STIMULATORY EFFECT OF VERMICOMPOST ON THE ANAEROBIC DIGESTION OF CAFETERIA FOOD WASTE

By

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ABSTRACT

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The overall objectives of this study were to evaluate the effectiveness of utilizing manure vermicompost as an additive to enhance anaerobic digestion of post-consumer cafeteria food waste in a single-stage digestion system and investigate the mechanisms associated with such enhancement. Vermicompost was chosen because of its buffering capacity, abundance of humic substance, and variety of trace metals, all of which may enhance the digestion process. The experiment was first conducted using a batch-scale biochemical methane potential assay and found that manure vermicompost added to the food waste reactors at concentrations of 2 g/L and 6 g/L both significantly increased ultimate methane yield and methane production rate. Then, a long-term study was conducted using twelve semi-continuous single-stage reactors to confirm such enhancement and further investigate the associated mechanism. The specific methanogenic activity and trace metal (iron, nickel, and cobalt) bioavailability were also evaluated. Results showed that the food waste digester without any supplement (control) had unstable and low methane production (254 mL/g VS $_{added}$/day and 455 mL/g VS $_{destroyed}$/day). During the experimental period, the control reactor experienced a dramatic reduction in pH (less than 6) due to a significant accumulation of volatile fatty acids (more than 2,600 mg/L). The trace metal bioavailability tests further demonstrated that the control digester could be deficient in nickel and iron. In contrast, the food waste digesters supplemented with manure vermicompost (2 g/L), trace metals (a mixture

of 0.01 mg/L nickel, 0.5 mg/L Fe, and 0.01 mg/L Co) or humic acids (0.4 g/L) all had stable and significantly greater methane production compared to the control. The pH was approximately 7 and volatile fatty acids were less than 200 mg/L. Among all treatments, the food waste digesters supplemented with manure vermicompost had the greatest methane production (625 mL/g VS $_{destroyed}$/day). In comparison to the control, supplementation of manure vermicompost also nearly doubled the acetate utilization rate and enhanced the propionate utilization rate by 60%. It was found that such enhanced digestion performance was likely related to the trace metals (particularly iron and nickel) provided by the vermicompost. Humic acids, naturally presented in mature vermicompost, also contributed to the enhanced performance of food waste digestion. In summary, manure vermicompost (without any additional chemical amendments) stabilized and increased methane production from anaerobic digestion of food waste in the single-stage digestion system.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AD: Anaerobic Digestion

AVG: Average

BMP: Biochemical Methane Potential

CH_4: Methane

Co: Cobalt

CO_2: Carbon Dioxide

COD: Chemical Oxygen Demand

CSTR: Continuous Stirred Tank Reactor

DI: Demonized

Fe: Iron

FW: Food Waste

FWVC: Food Waste with Vermicompost

HA: Humic Acids

H_2S: Hydrogen Sulfide

HRT: Hydraulic Retention Time

LCFA: Long Chain Fatty Acids

MAUR: Maximum Acetate Utilization Rate

MPUR: Maximum Propionate Utilization Rate

MSW: Municipal Solid Waste

N: Nitrogen

N_2: Nitrogen Gas

Ni: Nickel

OLR: Organic Loading Rate

P: Phosphorous

SEM: Standard Error of the Means

SRT: Solid Retention Time

STD: Standard Deviation of the Means

TE: Trace Elements

TS: Total solids

VC: Vermicompost

VFA: Volatile Fatty Acids

VOC: Volatile Organic Compounds

VS: Volatile solids

CHAPTER 1 INTRODUCTION

This chapter contains the background, problem statement, hypothesis, rationale, and objectives of the dissertation. Chapter 2 is a literature review which is followed by a description of the four stage of the research (Chapter 3, 4, 5, and 6). General conclusion and suggestions for future research then follow in Chapter 7.

1.1 Background

The term “food waste” is defined by the U.S. Environmental Protection Agency (EPA) as any food substance, raw or cooked, which is discarded, or intended or required to be discarded (US EPA, 2012). In 2010, more than 34 million tons of food waste was generated in the U.S., the second-largest component of municipal solid waste stream (US EPA, 2012). Only less than 3% of the total food waste was recovered and recycled, while the remaining 97% was simply thrown away (US EPA, 2012). This makes food waste the single largest component of municipal solid waste reaching landfills (US EPA, 2012). Food waste decomposition in landfills produces significant amounts of methane gas (CH_4), a greenhouse gas (GHG) with 21 times the global warming potential (100 year) of carbon dioxide (CO_2) (US EPA, 2012). An estimated 117.5 Tg CO_2 (or million metric tons of CO_2 equivalent) of methane were generated from landfills in 2009, the third- largest human-related source of methane in the U.S. (US EPA, 2012). The negative environmental impact and rising costs associated with landfill disposal have led to the development of alternative technologies for food waste management (Arvanitoyannis and Varzakas, 2008). The implementation of government initiatives, for example the European Union (EU) Landfill Directive (1999/31/EC), will further promote the diversion of food waste from landfill in pursuit

of alternative technologies such as composting, thermochemical conversion, and anaerobic digestion (AD).

