8-1-2012

Effects of Moisture Augmentation of Municipal Solid Waste Through Addition of Food Waste or Wastewater Treatment Biosolids on Bio-Gas Formation for Power Generation

Jared Michael Gore
University of Nevada, Las Vegas, jaredgore@gmail.com

EFFECTS OF MOISTURE AUGMENTATION OF MUNICIPAL

SOLID WASTE THROUGH ADDITION OF FOOD

WASTE OR WASTEWATER TREATMENT

BIOSOLIDS ON BIO-GAS FORMATION

FOR POWER GENERATION

By

Jared Michael Gore

Bachelor of Science in Civil Engineering
University of Nevada, Las Vegas
2002

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science in Engineering

Department of Civil and Environmental Engineering
Howard R. Hughes College of Engineering
The Graduate College

University of Nevada, Las Vegas

August 2012

We recommend the thesis prepared under our supervision by

Jared Gore

entitled

Effects of Moisture Augmentation of Municipal Solid Waste Through Addition of Food Waste or Wastewater Treatment Biosolids on Bio-Gas Formation for Power Generation

be accepted in partial fulfillment of the requirements for the degree of

Master of Science in Engineering
Department of Civil and Environmental Engineering

Jacimaria Batista, Committee Chair

David James, Committee Member

Thomas Piechota, Committee Member

Brian Hedlund, Graduate College Representative

Ronald Smith, Ph. D., Vice President for Research and Graduate Studies
and Dean of the Graduate College

August 2012

ABSTRACT

An investigation into the effect of moisture augmentation by manipulation of food waste proportion or wastewater treatment plant biosolids proportion was undertaken to determine the effects on production of methane and other biogases from municipal solid waste (MSW). Laboratory microcosm experiments were performed to determine the effect of various proportions of influent waste streams on the production of biogas. Results indicated that moisture augmentation through the addition of food waste to MSW increases the overall bio-gas and hydrogen gas formed during fermentation. Moisture augmentation through addition of wastewater treatment bio-solids lead to inconclusive results. Addition of food waste to MSW would allow for an increase in combustible gas production through formation of additional hydrogen gas in arid region landfills.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

PROBLEM STATEMENT AND OBJECTIVES

Biodegradation of municipal solid waste in landfills has been well studied for

more than 30-years. Typical biodegradation pathways have been identified along

with respective biogas and leachate products at each stage of degradation. Research

into methane formation and control has gone in two opposing directions,

enhancement and reduction. As the main gaseous products of solid waste

degradation in landfills are carbon dioxide gas and methane gas, both identified as

potent greenhouse gases (IPCC, 1996), researchers have sought to reduce their

production, especially methane, and thus reduce the impact that landfills have on

global warming. Reduction of methane emissions is achieved using aerobic decay

mechanisms (Read et al., 2001, Fricke et al., 2005, Lou et al., 2009, Erses et al.,

2007). Aerobic degradation ideally does not lead to the production of methane gas,

leaving carbon dioxide gas as the main gaseous product. Aerobic degradation is

generally implemented through air injection or composting of waste matter. Air can

be injected into a landfill to inhibit the onset of anaerobic decay; this also causes the

overall rate of biodegradation to increase. Composting of waste allows for aerobic

biodegradation to occur as the waste is turned and mixed thus exposing the waste to

oxygen in the air. Enhancement of methane production in landfills is associated with

its subsequent use for power generation or heating, with final end products of energy,

water, and carbon dioxide (Themelis et al., 2006). Recent research has also

identified hydrogen gas production during waste fermentation as an important source

of clean energy (Dong et al., 2009). The organic fraction of MSW can be an

important source of hydrogen gas. Many of the parameters affecting gas generation

and leachate formation have been identified (Meima et al., 2008, Komilis et al., 1999, Barlaz et al., 1996). Of most importance to waste degradation and formation of biogas are moisture content, leachate pH, rate of hydrolysis, and waste temperature.

Biogas production in municipal solid waste (MSW) landfills requires moisture content above 20% (wt/wt) to drive waste biodegradation by microorganisms that contribute to methane production (Meima et al, 2008). In addition to the inherent moisture within MSW, wastes disposed of in landfills located in areas of the country with more reliable rainfall receive supplemental moisture helping to drive anaerobic biodegradation of waste matter. In the arid southwest, there is little reliable rainfall to bolster the moisture content of waste within the landfill, which is thought to cause slower rates of waste degradation and less production of methane for power generation. Present research does not directly address waste decomposition, biogas formation, and methane generation potential in arid region landfills. Many researchers have studied different methods of increasing moisture content through addition of potable water directly to the waste or addition of a non-potable water source (Sanphoti et al., 2006, Alkaabi et al., 2009). Water is a valuable commodity in arid regions of the country, and the addition of water from sources other than precipitation infiltration to increase methane production would be a considerable waste. Moisture content is a key factor effecting the growth of microorganisms responsible for biodegradation within a landfill; low moisture content can inhibit the growth of degrading microorganisms and thus completely stop production of biogas (MacLeod et al., 2008).

Little has been reported on waste degradation and biogas formation in low moisture conditions or arid climates. Experiments have been performed on solid waste extracted from a German landfill to develop a model to estimate methane formation at different moisture contents ranging from 27% to 84% (Mora-Naranjo et al., 2004). Typically, waste degradation studies performed in laboratories utilize high moisture conditions to model the degradation of waste materials, production of bio-gases and leachate formation. This is generally performed to mimic moisture conditions found in wetter climates, to study the effect of amplified moisture conditions, or to accelerate degradation rates (Filipkowaska et al., 2004, He et al., 2005, Valencia et al., 2008, Hernandez-Berriel et al., 2008, Sanphoti et al., 2006, and others). Other studies performed in arid regions have focused on leachate quality and give little applicable information on biogas formation or waste degradation. Studies on a landfill located in arid Kuwait did not address gas formation and the results are generally not applicable due to the large amount of liquid wastes disposed of at the landfill (Yaqout et al., 2003). Research performed herein addresses the effects of moisture augmentation with high moisture content wastes namely food waste and biosolids from wastewater treatment, on biogas formation in arid region landfills.

Numerous studies have been performed analyzing microbial populations responsible for methane production within landfills (Laloui-Carpenter et al., 2006, Staley et al., 2011, Chen et al., 2003, and others). These studies have identified archaea responsible for methane production from acetate and hydrogen gas through genetic analysis and fluorescent in-situ hybridization. It has been shown that archaea

3

present in landfills throughout the world are genetically similar. Research has also

been undertaken identifying the relative proportion of acetotrophic and

hydrogenotrophic archaea present within landfills (Laloui-Carpenter et al., 2006).

These two metabolic pathways are responsible for the majority of methane

production within the landfill environment. End products of the two main metabolic

pathways are methane and carbon dioxide gas for acetotrophic archaea and methane

for hydrogenotrophic archaea. Ideally, degradation end products would include

methane alone without significant release of carbon dioxide; this would give a

greater energy potential to the biogas formed within the landfill and lessen the

impact on the environment. The ratio of organisms with the two main metabolic

pathways should be indicative of the ratio of gases formed during methanogenesis.

Organisms responsible for hydrogen production have also been identified (Dong et

al., 2008, Lay et al., 2009, Karadag & Puhakka, 2010, and others). The most

productive organisms have been identified as belonging to the genus *Clostridium.*

Several species have been identified in various studies and appear to be ubiquitous in

the environment.

The Las Vegas Valley in Nevada has become a large urban center of the

Southwestern United States in the last several decades, with a population of over 1.9

Million (U.S. Census, 2010). The Las Vegas Valley produces more than 11,000 tons

of municipal solid waste every day. The majority of this waste is disposed of at the

Apex Landfill located northeast of the Las Vegas Valley. Along with municipal

solid waste (MSW) (from homes, businesses and industry) are two other streams of

waste, bio-solids from local wastewater treatment plants and food waste from local

casinos. At present, food waste and wastewater bio-solids make up a relatively small proportion of the overall waste produced in the United States (EPA, 2010). Over time, as recycling rates increase, the proportion of food waste and wastewater bio-solids in waste will increase as paper, plastic, and metal content is reduced. Recycling programs in Clark County aim to increase the amount of overall waste diverted from the landfill to 35% from a level of only 10.9% in 2000 (Tellus Institute, 2002). The Las Vegas Valley generates approximately 450 tons of wastewater biosolids daily; these biosolids are disposed of at the Apex landfill (personal communication with CCWRD staff). The biosolids have a high moisture content, at around 70%; the solid portion consists of 7-30% grease and fats, 20-30% proteins, 8-14% cellulose, 15-20% silica, 2-4% iron, 0.8-2.8% phosphorus, and 1.5-4% nitrogen (Metcalf and Eddy, 2003). Wastewater biosolids provide a significant amount of moisture as well as biodegradable organic matter contributing to biogas formation.

Despite the lack of additional moisture from rainfall, the Apex landfill produces methane. Bio-gases produced at the Apex Landfill are currently not used for power generation; excess methane gas released from the Apex Landfill is burned in lieu of releasing methane directly to the atmosphere. This method of off gas disposal does reduce the landfill's impact on the environment, as carbon dioxide has less of a green house effect than methane (IPCC, 1996), but produces little benefit. Utilizing this source of energy to produce electricity is a simple way to benefit from a usually wasted resource. Recently, Republic Services, the waste hauler for southern Nevada has joined with Nevada Energy to harvest, treat, and use landfill

gas to produce electricity

(http://www.republicservices.com/Corporate/MediaRoom/landfill-renewable-energy-facility.aspx). Maintaining adequate moisture content at Apex is vital to assure sufficient methane is generated.

In this research, experiments have been formulated to determine the effects of moisture augmentation through addition of food waste or biosolids from wastewater treatment to typical MSW. In addition, Fluorescent in-situ Hybridization (FISH) is used to determine the ratio of archaea and bacteria within the experimental vessels. The specific objectives of this research were:

1) Determine the effect of moisture augmentation by manipulation of food waste content on degradation of municipal solid waste and bio-gas production in arid regions.

2) Determine the effect of moisture augmentation by manipulation of wastewater treatment plant biosolids content on municipal solid waste degradation and bio-gas production.

3) Investigate the proportion of hydrogenotrophic and acetotrophic methanogens present in experimental reactors.

.

CHAPTER 2

STATE OF KNOWLEDGE

2.1 Background Introduction

Several key parameters have been identified as contributing factors to the

degradation of solid waste and methane production. Moisture content, temperature,

pH and rate of hydrolysis have been identified as having the greatest effect on waste

degradation and methane production. Methods of controlling the various parameters

effecting waste degradation and biogas formation have been studied and

implemented in modern landfill operation to increase the rate of degradation and

methane production (Komilis et al., 1999, Pacey et al., 1998). Laboratory

experiments performed to analyze the effect of various parameters of waste

degradation have relied on accelerating the rate of decomposition to reach results in a

shorter time period (Barlaz et al, 2002). Typically, leachate recirculation is used

with initial water and nutrient addition to accelerate degradation in laboratory scale

experiments (Barlaz et al., 1991, Sanphoti et al., 2003, He et al., 2005, Valencia et

al., 2008, and others). Modern landfills are designed and operated as bioreactors;

degradation and methane production are enhanced through recirculation of leachate

with and/or without chemical or biological modification (Pacey et al., 1996).

Significant research has also been conducted on the composition of microbial

communities present during waste degradation (McDonald et al., 2009, Sawamura et

al., 2009, McDonald et al., 2008, Barlaz et al., 1991, Luton et al., 2002, and others).

Several study methods have been employed to identify and enumerate the different

microbes responsible for the degradation of solid waste in landfills and other similar

environments (Barlaz et al., 1996). Genetic studies of microbes present in landfill environments have shown the presence of eukaryotes, bacteria, and archaea within landfills (MacDonald et al., 2009). Other studies (Sawamura et al., 2009, Luton et al., 2002, and others) have shown that archaea responsible for methane production in different landfills around the world are genetically similar. Culture-independent methods are generally enlisted to allow identification of community members that are difficult to grow and isolate pure culture; often polymerase chain reaction (PCR) and subsequent analysis of DNA, fluorescence in-situ hybridization (FISH), or lipid analyses are performed to study the microbial communities present in landfill samples.

2.2 Biodegradation Process

Biodegradation in landfills goes through four primary phases prior to reaching a stabilized state. Degradation phases include initial aerobic degradation, anaerobic fermentation and acidogenesis, high rate methanogenesis, and final declining methanogenesis (Barlaz et al, 1996).

2.2.1 Initial Degradation

Initially, oxygen trapped in voids within the landfill is utilized by microorganisms to oxidize readily degradable matter, mainly components of food waste. Readily degradable waste is easily hydrolyzed by microbes present in the landfill. Sugars found in food waste can be readily utilized by microbes, while proteins, fats, and longer chain carbohydrates require microbes to exude extracellular enzymes to break down this matter into smaller molecules capable of being

metabolized (Barlaz et al., 1996). This phase of degradation is usually short and marked by production of carbon dioxide gas and depletion of oxygen. Also included in the initial degradation phase is nitrate reduction; although this is considered anaerobic respiration, nitrate must be consumed prior to subsequent degradation stages. Microbes present in many environments often have the ability to use multiple electron acceptors in their metabolic functions (Madigan et al., 2008). Microbes utilize higher energy electron acceptors like oxygen and nitrate prior to initiating fermentation and utilizing lower energy electron acceptors.

2.2.2   Fermentation

Fermentation of readily degradable matter begins to occur after consumption of the majority of oxygen and nitrate. Fermenting microorganisms and cellulose degraders begin to hydrolyze more complex organic matter and consume the hydrolysis products which are readily absorbed and utilized. Volatile fatty acids, carbon dioxide, ethanol, lactate, and hydrogen gas are among the products created from microbial fermentation of organic waste. Throughout initial fermentation, pH drops due to the production of fatty acids and depletion of alkalinity. The pH can drop to between 5 and 6, which can inhibit further microbial growth and waste degradation. Bacteria responsible for the consumption of many fatty acids are dependent on the presence of symbiotic organisms to reduce the concentration of the associated degradation products (Voopali et al., 1999). Degradation of butyrate and propionate by bacteria is generally unfavorable from an energy standpoint. Energy can only be obtained from degradation of butyrate and propionate when degradation products, hydrogen gas and acetate, are at low concentrations. If methanogenic

archaea are not present in sufficient amounts to consume hydrogen gas and acetate, these products build up within the landfill and inhibit further microbial growth and waste degradation. Fermenters and methanogenic archaea can both be inhibited by low pH. Recently, an archaeon has been identified that is capable of growing in low pH environments (Barlaz et al., 2011). This archaeon consumes organic acids within the landfill and produces methane; this process begins to increase the pH of the system and allows for the growth of other methanogenic archaea aiding the transition from acidogenesis to methanogenesis.

2.2.3   Methanogenesis

Methanogenesis then starts as other methanogenic archaea establish populations capable of utilizing sufficient amounts of hydrogen, acetate and other fermentation products. Reduction of the concentration of hydrogen and acetate increases degradation rates of fatty acids like propionate and butyrate by bacteria. During the high rate methanogenic stage, archaea utilize acetate, formate, or hydrogen and carbon dioxide along with other single carbon substrates to create methane gas; the pH continues to rise to about 7.0. This stage of degradation continues until the majority of the readily degradable organic matter is consumed. When there is little degradable matter left, the final stage methanogenesis starts. This stage of degradation is marked by trailing amounts of methane gas formation which diminish over time as remaining waste becomes less and less degradable. A landfill is generally considered stabilized at this point. Figure 1 depicts conversion pathways of organic matter to methane and other products through biodegradation.

Cellulose, Protein and other complex organics

Hydrolysis/Saccharification

Cellulose degrading bacteria

Simple sugars, Amino acids and other simple organics

Fermenting microorganisms

Fermentation

H_2 and CO_2

Fermentation

Fermenting microorganisms

Propionate, Butyrate, and other carboxylic acids and alcohols

Fermentation

Fermenting microorganisms

Acetate

Acetogenesis

Acetogens

Methanogenesis

Methanogenic archaea

Methane

Methanogenesis

Methanogenic archaea

Figure 1. Biodegradation Pathway to Methane Formation, modified from Barlaz et al., 1996

2.3 Parameter Sensitivity

Sensitivity analysis of the several parameters affecting the degradation process identified moisture content, leachate pH, temperature, and rate of hydrolysis as main factors in the overall degradation rate in anaerobic environments such as landfills (Meima et al, 2008). The rate of methanogenesis is also a significant factor, but less so than the others.

2.3.1   Moisture Content

Moisture content of waste in a landfill is the most important parameter involved in the degradation process. Loss of moisture during the degradation process can completely stop biological activity short of complete stabilization (MacLeod et al, 2007). Analysis of a landfill with low biological activities showed

that large amounts of biological oxygen demand (BOD) and chemical oxygen demand (COD) remained, but the moisture content of the waste had dropped below 20% thus inhibiting further biological decay. Other sources confirm that biological activity is limited by moisture content; biological activity has been measured occurring at moisture contents of 24%, but no activity was found at 16% (Danhamer et al, 1998). Biological activity increases with moisture content up to a limit that is slightly below complete saturation. Some suggest that adding water to landfills will further enhance the rate of degradation and increase methane production (Pacey et al, 96). Other sources compiled by Komilis et al, 1999, come to conflicting conclusions about the addition of water. Laboratory studies have shown that adding water increases the rate of degradation well beyond leachate recirculation alone (Sanphoti et al, 2006). In an arid region, addition of water may increase degradation rates, but would be an unacceptable use of a very valuable resource. Saline or brackish water addition to a landfill was shown to inhibit methane gas production; when wastewater treatment plant bio-solids were mixed into the leachate along with the brackish water, gas production rates went up despite the high salinity (Alkaabi et al, 2009). This would seem to indicate that brackish water could be used to increase moisture content, but operation of such a bioreactor landfill would require constant input of activated sludge to the leachate recycle to counteract the effect of increased salinity.

2.3.2   Leachate pH

Leachate pH is ideal at levels around neutral (7.0) (Barlaz et al, 1996). All of the microbial communities involved in the decay process thrive at pH 7. Actual measurements during the methanogenic stage of degradation range from between

about 6.5 to 8. Low pH associated with the acidogenic phase of degradation has an inhibitory effect on the majority of organisms responsible for waste decay (Barlaz et al, 1996).

2.3.3   Waste Temperature

Landfill temperature is mainly a function of biological activity and ambient temperature. Increases in landfill temperature can cause gas production to triple; thermophilic microorganisms are capable of degrading waste at a much greater rate (Barlaz et al, 1996). Methanogenesis by certain archaea optimally occurs in a mesophilic temperature range, ideally around 40° C; other populations operate in the thermophilic temperature range, above 50° C, and metabolize much faster. Landfills have been found to have internal temperatures ranging from around 9° C to as much as 60° C (Barlaz et al, 1996); this temperature range can be found in a single landfill indicating areas of high biological activity in hotter areas and less in colder areas.

2.3.4   Hydrolysis Rate

During the high rate methanogenic phase of degradation, the rate of hydrolysis becomes the main limiting factor. Precursors to fermentation need to be supplied in order to support the degradation process. Fermenting microbes will consume hydrolysis products as fast as cellulose-degrading bacteria can produce them. Methanogenic archaea living in symbiosis with the fermenting bacteria are able consume products of fermentation as fast as they are created. All of the microbial populations responsible for waste degradation are dependent on the amount of simple sugars and other hydrolysis products provided by the cellulose degrading

13

bacteria. As the amount of readily degradable matter falls and bacteria are forced to

feed on less degradable matter, the overall rate of decay begins to drop. This signals

the end of the high rate methanogenic stage and the beginning of the final stage of

methanogenesis.

2.4 Landfill Operation

Operating procedure can have a large effect on stabilization rate and methane

gas production in landfills. Providing leachate recirculation has been shown to

increase the rate of organic decay and increase the rate of methane production; this

type of landfill is known as a bioreactor landfill. Bioreactor landfills allow for many

of the parameters of the biodegradation process to be manipulated to achieve a

higher degradation rate and thus reach final stabilization faster. This concept was

introduced by Pohland in 1975. Leachate recirculation also serves to maintain

moisture content of the waste throughout the degradation process and increase water

contact with the waste through percolation (Komilis et al, 1999). Fermentation of

hydrolyzed cellulose and other polysaccharides creates acidic end products like

acetic acid, propionic acid, butyric acid, and other volatile fatty acids (Klass, 1984).

Neutralizing leachate during recirculation has the effect of increasing degradation

rates (Barlaz, 1990) and methane production. Methanogenic archaea perform best at

a neutral pH; numerous sources show that methanogenesis is inhibited at an acidic

pH (Meimi et al, 2008). Buffering leachate serves to reduce the length of the

acidogenic phase of landfill degradation by allowing for greater growth rates of

methanogenic archaea earlier in the degradation process. Further degradation of

higher molecular weight volatile fatty acids requires the symbiotic presence of

hydrogen gas and acetate-utilizing archaea to reduce hydrogen gas partial pressure (Klass, 1984 and Voolapalli et al, 1999). Maintaining environmental conditions favorable for methanogenic archaea is essential to increasing methane production.

