 d

3 and 4 produced 19.2±3.5 and 19.0±4.5 mL CH_4/gVS, respectively, while both Trials 1 and 2 produced less than 0.50 mL CH_4/gVS (Table 4.5).

The %VS reduction was calculated to reflect the amount of organics degradation accompanying CH_4 production. The mass of VS in each bottle was measured at the start of incubation (and expressed as the %VS relative to the TS in the bottle). This value

TABLE 4.5: Experiment 1 results

	Initial pH	Final pH	CH$_4$ Yield (mL/gVS)	VS Loading (gVS/L)
Trial 1	6.9±0.1	5.3±0.3	-0.1±0.1	88.6
Trial 2	7.4±0.5	6.3±0.1	0.2±0.4	86.9
Trial 3	7.4±0.6	7.9±0.1	19.2±3.5	89.2
Trial 4	7.9±0.8	7.8±0.1	19.0±4.5	87.2

represented the total organic matter available from the feedstock and inoculant biomass. A repeat measurement at the end of incubation allowed the percent change to be assessed. Although the biomass population is presumed increasing as food is consumed, slow anaerobe growth rates justify neglecting changes in biomass growth and assuming change is due to substrate consumption. Only Trials 3 and 4 showed CH_4 production, and the %VS reductions were similar (p=0.94 according to Student's t-test), with values of 42.0% and 43.6%, respectively.

The test vessels piloted in Experiment 1 were plastic Erlenmeyer-shaped flasks with internal baffles at the bottom. They were selected because it was anticipated they would enhance mixing and prevent vortexing. However, they proved to fracture easily at the baffle (Figure 4.2), which was unacceptable. Alternate vessels were used in subsequent experiments.

4.2.2. Experiment 2

When Catawba County introduced three new feedstocks (BG, GLY, and CM) for investigation at the conclusion of Experiment 1, these and new samples of PL and DAF were characterized and stored for subsequent experiments (Table 4.6). The PL was sourced from a facility that stored its material in an open container outdoors. It was much drier and lower in organic content when collected for Experiment 2 in mid-summer than Experiment 1 samples collected in spring. The new DAF, collected as a grab sample from the same supplier, was much lower in solids than the earlier sample, and this was likely the result of the collection technique.

In addition to formulating an experimental design that would answer the fundamental methodological research questions, mixes were prepared to determine how

the feedstocks newly available to Catawba County might affect CH_4 yields. Also, a trial was conducted to assess how CM compared to PL as a manure feedstock with FW. Two operational changes were made in Experiment 2 based on Experiment 1 outcomes. First, the reaction vessels were changed to 1 L square glass bottles, and the caps were modified

TABLE 4.6: Experiment 2 feedstock characteristics.

Feedstock	Total Solids (%)	Volatile Solids (% of TS)	Carbon† (%)	Nitrogen† (%)	Carbon$^\beta$ (%)	Nitrogen$^\beta$ (%)
Column #	1	2	3	4	5	6
Food Waste[a]	14.8	92.1	48.9	3.3	52.5	2.5
Poultry Litter	69.5	47.0	43.9	3.2	39.1	9.2
Dissolved Air Flotation	28.8	99.0	55.0	3.7	65.0	4.5
Brown Grease	95.3	99.8	55.5	3.5	63.2	1.2
Glycerin	56.3	93.1	51.7	0.1	48.8	0.01
Cow Manure	8.50	83.5	46.4	0.3	43.1	2.7

†Theoretical values based on VS

$^\beta$ Experimental values

[a] Based on total nitrogen values of chicken, potatoes, cabbage by (Sosulski and Imafidon 1990) and lard by (Badger and Miller, 2000)

FIGURE 4.2: Stress cracks at molded vanes led to bottle leaks

with 1/8-inch NPT Swagelok fittings to accommodate a septum for gas sampling. The square bottle shape offered some protection against votexing. Second, the continuous flow digital flow meter, an alternate gas volume measurement method, was employed to reduce collection time and improve accuracy.

The mixes were prepared as shown in Table 4.7. The nutrient ratios of the mixes in Experiment 2 were adjusted to ensure that the C:N ratio fell within an optimum C:N range of 15–30 (Monnet 2003). Values for %C and %N were developed from literature.

TABLE 4.7: Experiment 2 trial mixtures

TRIAL	Poultry Litter	Food Waste	Brown Grease	DAF	Glycerin	Cattle Manure	C:N[L] (lit)	C:N[A] (lab)
			Percent Total Solids (w/w)					
1	50	25	25	0	0	0	15	8
2	60	15	25	0	0	0	15	7
3	50	25	0	25	0	0	14	8
4	60	15	0	25	0	0	14	7
5	50	25	0	0	25	0	19	9
6	60	15	0	0	25	0	19	7
7	0	50	0	0	0	50	15	18

[L]C:N ratios calculated based on literature values.
[A]C:N ratios calculated based on analytical values.

Published C:N ratios for similar materials that converged around common values were identified. These were used with a %C value deduced from %VS measurements (%VS/1.8) (Adams et al. 1951) (Table 4.6, Col. 3) to back-calculate %N values (Table 4.6, Col. 4). The resulting %N values were compared to literature values to ensure that they were plausible. The mixes were prepared as described previously and monitored for 120–140 d until day-to-day changes became negligible. After the start of Experiment 2, feedstock samples were sent to an external laboratory for total carbon and total nitrogen analysis to confirm that use of literature values was a satisfactory method for estimating C:N ratios of mixes.

Because there was high variability among the codigestion replicates, a Dixon's Q analysis was used to remove outliers (Rorabacher 1991), and statistical analyses were conducted with n=2. The final average pH values (Table 4.8) showed that trials with >15% FW or with GLY ended with pH levels below 6, while those with high CH_4 yields were above 7. Trials 2 and 4 had the highest CH_4 yields (Figure 4.3, Table 4.8). Figure 4.4 shows the CH_4 production curve for Trial 2. They were significantly different from all other trials (p<0.005), but not from each other. Both of these mixes had lower fractions of FW (15%) and either BG or DAF addition. Trial 6 had the same fraction of PL and FW as Trials 2 and 4 with GLY instead of BG or DAF. Its performance and final pH (Table 4.8) resembled that of a mix with higher FW levels. The %VS reduction values for those samples yielding CH_4 were 64.9±11.7% in Trial 2 and 51.3±2.3% in Trial 4. The substitution of CM for PL (Trial 7) did not prevent the pH decline associated with 50% FW observed in Experiment 1.
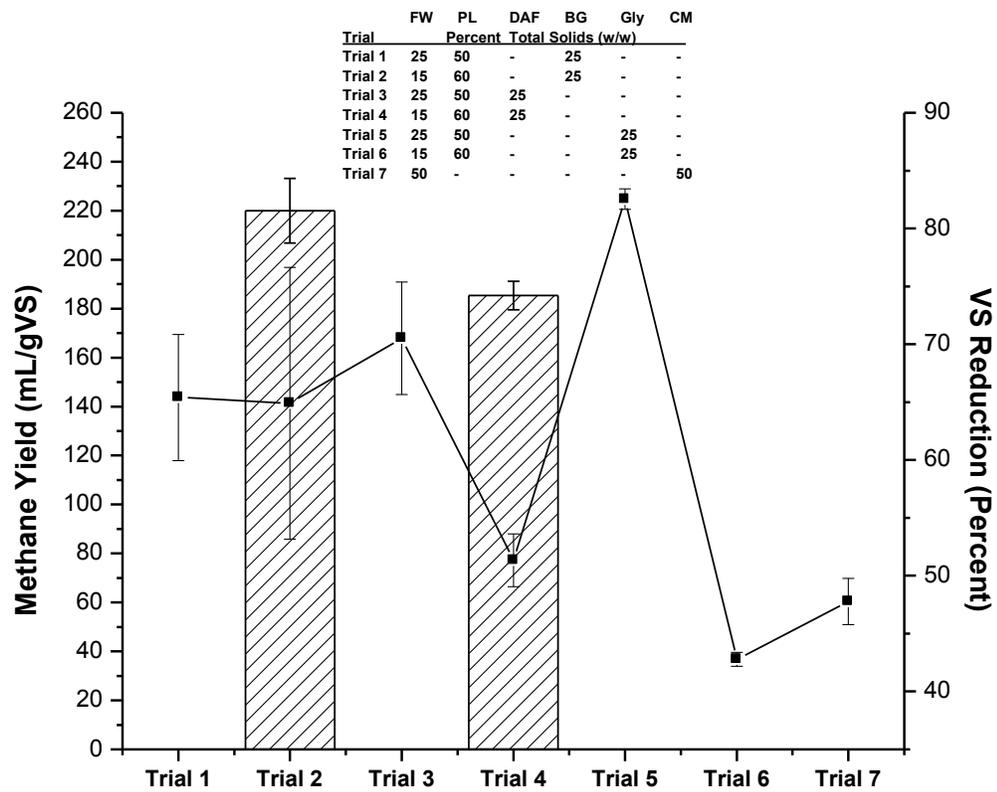
FIGURE 4.3: Experiment 2: methane yield (bar graph) and %VS reduction (line graph)
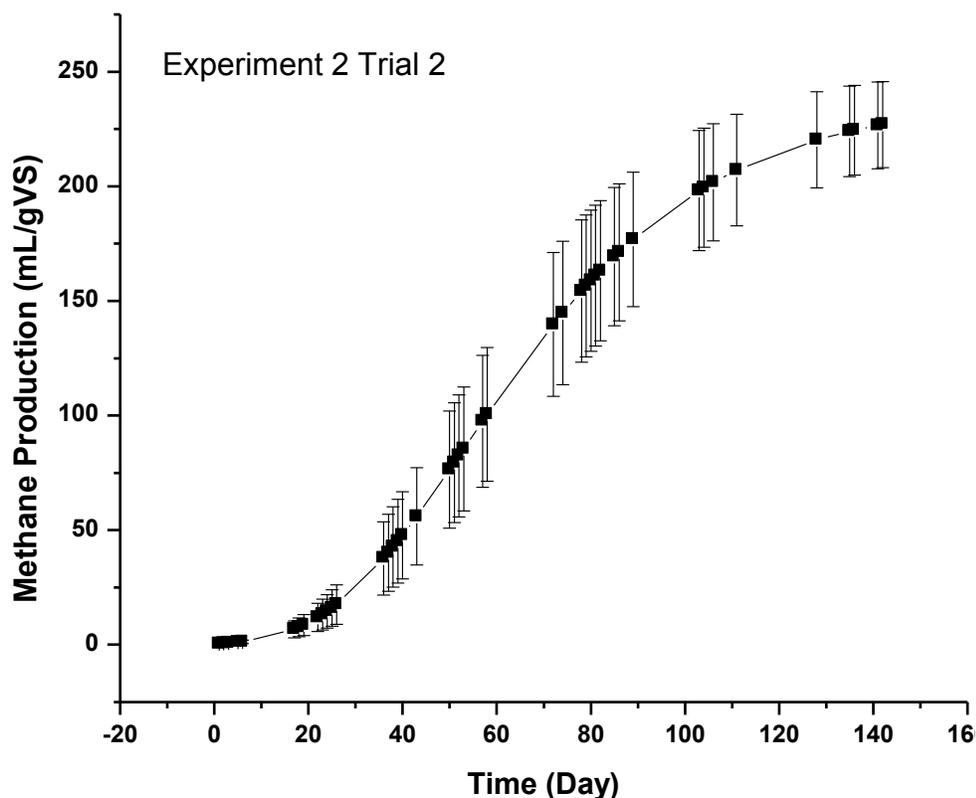
FIGURE 4.4: Typical methane production curve

Table 4.6 shows the %C (Col. 5) and %N (Col. 6) for both literature and analytical data. As noted, the values do differ, largely due to the high nitrogen content of the PL, which was unanticipated. These levels of nitrogen in poultry waste are atypical. The average range of nitrogen content is 1.6–3.9% (Richard 1996) with an average content range of 3.2–5.7 mg/L (Singh et al. 2010). Experimental feedstocks are shown in Table 4.7 (last two columns) and suggest that most of the trials operated at sub-optimal C:N levels. Additionally, it was discovered that due to a preparation error, only Trials 1–4 operated at 10% TS; while Trials 5 and 6 were prepared at 5% TS, and Trial 7 was 8% TS.

TABLE 4.8: Experiment 2 trial results

Trials	Initial pH	Final pH	Cum. CH$_4$ Yield[a] (mL/gVS)	VS Loading (gVS/L)
1	6.0±0.2	6.0±0.7	-2.0±2.1	71.5
2	6.1±0.2	7.4±0.2	220±13.2	67.0
3	6.0±0.1	5.4±0.1	-0.96±0.73	71.3
4	6.2±0.2	7.6±0.1	185 ±5.9	66.8
5	6.8±0.5	5.3±0.2	-3.3±2.5	69.8
6	6.8±0.5	5.4±0.3	-2.6±3.0	65.3
7	6.8±0.0	5.0±0.0	-2.0±1.3	87.8

Average ± Standard Deviation (n=2)
[a] Mean of controls subtracted from mean of trials

4.2.3. Experiment 3

Experiment 3 trials included FW with levels set below 15%, because Experiment 2 trials showed that this range of FW inclusion could produce CH$_4$ yields without digestion failure from rapid acidification and pH decline. New feedstocks were added in Experiment 3 along with strict digestion parameter limitations. The new feedstocks included CS and GLY from the Catawba County biodiesel production facility as well as paper and compostable tableware. The latter two feedstocks representing materials that would likely accompany fast food restaurant waste if it was collected post-consumer (Table 4.11).

Napkins were used as a surrogate for all paper products, and compostable cups were used to represent the compostable products available in fast food restaurants and for take-out service. Interviews were conducted at one popular fast food restaurant chain in the Hickory (NC) region, and it was learned that paper and packaging constitute twice the mass of FW. Therefore, such materials were included in a similar ratio in the experimental mixes.

Carbon to nitrogen ratios were calculated based on analytical data and combined with solids characteristics for each feedstock (Table 4.9) to determine mix ratios. The data from the table were inputted to a computer program written in Visual Basic (v6.0, Microsoft, Inc.) that took inputted values about feedstocks' moisture, carbon, and nitrogen content and would deliver output that met the goal and constraints shown in Table 4.10. These constraints permitted run times that were reasonable, ranging from 8-24 h. These constraints were informed by previous observations in the laboratory and literature review (Table 4.10). Inoculant additions were apportioned so that each trial was conducted with a food-to-microorganism (F:M) ratio of 0.6. Instead of digestate sludge, dewatered and press-dried 'cake' sludge was used as inoculant, and it was sourced from the same WWTP (Mallard Creek Reclamation Facility).

The yield calculations from Experiment 3 showed that none of the trial mixes showed any CH_4 production that exceeded that of the controls (Table 4.12) (there was no significant difference among the trials ($p > 0.05$)). The final pH levels ranged from 5.1–4.5. In analyzing the causes for failure of these trials, it was recognized that an erroneous deduction had been made about the cause of the poor performance of the GLY-containing trials of Experiment 2. Their low CH_4 production was attributed to the low solids

TABLE 4.9: Experiment 3 trial mixtures

Trial	Food Waste	Poultry Liter	DAF	Glycerin	Canola Seeds	Paper	CUPs	C:N
Percent Total Solids (w/w)								
1	15	15	5	35	-	30	-	20
2	15	15	5	35	-	-	30	20
3	15	15	20	20	-	-	30	20
4	-	10	-	40	35	15	-	20

(<10%TS) they contained (the result of a preparation error), when in fact, failure to produce CH_4 may have been due to the presence of GLY.

The mixes identified by the computer program in Experiment 3 were those that (i) contained the feedstocks required; and (ii) met all of the input criteria. Glycerin is high in organic carbon and evidently was required at a fairly high level to meet the C:N criterion of 20:1. Every mix failed, which made the presence of GLY highly suspect. After this mis-step, and as the list of feedstocks for testing expanded, a series of individual substrate tests was conducted to further inform the formulation of semi-continuous batch studies. The individual tests included BMPs and ATAs.

4.3.   Biochemical Methane Potential

Samples for the BMPs were drawn from those reserved for Experiments 2 and 3. Also, new feedstocks not tested in those experiments were evaluated, which included leaf waste (LM) from three different locations, feed meal (FM), and hatchery waste (HW). Characteristics of all BMP samples tested are shown in Table 4.11. The feedstocks were tested at 10% TS, and because most of the substrates were received at high solids content,

TABLE 4.10: Program design goal and constraints

Goal:	**Determine the combinations of substrate fractions that produce a C:N ratio of 20. Conform to the following constraints:**
Manipulation:	Substrate percentages
Constraint 1:	FW must be ≤ 15%.
Constraint 2:	TS must be 10% of the total volume.
Constraint 3:	If paper is used in the mix, then compostables cannot be used (vice versa).
Constraint 4:	The sum of all substrate fractions must equal 1.
Constraint 5:	C:N ratio must equal 20.
Constraint 6:	Let each substrate be 0% or ≥5% but not 1% > substrate > 4%

they were diluted to this concentration for testing. However, CM and SS were below 10% TS and had to be sieved to provide sufficient concentration for the CM (12.8±0.2% TS), but the SS had to be centrifuged (4,000 x g for 4 h) to achieve the 10% TS target needed. Filter cake from MCWRF was used as seed, and samples were prepared in duplicate and tested at an F:M of 1 and incubated for 66 d.

A typical curve showing cumulative CH_4 over the incubation period is shown in Figure 4.5. Cumulative CH_4 production was adjusted by subtracting contributions from the inoculant and then normalizing for the VS of the feedstock added (Figure 4.6,Table 4.12). Negative values in Table 4.12 reflect feedstock performance levels that were *below* that of the blank. Methane yield ranged from -65–371 mL CH_4/gVS, relative to the blanks, with BG showing the highest yield (Table 4.12). VS reduction from the substrates that produced more CH_4 than the blanks ranged from 27–7%. The two highest VS reductions occurred with SS and LM 3 at 27% with paper having 26% reduction. LM 3

TABLE 4.11: Characteristics of substrates

	%TS	%VS
Brown Grease	31.7±2.2	87.5±0.2
Cattle Manure	12.8±0.2	83.4±1.4
Canola Seed	90.3±0.2	90.1±0.4
DAF (H)	15.7±0.8	90.9±0.2
DAF (W)	37.6±1.7	99.1±0.0
Feed Meal	84.7±0.1	94.5±0.3
Food Waste	14.0±0.5	95.5±0.2
Glycerin	32.5±2.3	90.0±8.7
Hatchery Waste	25.9±0.4	95.1±0.1
LM 1	73.1±25	91.3±30
LM 2	87.4±0.7	76.1±0.5
LM 3	88.8±0.4	82.3±3.1
Paper	95.1±0.1	97.2±0.1
Poultry Litter	45.7±1.3	83.2±2.4
Sewage Sludge	10.9±0.1	89.6±0.1
Seed	20.3±0.1	81.0±0.1
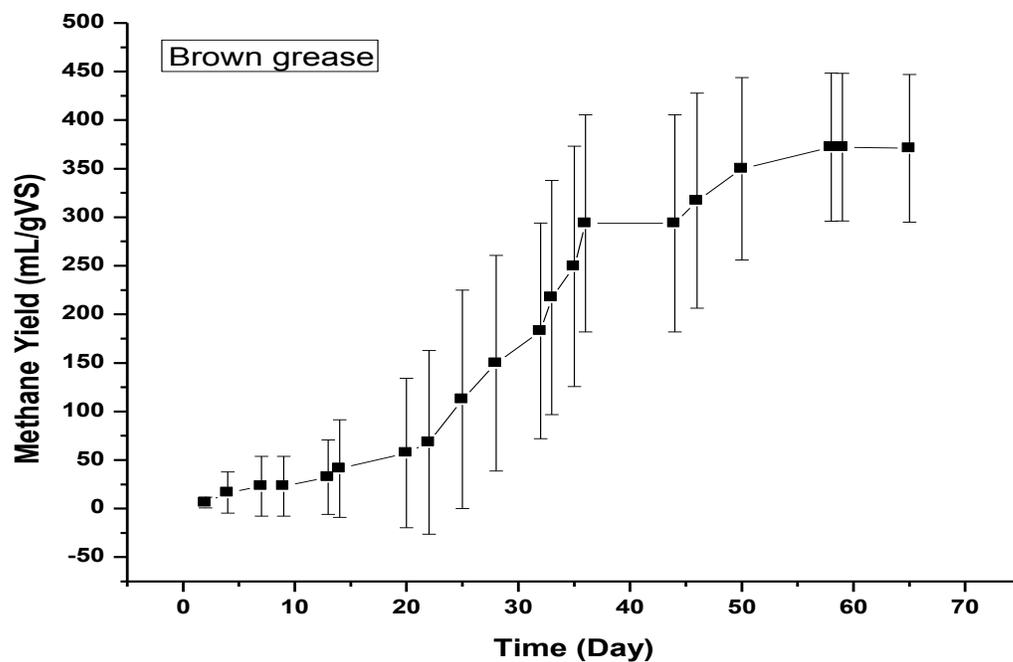
FIGURE 4.5: A typical methane production curve

and PL had the lowest VS reduction at 7%, while CM and BG had only 10% and 17% VS

reduction, respectively.

TABLE 4.12: Summary of BMP results

	Initial pH	Final pH	Cum CH$_4$ yield (mL/gVS) (n=2)	VS loading (gVS/L)
Brown Grease	8.1	7.6	371±76	58.3
Cattle Manure	8.4	6.2	125±11	55.6
Canola Seed	7.6	7.2	-65±0.42	60.1
DAF (H)	7.0	5.9	-18±2.9	60.6
DAF (W)	7.1	5.3	-58±0.41	66.1
Feed Meal	8.5	5.3	-30±0.92	63.0
Food Waste	7.0	5.8	-37±1.5	63.7
Glycerin	8.8	6.5	-35±2.9	60.0
Hatchery Waste	7.4	6.9	-49±1.3	63.6
LM 1	7.9	6.6	42±19	60.2
LM 2	7.5	7.6	-12±9.2	50.7
LM 3	7.3	7.4	18±5.3	54.9
Paper	8.6	6.6	150±33	64.8
Poultry Litter	8.3	7.0	120±27	55.5
Sewage Sludge	7.1	7.7	193±31	59.7
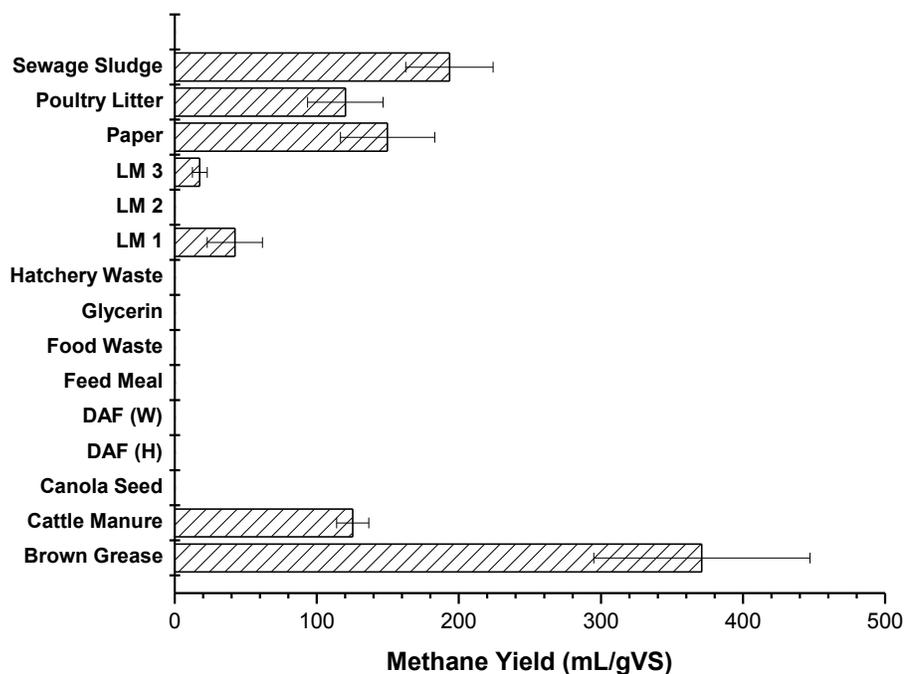
FIGURE 4.6: BMP yields for individual feedstocks incubated 65 d

4.4.   Anaerobic Toxicity Assays

Each ATA assay yielded a series of curves, and the relationship of each inclusion rate curve to the control curve (black) (Figure 4.7); rate curves that fall below the blank are inhibitory. Equation 3.1 was used with Day 3 data to calculate I, the degree of inhibition associated with each feedstock (Table 4.13). While the blanks performed at about the same level in each ATA study, the shape and spread of each feedstock curve set varied by substrate. Two typical sets of curves are shown in Figure 4.7, and a complete set of graphs is provided in Appendix B. Table 4.14 summarizes the initial and final pH values obtained for the ATAs.