Composting is a common alternative to landfill disposal of food waste, however, it requires large areas of land, emits volatile organic compounds (VOCs), and consumes energy (Mata-Alvarez et al., 2000). Food waste generally contains 74-90% mositure which makes thermochemical conversion technologies such as direct combustion or gasification undesirable due to the considerable decrease in energy efficiency (> 60%; Appels et al., 2011). In contrast, anaerobic digestion produces energy and reduces the emissions of CH_4 gas and VOCs (Mata-Alvarez et al., 2000). The residual material (digestate) contains the entire complement of nutrients originallly in the raw feedstocks which can be directly used or further composted and then used as nutrient soil amendments (Tambone et al.,2009). With such potential benefits, AD should be explored as a better recycling alternatives to landfill disposal of food waste.

What is AD? Anaerobic digestion (also called anaerobic fermentation) is a biological process that converts organic material at a modest temperature, ambient pressures, and nearly neutral pH to biogas in the absence of external electron acceptors (such as free molecule oxygen) (Klass et al., 1984). Biogas consists largely of CH_4 and CO_2 and trace amount of nitrogen (N_2), nitrogen oxides, and hydrogen sulfide (H_2S). Anaerobic digestion is a highly complex and dynamic system where microbiological, biochemical, and physical–chemical reactions are closely linked (Klass et al., 1984). If the substrate consists of high molecular weight carbohydrates, fats, and/or protein, it is first hydrolyzed to soluble polymers (simple sugars, fatty acids, alcohols, and amino acids) by

enzymatic reactions from hydrolytic bacteria. These soluble polymers are then fermented into volatile fatty acids (VFA_s), alcohols, hydrogen (H_2), and CO_2 by acidogenic bacteria. The VFAs longer than two carbons are converted to acetate and H_2 gas by the obligate hydrogen-producing acetogenic bacteria. Finally the acetate, CO_2, and H_2 are converted to CH_4 by methanogens. As a result of the CH_4 and CO_2 formation, the originally organic bound, non-carbon compounds are released to their soluble inorganic forms (Angelidaki and Sanders, 2004). The stability of the process is dependent on the critical balance between the symbiotic growth rates of the principal microbial organisms (Speece, 1996). AD is a mature biological treatment method that can be cost effective, environmentally sound and a source of renewable energy when implemented correctly (Mata-Alvarez et al., 2000). Many types of biomass containing carbohydrates, proteins, fats, cellulose, and hemicelluloses can be used as substrates (Weiland, 2009) including sewage sludge (Chynoweth et al., 1993), animal manure (Al-Masri, 2001), dedicated energy crop and crop residue (Amon, 2007), grass (Wilkie, 1986), wastewater from food processing plants (Tekin and Dalgic, 2000), fruit and vegetable waste (Knol et al., 1978), and the organic fraction of municipal solid waste (Bouallagui et al., 2003; Han et al., 2005; Xu et al., 2002).

Food waste contains a high content of readily degradable organic matter and is a desirable substrate for AD (Zhang et al., 2011). Various types of food waste have been evaluated individually for their biochemical methane potential (BMP) and showed promising results including cooked meat (482 mL/g volatile solid (VS) $_{added}$), boiled rice (294 mL/g VS $_{added}$), fruits, (180 to 430 mL/g VS $_{added}$) and

vegetables (190 to 400 mL/g VS_{added}) (Cho et al., 1995; Gunaseelan, 2004). The reported BMP of the post-consumer food waste collected from restaurant and cafeterias ranged from 435 to 480 mL/g VS_{added} (Cho et al, 1995; Zhang et al., 2007; Zhang et al., 2011).