2.5 Hydrogen Production

Recent research has been conducted identifying the potential of hydrogen production from municipal solid waste (Dong et al., 2008). Hydrogen has a greater energy potential than hydrocarbons with a heat of combustion of 122 kJ/g (Dong et al., 2008). Hydrogen gas is produced during bacterial fermentation of waste along with carbon dioxide and other soluble substrates. Typically, hydrogen is utilized by methanogenic archaea to produce methane, but it can be alternatively utilized by acetogenic bacteria to produce acetate. Numerous studies have been performed to determine ideal conditions for the production of hydrogen from various substrates (Lay et al., 2009, Karadag & Puhakka, 2010, Li et al., 2008, Mu et al., 2006 and others). The main conditions investigated were temperature, pH, substrate and substrate concentration. Hydrogen is produced by bacteria over a large range of temperatures; hydrogen production increases with increasing reactor temperature up to thermophilic conditions (Mu et al., 2006, Karadag & Puhakka, 2010). The greatest production of hydrogen was identified between 45° and 50° C; at greater temperatures bacterial populations change and different metabolic processes reduce the amount of hydrogen produced (Karadag & Puhukka, 2010). Hydrogen production ideally occurs at a pH between 5.5 and 6; pH above 6 can lead to establishment of methanogen populations that feed on the hydrogen and thus reduce the amount produced. Numerous substrates are capable of producing hydrogen

15

during bacterial fermentation (Dong et al., 2008). Typically, glucose is used in laboratory experiments to identify other parameters effecting hydrogen production (Karadag & Puhukka, 2010, Li et al., 2008, Mu et al., 2006). Simple carbohydrates were identified as having the greatest hydrogen production potential among various food substrates tested (Dong et al., 2008). Meat was identified as having a low potential hydrogen yield, while fats and oils produced almost no hydrogen gas. Cellulose also had a very low potential hydrogen yield. These tests were performed using sludge that had been boiled, thus removing many other microbes that could be responsible for other metabolic processes associated with degradation of cellulose, proteins, fats and oils. Hydrogen is produced in excess during the fermentation phase of degradation before adequate methanogen populations have been established that can consume the gas.

2.6 Methane Production

Biogas from landfills in later stages of degradation is mainly comprised of methane gas and carbon dioxide gas (Barlaz et al., 1996). Methane gas generated within the landfill can be collected and used as a fuel source for heating or electrical power generation. Methane and carbon dioxide are typically found in a ratio of about 55% to 45% (Themelis et al., 2007, Barlaz et al., 1996, Demibras, 2006). The proportion of methane gas to carbon dioxide gas can be as high as 70% to 30% (Meima et al., 2008). Studies performed estimating methane production from solid waste have shown that about 70-liters of methane gas can be produced from each kilogram of waste based on the EPA's 2006 waste composition analysis and component specific methane yield (Staley et al., 2009). This value corresponds with

other estimates of 40-80 liters of methane per kilogram of waste (Themelis et al., 2006, Barlaz et al., 1996).

The amount of methane generated by municipal solid waste is highly dependent on waste composition. Only the organic fraction of waste can form methane gas (Staley et al., 2009, Demibras, 2006); metals, glass, and other non-degradable components like plastics do not contribute directly to the amount of methane produced. Of the organic components of municipal solid waste, food waste has the greatest potential to form methane gas. Paper wastes also produce a large fraction of the methane formed in landfills; the Table 1 shows some of the organic components of municipal solid waste and the methane yield per kilogram (Barlaz et al., 1996). Actual gas collected from land-filled waste can be quite lower than the theoretical yield from laboratory studies due to slow degradation rates in landfill environments (Morris et al., 2003).

Table 1. Methane Yield from Various Organic Wastes, modified from Barlaz et al., 1996

Component	Methane Yield (L per kg)
Grass	144.4
Leaves	30.6
Branches	62.6
Food Waste	300.7
Office Paper	217.3
Coated Paper	84.4
Newsprint	74.3
Corrugated Boxes	152.3

Typical gas composition emanating from landfills generally transitions through four phases. Figure 2 shows the typical gas composition within a landfill over time. During aerobic degradation, Phase I, oxygen and nitrogen are the predominant gases present within the landfill; as aerobic degradation continues, oxygen is consumed and carbon dioxide is formed. Nitrogen is displaced from the

17

landfill by formation of carbon dioxide. As fermentation begins, Phase II, hydrogen is produced along with increasing amounts of carbon dioxide; nitrogen continues to be displaced by gases formed through biodegradation. At the initiation of methanogenesis, Phase III, hydrogen levels drop as it is utilized by microbes to form methane; methane levels rise and carbon dioxide levels drop until the two gases are at relatively equal levels. During methanogenesis, Phase IV, gas composition is dominated by methane and carbon dioxide. Typically, methane levels range between 45% and 60% and carbon dioxide levels range between 40% and 60%. Gas composition remains consistent until the majority of organic matter is consumed.
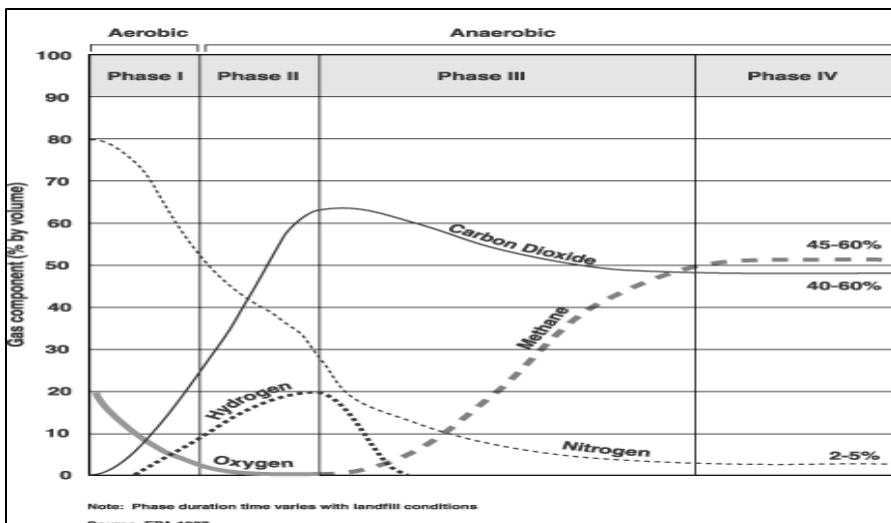
Figure 2. Typical Landfill Gas Composition, EPA 1997

2.6.1 General Degradation Equations

A number of generic chemical equations have been developed to simplify the overall degradable organic material's decomposition to methane gas and carbon dioxide gas. This allows for the estimation of total methane production from the

amount of input waste material. Table 2 shows a few of the various simplified

chemical formulae used to estimate the composition of input waste material:

Table 2. Empirical Waste Chemical Composition

Chemical Composition of Input Waste	Reference
$C_6H_{10}O_4$	Themelis et al., 2007
$C_{27}H_{41}O_{19}N$	Reichel et al., 2007
$C_{27}H_{43}O_{19}N$	Mora-Naranjo et al., 2004
$C_{13.99}H_{20.12}O_{9.5}N$	Behera et al., 2010

These formulae are coupled with main degradation products to determine basic

empirical chemical equations; Table 3 shows some empirical chemical equations

developed to determine methane yields from input waste:

Table 3. Chemical Equations for Methane Formation from MSW

Chemical Equation	Reference
$C_6H_{10}O_4 + 1.5*H_2O \rightarrow 3.25*CH_4 + 2.75*CO_2$	Themelis et al., 2007
$C_{13.99}H_{20.12}O_{9.5}N + 4.97*H_2O \rightarrow 6.76*CH_4 + 7.23*CO_2 + NH_3$	Behera et al., 2010

These equations are only a small sampling of the equations developed for the

purpose of methane gas quantification from input waste. More in-depth models have

been developed to model the degradation process of solid waste from initial

particulate substrates to final methane gas and carbon dioxide gas (Reichel et al.,

2007, Mora-Naranjo et al., 2004, and others).

2.6.2   Bio-Methane

The majority of methane is formed by archaea with one of two metabolisms,

acetotrophic and hydrogenotrophic (Demirel et al., 2008). Acetotrophic archaea

utilize acetate provided by fermenting microorganisms to produce methane and

carbon dioxide; the basic chemical equation follows:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \text{(Demirel et al., 2008)}$$
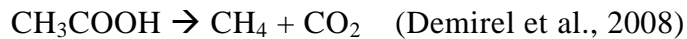
Hydrogenotrophic archaea utilize carbon dioxide and hydrogen gas provided by fermenting microorganisms to produce methane gas; the basic chemical equation follows:

$$4*H_2 + CO_2 \rightarrow CH_4 + 2*H_2O \qquad \text{(Demirel et al., 2008)}$$
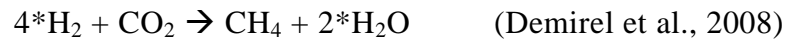
There are a number of other metabolisms utilized by archaea to produce methane; many methanogenic archaea can create methane utilizing a number of metabolic pathways (Demirel et al., 2008). Formate, methylamine, methanol, and other single carbon compounds can be converted to methane by various archaea. Acetotrophic and hydrogenotrophic metabolisms generally predominate within the landfill environment, as acetate, hydrogen and carbon dioxide are end products of fermentation by bacteria present within the landfill.

2.7 MSW Composition

The U.S. Environmental Protection Agency (EPA) creates annual reports regarding the composition of waste in the U.S. These reports detail the amount of wastes generated, recycled and sent to landfill. Data are created by estimating the production and lifespan of various products used in the U.S.; specific measurement of waste streams entering landfills is not analyzed. Material components of the various products are assumed to be disposed of at the end of their useful lifetime; portions of the materials are recycled and the remainder is sent to a landfill or incinerated. The material components of municipal solid waste are shown to fall within seven main categories including: paper and paperboard, yard trimmings, food waste, metals, glass, plastics, and "Other Wastes". "Other Wastes" are wastes that

could not be estimated using the methodology followed in the waste production

analysis utilized by the EPA in producing the data. These wastes include

construction and demolition debris and industrial wastes. Wastewater treatment bio-

solids disposal is covered in the "Other Wastes" category. Figure 3 illustrates the

composition of municipal solid waste disposed of in the U.S. in 2009.
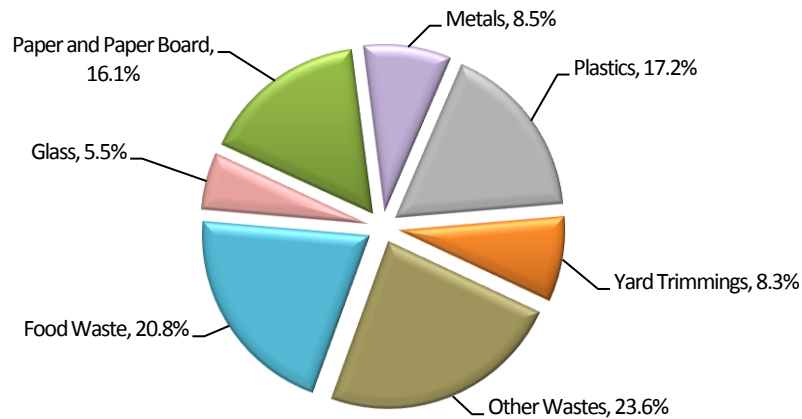
Figure 3, U.S. Typical Municipal Solid Waste Composition, Modified from Figure 13 USEPA, 2009

Figure 3 shows that food waste makes up a significant portion of degradable waste

disposed of in landfills. Other degradable organic materials are included in paper

and paperboard, yard trimmings, and the "Other Wastes" category. Approximately

69% of the total waste is at least partially comprised of biodegradable components.

Table 4 gives a breakdown of various wastes and their cellulose, hemicelluloses,

lignin and volatile solids (VS) content.

Table 4. Cellulose, Hemicellulose and Lignin Content of Paper Wastes, Modified from Barlaz et al., 1996

Component	Cellulose (%)	Hemicellulose (%)	Lignin (%)	VS (%)
Grass	26.5	10.2	28.4	85.0
Leaves	15.3	10.5	43.8	90.2
Branches	35.4	18.4	32.6	96.7
Food waste	50.8	6.7	9.9	92.0
Office Paper	87.4	8.4	2.3	98.6
Newsprint	48.5	9.0	23.9	98.5
Corrugated Boxes	57.3	9.9	20.8	98.2

Cellulose and hemicellulose content of the various wastes listed in Table 4 generally indicate their relative degradability. Food waste is comprised of other highly degradable components like carbohydrates, proteins, and fats. Lignin is generally much harder to degrade than cellulose. The EPA also provides data on the breakdown of the individual categories of waste. Plastics, paper and metals are broken down into various types. The metal category is mainly comprised of ferrous metals with a smaller portion of aluminum and the remainder is made up of all others. Paper and paper board is broken into many categories like computer paper, news paper, corrugated cardboard, magazine paper, and others. Plastics are broken into a number of different types of material such as high density polyethylene (HDPE), polyethylene terephthalate PET, poly vinyl chloride (PVC) and others.

Other studies of waste composition have shown that waste composition varies significantly between different regions of the country (Staley & Barlaz, 2009). Comparison of actual waste discarded into landfills and the EPA's waste estimation shows that the EPA overestimates some waste fractions and underestimates others. The most current EPA report from 2009 shows that food waste comprises about 20% of the overall waste, while actual waste analysis from various landfills shows that about 13.6% is food waste (Staley et al., 2009). Paper waste content from the EPA analysis is about 16% of the total waste, while an actual waste analysis shows that paper waste makes up about 35.5% of the total waste. Of the waste categories studied, food waste and paper waste have the greatest deviation between actual waste components and the EPA's estimation. Waste components in the Staley et al. study showed significant variation among the different landfills studied; some landfills

showed a good correlation with the EPA's component analysis, while others included in Staley et al.'s analysis showed significant variation from the EPA's analysis.

2.8 Landfill Microbiology

Bacteria are responsible for much of the degradation processes within a landfill; bacteria perform initial degradation steps needed for archaea to initiate methanogenesis including cellulose degradation and fermentation. Research into the microbial biota found in landfills has identified bacteria, archaea and fungus as the principal organisms responsible for waste degradation (McDonald et al., 2009). The main focus of study has been on the methanogenic microbial communities present in landfills. A number of studies have been performed analyzing archaea present in the leachate produced within landfills. One study retrieved 239 archaeal DNA sequences from a leachate sample (Laloui-Carpenter et al., 2006). The greatest fraction of DNA extracted belonged to family *Methanosaetaceae*, a group of methanogens that utilizes acetate to create methane and carbon dioxide gas. *Methanosaetaceae* DNA accounted for 65% of the archaea present within the leachate. Remaining archaea belonged to a number of different groups including *Methanosarcinaceae*, *Methanoculleus*, *Methanofollis*, *Methanomicrobiales*, uncultured Euryarchaeota and uncultured Crenarchaeota. Study of solid landfill samples shows a somewhat different archaeal community structure; some archaea can be found in solid landfill samples and are not found in leachate (Chen et al., 2003). More thermophilic archaeal species were found in the Taiwanese landfill studies by Chen et al. (2003). Archaea collected from the Taiwanese landfill included: *Methanosarcina, Methanoculleus, Methanosaeta,* and *Methanothermobacter*. The dominating archaea

were from thermophilic methanogens of the hydrogenotrophic variety. Incubations of the collected MSW samples indicated that the hydrogenotrophic metabolism dominated this landfill. Incubations using hydrogen and carbon dioxide gases produced more methane during the incubation than incubations with acetate as the substrate. Other studies on archaeal diversity agreed well with the previous two. Mori et al., 2003, sampled leachate from several collection pipes in a Japanese landfill. Each of the leachate pipes had a different relative diversity of archaea, overall, archaea were similar to other studies. This study found thermophilic methanogens as well as *Methanosaeta, Methanosarcina* and other Euryarchaeota and Crenarchaeota.

Bacteria present in landfills vary much more than archaea in differing landfills. A multitude of bacterial species can be found within in a landfill and species may vary from one location to another. Species present are more dependent on the region in which the landfill is located than on a type of metabolism. Numerous bacteria can have the same or similar metabolisms and different species can fill the metabolic niches required for degradation of waste. Many metabolic niches can also be filled by different species of fungus as well as bacteria; certain species of fungus and bacteria are capable of cellulose degradation, which is an important part of overall degradation within a landfill.

2.9 Fluorescence In-Situ Hybridization (FISH)

Among the many methods available for study of microbial communities found in landfills, FISH is one of the simplest to implement and least expensive (Kumar et

al., 2011). Using this technique, individual microbial populations can be identified

and enumerated to determine their relative abundance within a larger population of

microorganisms. FISH employs the use of a fluorescently labeled oligonucleotide

(probe) that binds with ribosomal rRNA of a target organism. A segment of rRNA

within the target organism is hybridized with the probe's nucleotide sequence.

Formamide, a chemical used in the hybridization process, disrupts hydrogen bonds

allowing a probe to bind only with a complementary strand of rRNA. A strand of

rRNA almost matching the probe will not bind with the probe given the proper

concentration of formamide. Probes are designed based on their specificity to target

organisms; nucleotide sequences can be formulated to be specific to a single

organism or include a sequence common to a large group of organisms.

Oligonucleotide composition is determined from analysis of 16S rRNA. The 16S

rRNA gene sequence of many organisms has been determined and is contained in

data bases. Probes are designed based on a short length of 16S rRNA contained

within the target organism. An oligonucleotide is chosen that corresponds to the

target organism, then it is compared to other organisms' 16S rRNA in the data bases.

If the probe is found to be unique to the target organism or group of organisms, it can

then be synthesized and tested. Testing of a probe consists of performing the

hybridization protocol on the target organisms to determine if the probe binds

properly; hybridization protocol is also performed on non-target organisms to show

that the probe does not bind with them.

2.9.2   Probe Dyes and Viewing

Probes are designed with a fluorescent dye attached at one or more positions along the oligonucleotide. A number of fluorescent labels are utilized in FISH analyses. Each label fluoresces under a different wavelength of light, emitting its own particular light wavelength. Numerous dyes are available for FISH analyses, but newer dyes give a better fluorescent response. After hybridization of the probe with a sample, individual fluorescing cells of the target organism can be viewed with a confocal laser scanning microscope or an epifluorescence microscope (Amann et al., 2008, Kumar et al., 2011). These microscopes emit specific wavelengths of light causing the probe labels to fluoresce.

The fluorescent dyes Fluorescein and tetramethylrhodamide emit green and red light respectively. These labels are used in standard FISH analyses and have a low fluorescent response. Indocarbocyanine labels have a much greater fluorescent response and are used when there are few ribosomal binding sites for probes or a stronger signal is desired; these labels are known as "Cy" labels and come in several colors. Indocarbocyanine labels have improved the sensitivity of FISH analyses substantially (Kumar et al., 2011). Multiple probes and fluorescent labels can be used in the same hybridization. This allows for the visualization and relative enumeration of a target organism within a heterogeneous population of organisms. A non-specific probe can be used for the overall microbial population and a highly specific probe with a different dye for the target organism.

Often times, a fluorescent cell stain is used to show total cells in conjunction with a FISH probe specific to target organisms. Multiple probes can also be used on a single target organism when ribosomal RNA is not present in great quantities. In this type of application, each probe is different, but is complementary to the target organism; each probe binds to different parts of the target organism's ribosomal RNA. Multiple labels are often used when the fluorescent response to a single label is low due to a small number of binding sites within the organisms studied (Amann et al., 2008). Longer oligonucleotides are used, which allows for the attachment of numerous fluorescent labels. A greater number of labels increase the fluorescent response of the probes.

2.9.3   FISH Limitations

Although FISH is an excellent method of analyzing microbial populations, certain conditions can render unusable results. FISH works by binding an oligonucleotide probe to the rRNA of a target microorganism (Amann et al., 2008). For the analysis to return results, rRNA must be present in adequate amounts to cause a significant fluorescent signal. *E. coli* cells can have 72,000 ribosomes during rapid growth, but this number can drop by one order of magnitude to around 6,000 ribosomes in a slower growth phase (Amann et al., 2008). Amann also notes that *E. coli* cells are large in comparison to other microbes; this limits the amount of rRNA that some cells can contain to just several hundred ribosomes. The amount of rRNA present within the cells of a target population can vary with growth conditions. Dormant or slow growing microbes will have less rRNA than populations experiencing significant growth. Many researchers study microbes during their

27

exponential growth phase to ensure an adequate amount of rRNA for FISH analyses (McDonald et al., 2010). McDonald et al. incubated leachate samples from a British landfill prior to performing FISH analyses and sequencing DNA. Microbes were provided with a food substrate, cotton, for two weeks prior to performing FISH analyses. The incubation time allowed for organisms present in the leachate to reestablish their symbiotic relationships and actively grow. This ensured an adequate amount of rRNA within the microbial population to perform FISH and receive adequate fluorescent signals, but may have selected for specific organisms present in the leachate.

2.9.4   Cell Permeability

Cell permeabilization is another common problem in FISH analyses. For the probes to bind with cellular rRNA, it must cross the cell wall. If the cell wall is not permeable, then the probes cannot enter and bind to RNA. Cell wall permeabilization is usually achieved during cell fixation (Amann et al., 1995). Fixation with paraformaldehyde (PFA) is used for Gram-negative cells. Ethyl alcohol is used to fix Gram-positive cells. These fixative agents react with proteins in the cell membrane. PFA causes cross linking among soluble proteins while ethyl alcohol precipitates soluble proteins in the cell wall. Disruption of cell membrane proteins reduces the hydrophobic nature of the cell wall, making it permeable to polar ionic compounds. Certain microbes still require additional treatments to allow for FISH probes to cross the cell wall. A number of chemical, enzyme or freeze and thaw procedures have been implemented to make cell walls permeable (Kumar et al.,

2011). There is concern that many of the techniques employed can cause cell lysis, making results from subsequent FISH analysis unusable.

Pseudomurein endoisopeptidase (PEI) is an enzyme used to permeabilize the cell wall of some archaea (Nakamura et al., 2006). PEI works by breaking chemical bonds within the cell wall, making them more permeable. Mutanolysin has also been successfully applied as an agent making cell walls more permeable. Researchers studying filamentous bacteria in sewage treatment plants used mutanolysin to allow for FISH probes to enter the cytoplasm and bind with rRNA (Marneri et al., 2009). Cell wall permeability of certain microbes can vary depending on the environment in which they are grown (Nakamura et al., 2006). *M. thermautotrophicus* cells develop a thicker wall when grown in mixed cultures as compared to pure culture growth. Harsh conditions can cause the development of thicker cell walls in microbes, making analysis of organisms from environmental samples more difficult than laboratory cultured organisms. Pure cultures of *M. thermoautotrophicus* hybridized easily with FISH probes, but the development of thicker cell walls when grown in mixed culture prevented hybridization without permeablization treatment (Nakamura et al., 2006).