Many of the feedstocks showed inhibitory effects, which was reflected by their curves falling below that of the blank. Values for I ranged from -26.9 to 99.9%. For some, such as CS and DAF, the effect was gradual, so that the curve heights fell as the feedstock concentrations rose. Other feedstocks, such as FW and LM, showed very discrete thresholds levels between low and higher inhibition. In the case of HW, the lowest feedstock concentration enhanced CH_4 production, but higher levels were inhibitory. Cow manure was the only feedstock to enhance CH_4 production across all concentrations tested, and PL stimulated gas production at lower concentrations but inhibited it at higher concentrations. Glycerin was the most toxic of the substrates tested, halting CH_4 production in all but the lowest concentration GLY sample, with the production level in the latter remaining well below that of the blank.
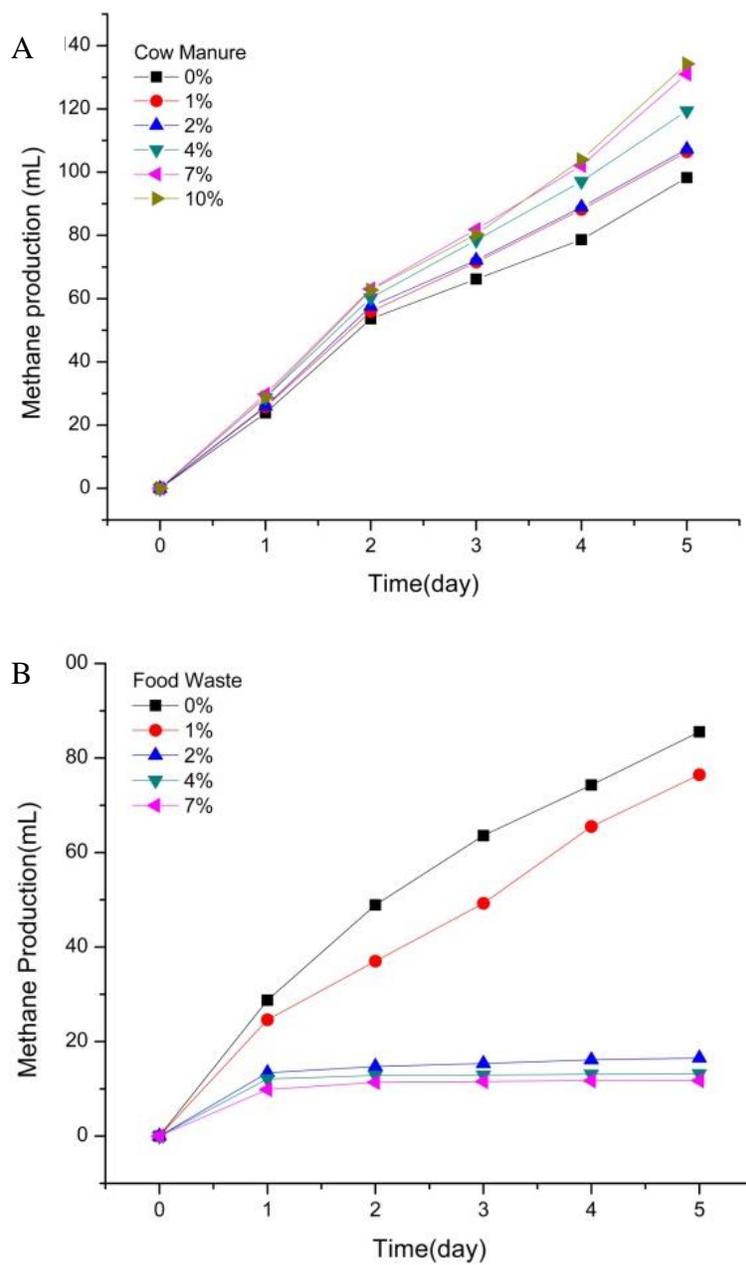
FIGURE 4.7: Typical positive (A) and negative (B) ATA graphs

109

TABLE 4.13: ATA results from the substrates analyzed

Percent Inclusion	Poultry Liter	Hatchery Waste	Paper Waste	Leaf Waste	Food Waste	Glycerin	Feed Meal	DAF	Canola Seed	Cattle Manure	Brown Grease
					Percent Inhibition						
0.5			77.0								
1	-17.9	-5.36	17.6	65.0	74.6	98.2	45.5	30.9	25.0	-8.03	7.6
2	-16.8	2.07	--	64.3	97.3	98.6	61.4	36.6	63.4	-9.01	10.6
3	-26.9	--	29.1	--	--	--	--	--	--	--	--
4	-14.2	43.7	10.4	69.8	98.5	98.6	70.0	52.4	87.6	-18.5	25.8
5	-11.5	--	--	--	--	--	--	--	--	--	--
7	3.13	47.9	-7.61	84.1	96.9	99.0	89.3	68.0	94.4	-23.7	23.6
10	55.1	59.6	--	87.4	--	99.9	87.5	--	94.3	-21.3	64.3

TABLE 4.14: ATA pH values

	Food waste		DAF		Cow manure		Glycerin		Canola Seed	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
0%	7.11	7.31	7.18	7.21	7.13	7.29	7.17	6.98	7.16	7.44
1%	7.24	7.08	7.22	6.67	7.13	7.34	7.53	5.89	7.2	7.21
2%	7.07	5.68	7.1	6.51	7.21	7.37	7.97	6.22	7.26	5.98
4%	7.08	5.33	7.11	6.05	7.28	7.41	8.07	6.46	7.3	5.93
7%	7.04	4.85	7.01	5.94	7.24	7.93	8.63	6.26	7.18	5.91
10%					7.33	8.18	8.81	7.38	7.18	5.97

	Paper		Poultry Litter		Leaf Waste		Hatchery Waste		Brown Grease	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Control	7.16	6.99	7.26	7.42	6.86	6.94	6.96	7.03	7.04	7.11
1%	7.25	6.64	7.06	7.34	6.86	6.75	7.00	6.94	7.15	6.52
2%	7.33	6.31	7.05	7.46	6.8	5.53	7.1	6.98	7.15	6.16
4%	7.25	6.19	7.05	7.47	6.75	4.83	7.05	6.4	7.12	6.31
7%	7.38	6.15	7.02	7.31	6.65	4.73	6.91	6.24	7.17	5.82
10%	7.33	6.16	6.94	6.98	6.51	4.83	6.88	6.04	7.20	5.91

pH for feed meal is not available.

4.5.   Semi-continuous Reactors

For the semi-continuous reactor studies, the following agricultural and industrial wastes were included (although not in the same reactors): DAF, PL, BG, SS, paper, HW, FM, LM, and FW. The DAF and BG had performed well in the batch tests, and the PL, SS, and HW remained in consideration because Catawba County anticipated that they would be likely waste streams available to them. The County also requested that we include LM, although it was less certain that they would routinely use it in an AD operation. The FW and paper were included by mutual agreement because of our shared interest in exploring the potential for post-consumer FW collection at restaurants.

The three mixtures tested are shown in Table 4.15. The two mixtures created as a research activity were designed to meet the preferences cited above using information pooled from computer program output (see Experiment 3) and BMP and ATA results. The computer program returned over 100,000,000 combinations. BMP and ATA data were used to narrow the results to a manageable number. The program identified a set of mixes that would satisfy the criteria, but there were no options that did not require one or another feedstock to dominate (Table 4.10) to counteract the high nitrogen content of the PL and HW. Table 4.16 is a summary of the feedstocks characteristics used in the program. Mix 1 permitted representation of a wide range of the feedstocks available to the County, although the computer program forced high use of HW and FM to achieve the proper C:N range. Mix 2 represented a mix that contained the PL the County wanted incorporated, but to balance the C:N ratio required high levels of BG and DAF. The third mix was defined by Catawba County and represented a mix formed according to the ratios that would be created if feedstocks were mixed according to the mass rates at which they were delivered.

The semi-continuous reactor experiments were conducted in two phases. In the

TABLE 4.15: Mix ratios tested in semi-continuous batch experiments

	Poultry Litter	Food Waste	Sewage Sludge	DAF (W)	DAF (H)	Paper	Hatchery Waste	Leaf Matter	Feed Meal	Brown Grease
Mix					**Fraction of TS (w/w)**					
1	0.05	0.10	0.05		0.05	0.05	0.35	0.05	0.30	
2	0.25	0.05			0.35					0.35
3	0.02	0.00	0.08	0.77	0.09	0.00	0.03	0.00	0.01	

first phase (pilot trials), the reactors were operated to ensure that feedstock inhibition was not occurring and that the protocol and equipment were performing as anticipated. Reactors had been started several weeks in advance of the pilot trials and were being maintained with WAS from MCWRF. The VS measurement of biomass from each reactor was used as the basis for a F:M calculation, with the reactor contents constituting the source of microbial inoculant. Each feedstock mix was added such that its addition (in gVS/d) created an F:M ratio of 0.5 relative to the biomass VS. This feed addition constituted the OLR in g VS/d (Table 4.17).

The second phase of the semi-continuous flow reactor trials were the experimental trials. These trials used acclimated seed that had been cultivated in each reactor during the pilot trials. At this point, the inoculant in each reactor was set such that

TABLE 4.16: Characteristics and design ranges

Feedstock	%TS	%VS	%C	N%	min value	max value	increase value
Poultry Litter	46.7	87.7	39.3	4.71	0	0.40	0.025
Food Waste	14	95.5	53	2.53	0	0.15	0.01
Brown Grease	31.7	87.5	63.2	1.24	0	0.40	0.025
Sewage Sludge	2.9	90.7	33.6	5.6	0	0.50	0.025
DAF (H)	21.3	92.6	61.1	3.02	0	0.50	0.025
DAF (W)	37.9	98.7	66.9	3.51	0	0.50	0.025
Leaf Matter	83.7	80.2	38.6	2.56	0	0.40	0.025
Paper	95.2	97.2	42.3	0.06	0	0.30	0.01
Hatchery Waste	25.9	95.1	59.1	8.17	0	0.50	0.025
Feed Meal	84.7	94.5	44.8	3.53	0	0.50	0.025

in every case, feeding volumes establish an SRT of 18 d and an OLR of 5 g VS/L-d (Table 4.17).

TABLE 4.17: Pilot and experimental trial operating conditions

		Organic loading (gVS/L-d)	SRT (d)	%TS
Pilot Trial	Mix 1	6.5	15	10
	Mix 2	3.2	27	10
	Mix 3	7.6	13	10
Experimental Trial	Mix 1	5.0	18	9.6
	Mix 2	5.0	18	9.7
	Mix 3	5.0	18	9.2

4.5.1. Pilot Trials

The larger gas production volumes generated in the semi-continuous flow reactor trials were beyond the range of the digital gas flow meter, and, once again, the salt solution-filled graduated cylinder system was employed. Often the overpressure generated in a single bottle required several fillings of the cylinder. Methane production in the pilot trials ranged from 12–165 mL CH_4/g VS-d (Figure 4.8). Mixes 1 and 3 had similar organic loadings (6.5 and 7.6 g VS/L-d, respectively) and SRTs of 15 and 13, respectively (FIGURE 4.17). However, the lipid content in Mix 3 was likely much higher because it contained 77% DAF, while Mix 1 included only 5% DAF. Mix 3 suffered from extensive foaming (Figure 4.9), and it became very viscous and difficult to mix. Mix 1 yielded 52.2 mL CH_4/gVS-d, while Mix 3 yielded only 12.8 mL CH_4/gVS-d. Despite these difficulties, Mix 3 showed higher VS reduction, suggesting that more of the initial organics present were consumed in Mix 3 than in Mix 1. Mix 2 had the longest SRT (27d) with the lowest loading rate (3.3 gVS/L-d), which resulted in the highest CH_4

production (165 mL CH_4/g VS-d). However, Mix 2 did not have the highest VS reduction.

Pilot Trials

Mix	Poultry Litter	Food Waste	Sewage Sludge	DAF(W)	DAF(H)	Paper	Hatchery Waste	Leaf Waste	Feed Meal	Brown Grease
			Fraction of TS (w/w)							
Mix 1	0.05	0.10	0.05	--	0.05	0.05	0.35	0.05	0.30	--
Mix 2	0.25	0.05	--	--	0.35	--	--	--	--	0.35
Mix 3	0.02	0.00	0.08	0.77	0.09	--	0.03	--	0.01	--
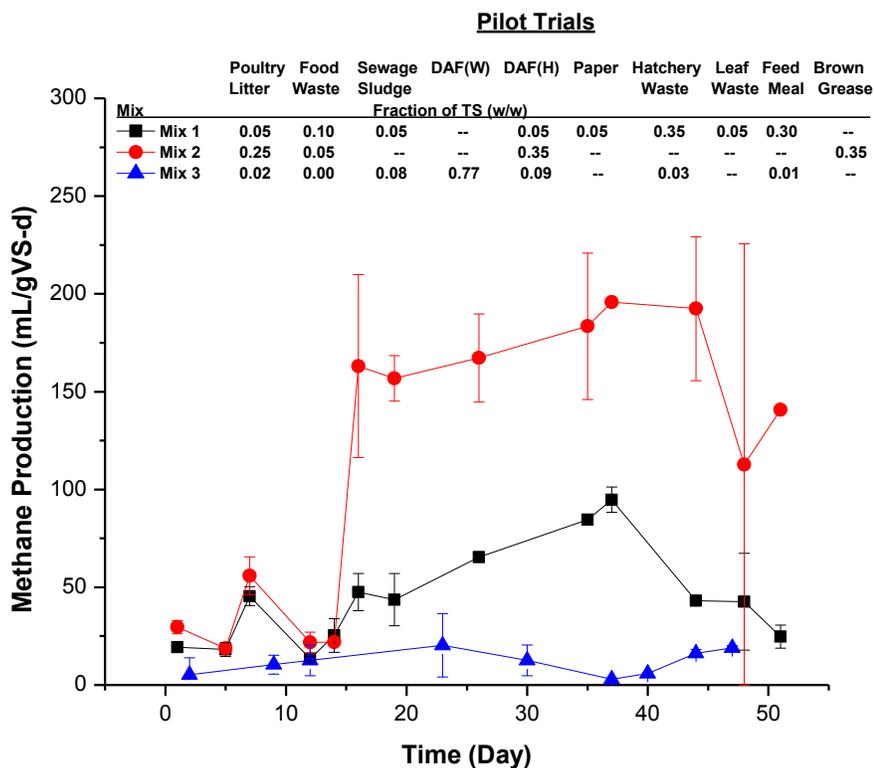
FIGURE 4.8: Semi-continuous pilot methane production over time

4.5.2. Experimental Trials

Mix 1, which contained a wider range of materials than the other mixes, showed a low CH_4 yield under the experimental trials regime (45 mL CH_4/g VS-d) (Figure 4.10, Table 4.18). This value was about 13% lower than Mix 1 performance in the pilot trials, where the organic loading was higher but the SRT was shorter. Mix 2 CH_4 yields declined sharply in the experimental trials, reaching only 15 mL CH_4/g VS-d compared to 165 mL CH_4/g VS-d. This represents a 91% decline in production. However, as noted

TABLE 4.18: Summary of pilot and experimental trial results

		Cum. CH$_4$ (mL/d)	Cum. CH$_4$ (mL/gVS-d)	%VS Reduction
Pilot Trial (50 d)	Mix 1	337±74.8	52.2±11.6	81±4.3
	Mix 2	528±117	165±36.6	44.±2.1
	Mix 3	97±53.6	12.8±7.1	93±0.90
Experimental Trial (36 d)	Mix 1	223±86.2	45±17.2	83±1.3
	Mix 2	75±11.6	15±2.3	28±0.2
	Mix 3	111±14.6	22±2.9	88±1.3

n=3; ±s.d.

previously, the pilot trial had low organic loading and high SRT. In the experimental trial, the lower SRT of 18 d resulted in foaming and increased viscosity (Figure 4.9) within 12–15 d after startup despite the use of acclimated seed. The appearance of Mix 2 in the experimental trial resembled that of Mix 3 pilot trial reactors; that is, the reactors were foaming and viscous. As in the pilot trials, Mix 2 had the lowest level of VS reduction. Mix 3 was more than 85% DAF and performed better at the longer SRT provided by experimental conditions. However, it did succumb to foaming and high viscosity at Day 20, which was about 10 d later than such problem occurred in the pilot experiment. The CH$_4$ yield was still low (22 mL CH$_4$/g VS-d), although it represented an increase from the pilot trial conducted at a shorter SRT with higher organic loading. As

FIGURE 4.9: Foam formation during digestion

with the pilot, Mix 3 showed the highest VS reduction level among the mixes.

Experimental Trials

Mix	Poultry Litter	Food Waste	Sewage Sludge	DAF(W)	DAF(H)	Paper	Hatchery Waste	Leaf Waste	Feed Meal	Brown Grease
			Fraction of TS (w/w)							
Mix 1	0.05	0.10	0.05	--	0.05	0.05	0.35	0.05	0.30	--
Mix 2	0.25	0.05	--	--	0.35	--	--	--	--	0.35
Mix 3	0.02	0.00	0.08	0.77	0.09	--	0.03	--	0.01	--
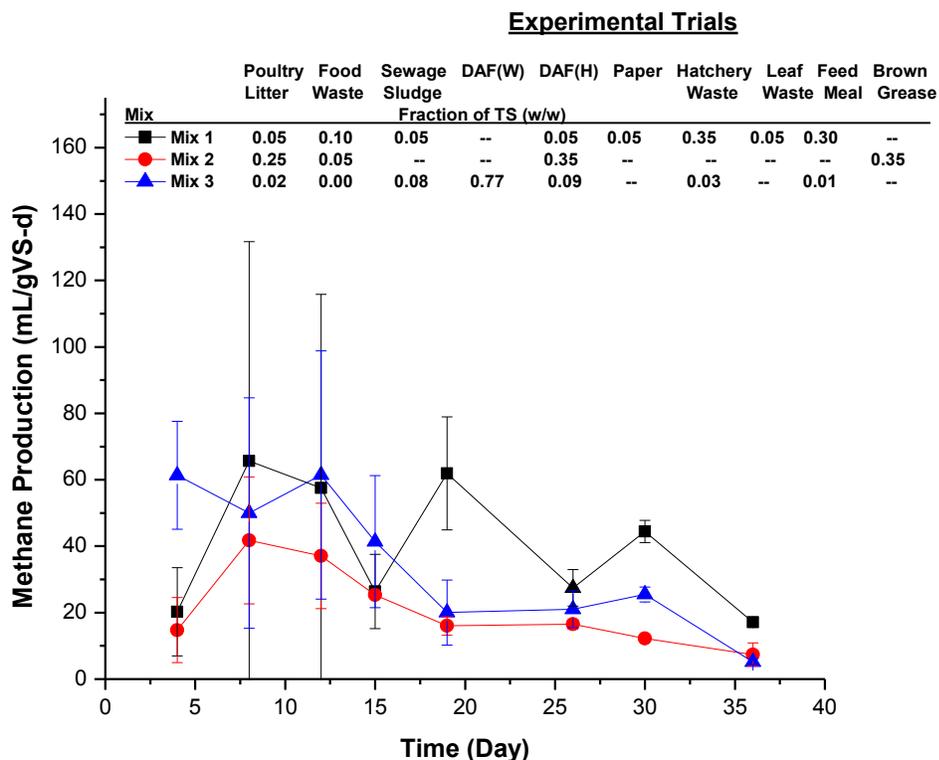
FIGURE 4.10: Experiment trial daily methane production

4.6.   Cell Counts

The F:M ratio utilized in AD design has traditionally assumed that the VS present in the feedstock is food and the VS from the inoculum is microorganisms. For some feedstocks, additional bacteria added by the feedstock such as WAS, CM, and PL. These feedstocks have live bacteria capable of aiding the microorganisms present in the inoculum, thereby altering the designed F:M ratio. Cell counts were performed on six feedstocks and one inoculum source using CTC-DAPI counterstaining: CM, CS, DAF (H), DAF (W), PL, PL (N), and seed cake. Table 4.19 presents the feedstock characteristics. The range of live cells was 1.8×10^8–4.2×10^{10} cells/gVS with CM having

the highest amount of live cells present (Table 4.19). CM also had the highest amount of total cells, but only 57% were alive. For this study, more PL had to be collected, designated as PL (N). This new PL had a higher moisture content, possibly due to the rain event just prior to collection, as well as more bedding and feathers (Table 4.19). As noted bacterial counts were obtained from the original PL and the new PL, PL had higher live and total bacterial counts (1.56×10^{10} cells/gVS and 2.43×10^{10} cells/gVS, respectively); however, PL (N) had a higher percentage of live to total bacteria (76%).

To evaluate if the additional bacteria affect CH_4 production, BMPs of PL (N), DAF (W), and paper were conducted for 43 days. These feedstocks represented high, mid, and low levels (PL (N), DAF (W), and paper, respectively) of bacteria ranging from $0–8.7 \times 10^{10}$ cells/gVS (Table 4.21). They were digested according to previously described BMP protocols with a designed F:M of 1. Additionally, live and dead bacterial counts were obtained for each BMP pre- and post-digestion (Table 4.21). Biomass was calculated based on an assumed conversion (20 fg C/cell) and the abundance determined

TABLE 4.19: Feedstocks characteristics

	%TS	%VS	Live (cells/gVS)[a]	Dead (cells/gVS)[a]	Total (cells/gVS)[a]
Cattle Manure	10.6±0.4	82.3±6.8	4.16E+10	3.1E+10	7.27E+10
Canola Seed	90.3±0.2	90.1±0.4	1.82E+08	7.9E+06	1.9E+08
DAF (H)	11.1±0.6	89.8±0.5	1.02E+10	7.51E+09	1.77E+10
DAF(W)	29.0±1.1	99.2±0	7.91E+08	4.59E+08	1.25E+09
Poultry Litter	52.9±5.2	68.5±6.2	1.56E+10	8.7E+09	2.43E+10
Poultry Litter (N)	37.9±0.8	62.1±1.2	2.51E+09	7.74E+08	3.29E+09
Paper	95.1±0.1	97.2±0.05	0	0	0
Seed	20.3±0.1	81±0.1	3.41E+09	1.82E+09	5.23E+09
[a] Average (n=3)					

from each BMP (Bratbak 1985). The live biomass from the BMPs ranged from 0–38.7 µg

C/mL $_{sample}$ (feedstock and seed) with PL and PL + seed having the highest at 38.7 µg

C/mL $_{sample}$ and 32.8 µg C/mL $_{sample,}$ respectively (Table 4.20).

TABLE 4.20: BMP biomass and methane production per abundance

	Live biomass (µg C/mL)[a]	Total biomass (µg C/mL) [a]	Cum CH_4 (mL CH_4/µg C)[b]	Cum CH_4 (mL CH_4/cell)[b]
Blank	16.26	30.35		
DAF (W)	4.83	5.25	0.84	1.7E-08
DAF (W) + Seed	6.77	13.07	0.67	0.85E-08
Paper	0.00	0.00	0.00	0
Paper + Seed	0.11	0.23	80	2.13E-08
Poultry Litter (N)	38.66	57.14	0.0090	0.018E-08
Poultry Litter (N) + Seed	32.80	56.06	0.014	0.033E-08
[a] Calculated using 20 fg of C/cell conversion				
[b] biomass and cell count based on total live cell abundance (seed and feedstock)				

TABLE 4.21: Summary of CTC/DAPI Results

Trials	Initial Live (cells/gVS)[a]	Initial Dead (cells/gVS)[a]	Initial Total (cells/gVS)[a]	Final Live (cells/gVS)[a]	Final Dead (cells/gVS)[a]	Final Total (cells/gVS)[a]
Blank	2.5E+10	2.1E+10	4.6E+10	1.3E+10	0.66E+10	1.9E+10
DAF (W)	0.73E+10	0.064E+10	0.80E+10	0.25E+10	0.71E+10	0.97E+10
DAF (W) + Seed	1.6E+10	1.1E+10	2.7E+10	0.75E+10	0.69E+10	1.4E+10
Paper	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Paper + Seed	1.2E+10	1.1E+10	2.3E+10	0.63E+10	0.33E+10	0.96+10
Poultry Litter (N)	5.9E+10	2.8E+10	8.7E+10	2.1E+10	2.0E+10	4.1E+10
Poultry Litter (N) + Seed	4.2E+10	2.5E+10	6.6E+10	1.7E+10	1.3E+10	3.0E+10
[a] Average (n=3)						

Each of the feedstocks was digested with seed and alone to see if the bacteria in the feedstock were bacteria necessary for AD. The CH_4 yield ranged from 0–263 mL CH_4/gVS (Figure 4.11,Table 4.22). Paper + seed produced the highest amount of CH_4, while the paper alone did not produce any CH_4. PL (N) + seed had the longest lag time. It did not produce more CH_4 than the blanks until day 40; however, PL (N) started to produce more CH_4 by day 11 (Figure 4.12).
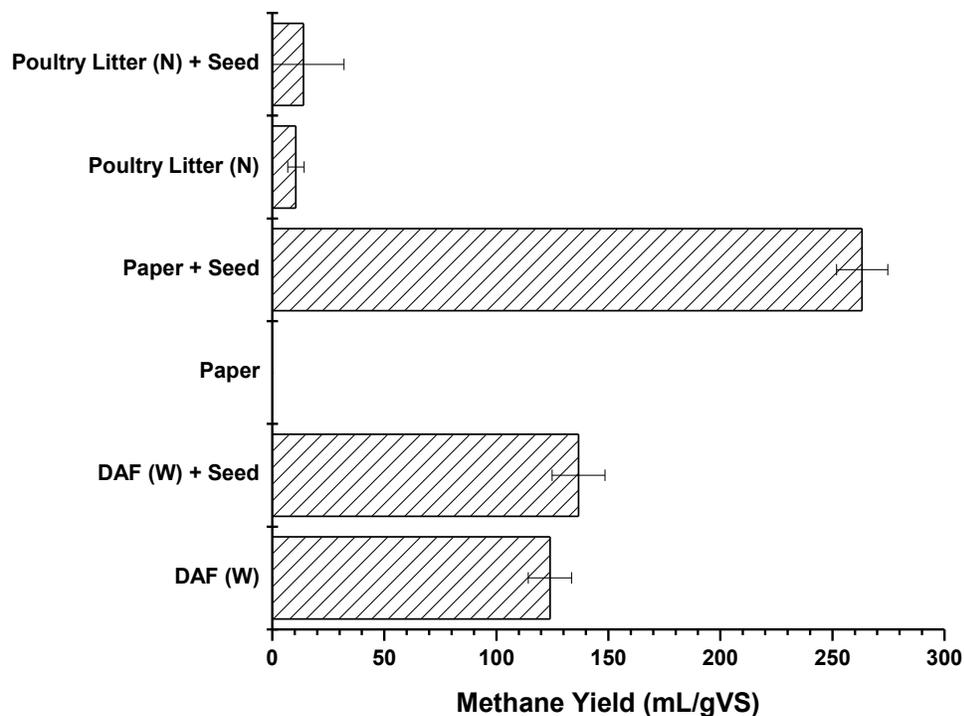
FIGURE 4.11: Normalized methane yield after 43 days.