1.2 Problem Statement

Despite the high methane potential, using restaurant and cafeteria food waste as a single substrate for AD was not very successful. Several researchers report elevated VFAs concentrations that resulted in digester instability and failure (El-Mashad et al., 2008; Climenhaga and Banks, 2008; Zhang et al., 2011; Banks et al., 2012). In a single-stage digestion system, food waste is often rapidly acidified to VFAs that accumulate and decrease the pH in the reactor, inhibiting the activity of methanogenic microorganisms. Recently, several studies reported that this accumulation of VFAs is likely caused by trace element deficiencies (Climenhaga and Banks, 2008; Zhang et al., 2011; Banks et al., 2012).

Previous research showed that a sophisticated two-stage digestion system can overcome these deficiencies (Lee et al., 1999; Xu et al., 2002; Wang et al., 2005). However, the application of a two-stage system is limited as the majority of full-scale anaerobic digesters around the world are in a traditional one-stage configuration (Zhang et al., 2011).

An alternative method is co-digestion with animal wastes that are rich in trace element (Liu et al., 2009; El-Mashad and Zhang, 2010; Zhang et al., 2011; Zhang et al., 2012). However, this strategy may not be practical in urban areas where most food waste is generated. Untreated animal waste and food waste both have a high moisture content preventing the economical long distant transport to a centralized

anaerobic digester. Additionally, having manure in highly urban areas may be unacceptable from a nuisance standpoint.

In summary, food waste has great energy potential and can be used as a substrate for AD to produce energy. However, there is a lack of practical and economical strategies to ensure stable and efficient digestion.

1.3 Hypotheses

The central hypothesis of this dissertation research is that the supplementation of manure vermicompost (VC) to a single-stage AD system using food waste as the sole substrate will stimulate methane production and enhance process stability.

1.4 Rationale

The rationale for this central hypothesis is that manure VC contains a wide range of trace minerals at concentrations favorable for AD (Heravs et al., 1989). Additionally, VC originating from animal manure contains high levels of humic acids (Canellas et al., 2000) that are reported to increase methane production and improve digestion stability (Hartung, 1989). A detailed literature reviews is in Chapter 2.

There are numerous reasons why manure VC was selected as the test nutrient-rich supplement for enhancing the AD of food waste, as discussed below.

1. Manure VC vs. raw manure. The earthworms used in the production of manure VC modify the physical, biological, and chemical properties of the original manure. The final product is an odor free, granular, and peat-like material with moisture content in the range of 45-60%. This makes it more suitable for transport and land application as a soil amendment. Moreover,

the concentrations of calcium (Ca), potassium (K), iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), and cadmium (Cd) increase (Yadav and Garg, 2010) as a result of carbon and nitrogen loss due to mineralization and decompositions of organic matter (Deolalikar et al., 2005).

2. Vermicompost vs. thermophilic compost. Vermicompost has much higher concentrations of available (water-soluble) nutrients, in comparison to traditional thermophilic compost derived from identical feedstocks (Subler et al., 1998; Short et al., 1999; Tognetti et al., 2005). Additionally, earthworm activity accelerates the humification of organic matter, producing a larger amount of humic acids compared to thermophilic composting (Edwards, 2004).
3. Vermicompost vs. commercial mineral nutrients. In recent years, several studies evaluated the feasibility of supplying commercially available, relatively pure trace elements to ensure stable and effective AD of food waste (Climenhaga and Banks, 2008; Zhang et al., 201; Banks et al., 2012). However, VC is potentially a more eco-friendly, economically viable, and sustainable alternative to commercial minerals, which are primarily produced from nonrenewable resources.

In summary, vermicompost serving as an AD supplement appears to be a viable, novel approach to improve the stability of AD and increase biogas production. However, its effectiveness is not demonstrated and the potential mechanisms of improvement not understood. In fact, there is no previously published research on the utilization of manure VC or conventional thermal compost to improve AD of food waste.

1.5 Objectives

The overall objectives of the study were to evaluate the effectiveness of utilizing dairy manure VC as an additive to enhance the AD of cafeteria food waste in a single-stage digestion system and investigate the associated mechanisms. To achieve these objectives, a four stage studies were conducted as described in Chapters 3-6. A brief summary of the structure of the dissertation research is shown in Table 1.1.