2.9.5   Probe Specificity

Results of probe specificity tests showed that some universal probes gave weak signals for certain target organisms (Mac Donald et al., 2009). Probes Univ 1390, EUB 338 and several others were tested on reference rRNA from a number of organisms with varying results. The Univ 1390 probe was supposed to bind to RNA

from any organism, but failed to hybridize with archaeal rRNA. This probe also gave relatively weak signals for many of the bacterial rRNA tested. EUB 338 is intended to hybridize with the majority of bacterial rRNA, but it also gave relatively weak signals for much of the bacterial reference rRNA tested. Other probes tested bound well with target organisms; a probe intended for eukaryotes showed a strong signal on reference rRNA and a general archaea probe also bound well with reference rRNA (Mac Donald et al., 2009).

CHAPTER 3

EXPERIMENTAL APPROACH

Variation in the levels of food waste and bio-solids are tested to determine the effect that initial moisture content and readily degradable matter has on bio-gas production rates and total bio-gas production. Food waste and bio-solids have high moisture content and are highly degradable by microorganisms present in landfill environments. Wastes such as paper, plastic and metals have very low moisture content; paper wastes biodegrade at lower rates than food and bio-solids, while plastics and metals are generally not biodegraded. Methanogenic organisms present within a landfill are also a determining factor of methane production. Methane is produced by archaea through two main metabolic pathways, acetotrophic and hydrogenotrophic; each metabolic pathway produces a different ratio of methane and carbon dioxide gas.

3.1 Experimental Procedure

Laboratory scale batch bioreactors were used to determine the effects that augmentation of moisture content through variation of food waste content and

30

wastewater treatment plant biosolids content have on bio-gas production and degradation of municipal solid waste. Input food waste and wastewater treatment plant biosolids were varied to determine the effects of moisture augmentation. Remaining waste component proportions were based on the typical EPA municipal solid waste analysis (EPA 2010). One-liter, brown, borosilicate glass bottles were used as reaction vessels to test each of the parameters. The experiments were housed within an incubator (Labline Environ-Shaker) set at 50° C through the duration of measurements. A total of six experiments were run in triplicate for a total of 18 reaction vessels; three (3) experiments had variable food waste content, and the remaining three (3) experiments had variable biosolids content. Food waste accounted for 20%, 30%, and 40% of the first three experiments while the remainder was comprised of typical EPA waste components. Biosolids accounted for 10%, 15%, and 20% of the waste mixture while the remainder was comprised of typical EPA waste components.

Food Waste Variation Experiments	WWTP Bio-solids Variation Experiments
F-1/S-1 5% WWTP Biosolids 20% Food Waste 75% Typical Waste (Three bottles)	**S-2** 10% WWTP Biosolids 20% Food Waste 70% Typical Waste (Three bottles)
F-2 5% WWTP Biosolids 30% Food Waste 65% Typical Waste (Three bottles)	**S-3** 15% WWTP Biosolids 20% Food Waste 65% Typical Waste (Three bottles)
F-3 5% WWTP Biosolids 40% Food Waste 55% Typical Waste (Three bottles)	**S-4** 20% WWTP Biosolids 20% Food Waste 60% Typical Waste (Three bottles)

Figure 4. Diagram of Experiment Setup for MSW Degradation

3.2 Waste Component Preparation

Various waste components were collected and blended to obtain the mixtures

required for each experiment. Food waste was collected from a typical residential

source and was mainly comprised of old, spoiled or stale food representative of food

that would be discarded. Individual items included old pizza, moldy sandwich meat,

stale bread, spoiled apples and oranges, chicken nuggets, flat soda, molded cheese,

stale tortillas, and aged frozen vegetables. The individual components were

combined and blended in a food processor (Black and Decker Model #FP16008) to a

paste consistency. Food was blended in small batches for about 5 minutes then

combined and mixed with a glass stir rod until the mixture was consistent.

Wastewater treatment plant biosolids were collected from two sources, the Clark

County Water Reclamation District's (CCWRD) main treatment facility and the Las

Vegas Water Pollution Control Facility (LVWPCF). CCWRD bio-solids used in the

experiment are typically a mixture of primary and secondary sludge that is

centrifuged to reduce water content; these bio-solids are ready for disposal in a

landfill. Bio-solids from the LVWPCF undergo anaerobic digestion prior to

centrifugation and disposal in the landfill. The two bio-solids sources were mixed to

approximate the relative amount disposed of in the Apex Landfill. Wastewater

treatment plant bio-solids account for between 5% and 6% of the waste entering the

Apex landfill. CCWRD bio-solids comprise the majority of bio-solids disposed of at

the landfill (as of the initiation of experiments in 2011), and LVWPCF bio-solids

make up a smaller portion due to anaerobic digestion and lower overall influent

wastewater flow. The proportion of each of the bio-solids samples was roughly

determined based on flow and further reduction of biosolids from the LVWPCF by anaerobic digestion. CCWRD bio-solids were used to represent typical un-digested bio-solids from local facilities, CCWRD, the Kurt R. Segler Water Reclamation Facility in the City of Henderson and the City of North Las Vegas Water Reclamation Facility. Total wastewater flow from the Las Vegas Valley is around 200 MGD; the LVWPCF treats about 60 MGD, and utilizes anaerobic digestion to reduce bio-solids volume by around two-thirds. The proportion of bio-solids produced at the LVWPCF is lower than its flow proportion by roughly 66%; the facility treats about 30% of the wastewater generated in the valley, but only produces about 13% of the total bio-solids disposed of at the Apex landfill. The final mixture was 87% CCWRD bio-solids and 13% LVWPCF bio-solids. Paper waste was derived from four main sources: junk mail, white computer paper with typical printing, corrugated card board boxes, and paper towels used in a bathroom. The paper wastes were shredded and combined in equal portions. Plastic waste was made from a number of plastic sources; the majority of plastic was from disposable plastic water bottles, while the remainder was comprised of milk bottles, HDPE plastic containers, grocery bags, PVC pipe, plastic soda cups, and Styrofoam. Plastic waste was shredded into small pieces no larger than 0.5 cm in width with varying lengths no longer than 3 cm. Shredded plastic was blended to obtain a relatively uniform mixture of the plastic sources. Metal waste was comprised of steel shavings from a metal lathe and aluminum cans. The steel shavings were washed and broken down to a size less than 0.5 cm; aluminum cans were cut into fine pieces no larger than 0.5 cm. The metals were combined to form a relatively uniform mixture. Glass was

obtained from beer bottles and broken laboratory glassware. Glass was smashed into small pieces less than 0.5 cm in size and uniformly mixed. Lawn waste was obtained from landscaped areas of the UNLV campus; grass trimmings comprised the majority, while leaves and shrubbery made up the remaining portions. Wastes included in the "other waste" category included crushed concrete, wood sawdust, vacuum cleaner residue, and garden soil. These wastes generally fall within the "other waste" category of the EPA's typical waste analysis. The following table presents proportions of the various waste components present in each of the experiments:

Table 5. Experimental Waste Proportions

Waste mixture	Food	Bio-solids	Other	Yard	Paper	Metals	Plastics	Glass
F-1 / S-1	20.00%	5.00%	19.00%	8.50%	16.00%	8.50%	17.00%	6.00%
F-2	30.00%	5.00%	16.47%	7.37%	13.87%	7.37%	14.73%	5.20%
F-3	40.00%	5.00%	13.93%	6.23%	11.73%	6.23%	12.47%	4.40%
S-2	20.00%	10.00%	17.73%	7.93%	14.93%	7.93%	15.87%	5.60%
S-3	20.00%	15.00%	16.47%	7.37%	13.87%	7.37%	14.73%	5.20%
S-4	20.00%	20.00%	15.20%	6.80%	12.80%	6.80%	13.60%	4.80%

3.2.1   Waste Mixture Preparation

Inverted, one (1) liter, brown, borosilicate glass reaction vessels (Figure 5) were used to carry out experiments. Two hundred grams of waste mixture was placed in each of the vessels. Butyl rubber septa were inserted into each of the reaction vessel lids allowing for gas extraction using a syringe needle connected to a Tedlar® gas bag (Cole-Parmer CAT# 01409). Leachate formed during the experiments was allowed to drain into a sand layer placed under the waste mixtures. Filter sand with a mean particle size of 0.25-0.5 mm was dried in a 103° C oven; 75

grams of sand was saturated with 37.5 ml of distilled water and placed on the waste

mixture. The sand was intended to allow leachate to collect and be extracted with a

syringe after bottles were inverted. Reaction vessels were incubated at 50° C

throughout the experiment to mimic thermophilic conditions. Inversion of the bottles

allowed for collection of any leachate formed during the experiment.
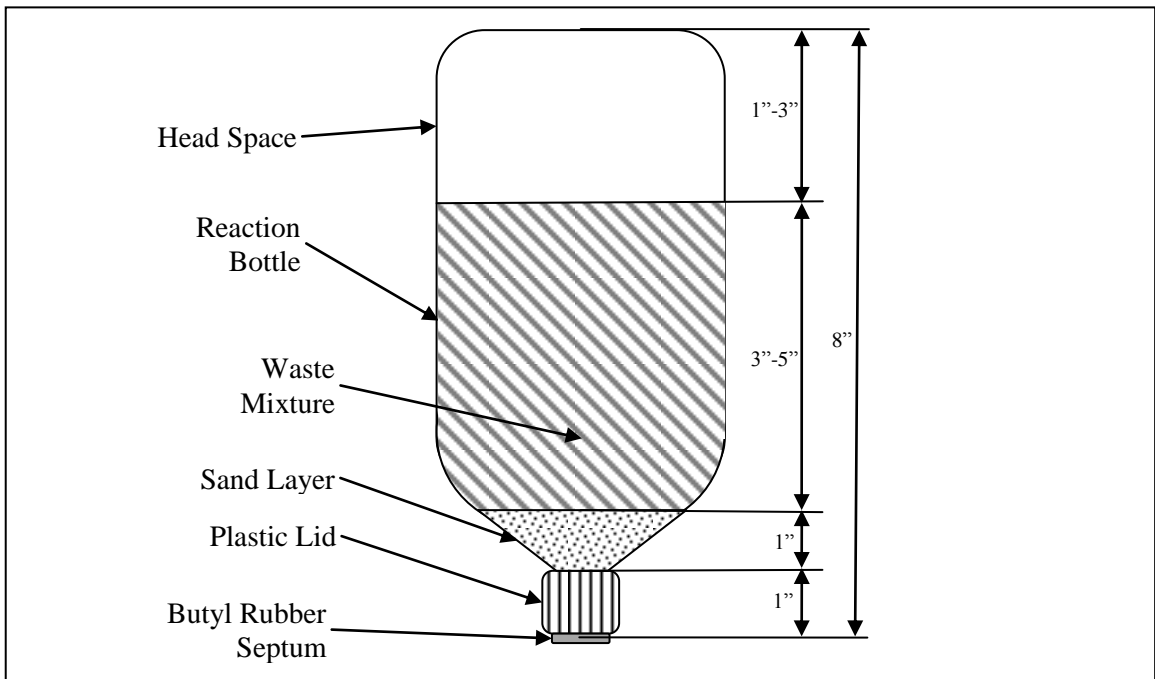
Figure 5. Reaction Vessel Schematic

Waste mixtures were prepared to ensure that each of the three vessels for each

experiment received the same amount of moisture laden waste fractions. Food waste,

bio-solids and lawn trimmings were mixed for each of the experiments in the

required proportions. This mixture was then divided into three parts. Dry waste

components were then weighed and mixed individually with the moisture-laden

waste. 200 grams of the prepared waste mixtures was then placed into each of the

glass reaction vessels then topped with the sand layer. The reaction vessels were

inverted and placed into a Styrofoam rack to hold the bottles in their inverted position. To mimic thermophilic conditions, vessels were incubated at 50° C throughout the experiment.

Figure 6. Incubator and Experiment Bottles

3.2.3 Experimental Measurements:

Initial measurements were taken prior to initiation of experiments to determine initial waste parameters. Initial measurements included: waste component moisture content and waste component elemental composition. Moisture content of the various waste components was determined using standard methods for solids content analysis, method 2540 B (Eaton et al., 2005). Samples of each of the waste components were weighed, dried at between 103° and 105° C, and weighed again. Difference in the initial component mass and final dried mass divided by the initial mass gave the component moisture content. Elemental component composition was determined for each of the biodegradable input wastes using dry combustion with

elementa® Vario MAX elemental analyzer. Dried samples of food waste, paper waste, yard trimmings, and wastewater bio-solids were analyzed.

3.2.4 Periodic Measurements

Periodic measurements were made to track the progress of degradation and gas formation. Periodic measurements included: gas volume produced, gas composition, leachate volume produced, leachate pH, and leachate volatile fatty acid content. Gas volume was measured every couple of days initially, and once the experiment was running for a number of weeks gas volume was measured on a weekly basis. Gas was extracted using a 6-inch septum piercing needle inserted through the butyl rubber septum into the headspace above the waste. A Tedlar® gas bag (Cole-Parmer CAT# 01409) attached to the needles by surgical tubing was used to collect gas from each of the bottles. Gas volume was measured using water displacement. Gas composition was measured initially every week and after rapid gas formation had ceased, gas composition was measured less frequently. A gas chromatograph (Shimadzu GC-2014) with a 15' (4.57-meter) packed column, Supelco CarboWax 1000, coupled to a thermal conductivity detector was used to determine the composition of the various gas components; hydrogen, oxygen, nitrogen, methane and carbon dioxide were tracked throughout. Leachate was collected when a sufficient amount had collected in the sand at the base of the experimental vessels. Leachate volume was determined using a syringe with a ½-inch 26-gage needle; leachate pH was then measured using pH strips. Small samples of leachate were collected from each of the experimental vessels that produced leachate and volatile fatty acids were measured using a gas chromatograph

(Shimadzu GC2014). A 30-meter, fused silica Supelco Nukol capillary column was used coupled with a flame ion detector for fatty acid analysis.

3.2.5   Final Measurements

Final measurements were made after 8-1/2 months of degradation and the first signs of methanogenesis were apparent. Final measurements included: residual waste moisture content and microbial population measurement. Representative waste samples were collected from each of the reaction vessels to determine the moisture content in the same manner described previously. Analysis of the final microbial population was accomplished using Fluorescence in-situ hybridization (FISH) as described in section 3.4.

3.3 Experiment Maintenance

Experimental reaction vessels were allowed to degrade with little disturbance. Measureable leachate was neutralized throughout the experiment in vessels producing adequate leachate (experiment series F-3). After gas production in many of the vessels had slowed or ceased, 10 ml of a methanogen medium was added to each of the vessels to ensure that adequate nutrients were present to stimulate the onset of methanogenesis. The methanogen medium consisted of a number of salts required for growth of methanogenic archaea. Table 6 presents the contents of the broth. The medium was slightly modified from The Handbook of Microbiological Media, Methanogen Medium, Zeikus (Atlas, 2004). Disodium EDTA was used as a substitute for nitrilotriacetic acid.

Table 6. Methanogen Nutrient Solution

Component	Concentration
K_2PO_4-$3H_2O$	1.45 g/L
NH_4Cl	1.0 g/L
KH_2PO_4	0.75 g/L
$MgCl_2$-$6H_2O$	0.2 g/L
Disodium EDTA	0.04 g/L
$CaCl_2$-$2H_2O$	0.2 g/L
$FeCl_2$-$4H_2O$	3.6 mg/L
$CoCl_2$-$6H_2O$	1.5 mg/L
$MnCl_2$-$4H_2O$	0.9 mg/L
$ZnCl_2$	0.9 mg/L
H_3BO_2	0.17 mg/L
Na_2MoO_4-$2H_2O$	0.09 mg/L
Na_2S-$9H_2O$	0.3 g/L
Wolfe's Vitamin Solution	10 ml/L

After addition of the broth, excess leachate was formed in all vessels. The pH of the leachate was measured and 5 ml of a phosphate buffer solution was added to each of the vessels to raise pH and buffer at levels required for growth of methanogenic archaea. The buffer solution was prepared by combining 56 ml of a 0.5 M NaH_2PO_4 solution with 144 ml of a 0.5 M Na_2HPO_4 solution. A 1 M solution of NaOH was used to bring the buffer solution to a final pH of 7.5.

Although not initially anticipated, gas production in many of the reaction vessels ceased and began to be consumed after several months of degradation. To ensure adequate gas pressure for growth, the partial vacuum that formed was pressurized to just above atmospheric pressure with ultra lift helium gas. Helium was used as it is inert and easily distinguishable from the other gases in compositional measurements.

3.4 FISH Procedure

A number of steps are involved in the hybridization of cells with oligonucleotide probes (Amann, 1995). Samples of cell material were taken from the bottles at the end of experimentation. Cell samples were then incubated over night in an anaerobic glucose based broth for analysis of bacterial cells, or in a methanogenic broth for analysis of methanogens. The cultured cells were sampled then "fixed" using either 4% paraformaldehyde (PFA) in a phosphate buffer saline solution (PBS) or 50% alcohol in the same buffer solution (Amann, 1995). The fixation solution used was determined by the microbes present in the sample; Gram negative bacteria and archaea are fixed with PFA solution, while Gram positive bacteria are fixed with alcohol solution. Cell walls of Gram positive bacteria are thicker than Gram negative bacteria; the thicker cell walls of Gram positive bacteria are more difficult to penetrate with fixing agents and subsequent hybridization probes. Three volumes of PFA or one volume of alcohol solution were mixed with one volume of sampled cells; the fixation mixture was then placed into a 4° C refrigerator for two (2) hours. Fixing the sample stops cellular metabolic activity, permeates cell walls and preserves cells for hybridization. The fixed samples were then centrifuged and fixing solution decanted. The centrifuged samples were rinsed with a triple concentration of a PBS (3xPBS) solution, centrifuged, and decanted. Samples were then rinsed, centrifuged and decanted twice with a single concentration of the PBS (1xPBS) solution. One volume of a 1:1 (v:v) alcohol and 1xPBS solution is added to the rinsed and decanted cell samples; this preserved the fixed cells allowing the

40

samples to be frozen at -20° C for an extended period of time prior to hybridization and microscopy.

3.4.2 Microscope Slide Preparation

Fixed cell samples were applied to microscope slides for drying, dehydration and treatment prior to hybridization. A small volume of the fixed cell solution, 10 μL, was applied to a microscope slide then air dried. The volume used was sufficient to ensure adequate cell density for analysis. The air dried slide was then dehydrated by subsequent dipping into 50%, 80% and 96% alcohol solutions for three minutes each, then drying.

3.4.3 Cell Hybridization

Four (4) probes were selected to determine the relative proportions of bacteria/archaea and acetotrophs/hydrogenotrophs. Slides were prepared for each of the selected experiments' cell samples. Four hybridizations were performed. Two slides were used for each of the hybridizations; one slide was used for paraformaldehyde fixed cells and the other for ethanol fixed cells. Hybridization solutions were comprised of formamide, saline solution, tris HCl buffer, RNA-ase free water and sodium dodecyl sulfate solution (SDS) in concentrations depending on the stringency required for binding to the target organism. FISH Probes used in the hybridization are shown in Table 7. Hybridization buffer solution compositions used are shown in Table 8.

Table 7. Hybridization Probes

Probe Name	Target Organisms	Label Sequence	Reference
EUB 338	Most Bacteria	5'-/5Cy3/GCT GCC TCC CGT AGG AGT-3'	Amann et al., 1990
ARCH 915	Most Archaea	5'-/5Cy3/GTG CTC CCC CGC CAA TTC CT-3'	Raskin et al., 1994
MX 825	Methanosaetaceae	5'-/5Cy3/TCG CAC CGT GGC CGA CAC CTA-3'	Raskin et al., 1994
MG 1200b	Most Methanomicrobiales	5'/56-FAM/CGG ATA ATT CGG GGC ATG CTG-3'	Crocetti et al., 2005

Table 8. Hybridization Buffer Solutions

Probe	5 M NaCl	1 M Tris HCl	H_2O	Formamide	10% SDS
EUB 338	180 µL	20 µL	749 µL	50 µl	1 µl
ARCH 915	180 µL	20 µL	799 µL	0 µl	1 µl
MX 825	180 µL	20 µL	299 µL	500 µl	1 µl
MG 1200b	180 µL	20 µL	599 µL	200 µl	1 µl

For each of the hybridizations, the slides and a piece of filter paper wetted with 0.5 ml of hybridization solution were placed into an airtight tube then incubated at 46° C for 1-5 hours or as long as overnight depending on the probe used (Amann et al., 1995, Nakamura et al., 2006, Chen et al., 2003). After incubation, hybridization solution was rinsed from the slides immediately upon removal from the incubation oven with a pre-warmed buffered wash solution.   Wash solution components include double distilled H_2O (ddH2O), saline solution, tris HCl and EDTA; concentrations of the components depend on the hybridization solution stringency used. Slides were then immediately placed into a 50 ml tube with warm wash solution and sealed; the tube with slide is placed into a water bath at 48° C for 10-15 minutes. Table 9 presents the different wash solutions used.

Table 9. Hybridization Wash Solutions

Probe	5 M NaCl	1 M Tris HCl	0.5 M EDTA	ddH₂O
EUB 338	6.3 ml	1 ml	0 ml	42.6 ml
ARCH 915	9 ml	1 ml	0 ml	40 ml
MX 825	0.18 ml	1 ml	0.5 ml	48.32 ml
MG 1200b	2.15 ml	1 ml	0.5 ml	46.35 ml

Slides were then removed from the wash solution and dipped in ice cold ddH$_2$O for 2-3 seconds and dried either with compressed, oil free air or just air dried.

3.4.4 Slide Viewing Preparation

Preparation for viewing was then undertaken. Slides were dipped into a DAPI solution to stain DNA of all cells on the slides; the DAPI solution was then rinsed by two (2) subsequent dips into ice cold water followed by drying. An antifadent, citifluor, was applied to each well and a cover slip was then placed on the slide. Clear nail polish was used to seal the cover slip to the slide to prevent movement. Slides were then viewed with an epifluorescent microscope and pictures were taken to allow for enumeration of the various cells.