TABLE 4.22: Summary of BMP results

Trials	Initial pH	Final pH	Cum. CH$_4$ Yield (mL)	Cum. CH$_4$ Yield[a] (mL/gVS$_{added}$)	VS Loading (gVS$_{added}$/L)	VS Reduction (%)
Blank	8.0±0.1	7.4±0	428±5.8			
DAF (W)	7.5±0.1	7.2±0	612±48.2	124±9.7	32.9	36±28.0
DAF (W) + Seed	7.4±0	7.5±0.1	1104±58.3	137±11.8	32.9	23±7.7
Paper	9.0±0.1	5.3±0.2	0±0	0±0	32.9	0±0
Paper + Seed	8.4±0.2	7.4±0	1729±56.4	263±11.4	32.9	79±32.3
Poultry Litter (N)	8.8±0.1	7.3±0.1	52±17.9	11±3.6	32.9	30±53.5
Poultry Litter (N) + Seed	8.5±0.1	8.1±0	497±89.6	14±18.1	32.9	32±1.2

Average ± Standard Deviation (n=3)
[a] Mean of blanks subtracted from mean of trials
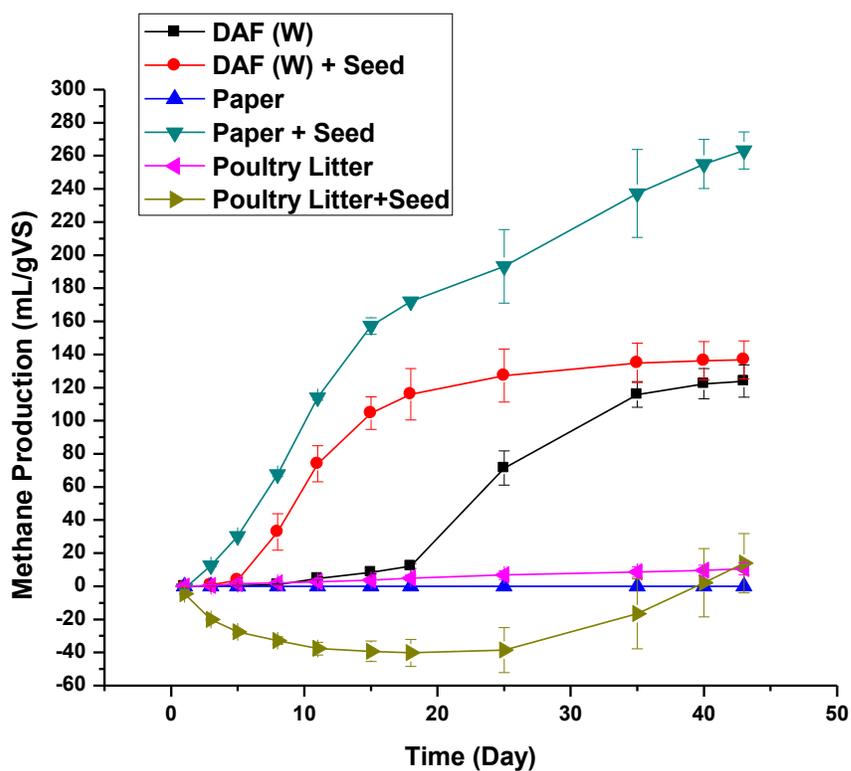
FIGURE 4.12: Normalized cumulative methane yield relative to blank mean

4.7. Pretreatment Results

From among the pretreatment categories considered (thermal, chemical, thermochemical, mechanical, and biological), chemical and biological pretreatment had the best outcomes, relative to net energy (Figure 4.13). All studies reporting these modes of treatment yielded analyses with positive E values. Half of the reports on mechanical pretreatment described systems that produced a net positive energy yield, while thermal and thermochemical pretreatment were the least reliable methods of providing net energy gains. Only 7% and 18%, respectively of applications of these methods resulted in positive E values.
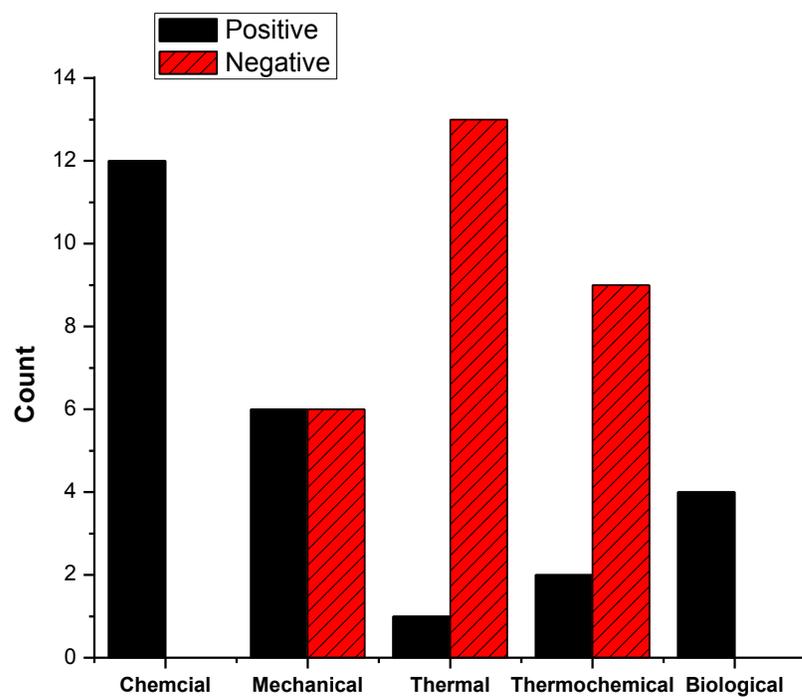
FIGURE 4.13: General count of positive and negative energy balances
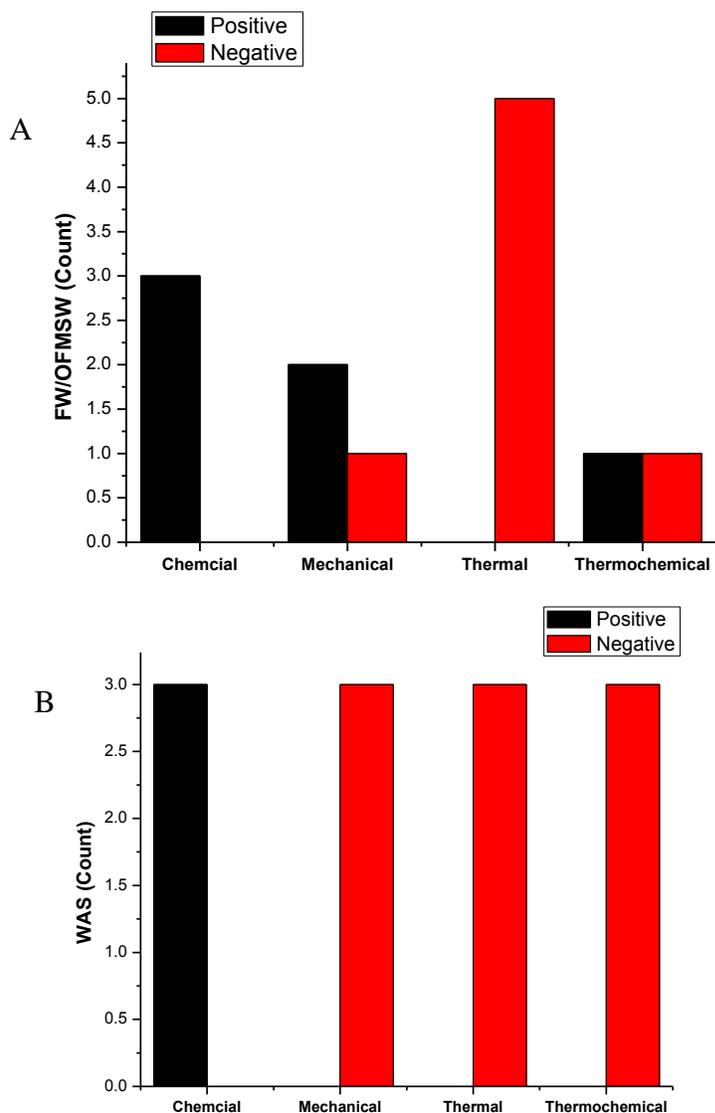
FIGURE 4.14: Energy balance counts for FW/OFMSW (A) and WAS (B)

WAS and FW/OFMSW proved to be the most difficult feedstocks to pretreat for positive energy yield (Figure 4.14). Chemical pretreatment proved effective for both of these feedstocks, while two instances of mechanical pretreatment resulted in a positive E for FW/OFMSW (Table 4.23, Table 4.24). Conversely, lignocellulosic materials and manures had a higher frequency of positive E values relative to the other feedstocks (Figure 4.15). Thermal and thermochemical pretreatment did not yield positive E values
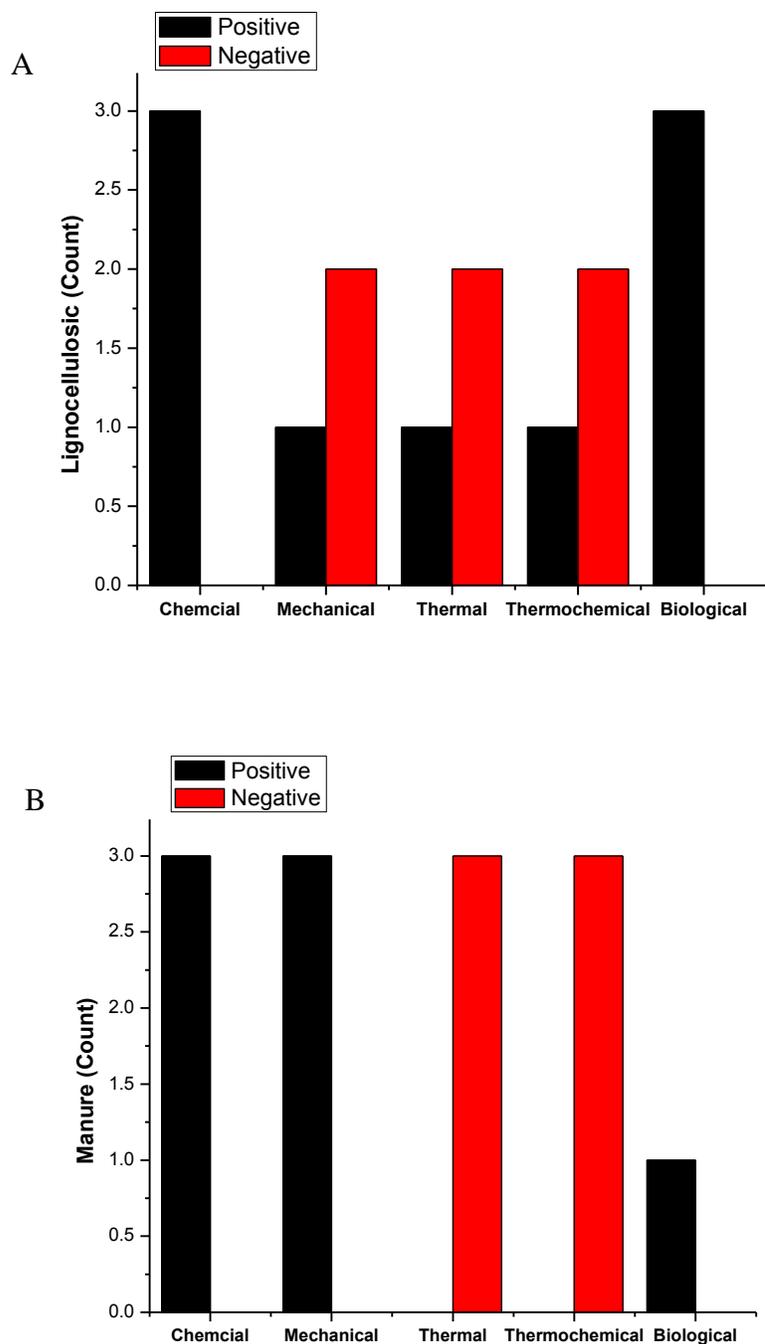
FIGURE 4.15: Energy balance counts for lignocellulosic (A) and manure (B)

for these feedstocks, nor did mechanical pretreatment of lignocellulosic material (Table 4.25, Table 4.26). Appendix A contains a table summarizing E for each category.

From the literature, the E_I/E_O ratios ranged from 0–1073 (Appendix A). The E_I/E_O values confirmed the results obtained from E calculations. Each of the observed positive E ratios had a E_I/E_O less than 1. The analyses conducted are only relative to the energy balance. It does not take into account other beneficial applications of pretreatment such as vector and pathogen reduction.

TABLE 4.23: Energy results from food waste and OFMSW

	Pretreat-ment Type	Sample	Net CH_4 (L) (out - in)	Energy Balance (out - in) (kJ)	E_I/E_O	Author(s)
Food waste and organic fraction of municipal solid waste	Chemical	Lime	1	11	0.00	(López Torres & Espinosa Lloréns 2008)
		H_2O_2	0.43	3.59	0.00	Shahriari et al. (2012)
		NaOH	0.01	23	0.00	Wang et al. (2009)
	Mechanical	Sonicated	0.14	-26	6	(Luste et al. 2009)
		Sonicated	10	137	0.00	(Elbeshbishy & Nakhla 2011)
		III - 1000; Bead Mill[a]	5030	69	0.00	(Izumi et al. 2010)
	Thermal	175°C	0.23	-29	10	(Marin et al. 2010)
		Microwave	0.65	-115	22	(Beszédes et al. 2011)
		175°C	0.07	-16	17	(Liu et al. 2012)
		175°C	0.15	-58	28	(Liu et al. 2012)
		170°C	0.01	-31	2	Wang et al. (2009)
	Thermo-chemical	85°C, H_2O_2	0.50	-30	8	Shahriari et al. (2012)
		130°C, 4gNaOH	0.02	1.79	1	Wang et al. (2009)

TABLE 4.24: Energy results from WAS feedstocks

	Pretreat-ment Type	Sample	Net CH$_4$ (L) (out - in)	Energy Balance (out - in) (kJ)	E$_I$/E$_O$	Author(s)
Waste activated sludge	Chemical	7g/L NaOH	0.85	12	0.00	Kim et al. (2003)
		H$_2$O$_2$ and FeCL$_2$	0.48	6.54	0.00	(Dhar et al. 2011)
		37% HCL	0.15	2.02	0.00	(Devlin et al. 2011)
	Mechanical	Sonicated	0.90	-492	41	Kim et al. (2003)
		Pump	0.47	-20	4	Dhar et al. (2011)
		Sonicated	0.58	-352	46	(Apul & Sanin 2010)
	Thermal	70˚C	0.01	-191	1073	(Kuglarz et al. 2013)
		Microwave	0.24	-33	18	(Eskicioglu et al. 2006)
		121˚C	1.02	-64	6	Kim et al. (2003)
	Thermo-chemical	121˚C,7g/L NaOH	1.01	-64	6	Kim et al. (2003)
		130˚C, pH10 (KOH)	0.05	-19	27	(Bougrier et al. 2006)
		90˚C, H$_2$O$_2$ + FeSO$_4$	0.06	-5.35	12	(Valo et al. 2004)

TABLE 4.25: Energy results from manure feedstocks

	Pretreat-ment Type	Sample	Net CH$_4$ (L) (out - in)	Energy Balance (out - in) (kJ)	E$_I$/E$_O$	Author(s)
Manure	Chemical	Alkaline	7.81	93	0.13	(González-Fernández et al. 2008)
		5% Ca(OH)$_2$	0.02	0.19	0.00	(Rafique et al. 2010)
		NaOH, pH 10	0.10	1.31	0.00	(Carrère et al. 2009)
	Mechanical	Sieved Solids	10	135	0.00	González-Fernández et al. (2008)
		Sonicated	731	0.69	1	(Elbeshbishy et al. 2011)
		Sonicated	775	6441	0.01	(Castrillón et al. 2011)
	Thermal	170˚C	0.24	-24	8	(Qiao et al. 2010)
		170˚C	2.22	-146	6	González-Fernández et al. (2008)
		80˚C	0.56	-6	2	(Bonmati et al. 2001)
	Thermo-chemical	Ca(OH)$_2$, 90˚C	0.05	-279	451	(Costa et al. 2012)
		100˚C, 5% Ca(OH)$_2$	0.04	-8	25	Rafique et al. (2010)
		190˚C, pH10	0.14	-13	8	Carrère et al. (2009)
	Biological	C. cellulolyticum	0.87	12	0.00	Costa et al. (2012)

TABLE 4.26: Energy results from lignocellulosic feedstocks

	Pretreat-ment Type	Sample	Net CH$_4$ (L) (out - in)	Energy Balance (out - in) (kJ)	E$_I$/E$_o$	Author(s)
Lignocellulosic feedstocks (grass and straw)	Biological	Mn Peroxidase	0.05	0.66	0.00	(Frigon et al. 2012)
		P. florida 50g/L	12	97	0.00	(Frigon et al. 2012)
		Novozyme (N342)	0.68	5.39	0.00	(Romano et al. 2009)
	Chemical	NaOH	0.06	0.76	0.00	(Frigon et al. 2012)
		8% NaOH	2.32	32	0.00	(Taherdanak & Zilouei 2014)
		3% NaOH	0.06	0.85	0.00	(Chandra et al. 2012)
	Mechanical	Sonicated	0.33	-1.11	1	(Fernández-Cegrí et al. 2012)
		Chopped	0.02	-150	539	(Frigon et al. 2012)
		Cut[a]		1839600	0.06	(Menardo et al. 2012)
	Thermal	180˚C	0.04	-33	40	Jackpwiak_2011
		120˚C	0.06	-58	78	(Antonopoulou et al. 2010)
		90˚C[a]		1360800	0.13	Menardo et al. (2012)
	Thermo-chemical	120˚C, Ammonium	1.34	-23	2	(Fernandes et al. 2009)
		85˚C, Ca(OH)$_2$	0.71	-19	3	Fernandes et al. (2009)
		100˚C, 7.5%NaOH	9.05	120	0.03	(Xie et al. 2011)

[a] Provided by Author (s)

4.7.1. BMP Tests With and Without Thermal Pretreatment

Pretreatment resulted in higher CH$_4$ yields for most of the samples; only the SS CH$_4$ production was inhibited by the heat treatment (Figure 4.16, Table 4.28). The yields
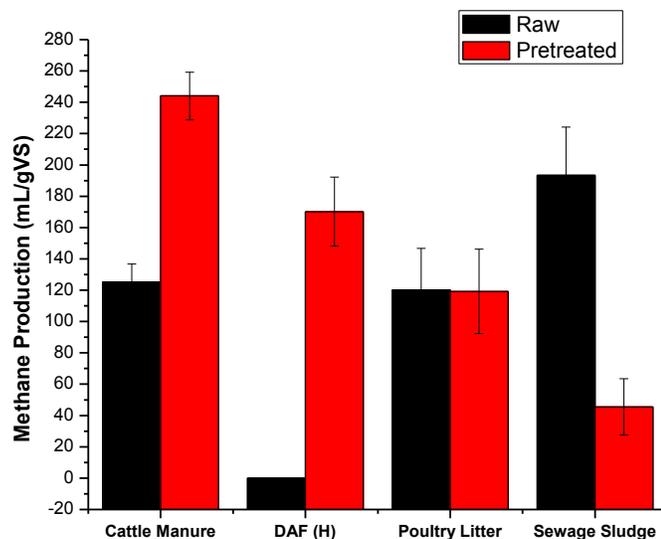
FIGURE 4.16: Pretreatment methane production

TABLE 4.27: Energy results for pretreated substrates

	Energy In (kJ$_{el}$)	Energy Out (kJ$_{el}$)	Energy Balance (kJ$_{el}$)	E$_I$/E$_O$
Cattle Manure	15.8	28.9	13.1	0.5
DAF (H)	7.8	21.6	13.8	0.4
Poultry Litter	2.0	12.7	10.6	0.2
Sewage Sludge	15.8	5.6	-10.2	2.8

ranged from 0–244 mL CH$_4$/gVS, with the highest yield obtained from pretreated CM (244±15 mL CH$_4$/gVS). The DAF (H) sample showed the largest change in CH$_4$ production after pretreatment (180 mL CH$_4$/gVS) (Figure 4.16). It was the only sample that failed to yield CH$_4$ when untreated (Figure 4.12), though none of the samples showed any signs of acidification. Net energy was determined for each of the BMP pairs according to the methods and equations used to analyze the literature data.

The analysis indicated that thermal pretreatment (70˚C) would be beneficial for CM and DAF (H) both in terms of CH$_4$ production and net energy yield (Table 4.27). PL was not affected by thermal pretreatment; however, it did have a positive E. But SS had

less CH_4 production after pretreatment (Figure 4.16). The E_I/E_O ratio ranged from 0.2–2.8, with PL at the lowest value (0.2), confirming a positive E and the largest different between input and output.
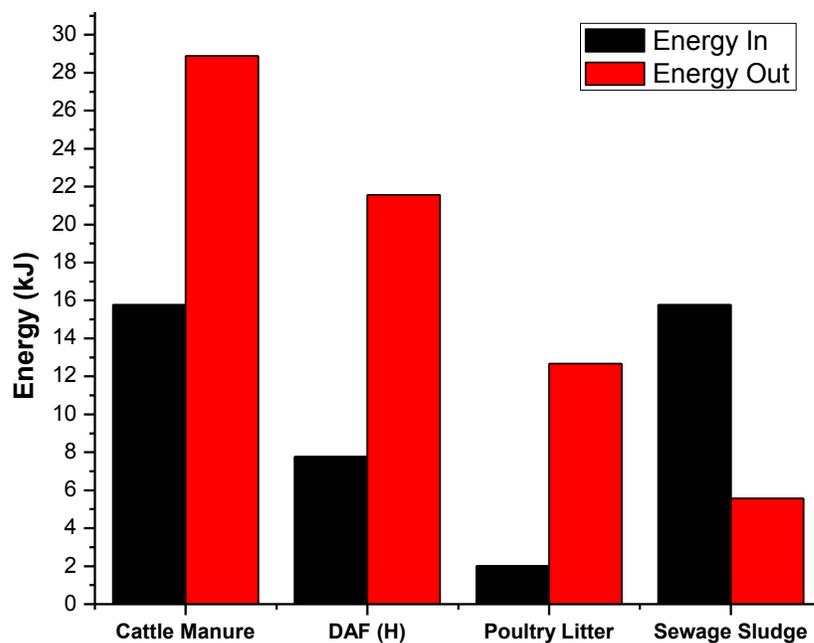
FIGURE 4.17: Energy in and out for the pretreated BMPs

TABLE 4.28: A summary of results for BMPs

	Initial pH	Final pH	Cum. CH_4 production (mL)	Cum. CH_4 yield (mL/gVS)	VS loading (gVS/L)
Cattle Manure	8.4	7.4	1045±95	125±11	55.6
Cattle Manure, 70°C	8.5	7.5	2112±131	244±15	57.7
DAF (H)	7.0	7.2	-163±27	-18±2.9	60.6
DAF (H), 70°C	7.0	7.7	1576±204	170±22	61.7
Poultry Litter	8.3	7.7	1000±221	120±27	55.5
Poultry Litter, 70°C	8.6	7.7	926±209	119±27	51.7
Sewage Sludge	7.1	7.6	1733±275	193±31	59.7
Sewage Sludge, 70°C	7.2	7.6	408±161	46±18	59.7

n=2, ±s.d.

CHAPTER 5: DISCUSSION

5.1.   Literature Database

The database showed variability in an author's preference for data reporting, which is typical for any area of investigation. For AD in the U.S. such variability is likely exacerbated by the fact that AD has evolved from being largely in the agricultural domain for several decades and is only now moving to the wastewater domain. There is an interesting dilemma in AD research, and that is the fact that each entity must take the time to test the feedstocks it intends to use. They are unique from source to source and it is not safe to rely on literature values. Yet the literature is extremely important in that it must serve as a high quality guide to how the testing is done.

The database analyses revealed that many of the studies available even since 2000 lack the proper controls and practices to be reliable. Less than a third of the articles attended to the ratio of microbes to substrate, only about a fifth noted nutrient ratios, and less than 10% tracked both. Measurement of biogas without differentiating between CH_4 and CO_2 is not useful information, because in the absence of methanogenesis copious amounts of CO_2 can be generated. Cumulative gas measurements unadjusted for controls can be misleading. Our results showed that controls generate CH_4, and this production should not be mis-attributed to feedstocks. Finally, yields should not be cross-compared without attention to the number of days of incubation over which the yield was generated. In some cases, it is implied that the yield represents the CH_4 produced at the time the

cumulative production curve plateaus, but the reader cannot assume this unless it is explicitly stated.

5.2.   Sample Characterization

Sample characteristic assessments confirmed that grab samples can vary widely from one collection event to another, depending on the source of the materials and how they are stored. This was anticipated well for the feedstocks most studied for codigestion: FW and BG. Samples sourced from kitchens with different cooking styles (e.g. high meat, high grease, and high fiber) or from different parts of a country or even different countries will not likely be comparable across experiments. The use of a replicable and representative FW feedstock reduced the variability of that feedstock from trial-to-trial. Likewise, the BG sample was from a large composited sample and was carefully managed, unlike samples that are used and reported in similar codigestion studies that are often single grab samples from a non-representative source.

For the more novel feedstocks, some of the experiments suffered from a lack of uniformity when multiple sampling events were necessary. For example, seasonal climate effects may have changed the nature of the PL collected for different experiments and also caused the nature of poultry bedding to deviate from expected carbon and nitrogen values predicted by the literature. A study monitored denitrification and ammonia volatilization relative to moisture content in PL over 13 d (Carballa et al. 2009). These researchers determined that the PL experienced higher losses of ammonia and nitrous oxide with increased water content. The water content of our two PL samples varied, which could alter the total nitrogen. The DAF material was originally collected as a single grab sample and reserved for use from trial-to-trial, but then additional source sites

were added. The multiple sites provided DAF samples, which did not yield similar results during testing.

5.3. Batch Codigestion Experiments

The batch studies provided a screening tool for the feedstocks. Despite being among the easier testing options, each set requires 2–3 months of data collection, and as Experiment 1–3 results demonstrated, there are many challenges to obtaining useful results. The long incubation period is required because the bioconversion of organic material to CH_4 in batch tests is typically conducted with unacclimated biomass, so that results can be considered "worst case" performance. Without seed acclimation, there is typically a long lag period before CH_4 production begins in part because cells will make some requisite enzymes for metabolizing the new food sources available (Chen et al. 2008). Figure 4.4 shows a typical cumulative CH_4 production curve for Experiment 2 Trial 2. Little activity occurred over the first 20 d of incubation. Lag times may also occur if a substrate creates early toxicity that is later overcome. Bujoczek et al. (2000) suggest this may be what occurs when the uric acid in PL is initially degraded to NH_3. If the NH_3 levels can be tolerated, once the uric acid metabolism is complete, methanogen activity may resume. Others have shown evidence of methanogen acclimation to NH_3 levels that were initially inhibitory (Abouelenien et al. 2009).

Experiments 1 and 2 showed that codigestion at high solids and OLRs was feasible with the right blends of PL, FW, DAF, and BG. The failure of mixes containing FW at levels of 25% and 50% (with the balance PL) in Experiment 1 was coupled with low final pH levels. Low pH is associated with a methanogen inhibition by the NH_3 introduced from the protein in FW and from the uric acid in PL. When methanogen

activity slows, VFAs accumulate, and it may or may not be possible for the process to recover (Chen et al., 2008; Bouallagui et al. 2005; Appels et al. 2008). In Experiment 2 when 25% FW was again tested with PL but in the presence of DAF or BG, low pH and no CH_4 production was observed; but when the FW level was lowered to 15% and blended with PL and DAF or BG, pH levels did not decline, and CH_4 production proceeded. This suggests that the presence of DAF or BG alone is not sufficient for success, but that FW levels also must be kept below a threshold level in combination with these materials. Together, these materials in batch screening yielded CH_4 equivalent to about 1.5 L/lb dry feedstock.