Table 1.1 Structure of dissertation research

Stage	Experiment	Objective
1	BMP assay	Preliminarily examine the feasibility of utilizing VC as an additive to enhance the AD of cafeteria food waste
2	Long term semi-continuous digestion trial	Examine the effectiveness of VC as an additive in a long-term operation and identify the stimulatory factors present
3	Specific methanogenic activity test	Determine the effect of VC on the acetate utilization rate and propionate utilization rate
4	Metal bioavailability study	Determine if the deficiencies of selected trace metals cause the low acetate utilization rate in a food waste digester

CHAPTER 2 LITERATURE REVIEW

In this chapter, the principles of the anaerobic process were briefly discussed first followed by in-depth reviews of: 1) the functions and requirements for trace metals; 2) the use of additives to stimulate AD; and 3) food waste digestion. A brief review of the VC process is also presented, including principles of its production, a comparison of VC and traditional composting, and the use of VC in the digestion process.

2.1 Overview of Anaerobic Digestion

Anaerobic digestion is the decomposition of organic matter by a microbial consortium in an oxygen-free environment (Ward et al., 2008). Organic carbon is converted by subsequent oxidation and reduction steps to its most oxidized state. CO_2, and its most reduced state. CH_4. In addition to CH_4 and CO_2, minor quantities of other gaseous products are generated such as N_2, nitrogen oxides, H_2, NH_4, and H_2S (Angelidaki and Sanders, 2004).

2.1.1 Historical Development and Present Status

Volta is recognized as the first to report the conversion of organic matter to CH_4 through an anaerobic digestion process (McCarty, 2001). In 1776 he showed that “combustible air” was derived from sediments in lakes, ponds, and streams. In 1856, Reiset reported that methane was formed from decomposing manure (McCarty, 2001). The first full-scale application of anaerobic treatment was a septic tank used for treating domestic wastewater, developed by Moigno in 1881 (McCarty, 2001). He named this system “Mouras’ Automatic Scavenger’’ and described this air-tight chamber in the French journal Cosmos. In 1890, Moncrieff constructed the first

hybrid anaerobic system that consisted of a tank digester and an anaerobic filter that was designed to decrease the volume of sludge (McCarty, 2001). Imhoff modified a septic tank to enable a longer solid retention time and, by the end of 1914, about 75 cities in the United States received a license to use the system, termed an Imhoff tank (McCarty, 2001).

Beginning in the 1920s, Bunswell and his colleagues conducted extensive research on applications of the anaerobic process for the management of industrial wastewater and agricultural residues (McCarty, 2001). Later, Stander discovered the importance of the solids residence time for reducing the reactor's size (McCarty, 2001). Taylor developed the first large-scale anaerobic filter to treat wheat starch wastewater in 1972 (McCarty, 2001). In 1970s, Lettinga developed the up-flow anaerobic sludge blanket reactor, which is now the one of the most successful new reactor designs because of its broad application to a variety of industrial and municipal wastewaters (McCarty, 2001).

By the end of 20th century, AD has become widely applied worldwide. In the U.S., AD is used at large farms for manure treatment, at municipal wastewater treatment plants, and to treat industrial wastewater. AD is more prominent in Europe, especially in Germany, Denmark, Austria, and Sweden because of strong government initiatives (Holm-Nielsen et al., 2009).

Although AD is a widely applied, the design is still generally empirical (De Baere, 2006). This is mainly due to the complexity of the biological process, that is still not fully understood, and the increasing range of feedstocks. Many problems associated with the AD technology such as poor operational stability and a long retention time limit its application and researchers are in agreement that more research is needed to further advance AD technology. Included are 1) improving

process efficiency by the pretreatment of substrates and the addition of biological and chemical additives; 2) identifying microbial community dynamics; 3) modeling of AD; and 4) upgrading and utilizing of biogas (Appels et al., 2011; Hom-Nielsen et al., 2009; Ward et al., 2008; Mata-Alvarez et al., 2000).

2.1.2 Principals of Anaerobic Digestion

Anaerobic digestion consists of a series of biochemical processes as illustrated in Figure 2.1.