3.4.5 Cell Proportion

A minimum of five pictures were taken of each of the slides using a camera attached to the epifluorescense microscope. Pictures of the wells probed with EUB 338 and ARCH 915 were taken with the DAPI filter and Cy3 filter. The DAPI filter caused all organisms present to fluoresce, while the Cy3 filter caused only the bacteria or archaea to fluoresce. These photos were then analyzed using ImageJ software, from the National Institute of Health, to determine the proportion of the target organisms to the overall organisms. Relative proportions of acetotrophic and hydrogenotrophic methanogens were determined similarly but using the 6-FAM filter and the Cy3 filter.

CHAPTER 4

RESULTS and DISCUSSION

4.1 Initial Measurements

Initial measurements were taken to establish the starting moisture content and organic content of the individual waste components prior to initiation of experiments. These measurements were used to correlate gas production and composition with moisture content and organic content.

4.1.1 Moisture Content

Prior to initiation of experiments, individual waste fraction moisture content was determined and an elemental analysis was performed. Moisture content of the various waste components was determined in triplicate to ensure accuracy of the measurements. Average moisture content and standard deviation of the various waste components are shown in Table 10.

Table 10. Moisture Content of Waste Components

Waste Component	Average Moisture Content	Standard Deviation
Paper	5.45%	0.32%
Plastic	0.17%	0.05%
Lawn Trimmings	72.06%	0.08%
Glass	0.00%	0.03%
Metal	0.00%	0.00%
Bio-solids	78.21%	0.52%
Food	71.20%	0.15%
Other Wastes	1.69%	0.16%

The highest moisture content was found in wastewater bio-solids samples followed closely by lawn trimmings and food waste. The remaining waste components had very little moisture ranging from 0% to just over 5% for paper waste. Based on the moisture content of the individual waste fractions and the component waste content

44

of each experimental series, the overall moisture content was determined for each

experiment (Table 11).

Table 11. Experiment Moisture Content

Experiment Series	Moisture Content
F-1	25.5%
F-2	31.64%
F-3	37.78%
S-2	28.92%
S-3	32.34%
S-4	35.76%

Moisture content of the experiments did not exceed 40%. The maximum moisture

content was in experiment series F-3 with an overall moisture content of 37.78%

followed closely by experiment series S-4 at 35.76%. The lowest moisture content

experiment series was F-1 with a moisture content of only 25.5%. Moisture content

in the food waste experiments increased by 6.14% in each experiment; biosolids

experiments have an incremental moisture content difference of 3.42% between

experiments.

4.1.2 Elemental Analysis

Degradable waste components were subject to elemental analysis to determine

the relative amount of carbon, nitrogen and sulfur present in the waste components.

Paper, food, biosolids and lawn trimmings were measured to determine their relative

elemental composition. Results of the analysis are shown in Table 12.

Table 12. Degradable Waste Elemental Composition

Waste Component	Carbon	Nitrogen	Sulfur
Food	47.46%	4.028%	0.43%
Bio-solids*	27.44%	3.31%	1.10%
Paper	41.96%	0.138%	0.612%
Lawn Trimmings	43.32%	2.216%	0.447%

*Weighted average of combined CCWRD and LVWPCF Bio-solids

Results for wastewater bio-solids were computed based on the weighted average of LVWPCF biosolids and CCWRD biosolids. Based on the elemental analysis and proportions of the individual waste components, the elemental content of degradable wastes was determined for each of the experiments (Table 13).

Table 13. Degradable Elemental Content

Experiment Series	Carbon	Nitrogen	Sulfur
F-1	21.26%	1.18%	0.28%
F-2	24.62%	1.56%	0.30%
F-3	27.98%	1.93%	0.33%
S-2	21.94%	1.33%	0.32%
S-3	22.62%	1.48%	0.37%
S-4	23.30%	1.64%	0.41%

Elemental content of degradable wastes shows that the ratio of carbon to nitrogen was within the range of empirically determined waste compositions. Empirical waste composition equations shown in Table 2, indicate that typical wastes have carbon to nitrogen ratios that range between 12:1 and 23:1. Experiment series S-4 had the lowest carbon to nitrogen ratio with a value of 14.2:1, while series F-1 had the highest ratio with a value of 18:1.

4.2 Experimental Measurements

Throughout the experiment gas volumes, gas composition, leachate volumes and leachate composition were measured. Results of the measurements concurred well with initial expectations and correlated well with moisture and organic content of the experiments.

4.2.1 Gas Volumes

. Gas volumes produced by each of the reaction vessels were measured periodically throughout the experiment. Figures 7 through 12 show the total gas volume produced in each experimental series. On average, experiments with the highest moisture and organic content (Figures 9 and 12) produced the greatest amount of gas.
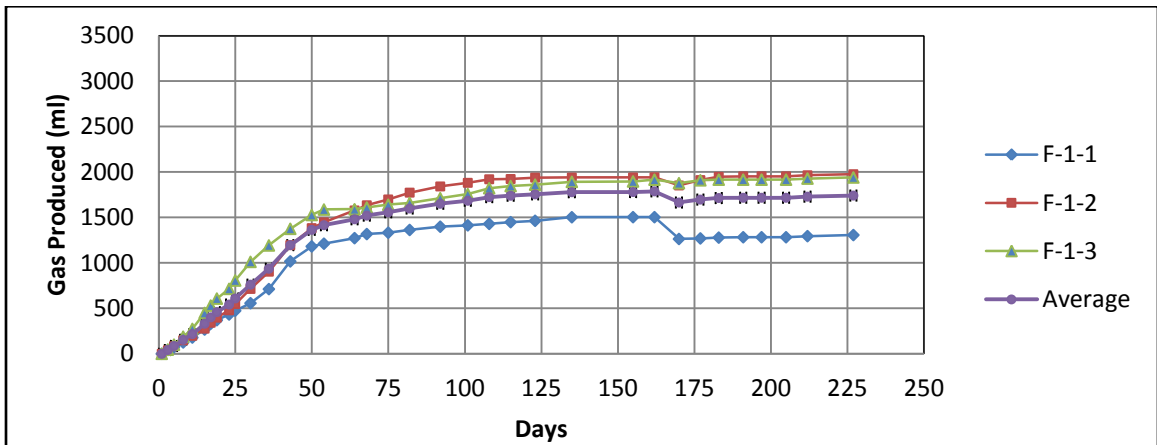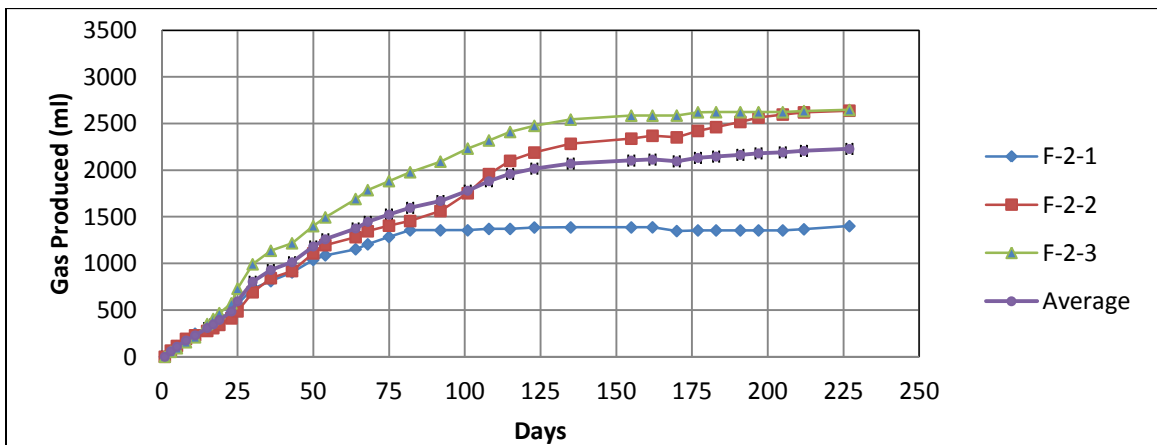

Figure 7. F-1 Gas Produced
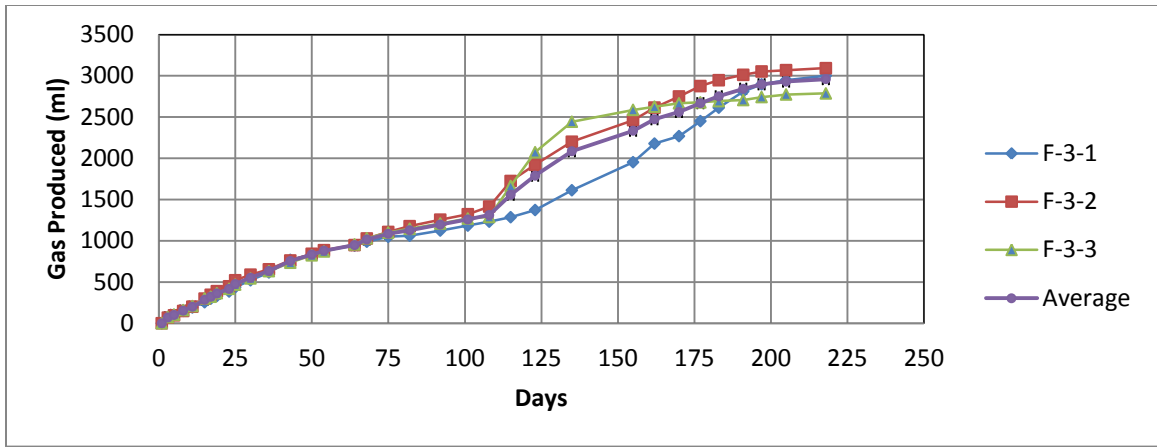

Figure 8. F-2 Gas Produced
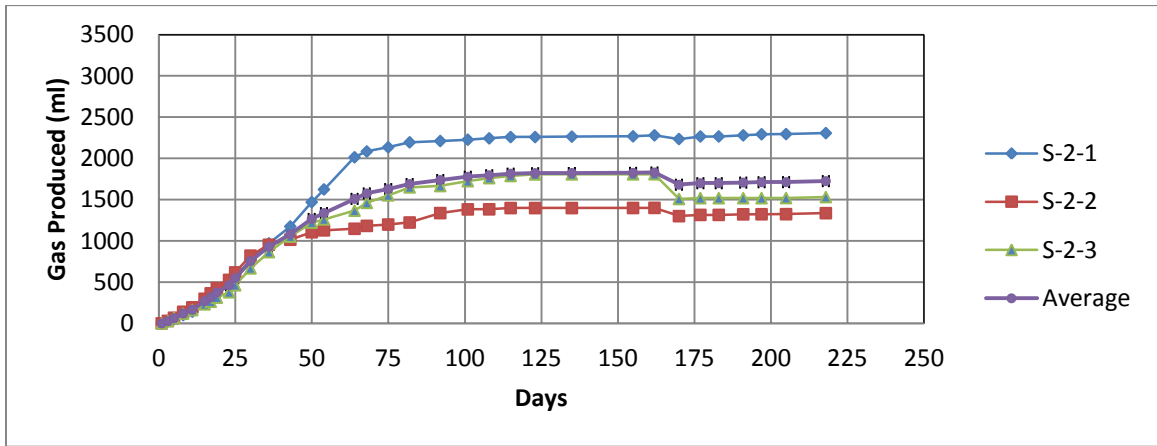
Figure 9. F-3 Gas Produced
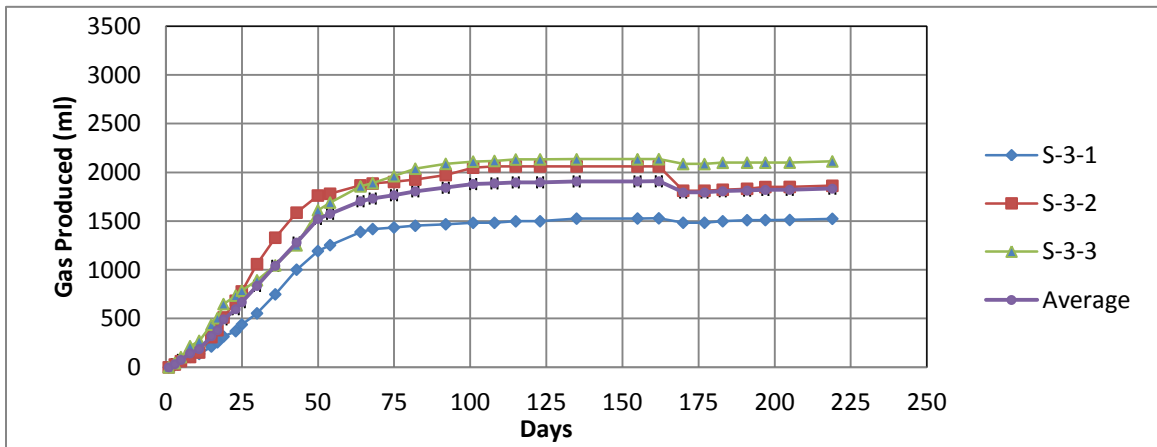

Figure 10. S-2 Gas Produced

Figure 11. S-3 Gas Produced

48
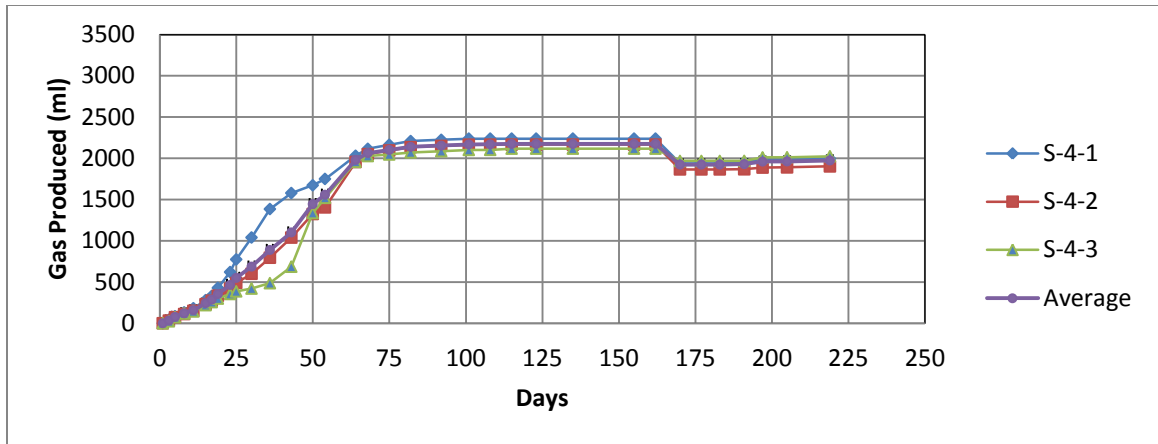
Figure 12. S-4 Gas Produced

Table 14 shows that the lower moisture content wastes have a much higher deviation between reaction vessels than those with higher moisture contents. The variation in cumulative gas volumes produced in each of the experiments can be seen in Figures 7-12. The least variable experiment series was S-4, shown in Figure 12, which had its greatest deviation in the first two months of measurements. After initial variation in measurements, each of the reaction vessels in experiment Series S-4 produced similar amounts of gas. Experiment series F-3, shown in Figure 9, had the second least standard deviation; its initial deviation was low until about three months into the measurements when gas production rate increased. The variation in experiment F-3 between the three reaction vessels grew during the phase of rapid gas production, but fell after another three months as the cumulative gas volume produced in each of the vessels became similar. Other experiments, with lower moisture, had gas measurements that varied greatly throughout. The food experiments had greater variation in cumulative gas production than the bio-solids experiments. Experiment series F-2 had the greatest variation at the end of

49

measurements (See Table 14). Two of the reaction vessels in series F-2 had comparable cumulative gas production values, but the remaining vessel produced just over half that amount.

Table 14. Average Cumulative Gas Production and Standard Deviation

Experiment Series	Average	Standard Deviation
F-1	1741.67	376.86
F-2	2229.00	717.08
F-3	2960.67	157.13
S-2	1724.67	513.65
S-3	1833.33	295.62
S-4	1974.33	64.30
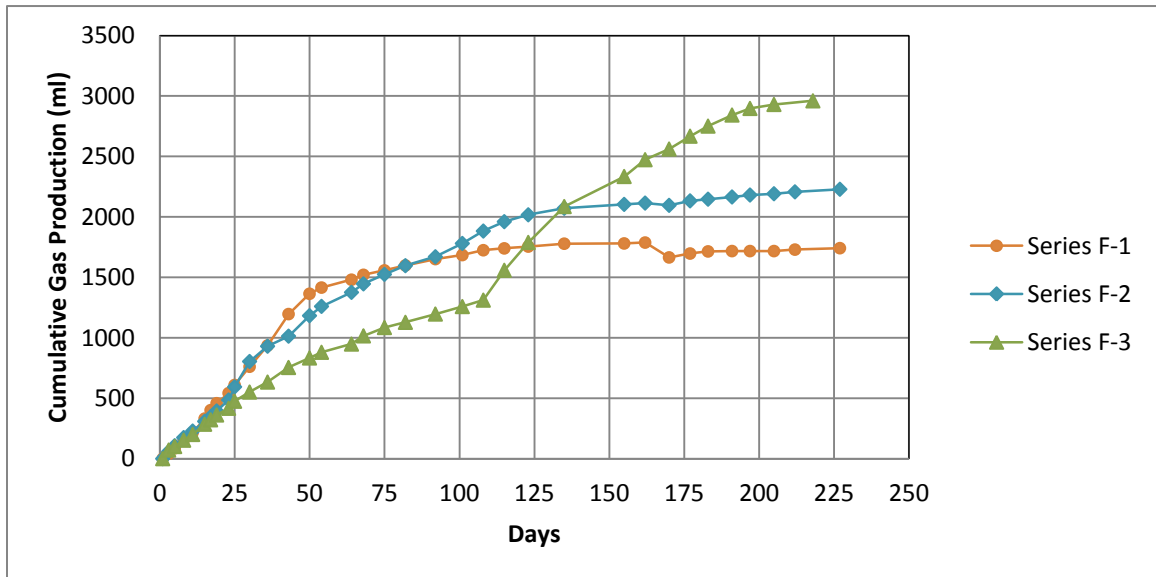
Figure 13. Food Experiments Average Cumulative Gas Produced

Within the food experiment series, there was an inverse relationship between initial cumulative gas production and moisture content. See Figure 13 for comparison of average cumulative gas production among the food experiments. Experiment series F-3, shown in Figure 9, had the highest initial moisture content, but its initial gas production was the lowest amongst the food experiments. Initial gas production in

series F-2, shown in Figure 8, was greater than series F-3, while series F-1, shown in

Figure 7, had the greatest initial gas production rate among the food experiments.

After 82 days, the inverse relationship between cumulative gas production and

moisture content began to disappear. Experiment series F-2 overtook series F-1 in

cumulative gas production. After 108 days, experiment series F-3 began a period of

rapid gas production and overtook Series F-1 after 125 days and Series F-2 after 135

days. Gas production started to drop off in the three food experiments in order of

their moisture content. After 100 days, gas production in series F-1 began to drop

off. Gas production in series F-2 began to drop off after 125 days. Gas production

in series F-3 continued through six months then began to slow down. After the drop

in gas production in experiments F-1 and F-2, gas began to be consumed in the

reaction vessels.

The bio-solids experiments had little variation in gas production rates during

the first 50 days of gas production measurements (Figure 14). After that point, the

cumulative gas production in the bio-solids experiments followed with moisture

content. Experiment series S-4, with the highest moisture content among the bio-

solids experiments, had the greatest cumulative gas production. Series S-3 and S-2

had lesser cumulative gas production. After 80 days of degradations, gas production

in the bio-solids experiments leveled off and slowly began to drop forming a partial

vacuum in the reaction vessels.
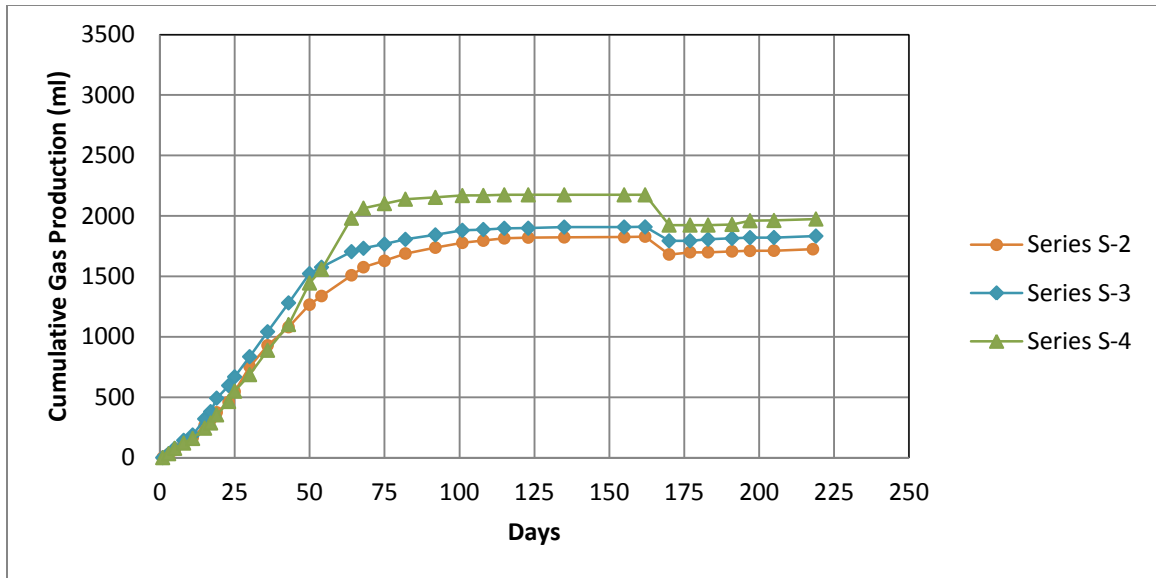
Figure 14. Bio-Solids Experiments Average Cumulative Gas Production

Gas consumption in the experiments is thought to have occurred due to acetogenesis, a process where carbon dioxide gas and hydrogen gas are combined by microbes to form acetate. Helium gas was injected into the reaction vessels to raise the internal pressure just above atmospheric; gas pressure in an actual landfill would not likely drop much below atmospheric pressure. It was also thought the low gas pressures may inhibit microbial activity. The greatest gas consumption among the food experiments was noted in experiment series F-1, Figure 7, with lesser consumption in series F-2, Figure 8; experiment series F-3, Figure 9, did not produce a vacuum during the experiments' observation period.