Glycerin and CS were judged to be the main source of failure in Experiment 3 trials. This conclusion was based on the high levels used and was supported by subsequent test results when the feedstocks were assayed separately. Others had reported success with GLY. Sell (2002) conducted batch experiments with crude GLY from a soybean and animal lard biodiesel-manufacturing facility. A range of concentrations (0.0175–1.225% by volume) was codigested with CM, and the bottles were supplemented with nutrients and alkalinity. The inoculum was digestate from a laboratory CSTR fed dog food and a nutrient medium. The tests were conducted at an F:M of 2 with an OLR of 5 g VS/L and the incubation lasted 30 d. For the bottles with less than 0.28% GLY, there was no significant difference from the controls (mean blank CH_4 production removed); however, at ≥0.28%, CH_4 production tripled relative to controls (28–52 mL $CH_4/mL_{substrate}$). Others tested GLY at fairly high levels. Amon et al. (2006b) codigested pig manure, corn and corn silage, and GLY in batch reactors. Without GLY, the codigested mixture (31% maize silage, 15% maize corn, and 54% pig manure) produced

335 mL CH_4/gVS after 42 d. They determined that GLY addition (3–6%) increased CH_4 production 9–31% (365–439mL CH_4/gVS with mean blank subtracted). The highest CH_4 production had 6% GLY addition. When they digested pig manure alone with 6% GLY, CH_4 production increased from 216 to 617 mL CH_4/gVS. Robra et al. (2010) digested CM with GLY at 5, 10, and 15% (wt) in semi-continuous reactors with an HRT of 23–25 d and an OLR of 3 gVS/L (0.3% VS/L). They saw increased biogas yields of 9.5% and 14.3% when GLY was added in ratios of 5 and 10%, respectively to CM.

However, there were reports that GLY could be problematic. Fountoulakis et al. (2010) codigested sewage sludge and GLY in semi-continuous reactors with an HRT of 24 d. They found that the GLY was quickly converted to propionic acid, but that propionic acid was metabolized to acetic acid much more slowly. When GLY was added at a level of 1% or below, CH_4 production was enhanced beyond theoretical values, but at levels above GLY 1%, pH levels dropped, and the system became unstable. They hypothesized that this was because the biomass was growing on the additional propionic acid produced from the GLY. This phenomenon may have occurred at the high GLY levels in our trials, where final pH levels ranged from 4.4–5.1, the lowest of all trials. Recently, Athanasoulia et al. (2014) digested GLY at 2–4% (v/v) with WAS in a two stage CSTR. For the 4% mixture (1g COD/L), some CH_4 production occurred early; however, this system never reached steady state. The authors attributed the early CH_4 production to the ready biodegradability of GLY and its failure to the higher organic loading causing a buildup of intermediate VFAs that lowered the pH.

There were no studies of CS in codigestion, although short-term digestion of dilute sunflower seed hulls in distilled water showed that oil seed hulls yielded CH_4 and

might be suitable AD feedstocks (De la Rubia et al. 2011). Finally, it is possible the carbohydrate-rich compostable tableware or paper may have had negative impacts, as pH decline is a possible inhibitory effect of cellulosic-based materials (Steffen et al. 1998). As was discussed in Sections 4.3 and 4.4, subsequent individual tests of GLY, CS, and paper provided some evidence that it was the GLY and perhaps the CS substrates that were the likely cause of failure in Experiment 3.

Although Experiments 1–3 were conducted at solids levels (10% TS) that were typical of many wet digesters in Europe (10–15%) (Vandevivere et al. 2003), it is difficult to find many high-solids laboratory studies for comparison. Callaghan et al. (1999) performed trials at 10% TS levels and mixed 20% (w/w) chicken manure (where an effort was made to remove the litter components); FW (random scraps collected by vegetarian students for the study); or DAF in codigestion trials with 70% (w/w) cattle slurry. Their inoculum (10% w/w) was not identified, and their controls were inoculum with 90% cattle slurry and therefore highly active. All of the additions (FW, DAF or chicken manure) increased the cumulative CH_4 production over 126 d of incubation relative to cattle slurry-only controls. Specific CH_4 yields were only provided for the poultry mix and DAF mixes, and they were compared to two different controls that yielded 0.15 and 0.3 m^3 CH_4/kg $VS_{removed}$. The two chicken manure trials ranged from 0.12–0.16 m^3 CH_4/kg $VS_{removed}$, suggesting they performed about as well as the least robust control. The DAF trial had a yield of 0.27 m^3 CH_4/kg $VS_{removed}$, suggesting it performed about as well as the best control.

Misi and Forster (2001) codigested 15% FW (from Zimbabwe markets) with 15% CM and 70% chicken manure (with bedding removed by hand) in duplicate batch trials.

Thickened digested SS was used as inoculant, and $NaHCO_3$ was used for pH control. After a 32 d incubation, the CH_4 yield was 240 ± 14.1 mL CH_4/gVS, which compares well with Experiment 2 Trial 2 (60PL:15FW:25BG) that yielded 220 ± 13.2 mL CH_4/gVS and with Experiment 2 Trial 4 (60PL:15FW:25DAF) that yielded 185 ± 5.9 mL CH_4/gVS. Experiments to test high solids digestion of FW with yard waste showed similar trends with increasing FW levels (Brown & Li 2013). Digestion at 20% TS were conducted with FW comprising 10% or 20% (by dry VS) of the feedstock mix (the balance was yard waste), with trials conducted at an F:M of 1 and C:N of 17–19. Initial and final pH levels of digestions fell with increasing FW percentages (0–20%), but were never below 7.2.

The stimulatory effects of BG have been documented in numerous studies, and their codigestion with wastewater solids has been well-reviewed by Long et al. (2012). These authors point out that while the effects of FOG addition are uniformly positive, the degree of stimulation varies widely depending on the many variables manipulated by the experimenter. In laboratory studies, its description is not always entirely indicative of its character. In some cases, dewatered solids (32.6% TS) (Kabouris et al. 2008b) or solids partially dewatered via polymer addition (42.4% TS) have been used (Kabouris et al. 2008a). In other instances where sludge was digested, in one case it was 25.4% TS (Luostarinen et al. 2009) and in another 17% TS that the authors note was thickened for experiments (Davidsson et al. 2008).

Kabouris et al. (2008b) used batch studies to codigest BG with SS at levels ranging from 10–41% (w/w as VS). For the 25% BG trial, the CH_4 yield after 44 d was 271 mL/g VS (after subtracting the performance of their no-feedstock blank). This value compares favorably with the 220 mL CH_4/g VS yield observed in Trial 2 of Experiment

2. Long et al. (2012) question the plausibility of the high yield Kabouris et al. (2008a) observed from FOG alone, because the remainder of their digestion mix was sewage. They point out that it is near or beyond the yield theoretically possible from the feedstocks tested. Of course, in our blend, PL and FW were present to contribute organic material to CH_4 production. Luostarinen (2009) tested BG at levels ranging from 10–70% in SS using digested SS inoculum, with some additional dilution with distilled water. No nutrients were added, but pH was modified to 7 with an acid or base. Their tests were conducted for 60 d at an F:M ratio of 1 and an OLR of 3 g VS/L (our BMPs were conducted at 10% TS; about 36 g VS/400 mL).

5.4.   Biochemical Methane Potential Tests

5.4.1. Food Waste

FW is easily biodegradable but quickly consumes alkalinity (Lin et al. 2009, Lisboa & Lansing 2013). The standard BMP protocol includes provisions for alkalinity supplementation, and it is presumed that the absence of CH_4 production and the low final pH levels in our FW BMP tests signified that there was insufficient alkalinity provided rather than an absence of activity in the FW samples tested. In other reports of FW BMPs, the solids loadings are substantially lower than levels used here because of different testing goals. The high variability of food mixes also requires that the protein and fat composition of the FW tested be scrutinized among various literature reports. Chen et al. (2010) tested commercial kitchen FW very similar to the mix simulated here at a loading rate of 1.5 g VS/L (0.16% TS) in tap water using digested SS inoculum and an F:M of 1. After 28 d, the CH_4 yield was 750±30 mL/g VS. In an alternate trial at F:M of 0.5, 600±40 mL CH_4/g VS was produced. Evans et al. (2010) used 15 FW samples

collected over five days from an institutional dining hall for BMP analyses. They tested the samples without nutrient addition and incubated them for 39 d. Samples were dosed by COD rather than VS. A sample concentration of 3 g COD of food/L yielded 190–570 mL of CH_4/g COD. The authors reported that the FW had a COD of 1400 mg/L and TS of 33 g/L (that was 85% VS). Translating the 3 g of COD to VS (~1.82 g VS/L) and converting the yield units to mL/g VS, the yield becomes 316–950 mL CH_4/gVS.

Zhang et al. (2013) tested FW from an institutional dining hall that was loaded at a rate of 8g VS/L (~0.87% TS) with acclimated inoculum and incubated about 27 d. No information is given about the proportional amounts of substrate and inoculum used. The authors report that 410 mL CH_4/gVS were produced, although no information is provided about controls for the inoculant alone. Li et al. (2009) used a replicable synthetic kitchen waste at a loading of 6 g VS/190 mL (3.42% TS) with digested sludge inoculum to give an F:M of 1.03. The samples were incubated 32 d and yielded 308 mL CH_4/gVS.

Moody et al. (2011a) tested food scrap and potato peel waste in BMP tests. They loaded their reaction bottles with substrate COD loadings that aimed to yield a target CH_4 volume of 100–150 mL. The equivalent VS of this substrate loading was calculated and matched with an accompanying amount of inoculum (measured as VS) from a laboratory digester (maintained with high protein dog food and nutrients) such that an F:M of 1 was achieved (in some cases an F:M of 2:1 was used). Nutrients and pH control amendments were added to the bottles. Their focus was not on a representative food sample but on testing foodstuff to compare it to a variety of other substrates. They found that their mix produced 241–289 mL CH_4/gVS. When Moody et al. (2011a) chose to standardize their loadings based on COD and gas production estimates, they precluded standardized TS or

VS loadings, so that direct comparisons with our experiments cannot be made. They report that they found COD to be an unreliable measurement of organics for solid and semi-solid substrates.

5.4.2. Poultry Litter

Poultry litter consists of poultry manure and bedding, and the nature and amount of each constituent will vary by farm and geographical source. The manure will be high in microbe populations, and the feathers will contain high lignocellulose content. Both of these will influence the rate and degree to which poultry waste feedstock will be digested (Singh et al. 2010). Many studies of poultry waste focus on the manure fraction, and it can be a dry feedstock or a wetted one (with urine and rainwater), depending on the way that the wastes are collected and stored at different farming operations. Costa et al. (2012) performed PL BMPs at an OLR of 6.6gVS/L but varied the %TS and F:M ratios. For raw PL at 5% TS and an F:M of 0.14, they observed a yield of 19 ± 3 mL CH_4/gVS after 80 d and ammonia levels of 1.28 ± 0.54 g/L, which were high enough to be inhibitory. When the solids were reduced to 1% TS and the F:M to 0.72, the yield was 145 mL CH_4/gVS, and the NH_3 levels were lower (0.25 ± 0.03 g/L), suggesting less NH_3 toxicity was occurring. Our PL BMP yielded more CH_4 production (120 ± 27 mL CH_4/gVS) over a shorter time period at a higher OLR (55.5 gVS/L) and %TS. Moody et al. (2011a) observed 245 mL CH_4/gVS for PL digestion.

5.4.3. Dissolved Air Flotation Sludge

DAF sludge varies depending on the wastewater feeding a particular DAF unit, but such operations are frequently employed for high protein wastes from meat processing operations. DAF sludge from a meat processing plant was tested by Woon and

Othman (2012) at a range of COD loadings, that when converted to VS loadings, ranged from 0.38–1.6 gVS/gVS inoculant (digested sewage sludge). For the loading that corresponded to a F:M of 1 (as used in our trials) the %TS was about 2.7, and they reported a yield of 550 mL biogas/gVS. The biogas measured an average of 71±5.4% CH_4, which translates to a yield of about 390 mL CH_4/gVS. Although controls and blanks were used in their experiments, it is not clear that they were used to normalize their yield calculations.

Luste et al. (2009) evaluated DAF from a slaughterhouse at a 0.3 gVS/L (0.03% TS) loading rate (F:M of 1) using digested SS seed. Adding 2M NaOH or 6M HCL adjusted the pH to 7.0, and sodium bicarbonate (3g/L) was added as buffer. After 70 d incubation, DAF samples produced 340 mL CH_4/gVS after the data was adjusted for the performance of seed-only controls. As with the FW trials, the DAF BMP results from other laboratories suggest robust activity while our BMP data show failed reactions. However, the levels of solids in our studies and in those cited for comparison are not the same. The low final pH values in our trials (<6) were a likely reason for the results we obtained, and they suggest that the alkalinity supplementation used in our BMP tests was insufficient for the high solids levels we tested.

5.4.4. Glycerin

The GLY BMPs did not show evidence of CH_4 production, but the final pH did not suggest that acidification was the cause (final pH of 6.5). Because this crude GLY is a by-product of biodiesel production, it is not pure. It may have contained materials that were toxic to the microbes as evidenced by a negative yield value, which suggests inhibition. If propionic acid accumulation was occurring, as suggested by Fountoulakis et

al. (2010), the inhibition might be less profound. Two other studies that were discussed in Section 5.3, Sell and Amon et al., also digested GLY. Sell (2011) conducted BMPs of GLY at (15.3gVS/L or 1.5%). After 30 d, GLY produced 54.1 ± 20 mLCH$_4$/gVS (23.6 ± 8.8 mL CH$_4$/g$_{substrate}$). However, after 42 d, Amon et al. (2006b) had CH$_4$ production of 750 mL CH$_4$/gVS.

5.4.5. Canola Seed

The CS samples showed no CH$_4$ production but also no evidence of acidification (final pH=7.2). The large negative yield value suggests that even baseline levels of CH$_4$ generation by the inoculum could not occur, and that some kind of inhibition was occurring. The closest reports to CS digestion were a pair of similar studies by De la Rubia et al. (2011) and Fernández-Cegrí et al. (2012). The former conducted BMPs on various size fractions of ground sunflower seeds, and the latter studied ultrasonic pretreatment effects but their protocols were the same. That is, they conducted testing at an OLR of 7.5 g/L (which translated to 0.7% TS and is 100-fold more dilute than our trials) and an F:M of 0.5. A nutrient supplement was provided, and samples were incubated for 7 d. De la Rubia et al. (2011) reported CH$_4$ yields that ranged from 182–213 mL CH$_4$/g VS, with the highest yield occurring in trials with the largest particle size. This size had the most soluble organics and the most protein. They reported stable final pH levels but noted that there was slower propionic acid removal in the smaller particle size fractions that were associated with lower CH$_4$ production. This may or may not be similar to the GLY inhibition phenomenon noted by Fountoulakis et al. (2010). Fernandez-Cegri et al. (2012) reported that their unsonicated controls produced 107mL CH$_4$/gVS.

A comparison of sunflower seed hulls and CS suggests that the two may not be similar enough to expect them to be similar in digestion. Table 5.1 compares hulls from sunflower and rapeseed that were pressed using conventional methods that included seed flaking and solvent extraction (methods not used by Catawba County). However the results provide a general comparison of the hull characteristics, including the fact that rapeseed hulls tend to hold more of the oil than do sunflower hulls after oil extraction. They also have much higher protein content than the sunflower hulls, which can generate NH_3 inhibition.

TABLE 5.1: Comparison of sunflower and rapeseed hull characteristics

Characteristic (% dry matter)	Sunflower Hulls	Rapeseed Hulls
Oil	2.5	12.0
Proteins (as Kjeldahl N)	6.2	15.2
Crude fiber	57.6	32.3
Ash	3.2	6.6
Neutral detergent fiber	83.9	50.7
Acid detergent fiber	64.9	41.8
Acid detergent lignin	22.3	23.1

Carre, Patrick. 2014. Personal Communication. Mr. Carre (Dipl. Ing. (ENTAB)) directs the French CREOL (Centre de Recherche et d'Experimentation sure Oleagineux and Proteagineux) pilot plant and is an authority on oil extraction techniques. He authored a paper for a work package on crop processing that fed into the work of a 22-member research team participating in the Sustoil Research Project to develop sustainable advanced biorefinery concepts (2008-2010). The data cited here was reported in that paper.

5.4.6. Paper

Pommier et al. (2010) investigated paper (e.g. magazines, newspapers, office paper, etc.) and cardboard mixtures sampled from a landfill. They created model substrates (100 x100 mm^2, 20 x20 mm^2, and thinly shredded paper <1 mm^2) as well as a landfill substrate that was 10x10 mm^2 and thinly shredded paper (<1 mm^2). The substrates (6 g_{ww}) were digested in 50 mL phosphate buffer solution and municipal SS

(4gVS/L). After 80–100 d of digestion, they reported a range of 160–188 mL CH_4/gVS (OLR of 5.7 gVS/L). Our paper showed a similar level of CH_4 production (150±33 mL/gVS by Day 66) but at a higher OLR (64.8gVS/L).

5.4.7. Brown Grease

Luostarinen et al. (2009) tested BG and SS individually using digested SS inoculum with additional dilution using distilled water. No nutrients were added, but pH was modified to 7 with an acid or base. Their tests were conducted for 60 d at an F:M ratio of 1 but at a much lower OLR (3 gVS/L, while our tests had about 58.3 gVS/L). The SS they tested yielded 263 mL CH_4/gVS and the BG yielded 918 mL CH_4/gVS. It is not clear whether or not these values were adjusted for contributions from the inoculum, although inoculum-only controls were run. Both of these yields are higher than ours, which were and 193 and 371 mL CH_4/gVS for SS and BG, respectively. When Evans et al. (2010) tested a grab sample of BG from the oil-grease separator of a grease trap in a BMP without nutrient addition incubated for 39 d. They loaded the sample by COD rather than VS. A sample concentration of 3 g COD of BG/L yielded 700 mL of CH_4/g COD (no statistics are provided, so it is not clear how much variability was present among replicates in these trials). These authors reported that BG had a COD of 1500 mg/L and TS of 68 g/L (that was 99% VS). Translating the 3 g of COD to VS and converting the yield units to mL/g VS, the yield becomes 1050 mL/gVS. This is three-fold higher than the level reported here, and the high value may stem from the rough conversion of COD to VS.

5.4.8. Cattle Manure

Moody et al. (2011a) tested a variety of beef and dairy CMs submitted to them, with much of the focus of their work on the methodology more than the features of their samples. They were able to offer little information about the characteristics of the samples they tested. Methane production from their beef manure samples after 30–40 d ranged from 84–264 mL CH_4/gVS, and within their trials, there was high variability. Our CM sample results (125±11 mL CH_4/gVS) fall within the range of their testing. Zhang et al. (2013) digested CM (C:N of 5.2) with acclimated digested sludge inoculum (fed FW and CM for 14 months). The OLR was 4 gVS/L, and samples were incubated for 28 d. They reported CH_4 production at 6 mL CH_4/gVS. Our BMP was conducted at a higher organic loading and yielded more CH_4 by Day 27 (23 gVS/L and 64±12.6 mL CH_4/gVS).

5.4.9. Leaf Waste

Liu et al. (2009b) reviewed the effects of green waste (grass clippings collected from the campus of University of California, Davis) digested in anaerobic sludge. After 25 d, reactors that received 12.5 gVS/L and operated at an F:M of 3.1 yielded 206 mL CH_4/gVS after being adjusted for controls. The leaf waste in our experiments was performing at about the same level as the blanks on Day 25 of the 66 d incubation. This may have been due to the higher OLR and to the higher lignin content of leaves relative to more readily degradable grass that Liu et al. (2009b) tested. Our BMP trials did exceed control level yields by Day 66, but among the three sources of leaf waste tested, there was high variability, and the yield levels (-12 to 42 mL CH_4/gVS) were not as high as those reported by Liu et al. (2009b).

5.4.10. Sewage Sludge

Sewage sludge is a well-documented feedstock. It is often used in codigestion due to its higher buffering capacity (Velmurugan et al. 2010). Kim et al. (2003a) digested SS with FW to determine how much FW increased CH_4 production with SS digested as a control. At an OLR of 2 gVS/L (C:N of 7.2), SS alone produced 116 mL CH_4/gVS after 16 d. Another study, noticed 278 mL CH_4/gVS after 50 d (ORL was 1.56–2 gVS/L) (Luostarinen et al. 2009). Davidsson et al. (2008) obtained higher CH_4 production after 37 d (325 mL CH_4/gVS at OLR of 3.8 gVS/L). The CH_4 production from the inoculum was withdrawn from this total. Our study observed 118±18 mL CH_4/gVS after 39 d (OLR of 59.7 gVS/L). Our CH_4 production was significantly lower than the sample at an OLR of 3.8 gVS/L; however, ours had a significantly higher OLR.

5.4.11. Feed Meal and Hatchery Waste

No comparable BMP studies were identified in the literature that was similar to the tests performed on FM and HW.

5.5.   Anaerobic Toxicity Assay

While ATA testing is not a new concept (Owen et al. 1979), it has not been widely utilized in anaerobic digestion research. There are limited known studies with which the ATA results presented here can be compared. The poultry and cow manures were the only substrates to show stimulatory behavior, which may be coincident with the fact that they were also the only substrates to bring additional active and acclimated biomass to the reaction. Callaghan et al. (1999) used CM as inoculant in codigestion studies. For the CM, all ATA inclusion levels tested were beneficial, with no statistically significant differences among the inclusion levels (p>0.05). For the PL, all but the two highest levels stimulated CH_4 production. Performance by the 10% inclusion rate was

statistically different from the others (p=0.003). The 4% inclusion level graph shows the telltale shape of initial NH_3 inhibition that is later overcome, such that in Days 1–2 performance is below that of the control but rises above it in Days 4–5.

The HW had positive effects at very low levels. The latter phenomenon may have been due to the organic material introduced by the slurry of material contained in the broken eggs mix, which at higher levels becomes inhibitory because of its high proteinaceous (i.e. NH_3) load. All of the other substrates showed inhibition at all inclusion levels tested despite evidence from the batch experiments that some of them, such as BG and DAF, enhanced CH_4 yields when codigested with other substrates. Moody et al. (2011b) refers to this phenomenon as "masking," when a substrate has some toxicity effects that are hidden in codigestion but revealed in ATA tests. Such masking may occur due to dilution or because alkalinity is provided by other substrates and can prevent rapid pH decline.

Some of the toxicity effects in the ATAs appeared to be quite linear, as was the case with BG, CS, DAF, PL, and PAP; but others appeared to have threshold values beyond which the toxicity effect was much more pronounced. These included FW, GLY, HW, and LW. Presumably some level of compound could not be neutralized or metabolized at a rate sufficient for any activity to occur. With LW and HW and even FW toxic effects were minimal at the lowest inclusion levels of feedstock, but with GLY, no inclusion level was non-inhibitory, and every level except the lowest level stopped all microbial activity completely. Only the highest levels of CS approached the levels of complete metabolic shutdown seen in the GLY ATA tests. The paper showed a reverse trend, with the lowest level added producing notable inhibition while the highest level

added tracked closely with the control. It may be that the higher solids present at higher loadings offered adsorption sites for microbes or for toxic compounds to sequester them and permit more reactor stability.

No diagnostics were performed to determine the source of inhibition that was revealed in the ATA tests, but final pH values provide some information about failure factors, because it is well known that methanogens are very sensitive to pH (Gerardi 2003), and have an optimum pH of about 6.6–7.0, (Monnet 2003, Appels et al. 2008). It is likely that in the ATA tests, which unlike the BMP tests received no alkalinity supplement, acidification would be even more severe for these substrates. Final pH values show that all FW samples >1% inclusion had pH levels below 6, as did all leaf waste samples >2% inclusion. These pH effects did not appear in the BMP tests, which was likely due to alkalinity supplementation. The CS hull sample pH levels fell to 5.98 in the 2% inclusion rate sample, but unlike FW and leaf waste, where pH continued to decrease with increasing substrate concentration, the pH remained at this level for all subsequent concentrations.

Sell (2011) reported an ATA for crude GLY in an inclusion range of 0.5–35% (v/v) that also showed strong inhibition; they attributed it to rapid acidification but did not report final pH values. Our final pH values after ATA testing for inclusion levels of 1–10% does not support the acidification hypothesis, although our 1% level was an anomaly, showing a low pH level (5.9). A build-up of propionic acid has been implicated in both GLY and rapeseed toxicity, but propionic acid has not been identified as a toxin of such potent capacity but rather one which slows methanogenesis (Hobson & Shaw 1976). High propionic acid has been shown to retard acetic acid degradation thereby

decreasing CH_4 production (Mawson et al. 1991). Because the GLY tested was not pure but the by-product of biodiesel production, it contained methanol and other contaminants and will need to be tested distilled and undistilled to further analyze sources of the toxicity.

The source of CS toxicity was not readily evident, as it is used as an animal feed supplement. They did not show the same propensity for acidification in the BMP test, where alkalinity was provided, but they did show the same failure to yield any CH_4. There has been some investigation into natural tannins in CS hull cakes (Naczk et al. 1994, Naczk et al. 2000) and other trace constituents (Zeb 1998) because they can interfere with animal digestion when this by-product is used for animal feed. Presumably if these compounds can interfere at the enzymatic level of biochemical processes, they might likewise be inhibitory for the microbes in the ATA test. On the other hand, CS is also known to be quite high in oil content due to the difficulty of capturing the oil efficiently from the small seeds. Canola along with BG and DAF were sources of long chained fatty acids (LCFAs) in the ATA tests, which have received significant research attention because they are associated with inhibition, floatation, and washouts in digesters (Chen et al. 2008).

LCFAs can be inhibitory to methanogens (Angelidaki and Ahring 1992). The inhibition is related to their propensity to associate with the solids fraction and coat the cells. The phenomenon was once thought to be irreversible but is now believed to be a function of a variety of factors that can be overcome operationally (Alves et al. 2009; Hwu and Lettinga 1997; Pereira et al. 2003, Shin et al. 2003; Pereira et al. 2004; Pereira 2005). Presumably the incoming LCFAs enmesh with the biomass and coat the cells. If

the solids are captured and permitted to digest the adsorbed LCFAs in batch while no new LCFAs are added, they will do so, and CH_4 will be generated (Alves et al. 2009). To produce CH_4, they must be metabolized by the H_2-producing acetogenic bacteria (Bryant 1979), which convert them to acetic acid and H^+. The methanogens then use acetic acid, formic acid, and hydrogen to make CH_4. The batch ATA incubation protocol actually mimics many of the early batch studies that generated the early erroneous notion that LCFAs were irreversibly inhibitory, although it would seem that an adsorption-metabolism sequence would have been possible in these incubations, because the substrate was added only once and not repeatedly. Further diagnostics would be needed about these complex wastes to discern the cause of their inhibition.