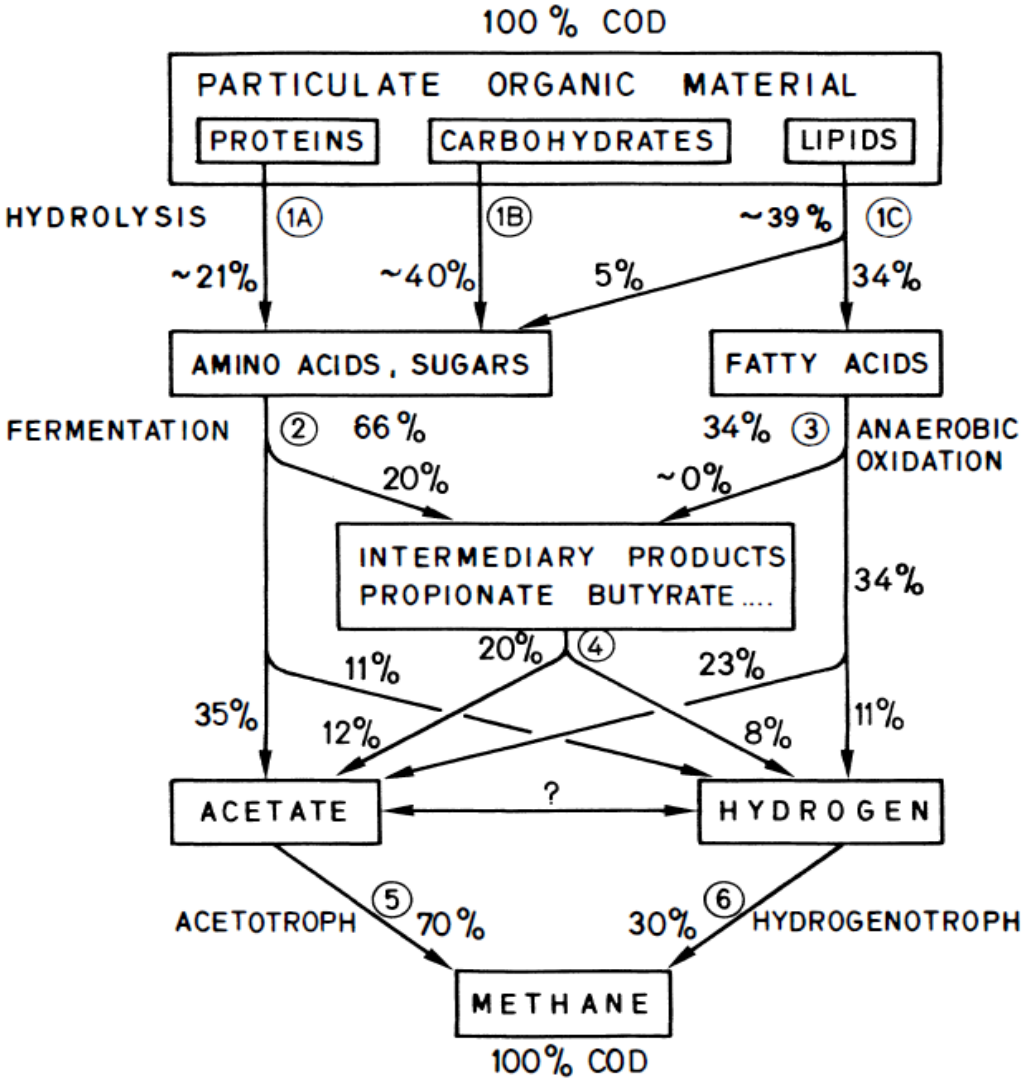


Figure 2.1 Anaerobic Digestion Process (Adapted from Gujer and Zehnder, 1983). Percentages indicate substrate flow (stoichiometrically) in the form of COD or CH_4, as described by Gujer and Zehnder, 1983.

Six distinct processes occur:

- Hydrolysis of complex polymers including proteins, carbohydrates, and lipids
- Fermentation of amino acids and sugars
- Anaerobic oxidation of long chain fatty acids
- Anaerobic oxidation of intermediary products such as VFAs (with the exception of acetate)
- Conversion of acetate to CH_4.
- Conversion of H_2 and CO_2 to CH_4.

Fermentation is defined as a microbial process in which organic matters serve both as electron donors and as electron acceptors. Anaerobic oxidation is defined as microbial process in which molecular H_2 is the main sink for electrons (Gujer and Zehnder, 1983).

These six processes are typically simplified to four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The trophic groups relevant for anaerobic process design and control are hydrolytic bacteria, acidogenic (or fermentative) bacteria, acetate-forming (also known as acetogenic) bacteria, and methanogens (archaea).

If complex insoluble compounds such as particulate and colloidal organic matter are used as substrates, the first stage of the AD process is hydrolysis. Hydrolysis is defined as the breakdown of organic substrates into smaller products, which then can be taken up and degraded by microorganisms (Morgenroth et al., 2002). Complex organic matter such as proteins, carbohydrates, and fats are complex polymeric substances which consist of many small molecules joined

together by unique chemical bonds. In general, most microorganisms are unable to directly use these substances, therefore, microorganisms first excrete extracellular hydrolytic enzymes to hydrolyze these complex polymer to soluble polymers or monomers such as amino acids, simple sugars (oligo- and monosaccharides), and long-chain fatty acids (Gujer and Zehnder, 1983). Typical hydrolytic enzymes include protease, cellulase, cellobiase, xylanase, amylase, and lipase. The soluble substrates entered the bacteria cells for ultimate degradation.