In the bio-solids experiments, series S-4 (Figure 12), had the most consistent gas consumption among its reaction vessels. Series S-3 (Figure 11), and S-2 (Figure 10), had individual vessels with substantial gas consumption that was not seen in the other vessels within the experiments.

To compare the data from each of the experiments based solely on moisture content, the gas volumes produced have been normalized with respect to carbon content. Figure 15 shows the average cumulative gas produced in each of the food experimental series based on carbon content. Figure 16 shows the average cumulative gas produced in the bio-solids experimental series based on carbon content.
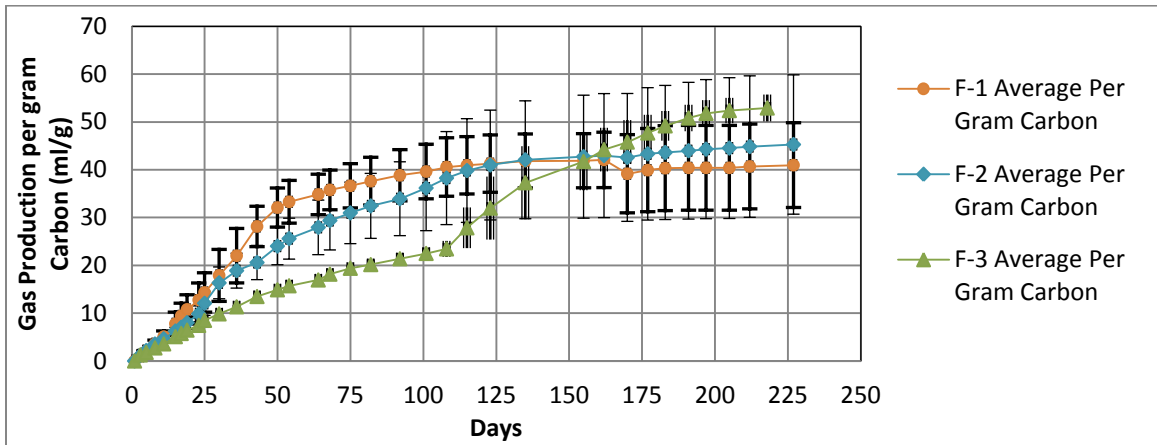
Figure 15. Food Experiments Gas Production per Gram Carbon
F-1 moisture content 25.5%, F-2 moisture content 31.64%, F-3 Moisture content 37.78%

Error bars shown on Figure 15 represent the standard deviation of each of the experiment series. Series F-1 error bars are medium thickness lines with end caps, F-2 error bars are thin lines with end caps, and F-3 error bars are thick lines with no end caps. Figure 15 shows that moisture content had an effect on the total gas produced in each of the experiments. Experiment series F-3 had the highest moisture content and produced the greatest amount of gas of all of the experiments by the end of the measurement period.

Statistical evaluation of the total gas production data did not prove a significant difference existed between all of the food experiments. An analysis of variance (ANOVA) was performed on the total gas production for the food experiments. The data are considered statistically different if the p-value is less than 0.05. The ANOVA showed that the p-value for the food experiments was 0.053, which is just above what would be considered statistically significant at the 95% confidence level.

Figure 16 shows a scatter plot of final cumulative gas volume produced vs. moisture content among the food experiments. Cumulative gas volume correlates fairly well with moisture content. The linear regression correlation coefficient of 0.6156 shows that cumulative gas volume produced correlates with moisture content. Analysis of the t variable shows that there is a non-directional probability of 0.012 indicating that the correlation is significant having a value less than 0.05.
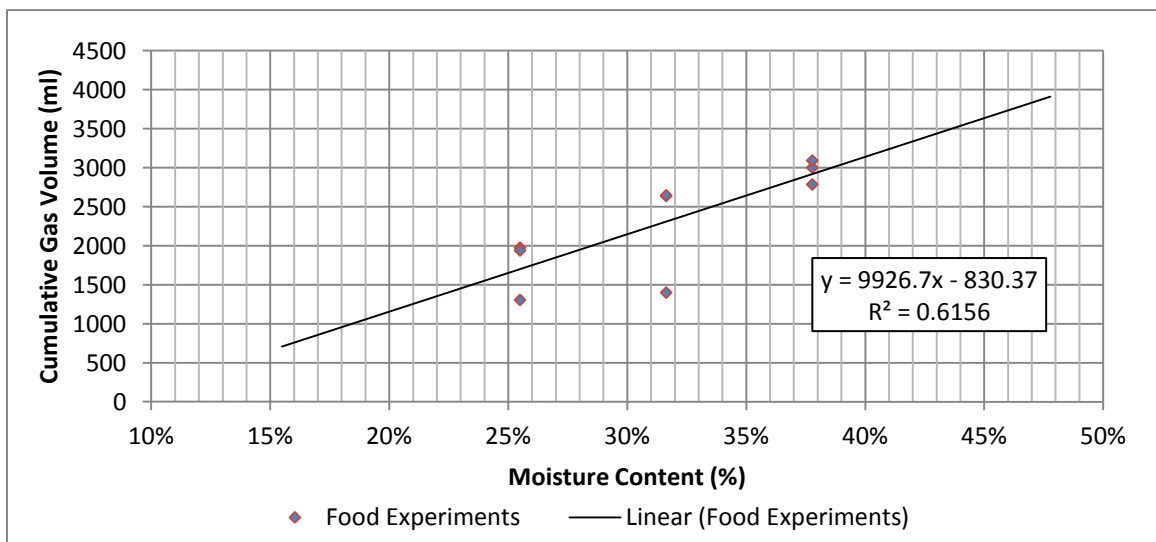
Figure 16. Food Experiments Cumulative Gas Volume vs. Moisture Content Correlation

The bio-solids experiments showed a slight correlation between moisture content and cumulative gas production (Figure 17); experiment series S-4 had the greatest gas production followed by S-3 then S-2. Peak gas production measurements in the bio-solids experiments, between day 75 and 160, showed a greater difference in total gas produced versus moisture content, but after the gas consumption period at about day 170, the difference in cumulative gas produced in the bio-solids experiments went down.
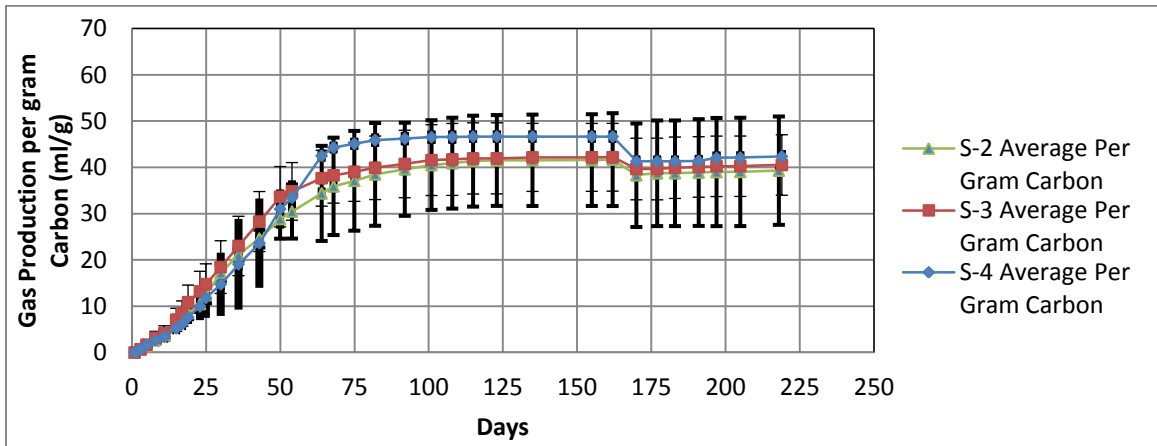
Figure 17. Biosolids Experiments Gas Production per Gram Carbon
S-2 moisture content 28.92%, S-3 moisture content 32.34%, S-4 moisture content 35.76%

Statistical analysis did not show a significant difference in the final cumulative gas production values for the biosolids experiments. The ANOVA showed a p-value of 0.68 for the bio-solids experiments. This means that the gas volumes produced in the different series of biosolids experiments were not statistically different; the volumes of gas produced by each of the experiments could have been produced by the same experiment.

55

Figure 18 shows a scatter plot of final cumulative gas volume produced vs. moisture content among the bio-solids experiments. The linear regression correlation coefficient of 0.1162 shows a low correlation between cumulative gas volume produced and moisture content. Analysis of the t variable shows that there is a non-directional probability of 0.0.37 indicating that the correlation is not significant having a value greater than 0.05. Series S-2, moisture content 28.92%, had the greatest difference in total gas volume produced among its reaction vessels. Series S-3, moisture content 32.34%, also had a large difference in total gas produced in its reaction vessels.

Figure 18. Bio-Solids Experiments Cumulative Gas Volume vs. Moisture Content Correlation

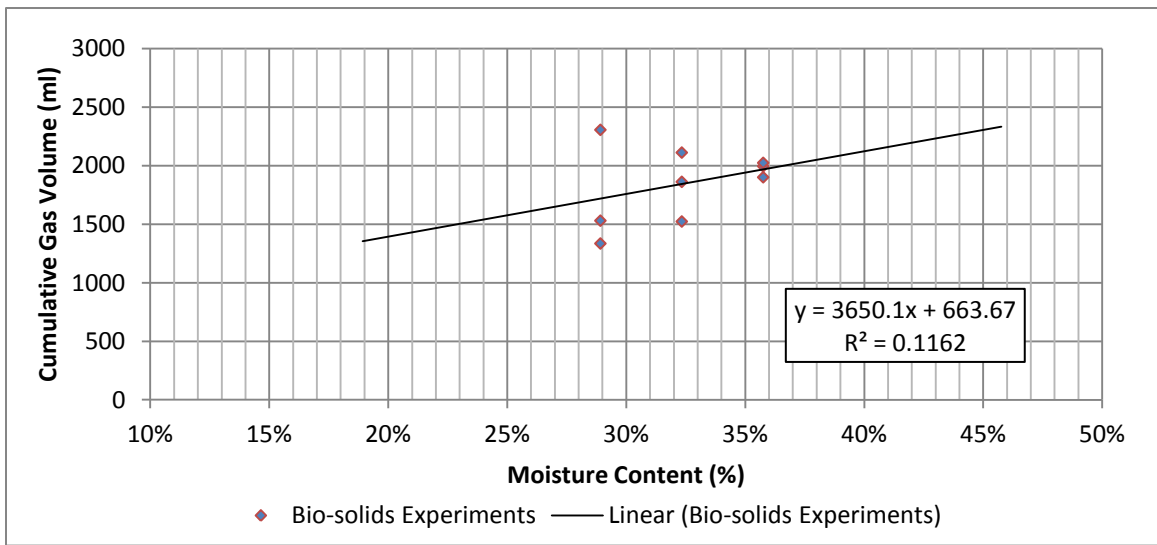The high variability among the lower moisture content experiments may be attributed to mixture consistency. During experiment preparation, waste mixtures with higher moisture content were more homogeneous and moisture laden wastes were well distributed amongst the dryer wastes. The lower moisture content waste

mixtures did not demonstrate the homogeneity of the higher moisture content mixtures. Moisture laden wastes in the low moisture content experiments may not have been distributed as well as the higher moisture content waste mixtures. The variation among the lower moisture content waste experiments presented difficulty in determining a clear statistical correlation based solely on moisture content. This is, however, typical behavior of landfills, which have a very heterogeneous mixture of waste components causing similar variation of gas production and waste degradation (Barlaz et al., 1996). In low moisture landfills, such as the ones found in the Southwest USA, this issue becomes more relevant as initial moisture within the waste is not evenly distributed throughout the landfill and precipitation infiltration does not provide additional moisture.

4.2.2 Gas Composition

Gas samples were taken periodically from each of the reaction vessels throughout the duration of experimental measurements. Five gases were measured with a gas chromatograph and included hydrogen, oxygen, nitrogen, methane and carbon dioxide. Results of gas composition measurements for each of the experiments are shown in Figures 19-24.
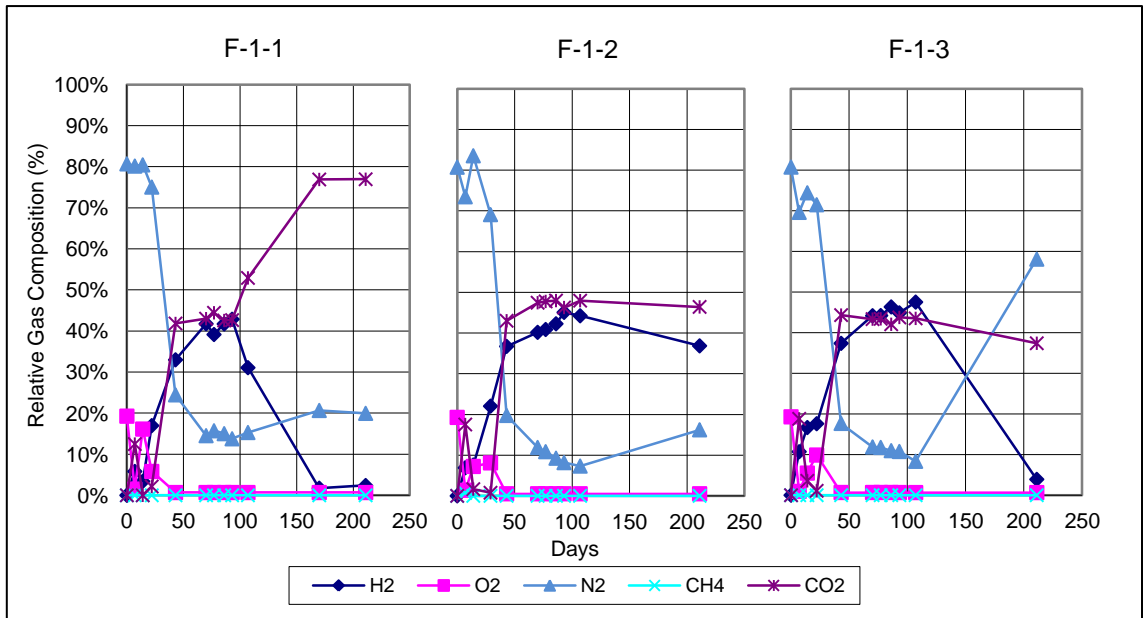
Figure 19. Experiment Series F-1 Relative Gas Composition
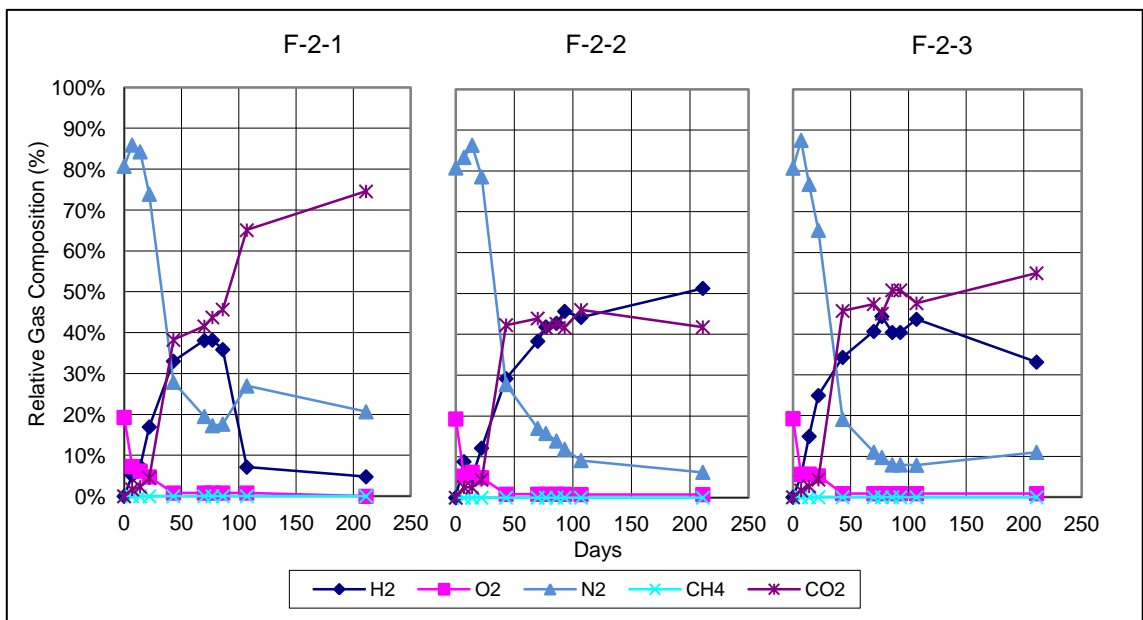
Figure 20. Experiment Series F-2 Relative Gas Composition
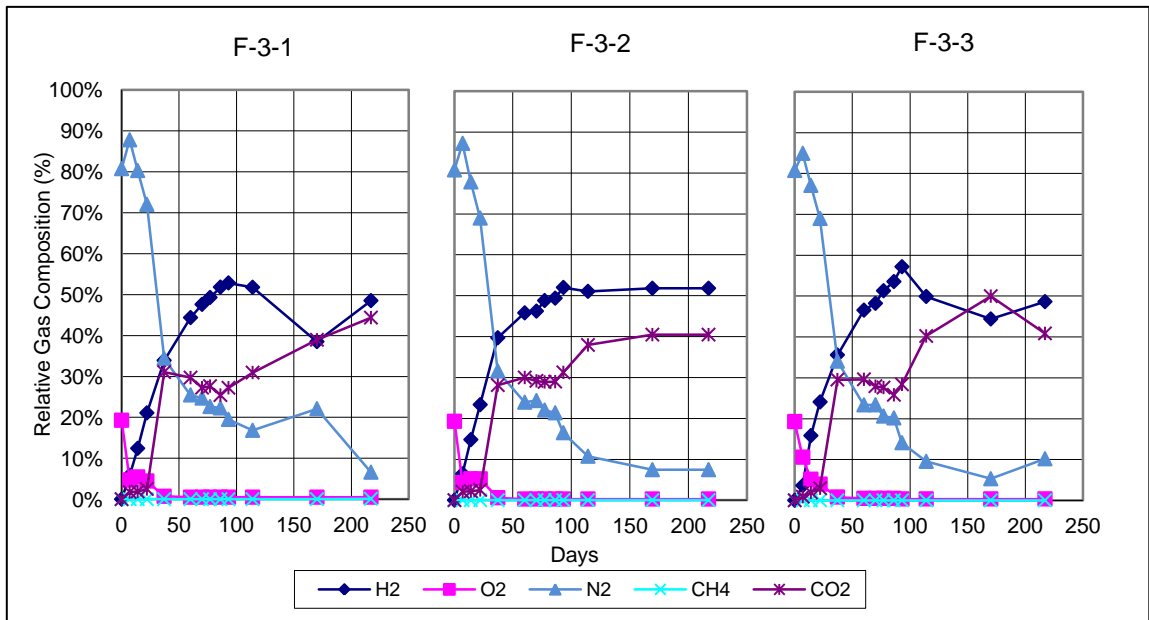
Figure 21. Experiment Series F-3 Relative Gas Composition
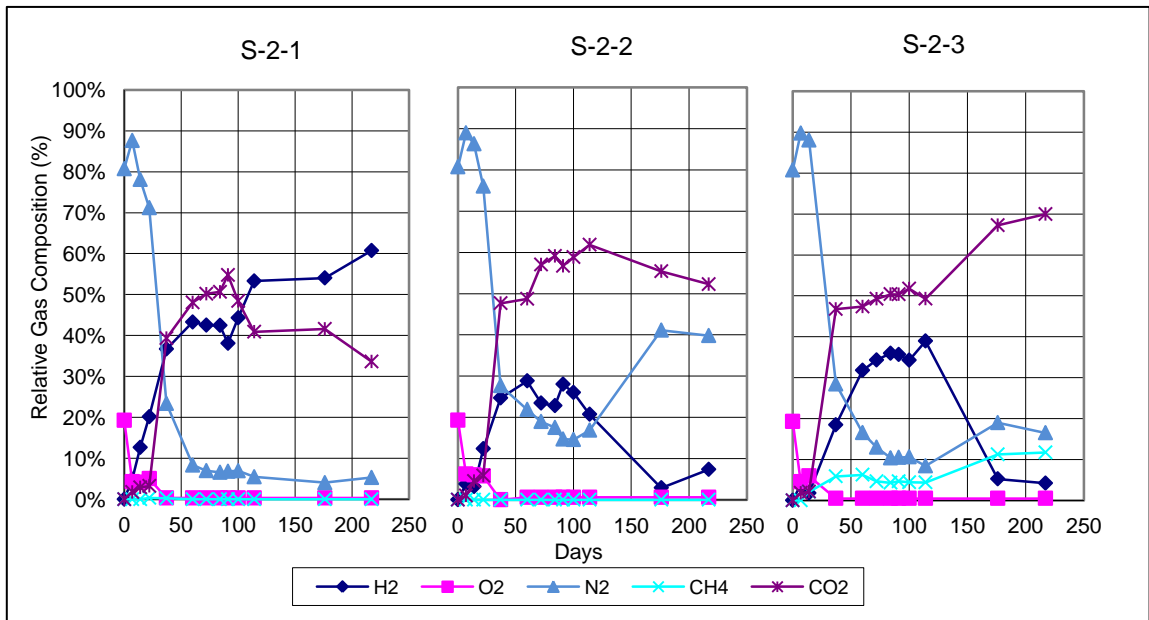
Figure 22. Experiment Series S-2 Relative Gas Composition
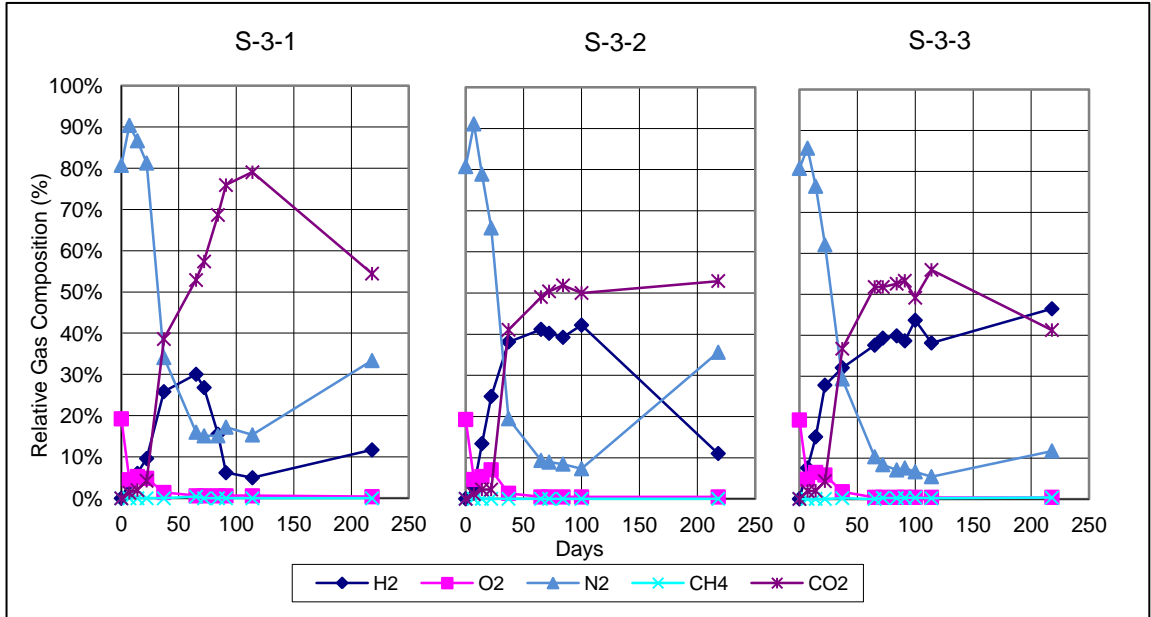
Figure 23. Experiment Series S-3 Relative Gas Composition
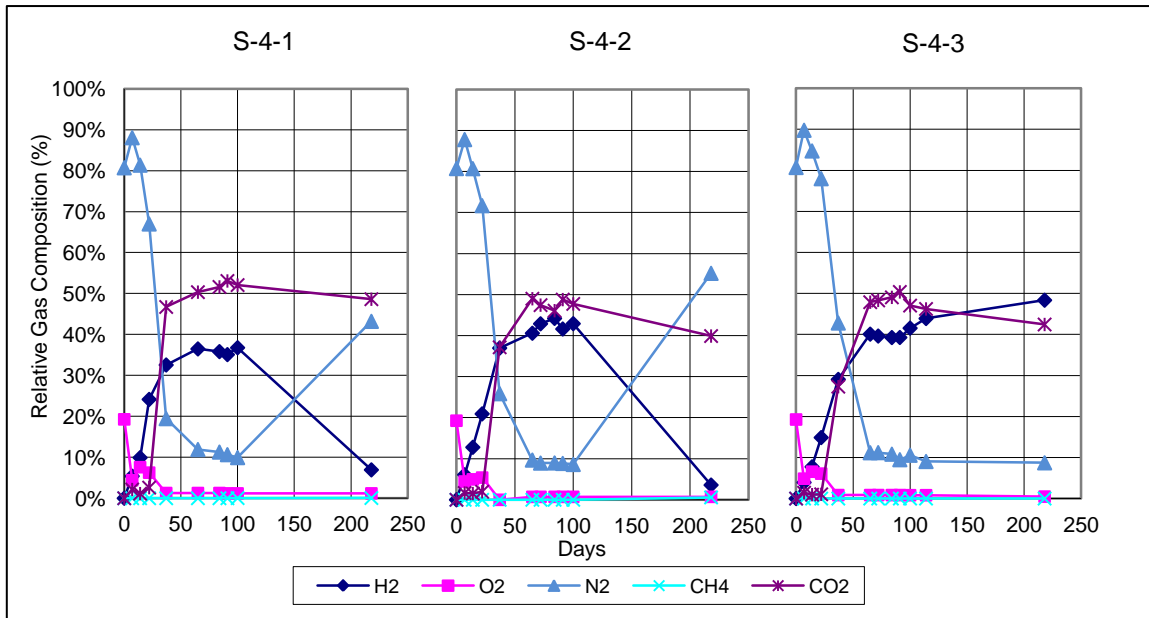
Figure 24. Experiment Series S-4 Relative Gas Composition