In addition to lipid content, (Steffen et al. 1998) lists high protein content as a trigger for instability in digesters. Both protein and the urea in the manures are sources of free NH_3, and its impact on digester stability has been well reviewed (Chen et al. 2008, Yenigün & Demirel 2013). Its effects vary with substrate, inoculum source, environmental conditions, and acclimation period. Free NH_3 may have contributed to the inhibition observed in FW, HW, PL, and CS samples, which was due to their protein or urea content. The range of NH_3 levels that are toxic, vary. The concentration at which a 50% reduction in CH_4 production activity occurs has been estimated to be anywhere from 1.7 to 14 g/L (Jarrell et al. 1987, Koster & Lettinga 1988, Wittmann C 1995, Steffen et al. 1998, Sung S 2003, Speece 2008, Buendia et al. 2009, Singh et al. 2010, Procházka et al. 2012). Because the ATA test does not allow for acclimation, NH_3 inhibition effects would be unmuted in FW, HW, PL, and CS and may account for some of the inhibition caused by these substrates. On the other hand, the lack of inhibition by CM and lower levels of

PL may have been due in part to the fact that these feedstocks were accompanied by acclimated microbes that entered with the manures and supplemented the inoculum (SS digestate). Additionally, PL tends to have higher ammonia levels than CM (Singh et al. 2010), which also may explain why higher levels of PL became inhibitory, while higher levels of CM did not.

A comparison of the ATA trials, where no alkalinity is introduced, and the BMP trials, where some alkalinity is provided, can be used to reflect the intensity of alkalinity consumption. In the ATA trials, the final pH levels of CS, BG, and leaf waste samples all fell well into the acid range. The final pH levels of these samples in the BMP trials all remained above 6. In contrast, in the ATA trials (without alkalinity) FW fell to below pH 6 quickly (at the 2% inclusion rate) and to a low of 4.9 at the 7% rate. In the FW BMP test, even with buffering present, the final pH was 5.8. In a different pattern, the ATA of one of the DAF samples (DAF-W) had a final pH that barely fell below 6 at the highest inclusion level (7%) but consumed enough alkalinity in the BMP test to yield a final pH of 5.3.

5.6. Semi-continuous Reactors

5.6.1. Pilot Trials

The variability observed day-to-day in these trials was likely due to the multiple measurements and data processing required, generating each data point. Slight variations in individual gas volume measurements along with similar variations in gas chromatography readings magnified fluctuation effects, as these values are multiplied to calculate CH_4 yield.

The high average CH_4 yield of Mix 2 was likely due to a combination of favorable operating factors. First, it had a lower organic loading so that foaming was less likely to occur. Second, the SRT of this mix was long, which undoubtedly allowed for good contact time between the microbes and their substrate. The good performance of DAF and BG was consistent with the batch experiments that showed these feedstocks to be promising CH_4 producers. The fact that Mix 2 did not have the highest rate of VS reduction suggests that much of the VS in this mix remained undegraded. This is consistent with Experiment 2 results in the batch codigestion experiments. When either DAF or BG was codigested with PL and FW, the %VS reduction of the DAF sample was a lower fraction of its CH_4 yield than was this same ratio for the BG sample (0.27 vs 0.30, respectively).

The foam formation in Mix 3 is typical of high-lipid feedstocks. It is sometimes related to an overgrowth of foam-forming organisms that thrive on lipids, prefer low pH environments, and produce exocellular products that build the foam structure (Ganidi et al. 2009). Sometimes intervals where mixing is ceased, can reduce such foam formation, although that was not done here in order to avoid introducing additional variables. High lipid feedstocks have also been implicated in digester inhibition due to the LCFAs they contain, but many authors now suggest that the presence of LCFAs may delay but not inhibit CH_4 production (Luostarinen et al. 2009).

5.6.2. Experimental Trials

Mix 1 proved to be a reliable mix with balanced C:N that suffered from no foaming or viscosity problems. We suspect the volatility evident in its day-to-day performance was due to high gas production that caused fatigue on the gas-tight bottle

fittings that led to hairline leaks that required frequent attention. While Mix 2 showed great promise at low organic loading and high SRT (pilot trial conditions), it could not sustain such performance under more moderate conditions. Luostarinen et al. (2009) reported finding reasonable CH_4 production from grease trap wastes from a meat-processing plant codigested with SS when grease comprised as much as 46% of the feed VS (HRT of 16 d; OLR of 3.46 gVS/L-d), but at higher levels of grease, the reactor failed. They observed an approximate cumulative CH_4 production of 278–463 mL CH_4/gVS after 50 d. We had a SRT of 18 d with an OLR of 5 g VS/L-d, where yields fell from 165 to 15 mL CH_4/g VS-d when the HRT was shortened and the OLR increased. A similar phenomenon was reported by (Noutsopoulos et al. 2013), who saw good grease digestion with SS at an HRT of 15 d in mixes up to 60% grease but failure when the grease component was raised to 90%, which had the highest OLR. They observed 10–700 mL CH_4/gVS. The lowest CH_4 production from 90% grease (OLR of 8.3 gVS/L) and the highest from 60% grease (3.5 gVS/L).

In Mix 2, BG made up 35% of the mix, but it was accompanied by a second lipid-based feedstock, DAF. Beyond the low CH_4 yield, this mix suffered from extensive foaming that was not observed in the pilot trial. The longer SRT and lower OLR that was allowed Mix 2 in the pilot trial was not a practical one, as it would require a large reactor volume. But a comparison of the two trials suggests that there is likely a proportion of BG and DAF that could be used with other modulating feedstocks, to avoid foaming but keep CH_4 yields high. It may be possible to make small mix modification, include amendments for pH control, and change other operational parameters to avoid the foaming and viscosity problems observed here.

5.7.   Cell Counts

Surprisingly, CS contained live cells (0.018×10^{10} cells/gVS), which is 96% of the total amount of cells. The cells present in the CS feedstock could have been acquired during processing or as part of its natural degradation during storage and the nature of these bacteria are unknown. However, this feedstock inhibited CH_4 production in previous BMPs.

DAF (W) brought in a lot of bacteria into the BMP, which could have affected the amount of CH_4 production (22% increase in live biomass). This assumption can be made because when digested alone (nutrients and water only), DAF (W) started to produce CH_4 by day 8 while DAF (W) + seed had a lag time of 5 days. DAF (W) performed well in terms of CH_4 production. It was thought that the PL would have performed better because it added more bacteria to the BMPs (74% increase in live biomass). As noted previously, CH_4 production did not start until day 40. One possible explanation for DAF (W) outperforming PL (N) is that DAF (W) + seed had a higher percentage of live bacteria per gVS in the bottle. There were probably complex microbial interactions occurring within the PL (N) + seed bottle to cause a longer lag time.

It was noticed that all of the feedstocks, except paper (alone), outperformed PL (N) + seed. However, the other feedstocks were starting to level in CH_4 production but PL (N) + seed was starting to produce more than the blank. If the test had continued for a longer time period, its cumulative CH_4 production would have been higher. In addition, PL contains more lignocellulosic material, which is harder to digest. DAF (W) was more of a liquid with higher lipid content. These differences could have contributed to the differences in CH_4 production. The exception to this was paper + seed. Because the

paper napkins used for this study are 100% recycled paper, the fibers in the paper are easier to breakdown. The paper could have added additional surface area for the microbes allowing more growth. In addition, the initial BMP counts may not reflect the true amount of bacteria especially for paper. When collecting the samples, 5g of seed and paper was collected in a sterile tube and then diluted. In the case with paper, the paper itself and the water added contributed to this mass. This mass, paper and water, did not contain bacteria. When the samples were diluted, the bacteria where dispersed within the dilution water but it did not have the same mass as the blank would have. The mass collected from the blank would have primarily been biomass and a little water, which is a more accurate cell count.

Additionally, the final count of live bacteria was less than the initial except for paper + seed. Bacteria will exhibit growth and decay rate in batch reactors (Tchobanoglous et al. 2003). Because no additional food and bacteria are entering the system as with continuous flow reactors, the bacteria will reach a point when decay occurs, this could explain why there was more dead bacteria after 43 days. However, CH_4 production was still going strong with the paper + seed, suggesting that the bacteria have not reached its decay cycle. In addition, because PL (N) + seed had just started to increase in its CH_4 production rate, the amount of bacteria may have been increasing. The decay shown could be attributed to a long acclimation period.

The study of live and dead microorganism counts aid in understanding the biomass within a system. The term biomass is measured and comprised of several different components: total cell biomass, dead cell biomass, and active cell biomass (Vollertsen et al. 2001). The benefit of determining the live microorganism biomass or

cell count versus the total amount is that the active portion allows researchers to determine the amount of microorganisms conducting respiration. CTC measures the amount of microorganisms that could be degrading and acting upon the feedstocks. However, it does not indicate the type or classification of microorganisms. Further testing would be required to determine the types of microorganisms present. Nonetheless, by performing bottle experiments with the substrate only, we were able to determine that at least some of the microorganisms present in the substrates for DAF are capable of CH_4 production, indicating that they are relevant to AD and can affect the F:M ratio calculated. Traditional F:M calculations assume that the organic material from the feedstock is strictly food; however, based on the BMP studies presented here, the feedstocks do add additional, beneficial microorganisms, which could affect the F:M calculations. Further testing would be needed to determine the extent of this addition.

5.8.   Pretreatment

Not surprisingly, thermal and thermochemical pretreatment methods required the most energy input, because many of the materials required heating large masses of water. Certainly in a pilot or full-scale system certain economies of scale and innovations could be evaluated and exploited to improve on the energy requirements used in laboratory settings. Chemical and biological pretreatment tended to require the least energy input, because they were associated with mixing and pumping, some of which were already required in the absence of pretreatment. There could be additional energy requirements for biological pretreatment, if the feedstock were to require incubation at a higher temperature for optimum growth; this would be treatment specific. Mechanical pretreatment is highly dependent on the nature and design of the system employed, which

in turn is often customized for specific feedstock categories. As rising costs drive rising efficiencies in equipment design, net energy calculations will likely become more positive. There are also a variety of waste-heat capture regimes that can be employed in full scale facilities, so that the amount of heat needed for thermal pretreatment could be reduced dramatically, making systems feasible both economically and energetically. Also, a life cycle analysis approach could change these numbers dramatically. If the energy input for chemical production or their transport was factored into chemical pretreatment; or if the energy to construct biological processing facility and make the materials used in its construction were factored into biological pretreatment, the analytics might change.

For instance, as ultilized in this experimental design, concentrated substrates were heated, which decreased the required energy for the substrates to heat to 70˚C; therefore, PL still has a positive E despite lower CH_4 production. PL and DAF (H) had higher solids content, which decreased the amount of water that needed to be heated. The water was added to the pretreated feedstock to create a 10% TS after it had been thermally pretreated. DAF (H) went from no CH_4 production (raw) to 170 mL/gVS of CH_4 production. (The energy required to heat 10 g TS was 0.68 kJ (Simpson & TenWolde 1999).)

Based on previous literature studies, the pretreated SS should have produced more CH_4. Research has shown that the optimal digestion of pretreated WAS occurs at pretreatments of 160–180°C for 30–60 min (Speece 2008, Sheng et al. 2011). The pretreatment presented here was below this optimal range. Qiao et al. (2011) found that treated SS (170˚C for 1 h) CH_4 production outperformed untreated SS by 67.8% after 15

d (1 g VS of substrate added). In another study by the same researchers, SS was pretreated by a microwave at 120–170°C for 5 and 10 min prior to digestion, resulting in increased biogas production for all of the pretreatments (Qiao et al. 2010). However, when reviewing VS dissolution of pretreated SS (80–170˚C), they found that at 80˚C had the lowest dissolution, indicating that the CH_4 production of the 80˚C would be lower than the other pretreatment temperatures. As thermal pretreatment works by breaking open the cells, the temperature may not have been high enough to lyses cells thereby decreasing CH_4 production.

Qiao et al. (2011) also analyzed thermally pretreated CM. Biogas production increased from 182 to 238 mL/gVS; however, the percent CH_4 decreased, which resulted in a decrease in CH_4 production. The pretreated CM in this study showed a two-fold increase in CH_4 production. However, PL did not show any difference in CH_4 production between raw and pretreatment. This may be due to the high lignocellulosic material in PL. The temperatures and time used in this study may not have been long and/or high enough to breakdown the lignin.

CHAPTER 6: CONCLUSION

There is great potential for AD to become a more frequent option among the suite of U.S. renewable energy options in the next few decades. The technology is familiar and well proven in the wastewater sector, and the U.S. is beginning the transition away from landfilling and seeking solid waste diversion options. Of those options, AD is the most biomimetic, producing a useable energy-rich gas, compostable solids, and nutrient-rich liquid. Many proof-of-concept initiatives are ongoing in Europe and Asia, so that the building blocks are in place for rapid technology transfer and emergence of a critical mass of solid waste AD facilities. Of course, the feedstocks will be unique to U.S. sites and situations, and each will require feedstock evaluation. The trend is toward codigestion, so that the synergies of multiple feedstocks can be exploited.

Despite what many see as a rising trend toward AD expansion in the U.S., federal funding for solid waste digestion research has remained low. The result is fewer research publications on the topic. Research performed in industrial laboratories is proprietary and remains unpublished. For AD to flourish as economic and feedstock opportunities make it a feasible and attractive option, a better roadmap is needed. This body of work was developed to guide feedstock feasibility and codigestion studies that are conducted in anticipation of new facility design. It grew out of literature review and work performed for one such facility in Catawba County, North Carolina.

6.1.   Anaerobic Digestion Literature Evaluation

A study of the AD literature that would be available to those beginning feedstock analyses revealed some deficits in the way that AD data is collected and collated. It is a body of work that is not always methodologically consistent and in some cases, not entirely reliable. There is a tendency even in the most current literature to cite the same few studies repeatedly on certain topics because they are the only ones available. Some of the deficiencies include failure to include controls or use the controls to adjust yield values; lack of replicates or use of statistics when replicates are present. Comparisons that fail to attend to differences in solids loadings, incubation period, or weight or volume percentage loadings are not very compelling. Some of these problems are highlighted in the literature review, but more importantly, such differences between studies make it difficult for true comparisons to be made going forward.

There is a need for more consistency among protocols so that data can be readily compared and shared if rapid advances of new enterprises are to occur. Those researching AD in the U.S. through a solid waste lens must create a new space for themselves in the AD literature. They will have an engineering perspective and be informed by European and Asian practice, where solid waste digestion has been underway for some time. Thus, through the database, this work offers streamlined access to existing codigestion literature. The database can serve as a starting point in experimental design. A query can direct users to research on particular feedstocks, the elements of design used, and the nature of the results. The opportunity to filter current available cross-disciplinary information for high quality guidance should expedite and advance new research. Further, if it is hosted and expanded by an appropriate agency, it can continue to serve as

a clearinghouse and communication tool for U.S. solid waste AD researchers. Agencies such as SWANA (the Solid Waste Association of North America) or EREF (the Environmental Research and Education Fund), an organization dedicated to funding solid waste research are possible candidates.

The batch studies performed here are of a style that is one of the most expedient available. Yet they remain time and labor intensive, requiring multiple replicates to accommodate the high variability inherent in testing heterogeneous substrates that require multiple and repeated measures. Direct literature comparison of the experimental mixtures performed in Experiments 1–3 was difficult because these codigestion mixtures were novel. Additionally, some of the feedstocks such as DAF and HW from a poultry processing facility, CS, GLY, and compostable cups have not been previously digested. Also, the higher %TS and OLR utilized in our experiments are not typically studied. Yet, high solids codigestion must be accomplished if stand-alone solid waste AD is to be economically feasible in the US. Clearly, if there is a desire to increase and advance solid waste AD research in the U.S., future study should be conducted in ways that allow ready comparisons to be made. Batch studies at high solids (10%) rather than at levels suitable for WWTP digester supplementation (2–3%) are quite challenging.

6.2.   Guidelines for Anaerobic Digestion Feedstock Analyses

The research elements of this work were used as a basis to create guidelines for batch codigestion studies that will foster more opportunity for cross-comparisons between laboratories. Those guidelines recommend use of proper controls; sample replicates and statistical analysis; nutrient balance as well as inoculum to feedstock balance; and digitally measured gas flow converted to volume. If these elements of

testing are included and reported in experiments, results can be credibly compared between one laboratory and another. The studies reported here yielded results that were not always consistent with literature values that were often, because samples were tested at high solids levels, characteristic of levels that would be used in the full scale digester. Many reports in the literature are based on experimental conditions that will produce results but that do not represent realistic field conditions.

One manipulation we elected not to make in the batch trials was to add alkalinity. Many researchers add alkalinity supplement to digestion reactions, especially when unacclimated seed is employed. When new feedstock and inoculum are first mixed, there tends to be a latency period while some of the microbes adjust to a new substrate. If acid producers outcompete the other microbes during this period, acidification can occur, which poisons the reaction environment for methanogens. While we used step feeding over 4–5 d to introduced the substrates in small loading increments to the inoculum, we did not add alkalinity but sought reactions robust enough to weather initial instability and continue on to stable CH_4 production, reasoning that under acclimated conditions, mixes would be even more stable and tolerant to ratio adjustments during operation. As a result, threshold levels of FW and high levels of GLY led to acidification failure in the batch studies. Some digesters will be designed to operate with alkalinity supplementation, and some experimenters may elect to use alkalinity supplementation to estimate conditions under more stable digester conditions.

The BMP and ATA screening tools provide good information for comparing one feedstock to another, but they are not necessarily predictive of a feedstock's value in a codigestion mix. The ATA test in particular, by its nature is changing nutrient balance

and substrate to microorganism ratio simultaneously, so it is not surprising that many substrates appear to have ill effects at high concentrations. Results from substrates that bring microbes to the mix (and thereby improved the otherwise imbalanced F:M ratio) are particularly suspect unless the new microbe counts are included, as demonstrated by the studies here. The feedstocks that performed best in our tests were manure-based substrates that included their own acclimated seed. One interesting use of the BMP and ATA tests compared against each other was their ability to show the intensity of alkalinity consumption by substrates. While the buffered BMP tests might show a similar final pH for two different feedstocks, comparisons of their ATA results showed that one quickly acidified while the other acidification was much more gradual.

Some interesting directions for future investigation can be gleaned from digestions that failed due to reasons other than acidification. As was learned from Experiments 1–3 with FW, acidification can sometimes be remedied by adjusting proportions, and as others have shown, further proportion adjustments will likely be possible after microbial acclimation occurs (Zhang et al. 2013). Alkalinity supplementation is another solution for acidification if the costs of chemical feed are not prohibitive. But in cases such as the BMPs of HW, CS, and GLY, where final pH levels were within normal range and yet samples performed below the levels of the controls; another cause for inhibition was responsible.

A number of explanations were offered for each of these substrates; including toxic contaminants; toxic naturally-occurring components; high proteinaceous content; or biochemical breakdown pathways that favor propionic acid production. If the latter occurs, then delicately balanced syntropic reactions with H_2-utilizing bacteria must occur

for the propionate to be consumed. If methanogens do not take up H_2 and HCO_3 to make

CH_4, and the H_2 accumulates, its presence (and its increasing partial pressure) will inhibit

the degradation of propionate. The reason is that H_2 is a *product* of propionate

degradation; the presence of H_2 drives the degradation reaction in the wrong direction

(Fukuzaki et al. 1990).

$$CH_3\,CH_2COO^- + 3H_2O \rightarrow CH_3\,COO^- + HCO_3^- + H^+ + 3H_2 \qquad \text{Eq. 6.1}$$
$$\Delta G = 76.1\ kJ$$

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \qquad \Delta G = -135.6\ kJ \qquad \text{Eq. 6.2}$$

$$CH_3\,COO^- + H_2O \rightarrow CH_4 + HCO_3^- \qquad \Delta G = -31.0\ kJ \qquad \text{Eq. 6.3}$$
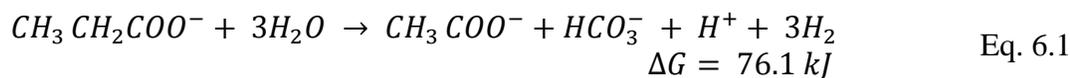
Both our batch experiments and semi-continous flow experiments demonstrated

that although BMP and ATA tests can aid in feedstock selection and in the diagnosis of

problems, they are only one element of decision making about feedstock mixes.

Feedstock availability and contractual arrangements and alternative uses for a feedstock

will also factor into the level of effort that needs to go into making a particular feedstock

useable. If a particularly problematic feedstock is considered worthy of persistent

investigation, then pursuit of the source of failure is justified. If not, but the conditions of

testing were rigorous, then the results may be useful to others with interests in the

feedstock.

The computer program, a third and important tool, used with the BMP and ATA

data for organizing the information about feedstock characteristics and creating balanced

mixes with respect to solids loading and C:N ratio. However, for the feedstocks available

to us, to achieve nutrient balance required creation of a skewed blend of feedstocks. In

one case a semi-continuous flow mix contained 35% HW and 30% FM, and in another

case a mix contained 25% PL, 35% DAF, and 35% BG. The performance of the high-

DAF high BG mix was only satisfactory under low loading conditions and a long SRT, because foaming and high viscosity impeded the digestion. The third trial run in this series was also a worthy one, in that it helps an entity evaluate an operational trade-off. That trade-off is between the revenue generated from CH_4 production by an optimized mix formula and the revenue needed to store materials for that mix rather than digesting a less-than-optimal mix in the proportions it arrives on-site.

6.3.   Feedstock Microbial Loadings

At the heart of the proposed guidelines is the aim to keep as many conditions constant as possible during testing, such as the solids loading, the organic loading, C:N ratio, and the F:M ratio. However, when several of our feedstocks were microbe-rich and used as inoculants themselves (e.g. cow manure), we questioned whether or not it was valid to assume that an F:M ratio based on VS measurements was reliable. Testing to measure live cell counts revealed that both poultry manure and DAF increased the total cells approximately 80% and 20%, respectively over those of the inoculant alone. The impact of these cells was not evaluated, however, and this will be the topic of future study. The results do not lead to the recommendation that live cell counts be conducted routinely, as they are not simple to perform, and the implications of the results are not yet clear. The results do suggest that trials conducted such that all samples are tested at the same F:M ratio may not actually be at similar ratios if certain feedstocks are contributing significant portions of microbes to the mix.

6.4.   Net Energy Benefits of Pretreatment

Feedstock pretreatment is employed for a variety of reasons, such as size reduction to ensure efficient mixing or heating to stabilize wastes that might carry

pathogens. Here we looked only at pretreatment used solely to enhance CH_4 production, because there are few reports of such analyses to date. Energy balances have been used to decide whether or not AD treatment or a particular AD design is feasible (Seppälä et al. 2008, Uellendahl et al. 2008, Pöschl et al. 2010, Yang et al. 2010) and for transport decisions related to AD (Uellendahl et al. 2008). There has been some investigation of thermal and mechanical pretreatment of FOG and some cellulosic materials (Menardo et al. 2012, Moisan 2013), and this work was greatly expanded here to assess whether or not various pretreatment modes were likely to produce a net energy yield.

Interestingly, although thermal and thermochemical options are the most common ones tested, they proved to be the least efficient options in our analysis. However, as pointed out here, on-site innovation, economies of scale, and more expansive life cycle analyses may change the outcomes of energy analyses. Direct comparison between laboratory and full scale systems is approximate at best, because laboratory devices tend to be less efficient (Pérez-Elvira et al. 2009), and equipment is usually different in laboratory and full-scale systems (Pérez-Elvira et al. 2009). Thermal pretreatment accomplished using waste heat from a combined heat and power system in the field will lower energy use predicted by laboratory tests.

In the pretreatment conducted in our laboratory, the concentrated substrates were pretreated prior to dilution to 10% TS. This reduced the amount of water that required heating, an energy intensive step in the pretreatment process. As AD facilities look towards thermal pretreatment as a means of increasing CH_4 production, finding alternative ways to heat the feedstocks and/or heating a more concentrated feedstock would aid in making the facility more economically feasible.

6.5. Major Contributions and Research Implications

In summary, the major contributions of this research are the following:

- A searchable database that expedites access to AD articles by feedstock(s); parameters reported (e.g. C:N ratio, F:M ratio); and statistical practices employed

- Guidelines for producing reproducible feedstock study assessments that can be compared from laboratory-to-laboratory

- Evidence of significant biomass contributions from feedstocks. These findings should begin assessments of the degree to which these microbial loadings skew F:M ratios (usually considered to be roughly constant) across trials

- Analyses of feedstock pretreatment data that suggest that energy inputs for pretreatment used solely to enhance CH_4 production should be carefully evaluated against net energy gains.

Some of implications of this research rest, in part, on the premise that the body of knowledge available to an emerging industry can impact the rate at which the industry grows and flourishes. The database and guidelines produced here, as well as the specific data reported should advance the rate at which others are able to move into this domain and begin testing with confidence and available resources. It is not clear whether or not the individual efforts at new AD facilities will be proprietary or publishable ones, but if they are the latter, new information may rapidly become available and accumulate to inform future experimenters.

The feedstock cell count studies exploited culture-independent techniques that are becoming more widely used among microbiologists but are still not ready for routine use by a plant operator. They required finesse and repetition and a trained eye. The studies

told us something that was not a great surprise to learn – that PL brings a significant microbial load to an F:M ratio. However, to control for the implications of this finding will be challenging, as it is difficult to inactivate the cells in a feedstock sample before testing it. Heating will serve as a form of pretreatment; fumigation will lyse the cells and make new carbon available. Fumigation is also complicated for routine work. There are also many interesting questions to be answered about the degree to which the new microbial loading participates in the biodegradation relative to the inoculum microbes, and there is the long-term question of whether or not F:M ratio is the appropriate parameter for use in calibrating AD feed.

The pretreatment assessments represent a very broad stroke effort to look at the wisdom of various pretreatment schemes for releasing additional energy from feedstocks. These analyses were based on laboratory evaluations and some of the numerical relationships may change with economies of scale and the equipment employed in full scale systems. Also, if one of the less feasible treatment schemes, such as thermal treatment, happens to be employed for other reasons, such as waste stabilization, and proves to be beneficial for energy production as well, then the energy investment may be more than justified, and such cases have yet to be analyzed. Conversely, the biological treatment that proved most likely to have a positive energy balance may not be one that could be leveraged for multiple purposes. We can envision the net energy analyses performed here being transformed for use in life cycle analyses and sustainable infrastructure analyses (e.g. ASCE's ISI rating system) for a more complete systems analysis of overall environmental, economic, and social costs and benefits.

REFERENCES

Abbasi, T., Tauseef, S.M., Abbasi, S.A. 2012. Biogas Energy. Springer New York.

Acharya, B.K., Mohana, S., Madamwar, D. 2008. Anaerobic treatment of distillery spent wash - a study on upflow anaerobic fixed film bioreactor. Bioresour Technol, 99(11), 4621-6.

Acharya, C., Kurian, R. 2006. Anaerobic Co-Digestion of a Single Source Oily Waste and High Strength Pet Food Wastewater: A Study of Failure and Revival of a Full Scale Digester. Proceedings of the Water Environment Federation, 2006, 5066-5073.

AD-Community. 2008. Anaerobic Digestion Plants in the British Isles. January 20, 2011. http://www.anaerobic-digestion.com/html/ad_plants_in_the_uk.html

Adams, R., Bennett, F., Dixon, J., Lough, R., Maclean, F., Martin, G. 1951. The utilization of organic wastes in NZ. New Zealand Engineering, 6(11), 396.

Agamuthu, P. 1999. Specific biogas production and role of packing medium in the treatment of rubber thread manufacturing industry wastewater. Bioprocess Engineering, 21(2), 151-155.

AgStar. 2013. Operating Anaerobic Digester Projects. April 21, 2013. http://www.epa.gov/agstar/projects/index.html

Alexander, R. 2012. Digestate Utilization in the US. in: *BioCycle*, Vol. 53, pp. 56.

Alves, M.M., Pereira, M.A., Sousa, D.Z., Cavaliero, A.J., Picavet, M., Smidt, H., JStams, A.J.M. 2009. Waste lipids to energy: how to optimize methane production from long-chain fatty acids (LCFA). Microbial Biotechnology, 2(5), 538-550.

Amon, B., Kryvoruchko, V., Amon, T., Zechmeister-Boltenstern, S. 2006a. Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. Agriculture, Ecosystems & Environment, 112(2–3), 153-162.

Amon, T., Amon, B., Kryvoruchko, V., Bodiroza, V., Pötsch, E., Zollitsch, W. 2006b. Optimising methane yield from anaerobic digestion of manure: Effects of dairy systems and of glycerine supplementation. International Congress Series, 1293(0), 217-220.

Angelidaki, I., Ahring, B.K. 1992. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Applied Microbiology and Biotechnology, 37(6), 808-812.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J., Guwy, A., Kalyuzhnyi, S., Jenicek, P., Lier, J.B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays.

Angelidaki, I., Ellegaard, L., Ahring, B. 2003. Applications of the Anaerobic Digestion Process: Biomethanation II, (Eds.) B. Ahring, B. Ahring, I. Angelidaki, J. Dolfing, L. Euegaard, H. Gavala, F. Haagensen, A. Mogensen, G. Lyberatos, P. Pind, J. Schmidt, I. Skiadas, K. Stamatelatou, Vol. 82, Springer Berlin / Heidelberg, pp. 1-33.

Antonopoulou, G., Stamatelatou, K., Lyberatos, G. 2010. Exploitation of rapeseed and sunflower residues for methane generation through anaerobic digestion: the effect of pretreatment. *Proceedings of the 2nd International Conference of Industrial Biotechnology*.

APHA, AWWA, WEF. 1998. Standard Methods for the Examination of Water and Wastwater. 20th ed. APHA, Washington, D.C.

Appels, L., Baeyens, J., Degrève, J., Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 34(6), 755-781.

Apul, O.G., Sanin, F.D. 2010. Ultrasonic pretreatment and subsequent anaerobic digestion under different operational conditions. Bioresource Technology, 101(23), 8984-8992.

Arora, S. 2011. Poultry Manure: The New Frontier for Anaerobic Digestoin.

Athanasoulia, E., Melidis, P., Aivasidis, A. 2014. Co-digestion of sewage sludge and crude glycerol from biodiesel production. Renewable Energy, 62(0), 73-78.

Azbar, N., Ursillo, P., Speece, R.E. 2001. Effect of process configuration and substrate complexity on the performance of anaerobic processes. Water Research, 35(3), 817-829.

Backus, B., Clanton, C., Goodrich, P., Morris, H. 1988. Carbon-to-nitrogen ratio and hydraulic retention time effect on the anaerobic digestion of cheese whey. Transactions of the ASAE, 31(4), 1274-1283.

Battimelli, A., Carrère, H., Delgenès, J.-P. 2009. Saponification of fatty slaughterhouse wastes for enhancing anaerobic biodegradability. Bioresource Technology, 100(15), 3695-3700.

Besnard, V., Federighi, M., Cappelier, J.M. 2000. Evidence of Viable But Non-Culturable state in Listeria monocytogenes by direct viable count and CTC-DAPI double staining. Food Microbiology, 17(6), 697-704.

Beszédes, S., László, Z., Szabó, G., Hodúr, C. 2011. Effects of microwave pretreatments on the anaerobic digestion of food industrial sewage sludge. Environmental Progress & Sustainable Energy, 30(3), 486-492.

BioCycle. 2011. Anaerobic Digest: State Funds 11 AD Projects. BioCycle, 52(1), 18.

Biogasdata.org. 2012. Biogas Data: Data to Inform and Inspire Sustainable Biosolids Management. April 16, 2016. http://www.biogasdata.org/home

Bodkhe, S., Vaidya, A. 2012. Complete recycle bioreactor for anaerobic digestion of organic substrates: Food waste. Research Journal of Chemistry and Environment, 16, 2.

Bolzonella, D., Pavan, P., Battistoni, P., Cecchi, F. 2005. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochemistry, 40(3–4), 1453-1460.

Bolzonella, D., Pavan, P., Mace, S., Cecchi, F. 2006. Dry anaerobic digestion of differently sorted organic municipal solid waste: a full-scale experience. Water Science and Technology, 53(8), 23-32.

Bonmati, A., Flotats, X., Mateu, L., Campos, E. 2001. Study of thermal hydrolysis as a pretreatment to mesophilic anaerobic digestion of pig slurry. Water science and technology: a journal of the International Association on Water Pollution Research, 44(4), 109.

Bouallagui, H., Rachdi, B., Gannoun, H., Hamdi, M. 2009. Mesophilic and thermophilic anaerobic co-digestion of abattoir wastewater and fruit and vegetable waste in anaerobic sequencing batch reactors. Biodegradation, 20(3), 401-409.

Bougrier, C., Delgenès, J.P., Carrère, H. 2006. Combination of Thermal Treatments and Anaerobic Digestion to Reduce Sewage Sludge Quantity and Improve Biogas Yield. Process Safety and Environmental Protection, 84(4), 280-284.

Bratbak, G. 1985. Bacterial biovolume and biomass estimations. Applied and Environmental Microbiology, 49(6), 1488-1493.

Braun, R., Wellinger, A. 2012. Potential of Co-digestion, IEA Bioenergy.

Brown, D., Li, Y. 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresource technology, 127, 275-280.

Bruni, E., Jensen, A.P., Angelidaki, I. 2010. Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. Bioresource Technology, 101(22), 8713-8717.

Bryant, M. 1979. Microbial methane production—theoretical aspects. Journal of Animal Science, 48(1), 193-201.

Buendia, I.M., Fernandez, F.J., Villasenor, J., Rodriguez, L. 2009. Feasibility of anaerobic co-digestion as a treatment option of meat industry wastes. Bioresource Technology, 100(6), 1903-1909.

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

Cappelier, J.M., Lazaro, B., Rossero, A., Fernandez-Astorga, A., Federighi, M. 1997. Double staining (CTC-DAPI) for detection and enumeration of viable but non-culturable Campylobacter jejuni cells. Veterinary research, 28(6), 547-555.

Carballa, M., Omil, F., Lema, J. 2009. Influence of Different Pretreatments on Anaerobically Digested Sludge Characteristics: Suitability for Final Disposal. Water, Air, & Soil Pollution, 199(1), 311-321.

Cardinali-Rezende, J., Debarry, R., Colturato, L., Carneiro, E., Chartone-Souza, E., Nascimento, A. 2009. Molecular identification and dynamics of microbial communities in reactor treating organic household waste. Applied Microbiology and Biotechnology, 84(4), 777-789.

Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F. 2012. The effects of substrate pre-treatment on anaerobic digestion systems: A review. Waste Management, 32(9), 1634-50.

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I. 2010. Pretreatment methods to improve sludge anaerobic degradability: A review. Journal of Hazardous Materials, 183(1–3), 1-15.

Carrère, H., Sialve, B., Bernet, N. 2009. Improving pig manure conversion into biogas by thermal and thermo-chemical pretreatments. Bioresource Technology, 100(15), 3690-3694.

Castrillón, L., Fernández-Nava, Y., Ormaechea, P., Marañón, E. 2011. Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin. Bioresource Technology, 102(17), 7845-7849.

Chandra, R., Takeuchi, H., Hasegawa, T. 2012. Hydrothermal pretreatment of rice straw biomass: A potential and promising method for enhanced methane production. Applied Energy, 94(0), 129-140.

Chang, J.-S., Lee, K.-S., Lin, P.-J. 2002. Biohydrogen production with fixed-bed bioreactors. International Journal of Hydrogen Energy, 27(11–12), 1167-1174.

Chen, X., Romano, R.T., Ruihong, Z. 2010. Anaerobic digestion of food wastes for biogas production. International Journal of Agricultural and Biological Engineering, 3(4).

Chen, Y., Cheng, J., Creamer, K. 2008. Inhibition of anaerobic digestion process: A review. Bioresource Technology, 99(10), 4044-4064.

Chivu, B. 2010. evaluation of dapi direct count, computer assisted anad plate count methods. in: *Water Science and Technology*, Vol. Master's, University of Stavanger. Stavanger, Norway, pp. 61.

Chouari, R., Le Paslier, D., Daegelen, P., Ginestet, P., Weissenbach, J., Sghir, A. 2005. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. Environmental Microbiology, 7(8), 1104-1115.

Chynoweth, D., Turick, C., Owens, J., Jerger, D., Peck, M. 1993. Biochemical methane potential of biomass and waste feedstocks. Biomass and Bioenergy, 5(1), 95-111.

Coello, M.D., Rodriguez-Barroso, M.R., Aragon, C.A., Quiroga, J.M. 2010. Use of microbial activity measurements for monitoring RBC biofilms. Environmental Monitoring and Assessment, 169(1-4), 451-455.

Costa, J.C., Barbosa, S.G., Alves, M.M., Sousa, D.Z. 2012. Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. Bioresource Technology, 111(0), 141-147.

Daims, H., Taylor, M., Wagner, M. 2006. Wastewater treatment: a model system for microbial ecology. Trends in biotechnology, 24(11), 483-489.

Davidsson, A., Lovstedt, C., Jansen, J.L., Gruvberger, C., Aspegren, H. 2008. Co-digestion of grease trap sludge and sewage sludge. Waste Management, 28(6), 986-992.

De Baere, L. 2000. Anaerobic digestion of solid waste: state-of-the-art. Water Science and Technology, 41(3), 283-290.

De la Rubia, M.A., Fernández-Cegrí, V., Raposo, F., Borja, R. 2011. Influence of particle size and chemical composition on the performance and kinetics of anaerobic digestion process of sunflower oil cake in batch mode. Biochemical Engineering Journal, 58–59(0), 162-167.

Delmer, D.P., Amor, Y. 1995. Cellulose biosynthesis. The Plant Cell, 7(7), 987.

Dereix, M., Parker, W., Kennedy, K. 2006. Steam-explosion pretreatment for enhancing anaerobic digestion of municipal wastewater sludge. Water Environment Federation. pp. 474-485.

Deublein, D., Steinhauser, A. 2008. Biogas from waste and renewable resources. Wiley Online Library.

Devlin, D.C., Esteves, S.R.R., Dinsdale, R.M., Guwy, A.J. 2011. The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresource Technology, 102(5), 4076-4082.

Dhar, B.R., Youssef, E., Nakhla, G., Ray, M.B. 2011. Pretreatment of municipal waste activated sludge for volatile sulfur compounds control in anaerobic digestion. Bioresource Technology, 102(4), 3776-3782.0

DOE. 2013. Hydrogen Production: Natural Gas Reforming. http://www1.eere.energy.gov/hydrogenandfuelcells/production/natural_gas.html

Dong, L., Zhenhong, Y., Yongming, S., Longlong, M. 2011. Anaerobic Fermentative Co-production of Hydrogen and Methane from an Organic Fraction of Municipal Solid Waste. Energy Sources Part A: Recovery, Utilization & Environmental Effects, 33(6), 575-585.

Eisenmann. 2013. Anaerobic Digestion. 2/27/13. http://www.eisenmann.us.com/anaerobic-digestion/

Elbeshbishy, E., Aldin, S., Hafez, H., Nakhla, G., Ray, M. 2011. Impact of ultrasonication of hog manure on anaerobic digestability. Ultrasonics Sonochemistry, 18(1), 164-171.

Elbeshbishy, E., Nakhla, G. 2011. Comparative study of the effect of ultrasonication on the anaerobic biodegradability of food waste in single and two-stage systems. Bioresource Technology, 102(11), 6449-6457.

EPA. 2013a. Methods for Calculating Efficiency. June 21, 2013. http://www.epa.gov/chp/basic/methods.html

EPA. 2013b. Sustainable Materials Management: Food Recovery Challenge. April 11, 2013. http://www.epa.gov/smm/foodrecovery/

EPA. 2010. Waste To Biogas Mapping Tool. February 14. http://134.67.99.137/myenvtools/biogas/index.html - instructions

Eskicioglu, C., Kennedy, K.J., Droste, R.L. 2006. Characterization of soluble organic matter of waste activated sludge before and after thermal pretreatment. Water Research, 40(20), 3725-3736.

Eskicioglu, C., Prorot, A., Marin, J., Droste, R.L., Kennedy, K.J. 2008. Synergetic pretreatment of sewage sludge by microwave irradiation in presence of H_2O_2 for enhanced anaerobic digestion. Water Research, 42(18), 4674-4682.

Evans, E.A., Evans, K.M., Ulrich, A., Ellsworth, S., Khan, W. 2010. Anaerobic Processes. Water Environment Research, 82(10), 1235-1287.

Fang, H.H., Chui, H., Li, Y. 1994. Microbial structure and activity of UASB granules treating different wastewaters. Water Science & Technology, 30(12), 87-96.

Fernandes, T.V., Klaasse Bos, G.J., Zeeman, G., Sanders, J.P.M., van Lier, J.B. 2009. Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. Bioresource Technology, 100(9), 2575-2579.

Fernández-Cegrí, V., de la Rubia, M.A., Raposo, F., Borja, R. 2012. Impact of ultrasonic pretreatment under different operational conditions on the mesophilic anaerobic digestion of sunflower oil cake in batch mode. Ultrasonics Sonochemistry, 19(5), 1003-1010.

Fountoulakis, M.S., Manios, T. 2009. Enhanced methane and hydrogen production from municipal solid waste and agro-industrial by-products co-digested with crude glycerol. Bioresource Technology, 100(12), 3043-3047.

Fountoulakis, M.S., Petousi, I., Manios, T. 2010. Co-digestion of sewage sludge with glycerol to boost biogas production. Waste Management, 30(10), 1849-1853.

Frigon, J.-C., Mehta, P., Guiot, S.R. 2012. Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switchgrass. Biomass and Bioenergy, 36(0), 1-11.

Frischmann, P. 2012. Enhancement and Treatment of DIgestates from Anaerobic Digestion. WARP.

Fukuzaki, S., Nishio, N., Shobayashi, M., Nagai, S. 1990. Inhibition of the fermentation of propionate to methane by hydrogen, acetate, and propionate. Applied and environmental microbiology, 56(3), 719-723.

Ganidi, N., Tyrrel, S., Cartmell, E. 2009. Anaerobic digestion foaming causes - A review. Bioresource Technology, 100(23), 5546-5554.

Garcia-Peña, E.I., Parameswaran, P., Kang, D.W., Canul-Chan, M., Krajmalnik-Brown, R. 2011. Anaerobic digestion and co-digestion processes of vegetable and fruit residues: process and microbial ecology. Bioresource Technology, 102(20), 9447-9555.

Gerardi, M.H. 2003. The Microbiology of Anaerobic Digesters. John Wiley & Sons, Inc., Hoboken, NJ.

Ghosh, S., Buoy, K., Dressel, L., Miller, T., Wilcox, G., Loos, D. 1995. Pilot-and full-scale two-phase anaerobic digestion of municipal sludge. Water Environment Research, 67(2), 206-214.

Glatz, P., Miao, Z., Rodda, B. 2011. Handling and treatment of poultry hatchery waste: A review. Sustainability, 3(1), 216-237.

Godon, J.J., Zumstein, E., Dabert, P., Habouzit, F., Moletta, R. 1997. Molecular microbial diversity of an anaerobic digester as determined by small-subunit rDNA sequence analysis. Applied and Enviornmental Microbiology, 63(7), 2802.

González-Fernández, C., León-Cofreces, C., García-Encina, P.A. 2008. Different pretreatments for increasing the anaerobic biodegradability in swine manure. Bioresource Technology, 99(18), 8710-8714.

Grady, C.P.L., Daigger, G.T., Lim, H.C. 1999. Biological wastewater treatment. CRC Press.

Griebe, T., Schaule, G., Wuertz, S. 1997. Determination of microbial respiratory and redox activity in activated sludge. Journal of Industrial Microbiology and Biotechnology, 19(2), 118-122.

Ha, M.-A., Apperley, D.C., Evans, B.W., Huxham, I.M., Jardine, W.G., Viëtor, R.J., Reis, D., Vian, B., Jarvis, Michael C. 1998. Fine structure in cellulose microfibrils: NMR evidence from onion and quince. The Plant Journal, 16(2), 183-190.

Haines, R. 2008. The BTA Process. 2/27/13. http://www.swea.co.uk/downloads/Biogas_ROBYN.pdf

Hamilton, D. 2013. Types of Anaerobic Digesters. 2/27/13. http://www.extension.org/pages/30307/types-of-anaerobic-digesters

Hansson, G.M.N. 1981. End product inhibition in methane fermentations: Effects of carbon dioxide on fermentative and acetogenic bacteria. European J. Appl. Microbiol. Biotechnol. European Journal of Applied Microbiology and Biotechnology, 13(4), 242-247.

Held, J., Mathiasson, A., Nylanders, A. 2008. Biogas from manure and waste products - Swedish case studies.

Hendriks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100(1), 10-18.

Heo, H.S., Kim, S.G., Jeong, K.-E., Jeon, J.-K., Park, S.H., Kim, J.M., Kim, S.-S., Park, Y.-K. 2011. Catalytic upgrading of oil fractions separated from food waste leachate. Bioresource Technology, 102(4), 3952-3957.

Hilger, H., Guyer, R., Parker, E., Wager, J., Deming, R., Clinton, S., Fagan, T., Jarrell, J. 2011. Greater Charlotte Region Biofuel Facility Project, Centralina Council of Governments for the Biofuels Center of North Carolina Charlotte, NC.

Hobson, P.N., Shaw, B.G. 1976. Inhibition of methane production by Methanobacterium formicicum. Water Research, 10(10), 849-852.

Hobson, P.N., Wheatley, A. 1993. Anaerobic digestion: modern theory and practice. Elsevier Applied Science.

Holm-Nielsen, J.B., Al Seadi, T., Oleskowicz-Popiel, P. 2009. The future of anaerobic digestion and biogas utilization. Bioresource Technology, 100(22), 5478-5484.

Horiuchi, J.I., Shimizu, T., Tada, K., Kanno, T., Kobayashi, M. 2002. Selective production of organic acids in anaerobic acid reactor by pH control. Bioresource Technology, 82(3), 209-213.

Hwu, C.S., Lettinga, G. 1997. Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. Enzyme and Microbial Technology, 21(4), 297-301.

IEA. 2005. Biogas Production and Utilisation, (Ed.) I.E. Agency. SWITZERLAND.

IEA. 2010. International Energy Agency Bioenergy Task 37. Febrary 15, 2011. http://www.iea-biogas.net/home.htm

Izumi, K., Okishio, Y.-k., Nagao, N., Niwa, C., Yamamoto, S., Toda, T. 2010. Effects of particle size on anaerobic digestion of food waste. International Biodeterioration & Biodegradation, 64(7), 601-608.

Jackowiak, D., Bassard, D., Pauss, A., Ribeiro, T. 2011. Optimisation of a microwave pretreatment of wheat straw for methane production. Bioresource Technology, 102(12), 6750-6756.

Jarrell, K.F., Saulnier, M., Ley, A. 1987. Inhibition of methanogenesis in pure cultures by ammonia, fatty acids, and heavy metals, and protection against heavy metal toxicity by sewage sludge. Canadian journal of microbiology, 33(6), 551-554.

Jihen, T., Hassib, B., Moktar, H., Said, N. 2010. Improvement of dairy manufacture effluent anaerobic digestion with biological waste addition using a Chinese dome digester. Bioresource Technology, 101(10), 3743-3746.

Jung, K.-W., Cho, S.-K., Yun, Y.-M., Shin, H.-S., Kim, D.-H. 2013. Rapid formation of hydrogen-producing granules in an up-flow anaerobic sludge blanket reactor coupled with high-rate recirculation. International Journal of Hydrogen Energy, 38(22), 9097-9103.

Kabouris, J., Pavlostathis, S., Tezel, U., Engelmann, M., Dulaney, J., Todd, A., Gillette, B. 2008a. Anaerobic Degradability of Pinellas County Sludge and FOG at Mesophilic & Thermophilic Temperatures. Florida Water Resources Journal, June

Kabouris, J., Tezel, U., Pavlostathis, S., Engelmann, M., Dulaney, J., Gillette, R., Todd, A. 2009. Methane recovery from the anaerobic codigestion of municipal sludge and FOG. Bioresource Technology, 100(15), 3701-3705.

Kabouris, J., Tezel, U., Pavlostathis, S., Engelmann, M., Dulaney, J., Gillette, R., Todd, A. 2007. The Ultimate Anaerobic Biodegradability of Municipal Sludge and FOG. Proceedings of the Water Environment Federation, 2007(11), 6776-6792.

Kabouris, J.C., Tezel, U., Pavlostathis, S.G., Engelmann, M., Todd, A.C., Gillette, R.A. 2008b. The anaerobic biodegradability of municipal sludge and fat, oil, and grease at mesophilic conditions. Water Environment Research, 80(3), 212-221.

Kayhanian, M. 1995. Biodegradability of the Organic Fraction of Municipal Solid Waste in a High-Solids Anaerobic Digester. Waste Management & Research, 13(2), 123-136.

Kim, H.W., Han, S.K., Shin, H.S. 2003a. The optimisation of food waste addition as a co-substrate in anaerobic digestion of sewage sludge. Waste Management & Research, 21(6), 515.

Kim, J., Park, C., Kim, T.-H., Lee, M., Kim, S., Kim, S.-W., Lee, J. 2003b. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. Journal of Bioscience and Bioengineering, 95(3), 271-275.

Kim, M., Speece, R.E. 2002. Reactor configuration - Part I comparative process stability and efficiency of mesophilic anaerobic digestion. Environmental Technology, 23(6), 631-642.

Koster, I.W., Lettinga, G. 1988. Anaerobic digestion at extreme ammonia concentrations. Biological Wastes, 25(1), 51-59.

Kuglarz, M., Karakashev, D., Angelidaki, I. 2013. Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. Bioresource Technology, 134(0), 290-297.

Li, R., Chen, S., Li, X., Saifullah Lar, J., He, Y., Zhu, B. 2009. Anaerobic Codigestion of Kitchen Waste with Cattle Manure for Biogas Production. Energy & Fuels, 23(4), 2225-2228.

Li, X.M., Cheng, K.Y., Selvam, A., Wong, J.W.C. 2013. Bioelectricity production from acidic food waste leachate using microbial fuel cells: Effect of microbial inocula. Process Biochemistry, 48(2), 283-288.

Li, Y., Sasaki, H., Yamashita, K., Seki, K., Kamigochi, I. 2002. High-rate methane fermentation of lipid-rich food wastes by a high-solids co-digestion process. Water Science & Technology, 45(12), 143-150.

Lin, Y., Wang, D., Wu, S., Wang, C. 2009. Alkali pretreatment enhances biogas production in the anaerobic digestion of pulp and paper sludge. Journal of Hazardous Materials, 170(1), 366-373.

Lisboa, M.S., Lansing, S. 2013. Characterizing food waste substrates for co-digestion through biochemical methane potential (BMP) experiments. Waste Management(0).

Liu, D., Liu, D., Zeng, R.J., Angelidaki, I. 2006. Hydrogen and methane production from household solid waste in the two-stage fermentation process. Water Research, 40(11), 2230-2236.

Liu, F.H., Wang, S.B., Zhang, J.S., Zhang, J., Yan, X., Zhou, H.K., Zhao, G.P., Zhou, Z.H. 2009a. The structure of the bacterial and archaeal community in a biogas digester as revealed by denaturing gradient gel electrophoresis and 16S rDNA sequencing analysis. Journal of Applied Microbiology, 106(3), 952-966.

Liu, G., Zhang, R., El-Mashad, H.M., Dong, R. 2009b. Effect of feed to inoculum ratios on biogas yields of food and green wastes. Bioresource Technology, 100(21), 5103-5108.

Liu, X., Liu, H., Chen, Y., Du, G., Chen, J. 2008. Effects of organic matter and initial carbon–nitrogen ratio on the bioconversion of volatile fatty acids from sewage sludge. Journal of Chemical Technology & Biotechnology, 83(7), 1049-1055.

Liu, X., Wang, W., Gao, X., Zhou, Y., Shen, R. 2012. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. Waste Management, 32(2), 249-255.

Liu, X.L., Liu, H., Du, G.C., Chen, J. 2009c. Improved Bioconversion of Volatile Fatty Acids from Waste Activated Sludge by Pretreatment. Water Environment Research, 81(1), 13-20.

Long, J.H., Aziz, T.N., Reyes Iii, F.L.d.l., Ducoste, J.J. 2012. Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. Process Safety and Environmental Protection, 90(3), 231-245.

López Torres, M., Espinosa Lloréns, M.d.C. 2008. Effect of alkaline pretreatment on anaerobic digestion of solid wastes. Waste Management, 28(11), 2229-2234.

Luostarinen, S., Luste, S., Sillanpaa, M. 2009. Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. Bioresource Technology, 100(1), 79-85.

Luste, S., Luostarinen, S., Sillanpää, M. 2009. Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry. Journal of Hazardous Materials, 164(1), 247-255.

Marañón, E., Castrillón, L., Quiroga, G., Fernández-Nava, Y., Gómez, L., García, M.M. 2012. Co-digestion of cattle manure with food waste and sludge to increase biogas production. Waste Management, 32(10), 1821-1825.

Marin, J., Kennedy, K.J., Eskicioglu, C. 2010. Effect of microwave irradiation on anaerobic degradability of model kitchen waste. Waste Management, 30(10), 1772-1779.

Mata-Alvarez, J. 2003. Biomethanization of the organic fraction of municipal solid wastes. IWA publishing.

Mawson, A.J., Earle, R.L., Larsen, V.F. 1991. Degradation of acetic and propionic acids in the methane fermentation. Water Research, 25(12), 1549-1554.