Overall, gas composition found in the reaction vessels differed somewhat from the typical gas composition found emanating from landfills (Figure 2). Initially, air, mostly nitrogen and oxygen, was present in the headspace of the reaction vessels. In all of the reaction vessels, the oxygen was quickly depleted and

nitrogen levels increased slightly then decreased sharply; this follows closely with

Phase I gas composition as shown in Figure 2. After 7 days of degradation,

hydrogen gas began to be produced by all of the experiments followed by carbon

dioxide at 21 days. This is indicative of Phase II gas composition, although,

typically carbon dioxide content increases before hydrogen content. Peak hydrogen

and carbon dioxide levels were reached at around day 65 in the biosolids experiments

(Figures 22, 23 and 24). Peak hydrogen and carbon dioxide levels were reached at

between 75 and 85 days for series F-1 (Figure 19) and F-2 (Figure 20), while series

F-3 (Figure 21) reached peak levels at 95 days. Figure 2 shows that hydrogen gas

accounts for a maximum of about 20% of the gas produced within a typical landfill

during Phase II. Most of the reaction vessels had hydrogen gas levels exceeding

30%, while series F-3 (Figure 21) had hydrogen gas levels exceeding 50%.

As the experiments produced more gas, remaining nitrogen levels dropped as

it was displaced from the reaction vessels following with typical gas composition

patterns during Phase II. Hydrogen and carbon dioxide continued to be the main

gases present in the reaction vessels, but at different levels than indicated by the

typical landfill gas composition shown in Figure 2. At this point, typical landfill gas

composition would have entered Phase III, where hydrogen levels drop with a

concurrent increase in methane levels. However, hydrogen and carbon dioxide

began to be consumed in many of the reaction vessels without a concurrent increase

in methane levels. During this gas consumption phase, many of the experiments

showed a greater proportion of nitrogen gas over previous measurements as

hydrogen and carbon dioxide were consumed. The increase in nitrogen content

among the component gases was not due to production of nitrogen, but, rather, the consumption of hydrogen and carbon dioxide caused the percentage of nitrogen present in the reaction vessels to increase as no excess gases were produced.

Typical landfill gas composition (Figure 2) shows that hydrogen gas levels should drop as methane production begins; some of the experiments showed a drop in hydrogen content near the end of the measurement period (F-1-1 and F-1-3 in Figure 19, F-2-1 in Figure 20, S-2-2 and S-2-3 in Figure 22, S-3-1 and S-3-2 in Figure 23, and S-4-1 and S-4-2 in Figure 24). However, methane was not produced in significant amounts in any of the reaction vessels with the exception of vessel S-2-3, Figure 22. As mentioned earlier, the drop in hydrogen levels in many of the experiments is thought to be due to acetogenesis, where carbon dioxide and hydrogen are used by acetogenic bacteria to produce acetate. Reaction vessel S-2-3 showed methane gas production well before other reaction vessels; this is thought to have occurred due to a small microcosm of methanogens present in the initial waste mixture. At the end of the measurement period, none of the experiments had reached Phase III gas composition. Hydrogen and carbon dioxide levels remained consistent or dropped due to consumption, and methane levels did not increase.

Cumulative gas volume produced in each of the experiments was used along with gas composition to determine the volumes of gas generated throughout the experiment. Table 15 presents the results of gas volume calculations.

Table 15. Cumulative Gas Component Production

Experiment	Hydrogen (ml)	Carbon Dioxide (ml)	Methane (ml)	Total (ml)
F-1-1	345.24	355.43	0.00	700.67
F-1-2	515.27	472.13	1.02	988.42
F-1-3	505.53	433.96	0.00	939.49
F-2-1	306.56	300.88	0.00	607.43
F-2-2	862.83	868.71	0.00	1731.55
F-2-3	816.76	860.12	0.00	1676.88
F-3-1	1158.66	880.85	0.36	2039.87
F-3-2	1331.26	914.35	0.00	2245.60
F-3-3	1134.35	864.25	0.00	1998.60
S-2-1	738.19	704.49	3.03	1445.71
S-2-2	220.20	383.66	0.00	603.86
S-2-3	336.54	606.74	78.60	1021.88
S-3-1	282.89	482.72	1.17	766.78
S-3-2	585.18	502.94	0.00	1088.11
S-3-3	589.95	577.74	1.78	1169.47
S-4-1	587.48	632.00	0.03	1219.51
S-4-2	687.04	675.29	0.21	1362.55
S-4-3	643.87	659.41	0.00	1303.28

Very little methane gas was produced in the majority of the reaction vessels. The greatest volume of methane was produced by experiment S-2-3. Methane production began in experiment S-2-3 in week two, while methane was not produced in any other experiment for over a month. The amount of methane produced in the majority of the experiments was nearly immeasurable and accounted for less than one percent of the total generated gas volume. Methane production in experiment S-2-3 indicates that a significant population of methanogens was present at the start of the experiment and an environment conducive to their growth was present in some part of that reaction vessel. The source of the initial methanogenic bacteria in the experimental vessels is likely biosolids from the wastewater treatment plant that uses anaerobic digestion. Onset of rapid stage methanogenesis was likely delayed in the rest of the experiments due to conditions within the reaction vessels. Only one

experiment series, F-3, produced any leachate; this made pH control in the rest of the reaction vessels nearly impossible. The low pH encountered throughout the majority of degradation likely inhibited growth of methanogens, and the possibility exists that no methanogens were present in the initial waste mixture.

Levels of hydrogen produced in many of the experiments were much more notable than methane. The pH of the experiments remained around 6 throughout. While this would inhibit methane production by methanogens, it is within the ideal range for hydrogen production (Mu et al., 2006). All of the experiments produced hydrogen gas in substantial amounts. The ratio of hydrogen to carbon dioxide produced in the majority of the experiments was around one, while series F-3 had ratios of over 1.3. The cumulative hydrogen gas produced by series F-3 was over 5 ml per gram of input waste and over 13 ml per gram of input food waste. Series F-3 continued to produce gas after the experiment was concluded so the final amount of hydrogen produced was not yet attained. Gas production trends followed with those reported for hydrogen producing bacteria grown on food substrates (Dong et al., 2008). In Dong et al.'s (2008) reporting, hydrogen producing bacteria were grown on pure food substrates and produced hydrogen gas composition ranging from 0% to over 70%. Ligno-cellulose, protein and oils had very low hydrogen gas production while carbohydrates like rice, potato and lettuce had very high hydrogen production. Hydrogen production also dropped off and was consumed in Dong et al.'s experiments; hydrogen consumption was thought to have occurred due to homoacetogens as input bacteria were boiled and no methane was produced. The amount of hydrogen produced in experiments performed herein is only a fraction of

the potential methane yield of 40-80 liters per kilogram (Themelis et al., 2006,

Barlaz et al., 1996).

Hydrogen production showed a better correlation with moisture content than overall gas production. Figures 25 and 26 show hydrogen gas produced vs. moisture content for the food and bio-solids experiments respectively. Non-directional probability for the food experiments was 0.003, which is much less than 0.05, indicating that the correlation is significant. The biosolids experiments had a non-directional probability of 0.19, which is greater than 0.05, indicating a non-significant correlation.
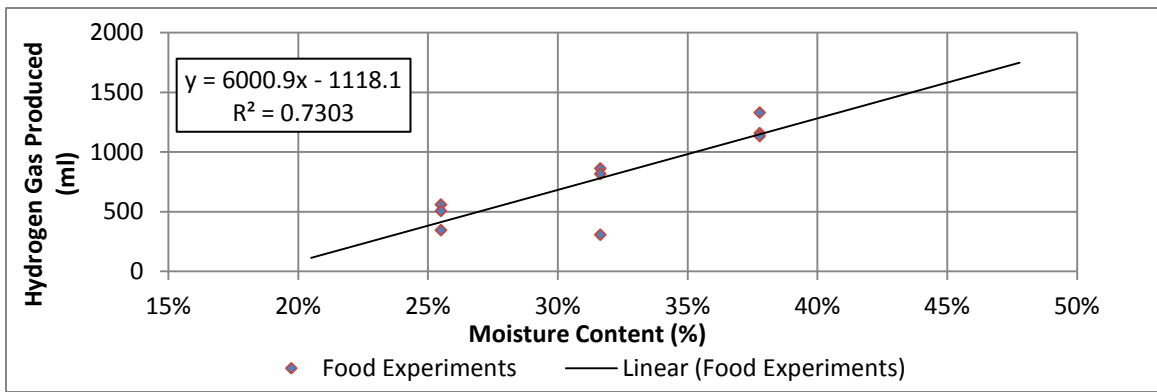

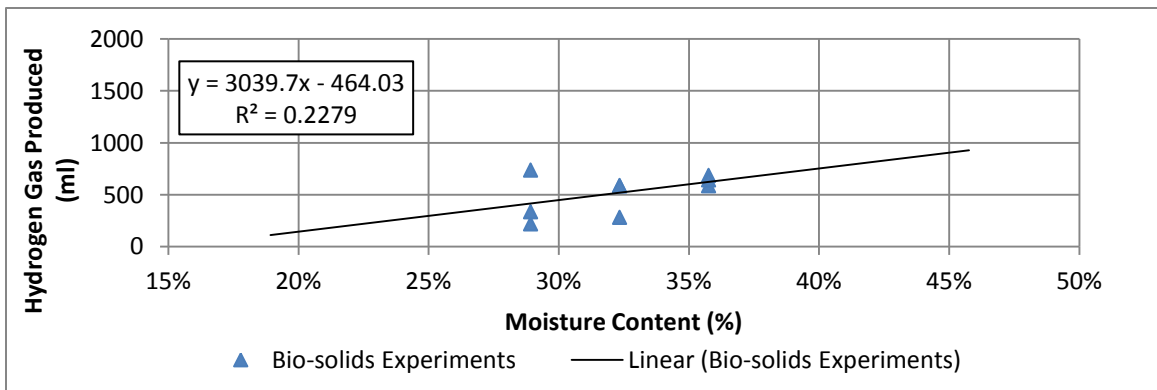Figure 25. Food Experiments Hydrogen Gas Production vs. Moisture Content

Figure 26. Bio-Solids Experiments Hydrogen Gas Production vs. Moisture Content

Both the food and bio-solids experiments showed a better correlation between hydrogen gas production and moisture content than between overall gas production and moisture content. Food experiments showed a better correlation between hydrogen gas production and moisture content than the bio-solids experiments. Statistical tests on the total hydrogen produced in the food experiments showed that there are significant differences in the total hydrogen produced in each of the experiment series. The ANOVA for hydrogen production in the food experiments showed a p-value of 0.00971; this indicates that hydrogen production among the different food experiments was statistically different. Increasing moisture content through addition of food waste causes an increase in the amount of hydrogen gas produced. Statistical tests performed on the bio-solids experiments' hydrogen production showed no significant differences. The ANOVA performed on the bio-solids hydrogen gas production results gave a p-value of 0.43; the difference in hydrogen gas production among the bio-solids experiments was not statistically significant.

The correlation of hydrogen gas production with moisture content is likely better than the correlation of overall gas production with moisture content because the overall volume contains gases from the head space above the waste that were not produced through degradation, but, rather, displaced as bio-gases were formed. Figures 27 and 28 show scatter plots of the sum of formed gases vs. moisture content; total hydrogen, carbon dioxide and methane formed in each vessel are added to determine the sum of the formed gases (Table 15). The correlation between the sum of formed gases and moisture content is better than the correlation between

66

overall gas production and moisture content. Non-directional probability for the food experiments was 0.005, which is much less than 0.05, indicating that the correlation is significant. The biosolids experiments had a non-directional probability of 0.25, which is greater than 0.05, indicating a non-significant correlation.
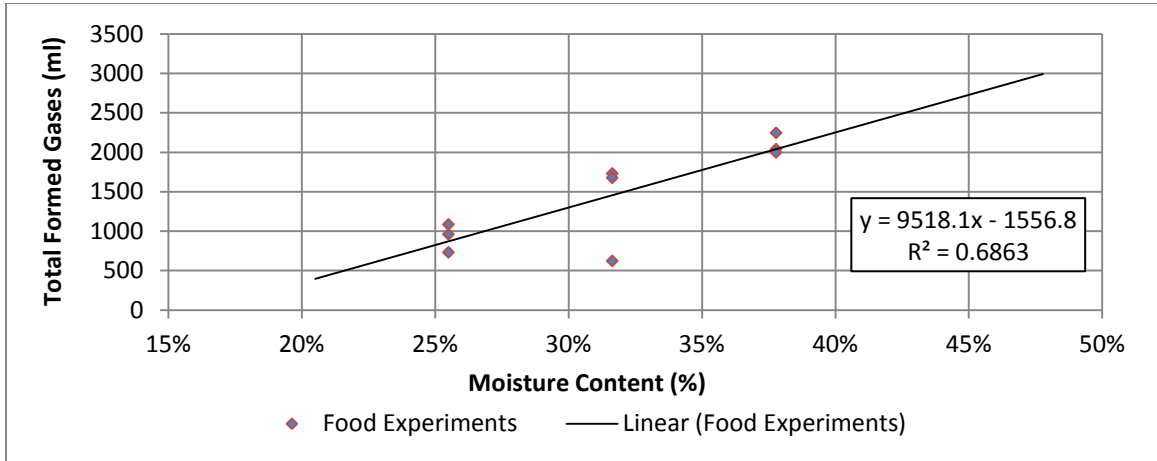
Figure 27. Food Experiments Total Formed Gases vs. Moisture Content
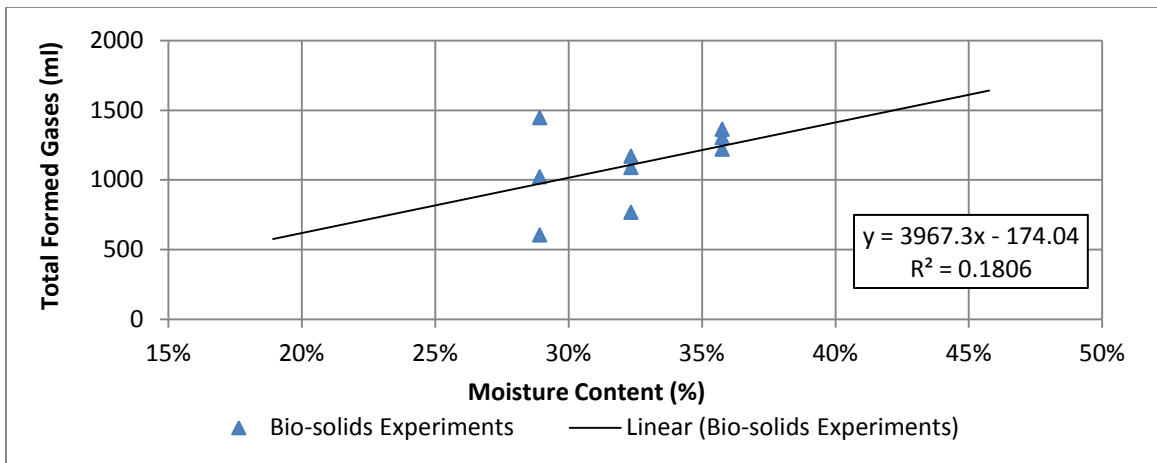
Figure 28. Bio-Solids Experiments Total Formed Gases vs. Moisture Content

Statistical analysis of the total gas formed showed that the food experiments had statistically significant differences in the total gas formed, while the bio-solids

experiments did not show statistically significant differences in the total gas formed. The ANOVA performed on the food experiments' total formed gas resulted in a p-value of 0.02569, while the bio-solids experiments had a p-value of 0.41. This indicates that increasing moisture content through addition of food waste likely causes an increase in the amount of gases formed. Food waste experiments also had significantly greater amounts of carbon present within the waste mixtures, which could indicate that carbon content may have also effected the formation of biogases.