Maya-Altamira, L., Baun, A., Angelidaki, I., Schmidt, J.E. 2008. Influence of wastewater characteristics on methane potential in food-processing industry wastewaters. Water Research, 42(8-9), 2195-2203.

McInerney, M.J. 1988. Anaerobic hydrolysis and fermentation of fats and proteins. Biology of anaerobic microorganisms, 373-415.

McInerney, M.J., Bryant, M.P., Hespell, R.B., Costerton, J.W. 1981. Syntrophomonas wolfei gen. nov. sp. nov., an Anaerobic, Syntrophic, Fatty Acid-Oxidizing Bacterium, Vol. 41, pp. 1029-1039.

Mehta, A. 2002. The economics and feasibility of electricity generation using manure digesters on small and mid-size dairy farms. Department of Agricultural and Applied Economics Energy Analysis and Policy Program. Madison, WI: University of Wisconsin.

Menardo, S., Airoldi, G., Balsari, P. 2012. The effect of particle size and thermal pre-treatment on the methane yield of four agricultural by-products. Bioresource Technology, 104(0), 708-714.

Merrylin, J., Kaliappan, S., Kumar, S.A., Yeom, I.-T., Banu, J.R. 2014. Enhancing aerobic digestion potential of municipal waste-activated sludge through removal of extracellular polymeric substance. Environmental Science and Pollution Research, 1-12.

Miltner, M., Makaruk, A., Bala, H., Harasek, M. 2009. Biogas Upgrading for Transportation Purposes–Operational Experiences with Austria's First Bio-CNG Fuelling Station. Chemical Engineering Transactions, 18, 617–622.

Minnesota, D.o.C. 2003. Minnesota's Potential for Electricity Production Using Biogas Resources.

Misi, S., Forster, C. 2001. Batch co-digestion of multi-component agro-wastes. Bioresource Technology, 80(1), 19-28.

Moisan, M. 2013. Ultrasonic Pretreatment for Anaerobic Digestion: a Study on Feedstock, Methane Yield, and Energy Balance.

Monlau, F., Barakat, A., Steyer, J.P., Carrere, H. 2012. Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. Bioresource Technology, 120(0), 241-247.

Monnet, F. 2003. An introduction to anaerobic digestion of organic wastes. Remade Scotland.

Moody, L., Burns, R., Bishop, G., Sell, S., Spajic, R. 2011a. Using Biochemical Methane Potential Assays to Aid in Co-substrate Selection for Co-digestion. Applied Engineering in Agriculture, 27(3), 433-439.

Moody, L., Burns, R., Sell, S., Bishop, G. 2011b. Using Anaerobic Toxicity Assays to Aid in Co-substrate Selection for Co-digestion. Applied Engineering in Agriculture, 27(3), 441-447.

Mshandete, A., Kivaisi, A., Rubindamayugi, M., Mattiasson, B. 2004. Anaerobic batch co-digestion of sisal pulp and fish wastes. Bioresource Technology, 95(1), 19-24.

Mucha, H., Lingens, F., Trösch, W. 1988. Conversion of propionate to acetate and methane by syntrophic consortia. Applied Microbiology and Biotechnology, 27(5-6), 581-586.

Muller, C.D., Abu-Orf, M., Blumenschein, C.D., Novak, J.T. 2009. A comparative study of ultrasonic pretreatment and an internal recycle for the enhancement of mesophilic anaerobic digestion. Water Environment Research, 81(12), 2398-2410.

Naczk, M., Amarowicz, R., Pink, D., Shahidi, F. 2000. Insoluble condensed tannins of canola/rapeseed. Journal of agricultural and food chemistry, 48(5), 1758-1762.

Naczk, M., Nichols, T., Pink, D., Sosulski, F. 1994. Condensed tannins in canola hulls. Journal of Agricultural and Food Chemistry, 42(10), 2196-2200.

Nagao, N., Tajima, N., Kawai, M., Niwa, C., Kurosawa, N., Matsuyama, T., Yusoff, F.M., Toda, T. 2012. Maximum organic loading rate for the single-stage wet anaerobic digestion of food waste. Bioresource Technology, 118(0), 210-218.

Nagase, M., Matsuo, T. 1982. Interactions between amino-acid-degrading bacteria and methanogenic bacteria in anaerobic digestion. Biotechnology and Bioengineering, 24(10), 2227-2239.

Nah, I.W., Kang, Y.W., Hwang, K.-Y., Song, W.-K. 2000. Mechanical pretreatment of waste activated sludge for anaerobic digestion process. Water Research, 34(8), 2362-2368.

Nayyeri, M., Kianmehr, M., Arabhosseini, A., Hassan-Beygi, S. 2009. Thermal properties of dairy cattle manure. Int. Agrophysics, 23, 359-366.

Nisman, B. 1954. The Stickland reaction. Bacteriological reviews, 18(1), 16.

Noutsopoulos, C., Mamais, D., Antoniou, K., Avramides, C., Oikonomopoulos, P., Fountoulakis, I. 2013. Anaerobic co-digestion of grease sludge and sewage sludge: The effect of organic loading and grease sludge content. Bioresource Technology, 131(0), 452-459.

Owen, W.F., Stuckey, D.C., Healy Jr, J.B., Young, L.Y., McCarty, P.L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Research, 13(6), 485-492.

OWS. 2013. DRANCO Technology. 2/27/13.
http://www.ows.be/pages/index.php?menu=85&choose_lang=EN

Park, C., Lee, C., Kim, S., Chen, Y., Chase, H.A. 2005. Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. Journal of Bioscience and Bioengineering, 100(2), 164-167.

Pereira, M., Cavaleiro, A., Mota, M., Alves, M. 2003. Accumulation of long chain fatty acids onto anaerobic sludge under steady state and shock loading conditions: effect on acetogenic and methanogenic activity.

Pereira, M.A., Sousa, D.Z., Mota, M., Alves, M.M. 2004. Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. Biotechnology and Bioengineering, 88(4), 502-511.

Pereira, M.A.A.d.S. 2003. Anaerobic Biodegradation of Long Chain Fatty Acids. in: *Chemical and Biological Engineering*, Vol. Doctorate, University of Minho. Minho, Portugal.

Pereira, M.A.P.O.C.M.M.A.M.M. 2005. Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. BIT Biotechnology and Bioengineering, 92(1), 15-23.

Pérez-Elvira, S., Fdz-Polanco, M., Plaza, F.I., Garralón, G., Fdz-Polanco, F. 2009. Ultrasound pre-treatment for anaerobic digestion improvement. Water Science & Technology, 60(6), 1525-1532.

Pinnekamp, J. 1989. Effects of thermal pretreatment of sewage sludge on anaerobic digestion. Water Science & Technology, 21(4-5), 97-108.

Pommier, S., Llamas, A.M., Lefebvre, X. 2010. Analysis of the outcome of shredding pretreatment on the anaerobic biodegradability of paper and cardboard materials. Bioresource Technology, 101(2), 463-468.

Pöschl, M., Ward, S., Owende, P. 2010. Evaluation of energy efficiency of various biogas production and utilization pathways. Applied Energy, 87(11), 3305-3321.

Prashanth, S., Kumar, P., Mehrotra, I. 2006. Anaerobic Degradability: Effect of Particulate COD. Journal of environmental engineering, 132(4), 488-496.

Procházka, J., Dolejš, P., Máca, J., Dohányos, M. 2012. Stability and inhibition of anaerobic processes caused by insufficiency or excess of ammonia nitrogen. Applied Microbiology and Biotechnology, 93(1), 439-447.

Pullen, D. 2004. Carcass Disposal: A Comprehensive Review.

Qiao, W., Wang, W., Zhu, C., Zhang, Z. 2010. Biogas recovery from microwave heated sludge by anaerobic digestion. SCIENCE CHINA Technological Sciences, 53(1), 144-149.

Qiao, W., Yan, X., Ye, J., Sun, Y., Wang, W., Zhang, Z. 2011. Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. Renewable Energy, 36(12), 3313-3318.

Rafique, R., Poulsen, T.G., Nizami, A.-S., Asam, Z.-u.-Z., Murphy, J.D., Kiely, G. 2010. Effect of thermal, chemical and thermo-chemical pre-treatments to enhance methane production. Energy, 35(12), 4556-4561.

Ramsay, I.R., Pullammanappallil, P.C. 2001. Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation, 12(4), 247-256.

Rao, P.V., Baral, S.S., Dey, R., Mutnuri, S. 2010. Biogas generation potential by anaerobic digestion for sustainable energy development in India. Renewable and Sustainable Energy Reviews, 14(7), 2086-2094.

Raposo, F., Borja, R., Martín, M., Martín, A., De la Rubia, M., Rincón, B. 2009. Influence of inoculum-substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation. Chemical Engineering Journal, 149(1-3), 70-77.

Rapport, J., Zhang, R., Jenkins, B.M., Williams, R.B. 2008. Current Anaerobic Digestion Technologies Used for Treatment of Municipal Organic Solid Waste. University of California, Davis.

Richard, T. 1996. Estimating Carbon Content. 10/7/13.
http://compost.css.cornell.edu/calc/carbon.html

Riviere, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., Li, T., Camacho, P., Sghir, A. 2009. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. Isme Journal, 3(6), 700-714.

Robra, S., Serpa da Cruz, R., de Oliveira, A.M., Neto, J.A.A., Santos, J.V. 2010. Generation of biogas using crude glycerin from biodiesel production as a supplement to cattle slurry. Biomass and Bioenergy, 34(9), 1330-1335.

Rodriguez, G., Phipps, D., Ishiguro, K., Ridgway, H. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. Applied and Environmental Microbiology, 58(6), 1801-1808.

Romano, R.T., Zhang, R., Teter, S., McGarvey, J.A. 2009. The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. Bioresource Technology, 100(20), 4564-4571.

Rorabacher, D.B. 1991. Statistical treatment for rejection of deviant values: critical values of Dixon's "Q" parameter and related subrange ratios at the 95% confidence level. Analytical Chemistry, 63(2), 139-146.

Rughoonundun, H., Mohee, R., Holtzapple, M.T. 2012. Influence of carbon-to-nitrogen ratio on the mixed-acid fermentation of wastewater sludge and pretreated bagasse. Bioresource Technology, 112(0), 91-97.

Sánchez, E., Borja, R., Weiland, P., Travieso, L., Martín, A. 2000. Effect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal

in the batch anaerobic digestion process of cattle manure. Bioprocess and Biosystems Engineering, 22(3), 247-252.

Sanz, J.L., Kochling, T. 2007. Molecular biology techniques used in wastewater treatment: An overview. Process Biochemistry, 42(2), 119-133.

Schumacher, B.A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. Ecological Risk Assessment Support Center, 1-23.

Sell, S.T. 2011. A scale-up procedure for substrate co-digestion in anaerobic digesters through the use of substrate characterization, BMPs, ATAs, and sub pilot-scale digesters.

Seppälä, M., Paavola, T., Lehtomäki, A., Pakarinen, O., Rintala, J. 2008. Biogas from energy crops—optimal pre-treatments and storage, co-digestion and energy balance in boreal conditions. Water Science & Technology, 58(9), 1857-1863.

Sheng, F., Lijuan, P., Zhixiang, Y., Jianwei, M. 2011. A review of different pretreatment techniques for enhancing biogas production. *Materials for Renewable Energy & Environment (ICMREE), 2011 International Conference on*, 20-22 May 2011. pp. 263-266.

Shin, H.S., Kim, S.H., Lee, C.Y., Nam, S.Y. 2003. Inhibitory effects of long-chain fatty acids on VFA degradation and beta-oxidation. Water Science and Technology, 47(10), 139-146.

Show, K.Y., Wang, Y., Foong, S.F., Tay, J.H. 2004. Accelerated start-up and enhanced granulation in upflow anaerobic sludge blanket reactors. Water Res, 38(9), 2292-303.

Simpson, W., TenWolde, A. 1999. Wood handbook: Wood as an engineering material. Gen. Tech. Rep. FPL–GTR–113, Madison, WI: US Department of Agriculture, Forest Service. Forest Products Laboratory.

Singh, K., Lee, K., Worley, J., Risse, L., Das, K. 2010. Anaerobic Digestion of Poultry Litter: A Review. Applied Engineering in Agriculture, 26(4), 677-688.

Sommer, S.G., Christensen, M.L., Schmidt, T., Jensen, L.S. 2013. Animal Manure Recycling: Treatment and Management. John Wiley & Sons.

Sousa, D.Z., Pereira, M.A., Stams, A.J.M., Alves, M.M., Smidt, H. 2007. Microbial communities involved in anaerobic degradation of unsaturated or saturated long-chain fatty acids. Applied and Environmental Microbiology, 73(4), 1054-1064.

Speece, R. 2008. Anaerobic Biotechnology and Odor/Corrosion Control for Municipalities and Industrial Wastewaters Archae Press, Nashville, TN.

Sri Bala Kameswari, K., Chitra, K., Porselvam, S., Thanasekaran, K. 2011. Optimization of inoculum to substrate ratio for bio-energy generation in co-digestion of tannery solid wastes. Clean Technologies and Environmental Policy, 1-10.

Stabnikova, O., Liu, X.Y., Wang, J.Y. 2008. Digestion of frozen/thawed food waste in the hybrid anaerobic solid–liquid system. Waste Management, 28(9), 1654-1659.

Steffen, R., Szolar, O., Braun, R. 1998. Feedstocks for Anaerobic Digestion. Institue for Agrobiotechnology Tullen.

Steinberg, L.M., Regan, J.M. 2009. mcrA-Targeted Real-Time Quantitative PCR Method To Examine Methanogen Communities. Appl. Environ. Microbiol., 75(13), 4435-4442.

Steinberg, L.M., Regan, J.M. 2008. Phylogenetic Comparison of the Methanogenic Communities from an Acidic, Oligotrophic Fen and an Anaerobic Digester Treating Municipal Wastewater Sludge, Vol. 74, pp. 6663-6671.

Steinberg, L.M., Regan, J.M. 2011. Response of lab-scale methanogenic reactors inoculated from different sources to organic loading rate shocks. Bioresource Technology, 102(19), 8790-8798.

Stroot, P.G., McMahon, K.D., Mackie, R.I., Raskin, L. 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—I. digester performance. Water Research, 35(7), 1804-1816.

Sung S, L.T. 2003. Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere, 53(1), 43-52.

Taherdanak, M., Zilouei, H. 2014. Improving biogas production from wheat plant using alkaline pretreatment. Fuel, 115(0), 714-719.

Taherzadeh, M., Karimi, K. 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. International Journal of Molecular Sciences, 9(9), 1621-1651.

Tanaka, S., Kobayashi, T., Kamiyama, K.-i., Lolita, M., Signey Bildan, N. 1997. Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. Water Science and Technology, 35(8), 209-215.

Tchobanoglous, G., Burton, F., Stensel, H.D. 2003. Wastewater Engineering: Treatment and Reuse. Fourth ed. McGraw-Hill Comp., New York, NY.

Uellendahl, H., Wang, G., Moller, H., Jorgensen, U., Skiadas, I., Gavala, H.N., Ahring, B.K. 2008. Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation. Water Science & Technology, 58(9), 1841-1847.

Valo, A., Carrère, H., Delgenès, J.P. 2004. Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion. Journal of Chemical Technology & Biotechnology, 79(11), 1197-1203.

Vandevivere, P., Baere, L.D., Verstraete, W. 2003. Types of anaerobic digesters for solid wastes. in: *Biomethanization of the organic fraction of municipal solid wastes*, (Ed.) J. Mata-Alvarez, IWA Publishing. London, pp. 111-140.

Velmurugan, B., Arathy, E., Hemalatha, R., Philip, J., Ramanujam, R. 2010. Anaerobic Co-Digestion of Fruit and Vegetable Wastes and Primary Sewage Sludge. Journal of Environmental Science & Engineering, 52(1), 19-22.

Voell, C. 2013. Cost Effective Conversion of Biofas to Bio-CNG (RNG). June 21, 2013. http://www.dec.ny.gov/docs/administration_pdf/rngweb.cv.pdf

Vollertsen, J., Jahn, A., Lund Nielsen, J., Hvitved-Jacobsen, T., Halkjær Nielsen, P. 2001. Comparison of methods for determination of microbial biomass in wastewater. Water Research, 35(7), 1649-1658.

Wang, L., Aziz, T.N., de los Reyes, F.L. 2013. Determining the limits of anaerobic co-digestion of thickened waste activated sludge with grease interceptor waste. Water Research, 47(11), 3835-3844.

Webb, A.R., Hawkes, F.R. 1985. The anaerobic digestion of poultry manure: Variation of gas yield with influent concentration and ammonium-nitrogen levels. Agricultural Wastes, 14(2), 135-156.

Weiland, P. 2010. Country Report Germany. Johann Heinich von Thunen-Institut.

Wellinger, A., Linberg, A. 2000. Biogas Upgrading and Utilization-IEA Bioenergy Task 24. International Energy Association, Paris, France, 20.

Wellinger, A., Linberg, A. 2005. Biogas Upgrading and Utilization-IEA Bioenergy Task 37. International Energy Association, Paris, France, 20.

Westlund, A.D., Hagland, E., Rothman, M. 1998. Operational aspects on foaming in digesters caused by Micothrix parvicella. Water Science and Technology, 38(8-9), 29-34.

Wiltsee, G. 1998. Waste grease resources in 30 US metropolitan areas. *Proceedings of the Bioenergy '98 Conference*, October 4-8, 1998, Madison, WI. pp. 956-963.

Wittmann C, Z.A.P.D.W.D. 1995. Growth inhibition by ammonia and use of a pH-controlled feeding strategy for the effective cultivation of Mycobacterium chlorophenolicum. Applied Microbiology and Biotechnology, 44(3-4), 3-4.

Woon, S., Othman, M. 2012. Anaerobic Digestion of Meat Wastes. TVS (mg/l), 49971(13417), 9028.

Xie, S., Frost, J.P., Lawlor, P.G., Wu, G., Zhan, X. 2011. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. Bioresource Technology, 102(19), 8748-8755.

Yang, X., Wang, X., Wang, L. 2010. Transferring of components and energy output in industrial sewage sludge disposal by thermal pretreatment and two-phase anaerobic process. Bioresource Technology, 101(8), 2580-2584.

Yen, H.-W., Brune, D.E. 2007. Anaerobic co-digestion of algal sludge and waste paper to produce methane. Bioresource Technology, 98(1), 130-134.

Yenigün, O., Demirel, B. 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochemistry, 48(5), 901-911.

Yi, H., Han, Y., Zhuo, Y. 2013. Effect of Combined Pretreatment of Waste Activated Sludge for Anaerobic Digestion Process. Procedia Environmental Sciences, 18(0), 716-721.

Zeb, A. 1998. Possibilities and limitations of feeding rapesseed meal to broiler chicks, Niedersächsische Staats-und Universitätsbibliothek Göttingen.

Zhang, C., Xiao, G., Peng, L., Su, H., Tan, T. 2013. The anaerobic co-digestion of food waste and cattle manure. Bioresource Technology, 129(0), 170-176.

Zhi, Y., Zhou, C. 2011. An Effective Way of Anaerobic Digestion of Chicken Manure with Plant Biomass. *Nanotechology Conference and Expo 2011*, June 13 - 16.

Zhong, W., Zhang, Z., Luo, Y., Sun, S., Qiao, W., Xiao, M. 2011a. Effect of biological pretreatments in enhancing corn straw biogas production. Bioresource Technology, 102(24), 11177-11182.

Zhong, W., Zhang, Z., Qiao, W., Fu, P., Liu, M. 2011b. Comparison of chemical and biological pretreatment of corn straw for biogas production by anaerobic digestion. Renewable Energy, 36(6), 1875-1879.

Zoetemeyer, R.J., van den Heuvel, J.C., Cohen, A. 1982. pH influence on acidogenic dissimilation of glucose in an anaerobic digestor. Water Research, 16(3), 303-311.

Zumstein, E., Moletta, R., Godon, J. 2000. Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. Environmental Microbiology, 2(1), 69-78.

APPENDIX A: A SUMMARY OF THE PRETREATMENT ARTICLES FOR ANALYSIS

TABLE A.1: A summary of pretreatment results for food waste (kJ of electrical energy)

	Pretreatment Type	Sample	Net CH4 (L) (out - in)	Energy In (kJ)	Energy Out (kJ)	Energy Balance (out - in) (kJ)	E_I/E_O	Author(s)
Food waste and organic fraction of municipal solid waste	Chemical	Lime	1	0	11	11	0.00	(López Torres & Espinosa Lloréns 2008)
		H_2O_2	0.43	0	3.59	3.59	0.00	Shahriari et al. (2012)
		NaOH	0.01	0	23	23	0.00	Wang et al. (2009)
	Mechanical	Sonicated	0.14	31	4.92	-26	6	(Luste et al. 2009)
		Sonicated	10	0	137	137	0.00	(Elbeshbishy & Nakhla 2011)
		III - 1000; Bead Mill[a]	5030	0	69	69	0.00	(Izumi et al. 2010)
	Thermal	175°C	0.23	32	3.14	-29	10	(Marin et al. 2010)
		Microwave	0.65	120	5.43	-115	22	(Beszédes et al. 2011)
		175°C	0.07	17	1.00	-16	17	(Liu et al. 2012)
		175°C	0.15	60	2.11	-58	28	(Liu et al. 2012)
		170°C	0.01	55	24	-31	2	Wang et al. (2009)
	Thermo-chemical	85°C, H_2O_2	0.50	34	4.15	-30	8	Shahriari et al. (2012)
		130°C, 4gNaOH	0.02	40	42	1.79	1	Wang et al. (2009)

192

TABLE A. 2: A summary of pretreatment results for waste activated sludge (kJ of electrical energy)

Pretreatment Type	Sample	Net CH4 (L) (out - in)	Energy In (kJ)	Energy Out (kJ)	Energy Balance (out - in) (kJ)	E_I/E_O	Author(s)
Chemical	7g/L NaOH	0.85	0	12	12	0.00	Kim et al. (2003)
	H_2O_2 and $FeCL_2$	0.48	0	6.54	6.54	0.00	(Dhar et al. 2011)
	37% HCL	0.15	0	2.02	2.02	0.00	(Devlin et al. 2011)
Mechanical	Sonicated	0.90	504	12	-492	41	Kim et al. (2003)
	Pump	0.47	26	6.42	-20	4	Dhar et al. (2011)
	Sonicated	0.58	360	7.88	-352	46	(Apul & Sanin 2010)
Thermal	70°C	0.01	191	0.18	-191	1073	(Kuglarz et al. 2013)
	Microwave	0.24	35	1.92	-33	18	(Eskicioglu et al. 2006)
	121°C	1.02	78	14	-64	6	Kim et al. (2003)
Thermo-chemical	121°C,7g/L NaOH	1.01	78	14	-64	6	Kim et al. (2003)
	130°C, pH10 (KOH)	0.05	20	0.72	-19	27	(Bougrier et al. 2006)
	90°C, H_2O_2 + $FeSO_4$	0.06	6	0.51	-5.35	12	(Valo et al. 2004)

Waste activated sludge

TABLE A.3: A summary of pretreatment results for lignocellulosic material (kJ of electrical energy)

Pretreatment Type	Sample	Net CH4 (L) (out - in)	Energy In (kJ)	Energy Out (kJ)	Energy Balance (out - in) (kJ)	E_I/E_O	Author(s)
Biological	Mn Peroxidase	0.05	0	0.66	0.66	0.00	(Frigon et al. 2012)
	P. florida 50g/L	12	0	97	97	0.00	(Frigon et al. 2012)
	Novozyme (N342)	0.68	0	5.39	5.39	0.00	(Romano et al. 2009)
Chemical	NaOH	0.06	0	0.76	0.76	0.00	(Frigon et al. 2012)
	8% NaOH	2.32	0	32	32	0.00	(Taherdanak & Zilouei 2014)
	3% NaOH	0.06	0	0.85	0.85	0.00	(Chandra et al. 2012)
Mechanical	Sonicated	0.33	6	4.52	-1.11	1	(Fernández-Cegrí et al. 2012)
	Chopped	0.02	150	0.28	-150	539	(Frigon et al. 2012)
	Cut[a]		108000	1947600	1839600	0.06	(Menardo et al. 2012)
Thermal	180°C	0.04	34	0.85	-33	40	Jackpwiak_2011
	120°C	0.06	59	0.76	-58	78	(Antonopoulou et al. 2010)
	90°C[a]		198000	1558800	1360800	0.13	Menardo et al. (2012)
Thermo-chemical	120°C, Ammonium	1.34	41	18	-23	2	(Fernandes et al. 2009)
	85°C, Ca(OH)2	0.71	29	10	-19	3	Fernandes et al. (2009)
	100°C, 7.5%NaOH	9.05	4	124	120	0.03	(Xie et al. 2011)

Lignocellulosic feedstocks (grass and straw)

[a] **Energy values provided by authors**

TABLE A.4: A summary of pretreatment results for waste activated sludge (kJ of electrical energy)

| Pretreatment Type	Sample	Net CH4 (L) (out - in)	Energy In (kJ)	Energy Out (kJ)	Energy Balance (out - in) (kJ)	E_I/E_O	Author(s)
Chemical	Alkaline	7.81	13	107	93	0.13	(González-Fernández et al. 2008)
	5% Ca(OH)$_2$	0.02	0	0.19	0.19	0.00	(Rafique et al. 2010)
	NaOH, pH 10	0.10	0	1.31	1.31	0.00	(Carrère et al. 2009)
Mechanical	Sieved Solids	10	0	135	135	0.00	González-Fernández et al. (2008)
	Sonicated	731	9	10	0.69	1	(Elbeshbishy et al. 2011)
	Sonicated	775	38	6479	6441	0.01	(Castrillón et al. 2011)
Thermal	170°C	0.24	28	3.26	-24	8	(Qiao et al. 2010)
	170°C	2.22	177	30	-146	6	González-Fernández et al. (2008)
	80°C	0.56	13	7.68	-6	2	(Bonmati et al. 2001)
Thermo-chemical	Ca(OH)$_2$, 90°C	0.05	280	0.62	-279	451	(Costa et al. 2012)
	100°C, 5% Ca(OH)$_2$	0.04	8.17	0.33	-8	25	Rafique et al. (2010)
	190°C, pH10	0.14	15	1.92	-13	8	Carrère et al. (2009)
Biological	C. cellulolyticum	0.87	0	12	12	0.00	Costa et al. (2012)

Manure

APPENDIX B: ATA GRAPHS
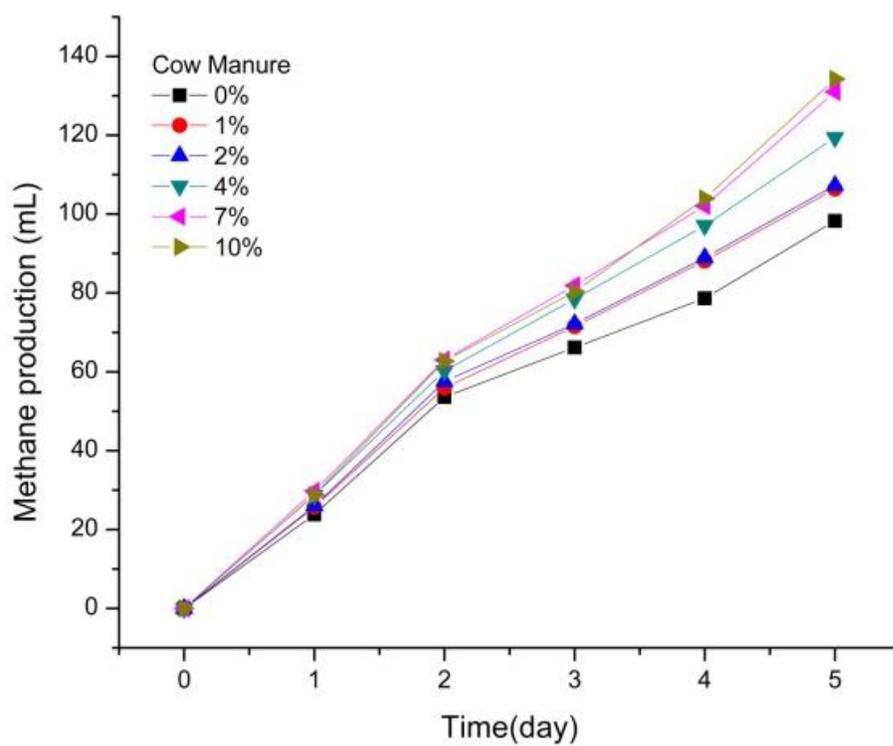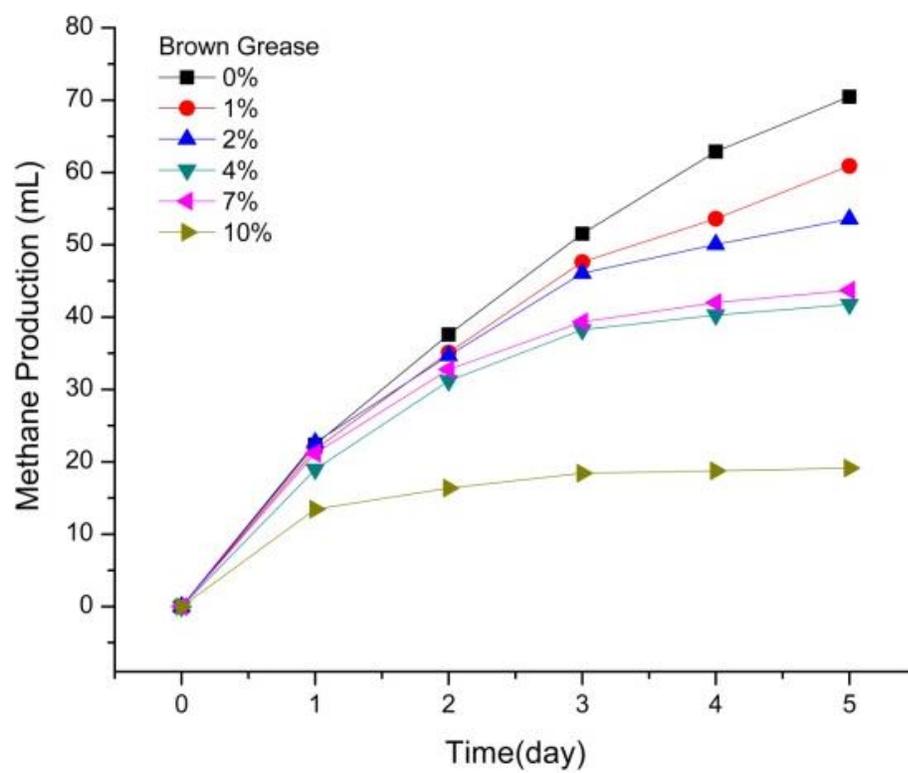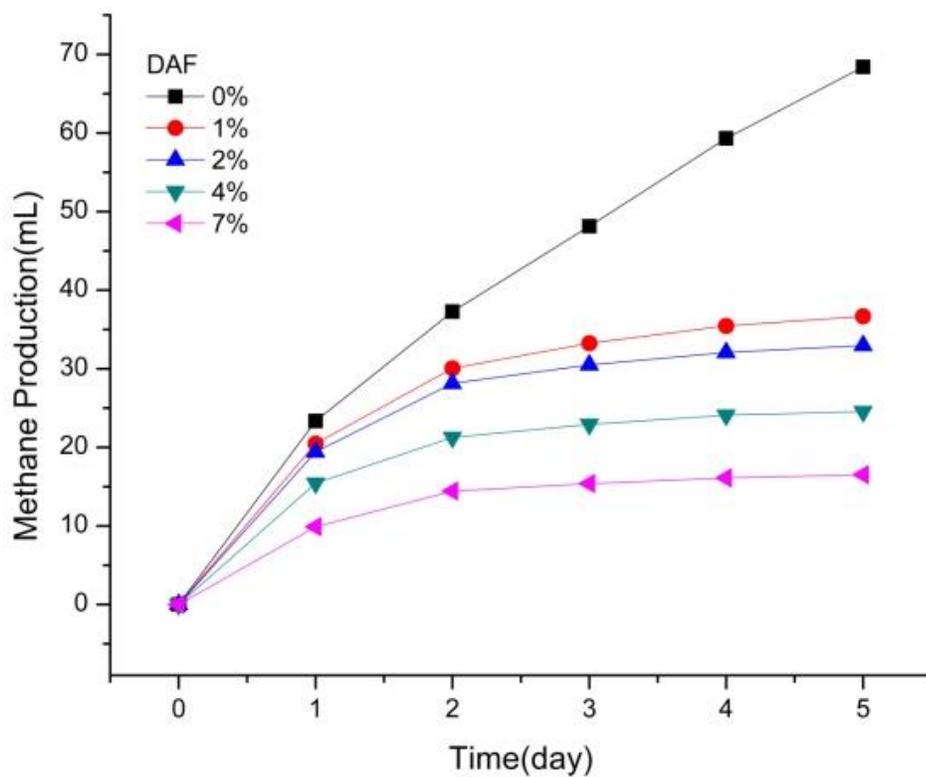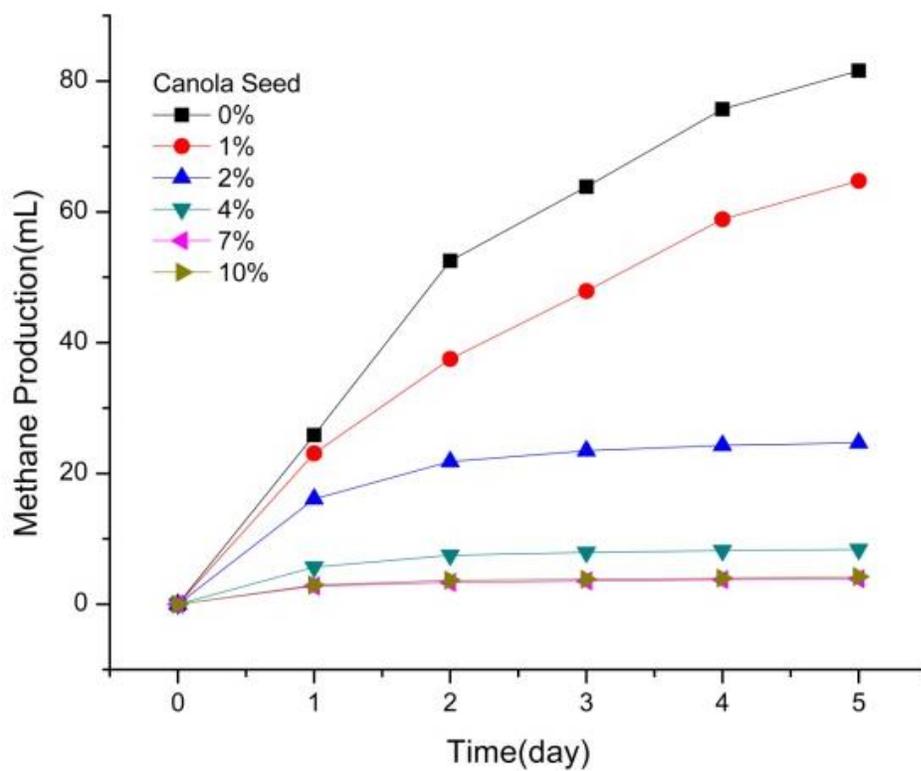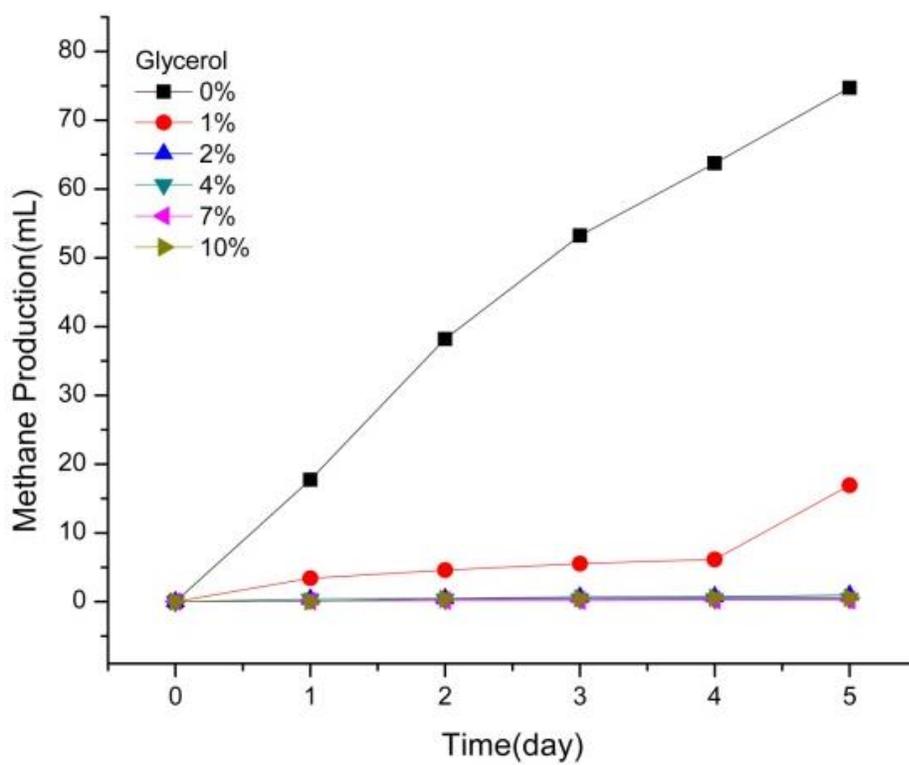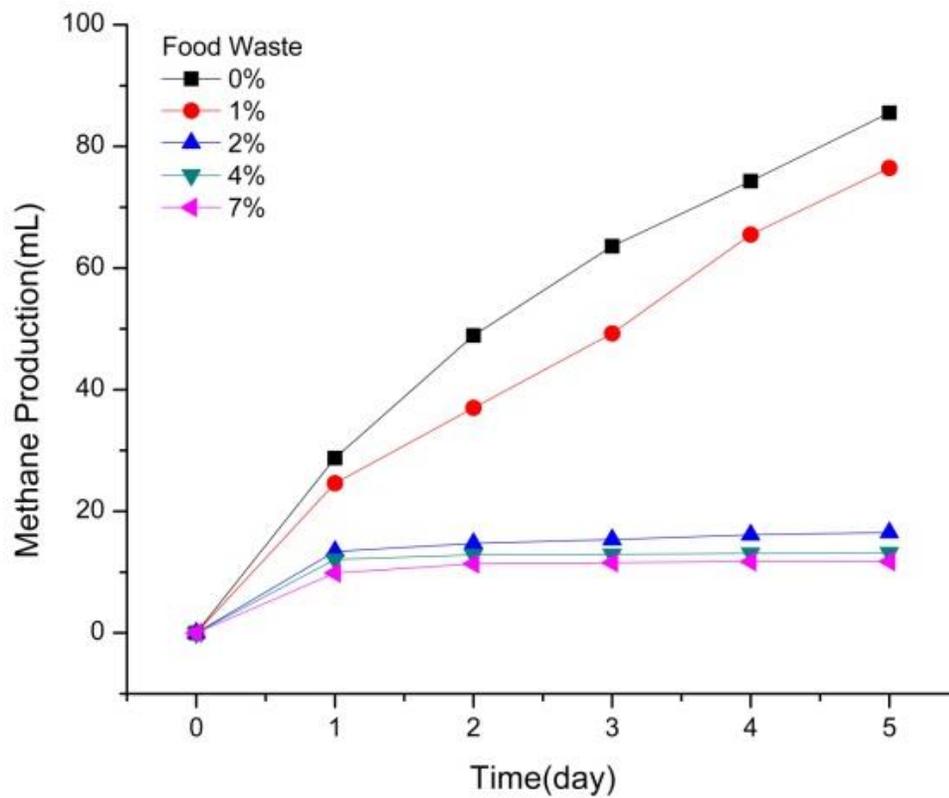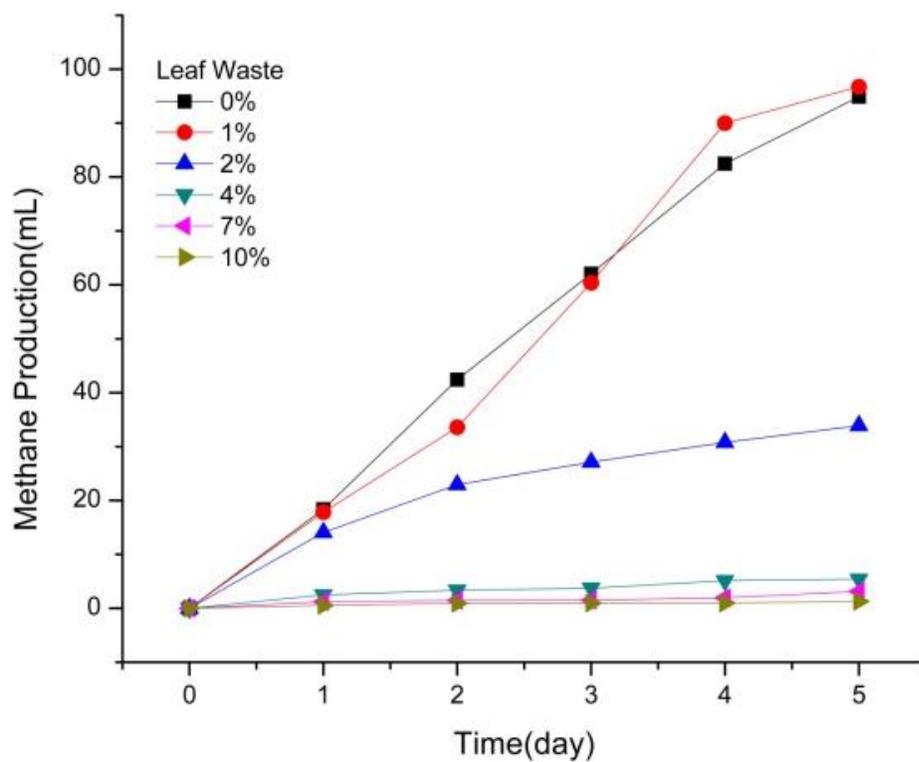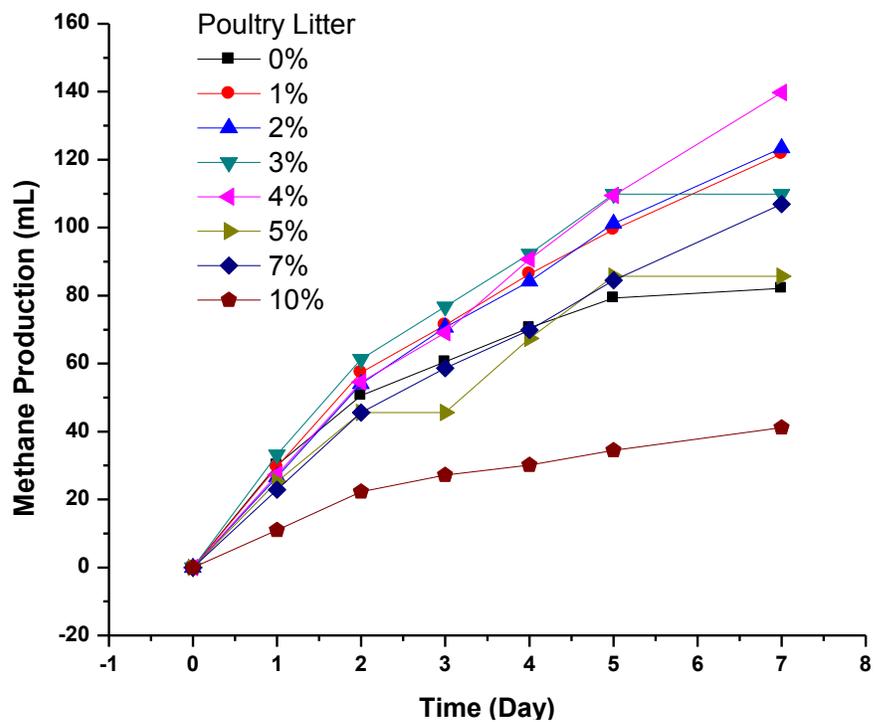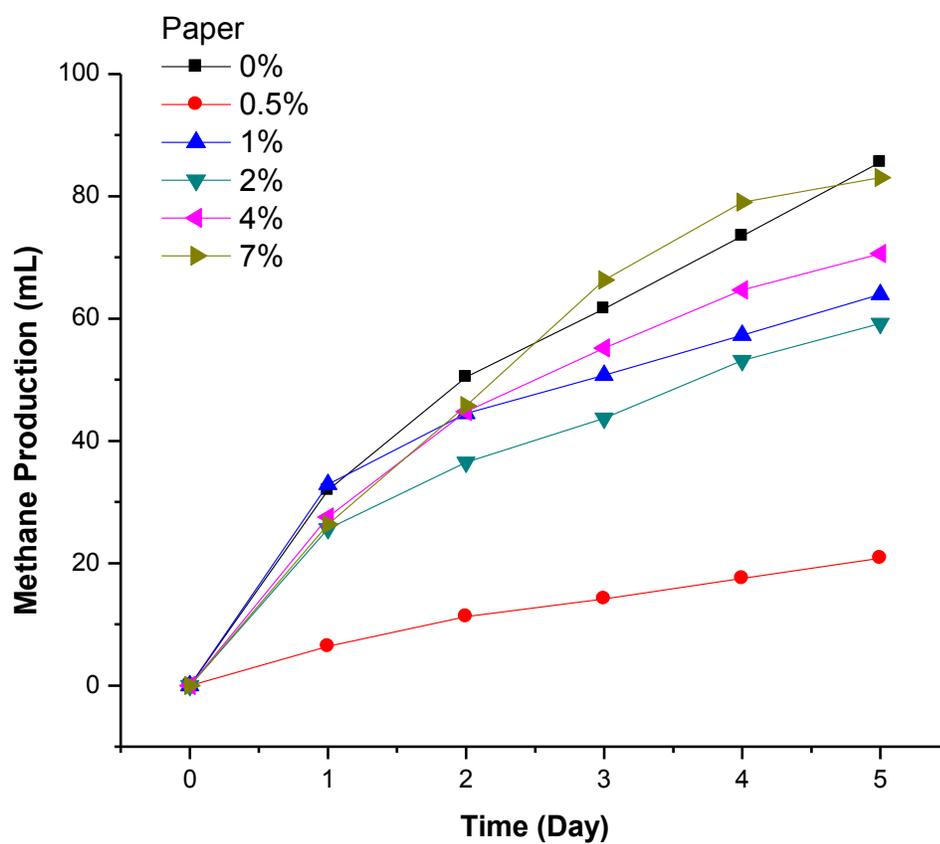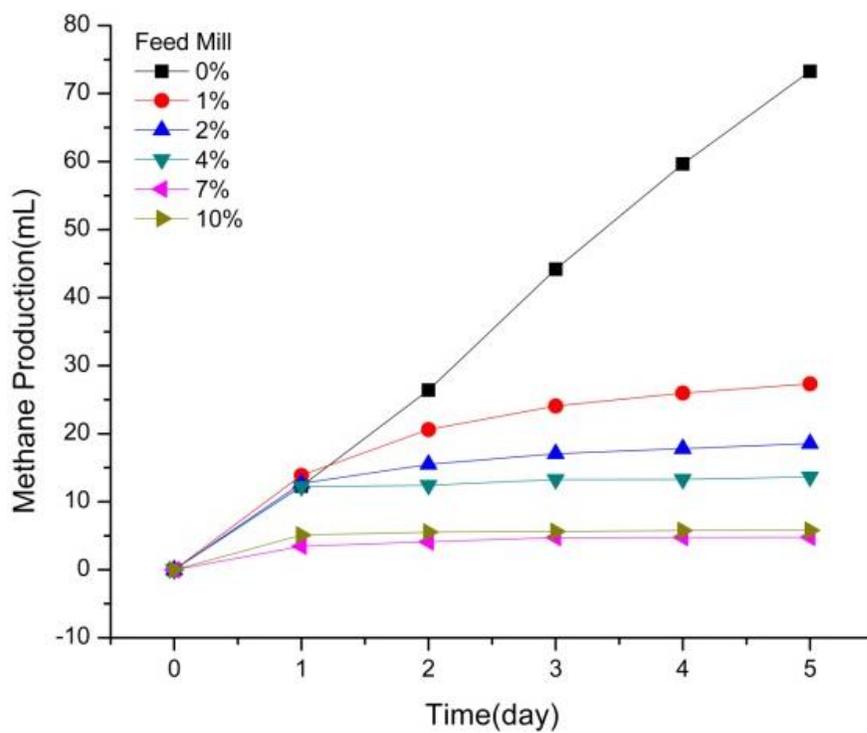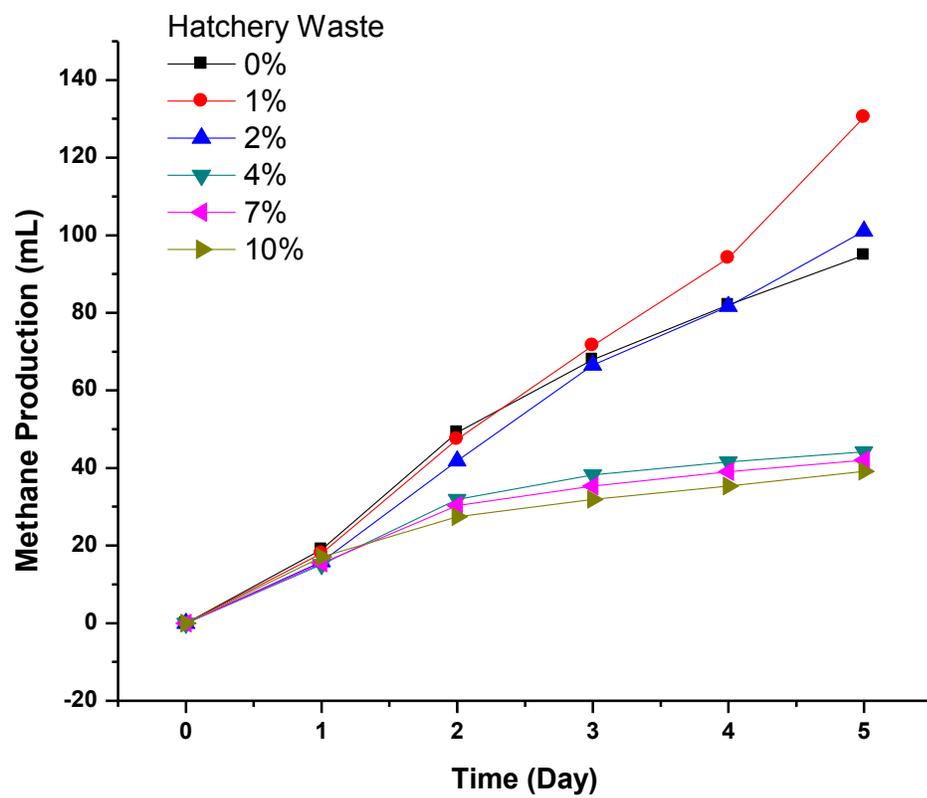
APPENDIX C: GUIDELINES FOR BATCH (INCLUDING BMP) AND SEMI-CONTINUOUS FLOW ANAEROBIC DIGESTER FEEDSTOCK TESTING

- Obtain representative samples for testing. Consider using composite samples or surrogate mixes for highly variable or heterogeneous samples (e.g. grease interceptor wastes, food waste).

- Store samples such that potential degradation is minimized. If samples are changing during storage, this can appear as a treatment change if subsamples are used several months apart for testing.

- Perform characteristic tests on the substrates, including
    o Total solids

    o Volatile solids

    o Total carbon and total nitrogen (for C:N ratio calculation)

- Biochemical methane potential (BMP) tests are most useful for comparing a set of samples that are tested against each other at the same time. A material's performance in codigestion will not necessarily be reflected in its BMP performance as an individual substrate.

    o Use replicates (triplicates or more are recommended) and perform statistical analyses

    o Consider C:N ratio, F:M ratio, percent total solids of feedstock slurry when designing BMPs and when comparing results across laboratories. Include test duration when comparing results across laboratories.

    o Measure CH_4 composition; biogas production does not mean that CH_4 has been produced

    o Use the best gas measurement equipment available. Small errors in gas volume measurements are magnified when multiplied by gas concentration levels, which tend to be variable day-to-day.

    o For batch studies and BMP tests, use controls that receive inoculum but no sample to assess CH_4 produce by the seed alone

    o Subtract CH_4 produced by the seed from that produced by test samples to calculate the CH_4 due to feedstock alone.

    o Normalize the feedstock CH_4 production for the amount of material tested: e.g. express it as a *yield* by dividing CH_4 produced by the grams VS added

- o Report testing parameters along with VS loading and incubation time (minimum of 30 d)

- Conduct anaerobic toxicity assays (ATA) to reveal problems such as rapid acidification or potential toxicities. A material's performance in codigestion will not necessarily be reflected in its ATA performance as an individual substrate. ATAs may be repeated on samples that rapidly acidified but with the addition of alkalinity to test for toxicities beyond those due to acidification.

- Semi-continuous reactors are useful for comparing how a mixture may perform in a full-scale reactor, though not scalable, revealing possible problems such as mixing, foaming, and reactor failure. Consider reporting and testing for the same parameters as suggested for BMPs and batch reactors, especially replication.