Figures 29 and 30 show scatter plots of total gas formed vs. moisture content and total gas formed vs. carbon content respectively for all of the experiments together. The total gas formed shows a better correlation with carbon content among all of the experiments than with moisture content. This would indicate that when comparing wastes of differing composition, the carbon content may have a greater effect on gas formation than moisture content. Non-directional probability for the moisture content correlation was 0.002, which is much less than 0.05, indicating that the correlation is significant. Non-directional probability for carbon content was 0.00005, indicating a more significant correlation.
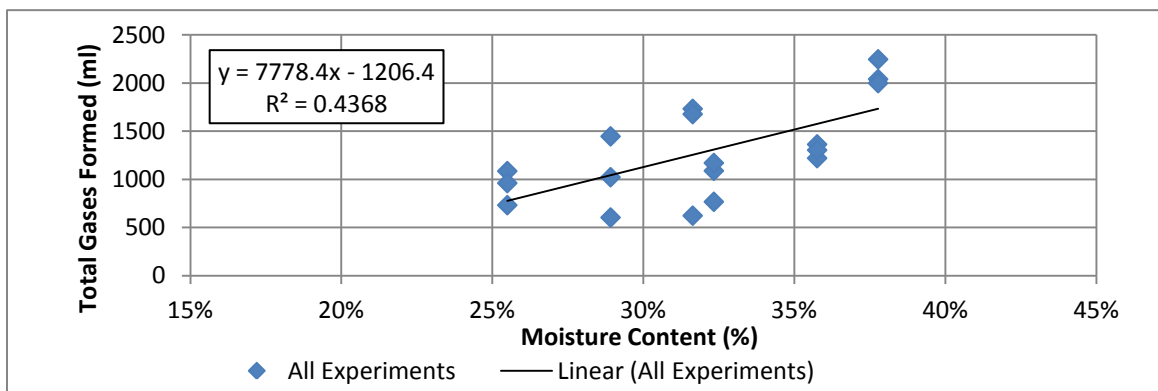
Figure 29. All Experiments Total Formed Gases vs. Moisture Content
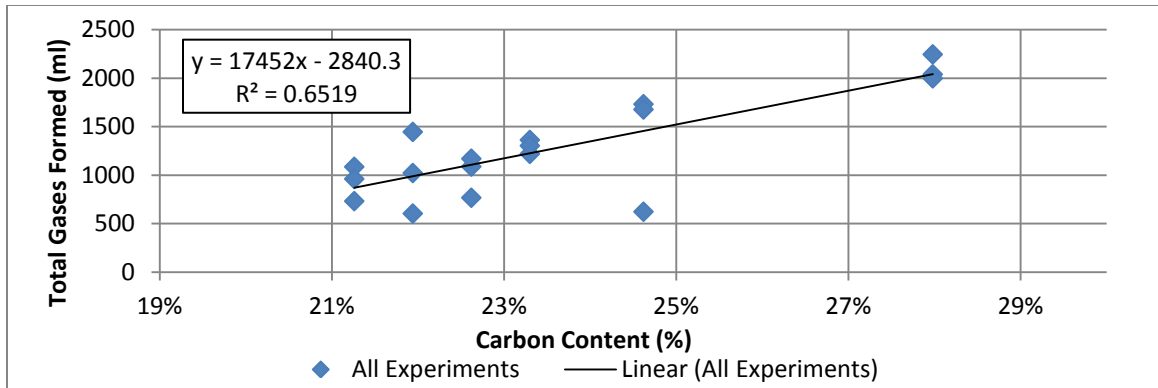
Figure 30. All Experiments Total Formed Gases vs. Carbon Content

When the food and biosolids experiments' total gases formed vs. carbon content are viewed individually, the correlation between total gases formed and carbon content is the same as the correlation between total gases formed and moisture content. Within the two main experiments, food or biosolids, carbon content and moisture content are directly related, but carbon/moisture relationship is different between the two main experiments.

4.2.3 Leachate Production and Composition

Only one experimental series produced leachate during the majority of the measurement period. Experiment series F-3 had the highest initial moisture content and produced small amounts of leachate throughout the experiment. Leachate was collected from the reaction vessels as it was produced and samples were taken for compositional measurements using a gas chromatograph. Table 16 shows the results of leachate volume measurements for each of the experiments in series F-3.

Table 16. Experiment Series F-3 Leachate Volumes and pH

Experiment:	F-3-1		F-3-2		F-3-3	
Day	Vol. (ml)	pH	Vol. (ml)	pH	Vol. (ml)	pH
8	0.2	6	1.4	6	0.65	6
22	0	6	0.18	6	0.11	5.5
50	0.8	5.5	0.6	5.5	0.8	5.5
63	0.8	5.5	0.5	5.5	0.4	5.5
86	0.7	6	1	6	0.6	6
98	0.1	6	1	6	0.3	6

The volume of leachate produced in each of the experiments varied greatly among reaction vessels. The pH measured in series F-3 was also lower than required for growth of methanogens. The high carbon dioxide partial pressure in the reaction vessels likely caused the pH to be low. This can be seen in gas composition Figures 19 through 24 when compared with typical gas composition in Figure 2. Carbon dioxide formation followed hydrogen formation in the reaction vessels while typical gas composition shows carbon dioxide formation prior to hydrogen. It is likely that initial carbon dioxide formed during Phase I aerobic degradation was dissolved causing the pH in the reaction vessels to be acidic.

After gas production ceased in most of the reaction vessels, on day 169, 10 ml of a micronutrient broth for methanogens was injected into each vessel to ensure that adequate nutrients were present to initiate methanogenesis. At this point, measureable leachate was formed in all of the reaction vessels. This allowed for neutralization of leachate prior to recycle. Addition of moisture and neutralization of the leachate formed thereafter appeared to have no effect on gas production or the onset of methanogenesis.

Leachate fatty acid content was determined for each of the leachate samples prior to addition of methanogen micronutrients. Six fatty acids, acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids, were measured with a gas chromatograph. All of the acids were found in the leachate samples. Interestingly, the gas chromatograph indicated the presence of ethanol and other unknown compounds. Ethanol was present in all of the samples as a sharp peak near the beginning of the chromatograph readout; the unknown compounds, likely other alcohols or products of fermentation, caused peaks on the gas chromatograph readout to be almost indistinguishable from one another. Figure 31 shows the fatty acid composition of the F-3 series of experiments. Results of the fatty acid measurements show that acetate and valerate remained at a relatively low concentration throughout the initial part of experimentation. Propionate, isobutyrate, butyrate and isovalerate concentrations increased as the experiment progressed. Fatty acids found in the leachate are degradation products of fermentation; these acids can be formed from degradation of a number of differing compounds. Degradation of the longer chain fatty acids is dependent on concentrations of hydrogen and acetate.
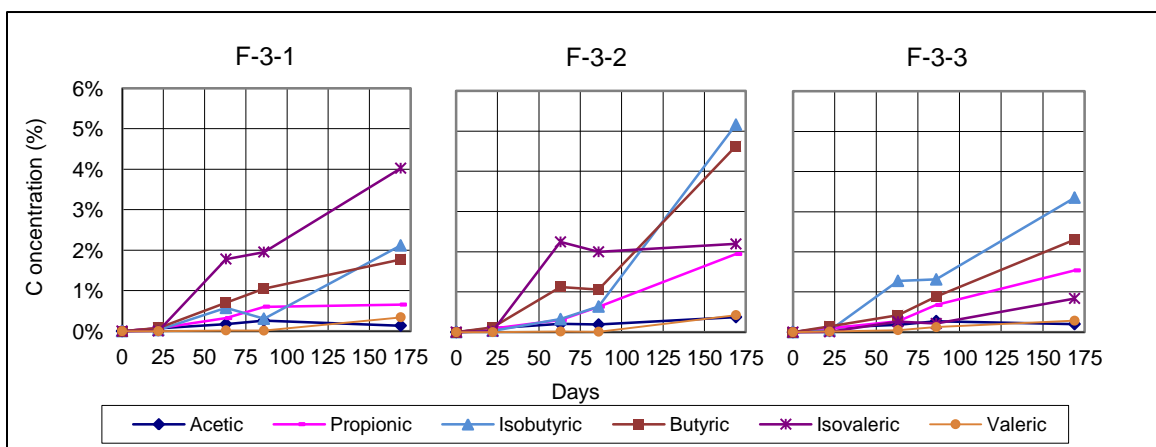
Figure 31. Experiment Series F-3 Fatty Acid Content

71

Energy cannot be derived from further degradation of these acids by microbes until degradation products, hydrogen and acetate, are at low enough concentrations (Voolapalli et al., 1999). Hydrogen gas was present throughout the experiment likely causing the buildup of the longer chain fatty acids. The ending concentration of fatty acids in the F-3 experiments varied widely as shown in Figure 29; each of the experiments showed different levels of the fatty acids tested.

4.3 Final Measurements

At the conclusion of the experiments, final moisture content and microbial measurements were undertaken. Results of final measurements tended to not match well with initial expectations.

4.3.1 Final Moisture Content

The contents of each of the experiments were emptied and random, representative samples were taken for moisture content analysis. Three samples from each experiment were analyzed and the final moisture content was taken as the average of the three samples. Results of the final moisture content calculations are shown in Table 17.

Table 17. Final Moisture Content Results

Experiment	Average Moisture Content	Standard Deviation
F-1-1	24.86%	28.42%
F-1-2	30.16%	6.59%
F-1-3	31.02%	0.14%
F-1 Average	**28.68%**	
F-2-1	35.68%	6.10%
F-2-2	35.56%	1.50%
F-2-3	33.26%	1.63%
F-2 Average	**34.83%**	
F-3-1	40.70%	2.93%
F-3-2	38.91%	1.52%
F-3-3	40.24%	0.54%
F-3 Average	**39.95%**	
S-2-1	30.38%	1.24%
S-2-2	34.55%	2.31%
S-2-3	37.85%	1.68%
S-2 Average	**34.26%**	
S-3-1	41.42%	3.83%
S-3-2	36.47%	5.73%
S-3-3	41.59%	3.62%
S-3 Average	**39.82%**	
S-4-1	39.57%	0.46%
S-4-2	40.43%	2.19%
S-4-3	38.58%	0.91%
S-4 Average	**39.53%**	

The final moisture content of all of the experiments went up. This is likely due to a combination of a number of factors. Some biochemical reactions result in the production of water; hydrolysis produces water as an end product. Other volatile compounds were formed during fermentation; gas chromatograph output of the fatty acid analysis showed that ethanol was present in the leachate. Although not specifically identified in the gas chromatograph output, isopropyl alcohol could also have been present in the leachate. The volatile fatty acids and possible longer chain alcohols present in the experiments should not have evaporated along with the water and shorter chain alcohols at $103\text{-}105^{o}\,$C.

Figures 32 and 33 show scatter plots of total gas production vs. final moisture content of the experiments. The correlation between final moisture content and total gas production is not as strong as the correlation between initial moisture content and total gas production. The food experiments showed a much stronger correlation between final moisture content and total gas produced than the bio-solids experiments. The non-directional probability for the food experiments was 0.015, for the biosolids experiments it was 0.69; the food experiments had a significant correlation with final moisture content and the biosolids experiments show little significance.
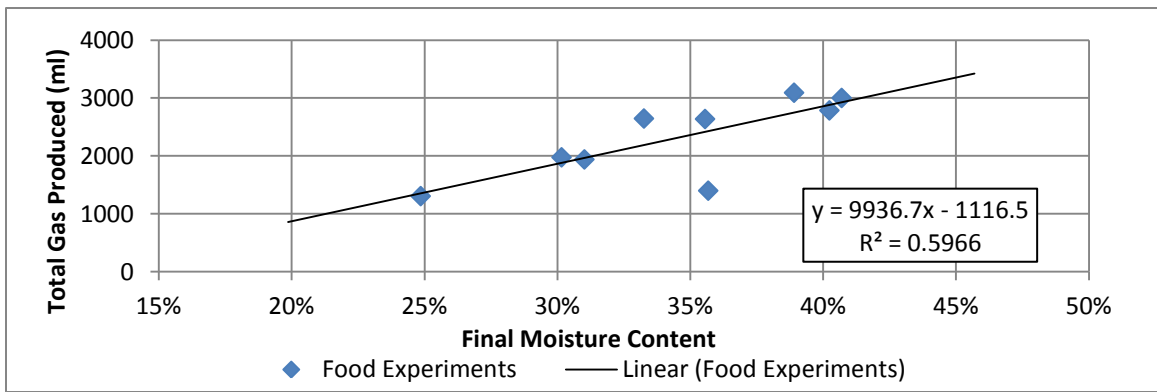
Figure 32. Food Experiments Total Gas Produced vs. Final Moisture Content
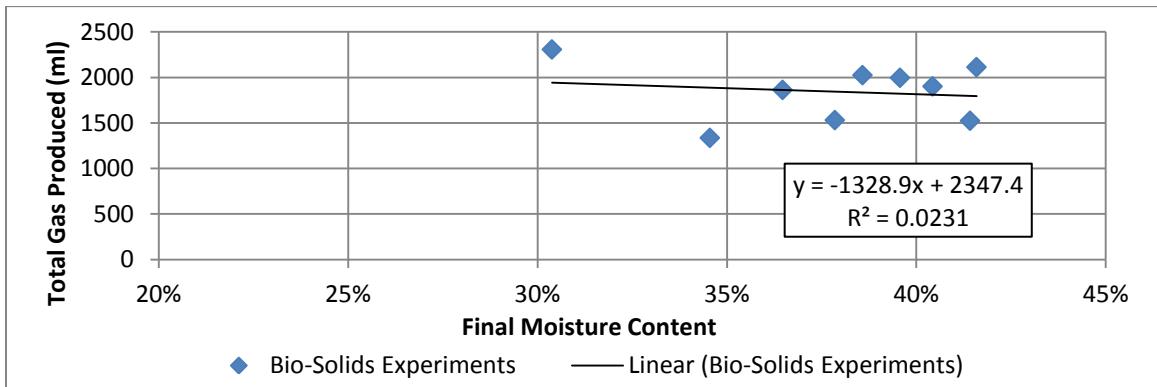
Figure 33. Bio-Solids Experiments Total Gas Produced vs. Final Moisute Content

Final moisture content showed similarly poor correlations with total hydrogen formed and total formed gases. Figures 34 and 35 show scatter plots of total hydrogen vs. final moisture content for food experiments and bio-solids experiments respectively. Figures 36 and 37 show scatter plots of total formed gas vs. final moisture content for food experiments and bio-solids experiments respectively.
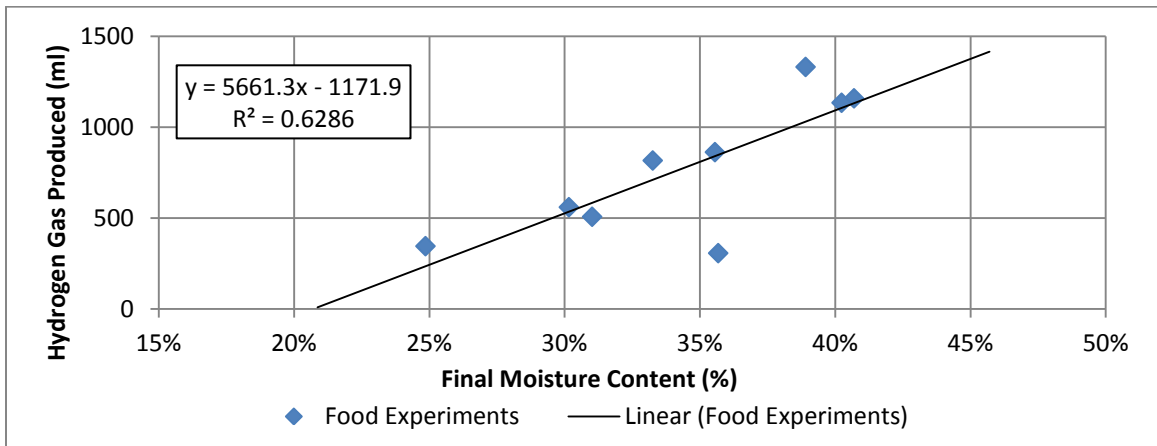
Figure 34. Food Experiments Hydrogen Production vs. Final Moisture Content
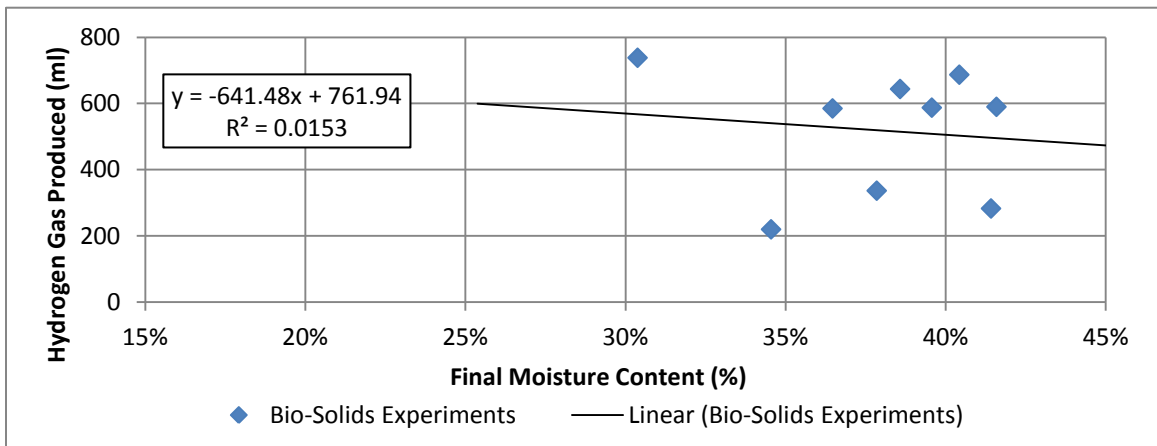
Figure 35. Bio-Solids Experiments Hydrogen Production vs. Final Moisture Content
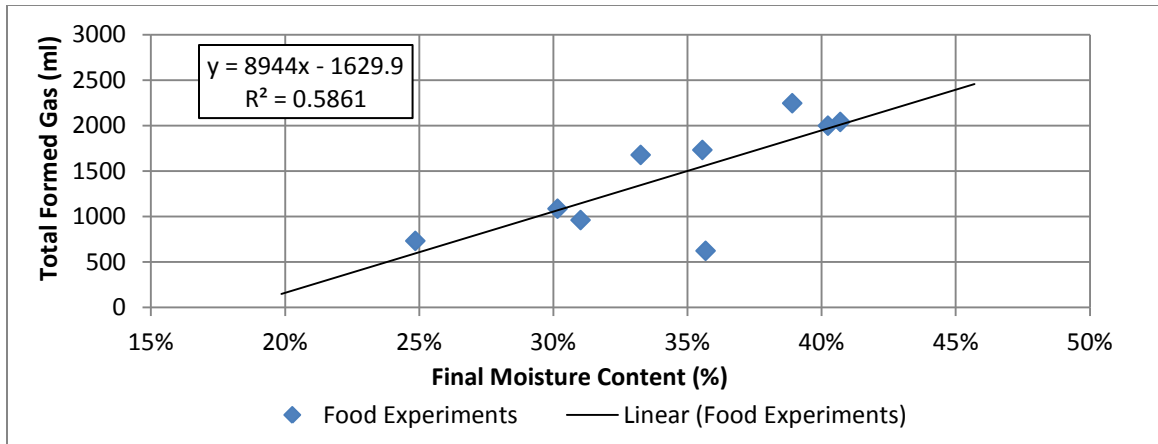
Figure 36. Food Experiments Total Formed Gas vs. Final Moisture Content
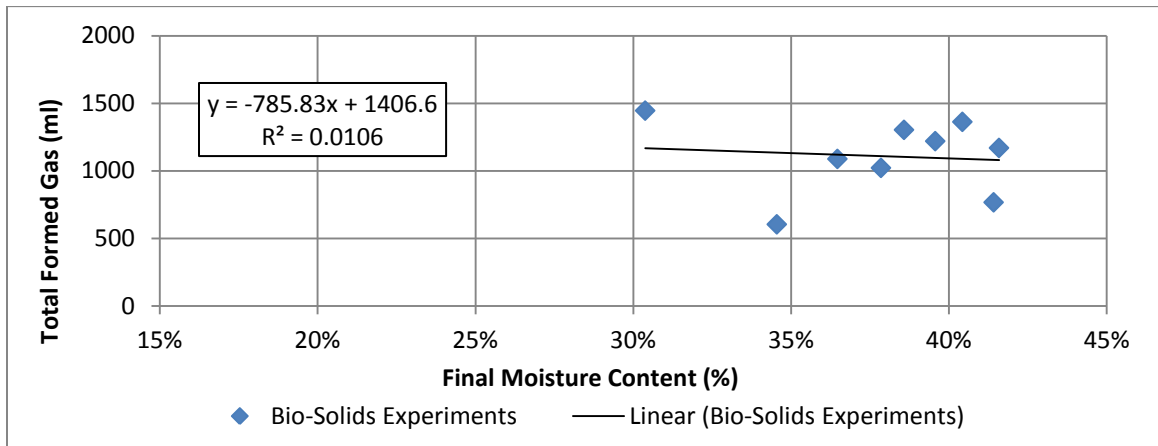
Figure 37. Bio-Solids Experiments Total Formed Gas vs. Final Moisture Content

Correlation of hydrogen production and total formed gas with final moisture content are not as strong as with initial moisture content. The food experiments showed fairly strong correlations with correlation coefficients close to those found for initial moisture content, but the bio-solids experiments showed almost no correlation. Non-directional probability for the food experiments' final moisture content vs. hydrogen production was 0.01; non-directional probability for the food experiments' final moisture content vs. total formed gas was 0.016. These values indicate that the correlation between hydrogen gas production and total gas

production vs. final moisture content is significant for the food experiments. Non-directional probability for the biosolids experiments' final moisture content vs. hydrogen production was 0.75; non-directional probability for the biosolids experiments final moisture content vs. total formed gas was 0.79. These values indicate that the correlation between hydrogen gas production and total gas formed vs. final moisture content is not significant for the biosolids experiments.

4.3.2 Biological Measurements

Cell counts for each of the hybridizations performed were conducted using ImageJ software. Four hybridizations were attempted on four of the six reaction series and on experiment S-2-3. Experiment S-2-3 was the only experiment to produce methane consistently; hybridizations were performed on this experiment to determine if there were significant differences in the proportion of archaea and bacteria present. The lowest and greatest moisture content experiments were hybridized to determine if a significant difference in microbial populations existed between the lowest and highest moisture content experiments. General bacterial probe, EUB 338, and archaeal probe, ARCH 915, bound well with cultured cells. Acetotrophic, MX 825 for *Methanosaetaceae*, and hydrogenotrophic, Mb 1200 for most *Methanomicrobiales*, probes did not bind with any of the cell samples. Due to the lack of methane production it can be assumed that significant methanogen populations were not present in the reaction vessels' leachate. Probe MX 825 may also have not been properly targeted at thermophilic members of *Methanosaetaceae*, as other probes have been developed to cover species that have been discovered since initial probe formulation. Results of cell counts for bacteria and archaeal probes are

shown in Table 18.   The analyses were somewhat difficult to perform; a number of

trial attempts failed to show hybridization with any of the cells. It was found that

cells from leachate samples would not hybridize with any of the probes; the exact

reason is not clear. FISH requires a sufficient amount of rRNA to bind with; cells

collected directly from leachate may not have had sufficient rRNA for hybridization.

To overcome the difficulty with hybridizing leachate samples directly, samples were

cultured in either an anaerobic or a methanogenic broth prior to hybridization. These

ensured that the cells would be actively growing and have sufficient rRNA for

hybridization.

Table 18. FISH Cell Count Results

Experiment	EUB 338 Ethanol Fixation	EUB 338 Paraformaldehyde Fixation	ARCH 915 Ethanol Fixation	ARCH 915 Paraformaldehyde Fixation
F-1	93.37%	87.14%	18.94%	6.15%
F-3	87.24%	66.67%	22.91%	10.17%
S-2	84.12%	91.82%	25.98%	17.95%
S-4	52.66%	93.45%	19.18%	19.86%
S-2-3	77.63%	82.28%	23.61%	15.70%

There is a slight difference in the number of archaea present in series F-3 as

compared to F-1, but the difference is small. The results of the hybridizations on

bio-solids experiments are fairly similar between each of the experiments. Series F-3

and S-4 show a greater variation in bacterial populations present in the

paraformaldehyde and ethanol fixed cells respectively. This is most likely indicative

of a poor hybridization rather than any kind of population difference among the

experiments. The other bacterial hybridizations show relatively similar percentages

between the paraformaldehyde fixed cells and the ethanol fixed cells. The results

may be somewhat skewed due to population selection and amplification during culturing. The broths used for culturing likely caused some microorganisms to grow at a much greater rate than others making the results of the FISH analyses inconclusive. Typical images of the EUB 338 hybridized cells are shown in Figure 38. The left image shows a typical EUB 338 Cy3 probe image and the right image shows a typical DAPI stain of the same microbes. DAPI stains the DNA of all organisms and results in a blue color.
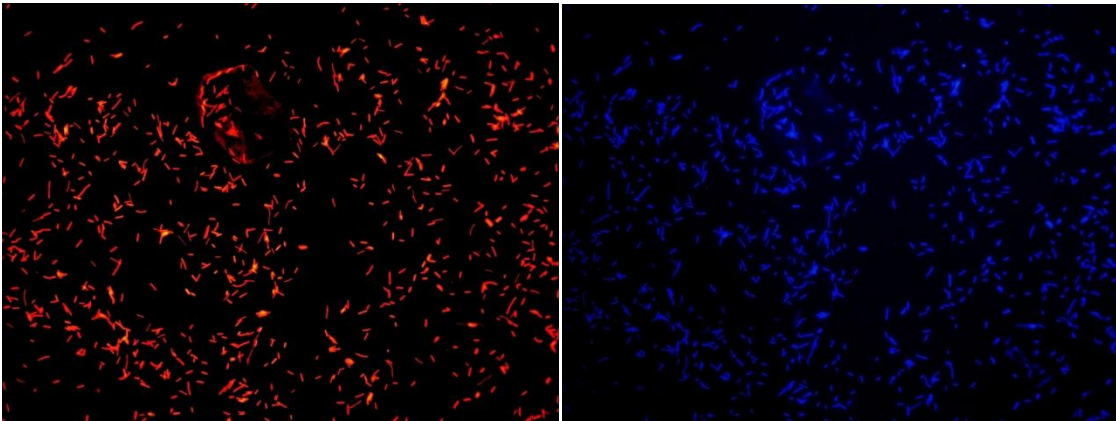
Figure 38. Typical Bacteria Cell Images, EUB 338 Cy3 left, DAPI Right

When the images are overlaid, the Cy3 image shows slightly less microbes than the DAPI image. This indicates that some of the microbes are not bacteria, or some of the bacteria that do not have rRNA that will bind with the EUB 338 probe. Figure 39 shows ARCH 915 hybridized cells.

Figure 39. Typical Archaea Cell Image, ARCH 915 Cy3 Left, DAPI Right

The ARCH 915 image shows much fewer hybridized cells, demonstrating that fewer archaea were present in the leachate samples.

4.4 Discussion

After an initial lag period, waste mixtures with the greatest moisture and organic contents produced the greatest amount of biogas. Total biogas production within the food experiment sets corresponded well with moisture content and organic content; lower moisture content experiments produced less overall biogas than the high moisture content experiments. Leachate formation also corresponded to moisture content; no measurable leachate was formed in any of the reaction vessels except in the experiment with the highest moisture content, food experiment series F-3. Moisture content and organic content of the experiments also had an effect on the composition of gases formed throughout the experiments. It was found that during initial waste fermentation higher moisture/organic content wastes produced a greater proportion of hydrogen gas than did wastes with lower moisture/organic contents.

Hydrogen gas made up more than 50% of the gas formed during fermentation within the highest moisture/organic content experiments, food experiment series F-3.

The onset of methanogenesis was quite slow with the exception of a single reaction vessel. It took nearly five (5) months for experiments to begin producing methane. A reaction vessel within one of the biosolids series of experiments, vessel S-2-3, was the first to produce methane gas on a regular basis; however, the two other replicate reaction vessels within that experimental series did not show the same levels of methane. This is thought to have occurred due to the presence of methanogens in the input waste mixture; a small micro-environment likely existed that allowed for the methanogens to grow and produce methane throughout most of the measurement period. The experiments were not able to reach full methanogenesis during the experimental period. The onset of methanogenesis was likely delayed due to one of two possibilities, environmental conditions within the reactions vessels were not correct for growth of methanogens, or a sufficient population of methanogens was not present in the input waste of all of the experiments. The length of time required to reach methanogenic conditions in the experiments was not anticipated at inception. Had the experiments been allowed to degrade for a much greater length of time, methanogenesis may have begun, allowing for an assessment of tested variables. Hydrogen production was quite notable; all of the experiments produced significant amounts of hydrogen gas. Hydrogen and fatty acid formation during fermentation caused the pH of the waste to drop to levels that inhibit the growth of methanogens; the low pH was nearly ideal for the growth of hydrogen producing bacteria.

The experimental design itself may have contributed to the slow onset of methanogenic conditions. Reaction vessels were intended to allow for the easy extraction of both gases and leachate formed during the experiment. The vessels did not, however, allow for complete compaction of the waste to a level consistent with a typical landfill with the amounts of waste used. While filling the reaction vessels, the lower moisture content waste mixtures tended to be "fluffy" and did not maintain any compaction. The headspace above the waste allowed for storage of produced gases; it is thought that exposure to an excess of hydrogen and carbon dioxide allowed for acetogenic microorganisms to grow to greater level than would occur in a system with no gas storage. A similar condition was noted by Dong et al., during hydrogen production experiments; peak hydrogen production was limited due to consumption of the gas by what was asserted to be acetogenic bacteria. Production of acetate could not be measured in the vessels that had large consumption of gases due to the lack of leachate production to verify this hypothesis. A lack of an initial thermophilic methanogen population could have also been the sole reason for the lack of methane production.

4.4.1 Recommendations for Future Research

Several issues that arose during experimentation could be resolved with some changes in experimental design and measurement methods. At the start of experimentation, more waste could have been used in the experiments. A larger amount of waste at a greater compaction level could allow for accumulation of leachate in some of the lower moisture content experiments, but leachate production would not be guaranteed. A greater amount of leachate would have allowed for

greater control of reactor pH; leachate pH measurements were all below that required for methanogen growth. A greater volume of leachate would have allowed for more rapid stabilization of reactor conditions, thus causing earlier onset of methanogenesis.

Some difficulties were encountered during periodic measurements of gases and leachate. During rapid gas production, gas volume production and component measurements were easily completed. As gas production ceased and consumption began, no mechanism was present to determine the amount of gas consumed other than injection of an inert gas. This also made collection of gas samples rather difficult. Gases present in the reaction vessels were below atmospheric pressure; this caused gas collection syringes to suck in air after being pulled from the septum. Many samples were fully contaminated with air and unreadable results were obtained from the gas chromatograph. This was solved by using a syringe valve to prevent air from infiltrating after removal from the septum. This still made gas component measurements somewhat erroneous; gas component measurements showed an increase in the amount of nitrogen present after gas consumption began. Nitrogen production is not thought to have occurred in any significant fashion, but the proportion of nitrogen in the vessels went up when the levels of hydrogen and carbon dioxide were depleted. A mechanism that would allow for the accumulation of gases outside of the reaction vessel and maintain separation of the produced gases from the degrading waste could alleviate gas consumption problems.

The lack of large volumes of leachate and inconsistent production made measurements difficult to obtain. Leachate composition made pH measurements and gas chromatograph readings inconsistent. Very small volumes of leachate were produced and this limited the methods of obtaining an accurate pH. Acidity was determined using pH strips, but leachate was brown in color and became much darker as measurements went on. Leachate color made reading the pH strips very difficult, and the small volumes obtained prevented use of a standard pH probe. Use of a needle pH probe in future research would allow for pH readings to be taken from all of the reaction vessels, regardless of excess leachate formation. The leachate was full of a number of solids and microorganisms. The small volumes obtained prevented filtration using syringe filters prior to analysis with the gas chromatograph. Solids present in the leachate tended to build up on the fused silica within the neck of the gas chromatograph causing a limited number of accurate readings prior to replacement of the silica. The gas chromatograph itself was quite touchy even with control samples and obtaining repeatable results was quite difficult when analyzing liquids. Multiple readings of the same sample were taken to ensure some accuracy. A number of other compounds were present in the leachate other than those intended for measurement. Unknown compound peaks made gas chromatograph readings difficult to interpret. Future attempts at analyzing leachate should be done using a different method.

The presence of thermophilic methanogens in the input waste could not be verified. To ensure that methanogens are present, leachate from an active landfill could be used to spike the initial waste mixture. This could be done by adding

84

leachate to the sand layer of the experiment without causing a change in the input waste moisture conditions. Ensuring the addition of methanogens at the beginning of the experiments would make reactor conditions (pH or moisture content) the primary reason for a lack of methane production.

An alternative method of analyzing the microbial communities present within the reaction vessels would allow for more precise quantification of bacteria, archaea and other microbes possible present. FISH probes failed to bind with microbes fixed directly from leachate samples. Leachate was used to seed culture broths with microorganisms so FISH analyses could be performed. Using the broths to grow microbes can cause selection of certain organisms that grow well within the broth environment and distort the actual amount found within the leachate. Using quantitative polymerase chain reaction directly on leachate would allow for a quantitative determination of the microbes present in the reaction vessels.

4.4.2 Conclusions

Most of the results of laboratory scale biodegradation experiments conformed to initial expectations, but some of the results presented herein did not produce conclusive correlations of the tested variables. Results of gas volume and component measurements allowed for determination of the effects of moisture augmentation using food waste on initial gas formation and composition during fermentation of MSW, demonstrating that statistically significant different hydrogen gas and total formed gases were found among the experiments. Results obtained from moisture augmentation using bio-solids demonstrated no statistically significant

85

difference in the end gas volumes, hydrogen gas produced or total gas formed among the experiments performed. It was found that the lower moisture content waste mixtures tended to have greater variability in gas volume produced, and that higher moisture content mixtures had less variable gas production. The highest moisture content food experiment produced more hydrogen gas than the other food experiments as well as a greater proportion of hydrogen to carbon dioxide. Methane formed during experimentation tended to be somewhat random. Experiment S-2-3 began methane production in week two of the experiment, while other experiments produced only small amounts of methane after several months.

Arid region landfills could use food waste to increase the moisture content of input MSW. Given sufficient environmental conditions within the landfill for methanogen growth, the additional hydrogen gas produced from additional food waste would allow for more methane production. The additional moisture from food waste would allow for an increase in the rate of overall bio-gas formation and waste degradation. Addition of bio-solids to MSW did not show any conclusive results; however, the higher moisture content experiments had more consistent gas production values than lower moisture content experiments. Increasing the moisture content of the input MSW through addition of food waste or bio-solids would make subsequent bio-gas production more consistent.

REFERENCES CITED

Amann R. I., Binder B. J., Olson R. J., Chisholm S. W., Devereux R. and Stahl D. A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56: 1919-1925

Amann, R. I., Ludwig, W., Shleifer, K. H., 1995. Phylogenetic Identification of Individual Microbial Cells without Cultivation. Microbiological Review 59:143-169

Atlas, R. M., 2004. Handbook of microbiological media. CRC Press LLC, Boca Raton, Florida

Barlaz, Morton A., Ham, Robert K., Schaefer, Daniel M., 1992. Microbial, Chemical and Methane Production Characteristics of Anaerobically Decomposed Refuse With and Without Leachate Recycling. Waste Management & Research 10:257-267

Barlaz, Morton A., Palmisano, Anna C., 1996. Microbiology of Solid Waste. CRC Press Inc., New York

Behera, S., Park, J., Kim, K., Park, H., 2010. Methane Production from Food Waste Leachate in Laboratory-Scale Simulated Landfill. Waste Management 30; 1502:1508

Chen, A., Imachi, H., Sekiguchi, Y., Ohashi, A., Harada, H., 2003. Archaeal community compositions at different depths (up to 30 m) of a municipal solid waste landfill in Taiwan as revealed by 16S rDNA cloning analyses. Biotechnology Letters 25: 719-724

Demirbas, A., 2006. Biogas Production From Organic Fraction of Municipal Solid Waste. Energy Sources, Part A, 28; 1127:1134

Dong, L., Zhenhong, Y., Yongming, S., Xiaoying, K., 2008. Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. International Journal of Hydrogen Energy 34: 812-820

Eaton, A. D., Clesceri, L. S., Rice, E. W., Greenberg, A. E., 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association, Washington, D.C.

Erses, A., Onay, T., Yenigun, O., 2007. Comparison of aerobic and anaerobic degradation of municipal solid waste in bioreactor landfills. Bioresource Technology 99; 5418:5426

Filipkowaska, U., Agopsowicz, M., 2004. Solids Waste Gas Recovery Under Different Water Conditions. Polish Journal of Environmental Studies 13; 663:669

He, R., Shen, D., Wang, J., He, Y., Zhu, Y., 2005. Biological degradation of MSW in a methanogenic reactor using treated leachate recirculation. Process Biochemistry 40; 3660:3666

Hernandez-Berriel, M., Marquez-Benevides, L., Gonzalez-Perez, D., Buenrostro-Delgado, O., 2008. The effect of moisture regimes on the anaerobic degradation of municipal solid waste from Metepec (Mexico). Waste Management 28; S14:S20

Intergovernmental Panel on Climate Change (IPCC), 1996. Report on the Twelfth Season of the Intergovernmental Panel on Climate Change, Mexico City, 11-13 September 1996

Karadag, D., Puhakka, J. A., 2010. Effect of changing temperature on anaerobic hydrogen production and microbial community composition in an open-mixed culture bioreactor. International Journal of Hydrogen Energy 35: 10954-10959

Klass, Donald L., 1984. Methane from Anaerobic Fermentation. Science, New Series 223:1021-1028

Komilis, D. P., Ham, R. K., Stegmann, R., 1999. The Effect of Landfill Design and Operation Practices on Waste Degradation Behavior: A Review. Waste Management & Research 17:20-26

Laloui-Carpenter, W., Li, T., Vigneron, V., Mazeas, L, Bouches, T., 2006. Methanogenic diversity and activity in municipal solid waste landfill leachates. Antonie van Leeuwenhoek 89:423-434

Lay, C. H., Wu, J. H., Hsiao, C. L., Chang, J. J., Chen, C. C., Lin, C. Y., 2009. Biohydrogen production from soluble condensed molasses fermentation using anaerobic fermentation. International Journal of Hydrogen Energy 35: 13445-13451

Lou, X., Nair, J., 2009. The impact of landfilling and composting on greenhouse gas emissions – A Review. Bioresource Technology 100; 3792:3798

MacLeod, I., Savage, A. L., Pahl, O., Baird, J., 2008. Decline in Microbial Activity does not Necessarily Indicate an end to Biodegradation in MSW-Biowaste: A Case Study. Bioresource Technology 99:8626-8630

Madigan, M., Martinko, J., Dunlap, P., Clark, D., 2009. <u>Brock Biology of Microorganisms</u> 12th edition. Benjamin Cummings

Marneri, M., Antoniou, K., Mamais, D., September 2009. Microthrix Parvicella in Mesophilic and Thermophilic Anaerobic Digestion Systems at Different Detention Times. International Conference on Environmental Science and Technology.

McDonald, James E., Allison, Heather E., McCarthy, Alan J., 2010. Composition of the Landfill Microbial Community as Determined by Application of Domain- and Group-Specific 16S and 18S rRNA-Targeted Oligonucleotide Probes. Applied and Environmental Microbiology 76:1301-1306

McDonald, James E., Lockhart, Robert J., Cox, Michael J., Allison, Heather E., McCarthy, Alan J., 2008. Detection of Novel *Fibrobacter* Populations in Landfill Sites and Determination of Their Relative Abundance via Quantitative PCR. Environmental Microbiology 10:1310-1319

McMahon, Katherine D., Stroot, Peter G., Mackie, Roderick I., Raskin, Lutgarde, 2001. Anaerobic Codigestion of Municipal Solid Waste and Biosolids Under Various Mixing Conditions—II: Microbial Population Dynamics. Water Resources 35:1817-1827

Meima J. A., Naranjo, N. Mora, Haarstrick, A., 2008. Sensitivity Analysis and Literature Review of Parameters Controlling Local Biodegradation Processes in Municipal Solid Waste Landfills. Waste Management 28:904-918

Mora-Naranjo, N., Meima, J. A., Haarstrick, A., Hempl, D. C., 2004. Modeling and Experimental Investigation of Environmental Influences on the Acetate and Methane Formation in Solid Waste. Waste Management 24; 763:773

Morris, J. W. F., Vasuki, N. C., Baker, J. A., Pendleton, C. H., 2003. Findings from Long-Term Monitoring Studies at MSW Landfill Facilities with Leachate Recirculation. Waste Management 23:653-666

Moter, A., Gobel, U., 2000. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. Journal of Microbiological Methods 41:85-112

Mu, Y., Zheng, X. J., Yu, H. Q., Zhu, R. F., 2006. Biological hydrogen production by anaerobic sludge at various temperatures. International Journal of Hydrogen Energy 31: 780-785

Nakamura, K., Terada, T., Sekiguchi, Y., Shinzato, N., Meng, X., Enoki, M., Kamagata, Y., 2006. Application of Pseudomurein Endopeptidase to Fluorescence in Situ Hybridization of Methanogens within the Family Methanobacteriaceae. Applied and Environmental Microbiology, Nov. 2006, 6907-6913

Osborn, A. Mark.; Smith, Cindy J., 2005. Molecular Microbial Ecology BIOS Advanced Methods. Taylor & Francis Routledge, New York, NY.

Pacey, J., Augestein, D., Morock, R., Reinhart, D., Yazdani, R., 1998. The Bioreactor Landfill – An innovation in Solid Waste Management. http://www.yolocounty.org/recycle/docs/Bioreactor_issues.pdf

Read, Adam D., Hudgins, Mark, Harper, Sid, Phillips, Paul, Morris, John, 2001. The Successful Demonstration of aerobic Landfilling The Potential for a More Sustainable Solid Waste Management Approach? Resources, Conservation and Recycling 32:115-146

Reichel, T., Ivanova, L. K., Beaven, R. P., Haarstrick, A., 2007. Modeling Decomposition of MSW in a Consolidating Anaerobic Reactor. Environmental Engineering Science 24 1072:1083

Sanphoti, N., Towprayoon, S., Chaiprasert, P., Nopharatana, A., 2006. The Effects of Leachate Recirculation with Supplemental Water Addition on Methane Production and Waste Decomposition in a Simulated Tropical Landfill. Journal of Environmental Management 81:27-35

Senior, Eric, 1994. Microbiology of Landfill Sites. CRC Press Inc., Boca Raton

Staley, B. F., de los Reyes, F. L., Barlaz, M. A., 2011. Effect of Spatial Differences in Microbial Activity, pH, and Substrate Levels on Methanogenesis Initiation in Refuse. Applied and Environmental Microbiology, 2011; 77 (7): 2381

Staley, Brian F., Barlaz, Morton A., 2009. Composition of Municipal Solid Waste in the United States and Implications for Carbon Sequestration and Methane Yield. Journal of Environmental Engineering October 2009:901-909

Stroot, Peter G., McMahon, Katherine D., Mackie, Roderick I., Raskin, Lutgarde, 2001. Anaerobic Codigestion of Municipal Solid Waste and Biosolids Under Various Mixing Conditions—I. Degester Performance. Water Resources 35:1804-1816

Themelis, N., Ulloa, P., 2007. Methane Generation in Landfills. Renewable Energy, 32, 1243:1257.

United States Environmental Protection Agency, Office of Solid Waste, 2010. Municipal Solid Waste in the United States, 2009 Facts and Figures. www.epa.gov

Valencia, R., van der Zon, W., Woelders, H., Lubberding, H., Gijzen, H., 2008. The effect of hydraulic conditions on waste stabilisation in bioreactor landfill simulators. Bioresource Technology 100; 1754:1761

Voolapalli, Ravi K., Stuckey, David C., 1999. Relative Importance of Trophic Group Concentrations During Anaerobic Degradation of Volatile Fatty Acids. Applied and Environmental Microbiology 66:5009-5016

VITA

Graduate College
University of Nevada, Las Vegas

Jared Michael Gore

Local Address:
        8805 Jeffreys St. #1096
        Las Vegas, Nevada 89123

Degrees:
        Bachelor of Science in Civil Engineering, 2002
        University of Nevada, Las Vegas

Special Honors and Awards:
        Renewable Energy Scholarship, GPSA Scholarship, 2011

Thesis Title: Effects of Moisture Augmentation of Municipal Solid Waste through Addition of
Food Waste or Wastewater Treatment Biosolids on Bio-gas Production for Power Generation.

Thesis Examination Committee:
        Chair Person, Dr. Jacimaria Batista, Ph.D.
        Committee Member, Dr. David James, Ph.D.
        Committee Member, Dr. Thomas Piechota, Ph.D.
        Graduate College Representative, Dr. Brian Hedlund, Ph.D.