In the acidogenesis stage, soluble compounds produced through hydrolysis or directly fed to the digester are degraded by acidogenic bacteria. The degradation of these compounds results in the production of CO_2, H_2, alcohols (such as butanol, ethanol, methanol, and propanol), organic acids (such as acetate, butyrate, formate, lactate, propionate, and succinate), organic-nitrogen compounds, and organic-sulfur compounds (Geradi, 2003). The presence of organic-nitrogen compounds and organic sulfur compounds is due to the degradation of proteins CO_2 and H_2 can be converted directly to acetate or methane.

Many alcohols and acids generated during the acidogenesis stage (such as propionate, butyrate, and ethanol) are further degraded to acetate, formate, CO_2, and H_2 during the acetogenesis stage, by acetate-and H_2-forming bacteria (also called acetogenic bacteria). The accumulation of hydrogen can inhibit the metabolism of acetogenic bacteria; therefore, the maintenance of an extremely low partial pressure of hydrogen is essential.

The final stage in AD is methanogenesis, where CH_4 is produced from acetate, CO_2, and H_2 by the methanogens. Methane can also be formed from

formate and methanol although this is not common. Acids, alcohols, and organic-nitrogen compounds not used by methanogens accumulate in the digester. Methanogens are classified as archaea, a biology domain distinct from bacteria. There are three principal groups of methanogens, acetotrophic, hydrogenotrophic, and methylotrophic, which will be discussed in more details in the next section. Although many details on the metabolic networks in a methanogenic consortium are not clear, present knowledge suggests that H_2 may be a limiting substrate (Bagi et al., 2007). This assumption is based on findings that the addition of H_2-forming bacteria to the natural biogas-forming consortium increases daily biogas production.

2.1.3 Anaerobic Microorganisms

Three groups of anaerobic microorganisms including acetate-forming bacteria, sulfate-reducing bacteria and methanogens are reviewed in this subsection.

2.1.3.1 Acetate-Forming Bacteria

Acetate-forming bacteria grow in a symbiotic relationship with methanogens. When acetate-forming bacteria produce acetate, hydrogen is also produced and used by methanogens for CH_4 production. Acetate-forming bacteria survive only if their metabolic waste—H_2—is continuously removed by methanogens or other hydrogen-utilizing bacteria. If H_2 accumulates, acetate-forming bacteria cease and depress acetate production, causing the AD to fail (Amani et al., 2010). Failure to maintain the balance between these two groups of microorganisms is the primary cause of reactor instability (Wang et al., 2009).

2.1.3.2 Sulfate-Reducing Bacteria

There are two groups of sulfate-reducing bacteria—incomplete oxidizers and complete oxidizers. Incomplete oxidizers degrade organic compounds to new bacterial cells, CO_2, and acetate, ethanol, formate, lactate, and propionate. Complete oxidizers degrade organic compounds to new bacterial cells and CO_2 (Geradi, 2003). If sulfates are present, sulfate-reducing bacteria compete with methanogens for the same substrates (H_2 and acetate) and reduce sulfate to hydrogen sulfide. At substrate-to-sulfate ratios <2 (mass basis), sulfate-reducing bacteria out-compete methane-forming bacteria for acetate while at substrate-to-sulfate ratios between 2 and 3, competition is very intense (Geradi, 2003). At substrate-to-sulfate ratios >3, methane-forming bacteria are favored (Geradi, 2003).

2.1.3.3 Methanogens

Methanogens are a morphologically diverse group of the archaea that have many shapes, growth patterns, and sizes but unified by their ability to gain energy by reducing carbon monoxide (CO), CO_2, formate, methanol, methylamines, or acetate to CH_4. Methanogens employ hydrogenase, formate dehydrogenase, carbon monoxide dehydrogenase, methyl reductase and secondary alcohol dehydrogenase to obtain reducing equivalents for generating methane from molecular hydrogen, formate, acetate, methyl groups and secondary alcohols, respectively (Reeve, 1992). Coenzymes that are unique to methanogens are coenzyme M and the nickel-containing coenzymes F_{420} and F_{430} (Geradi 2003). Coenzyme M is used to

reduce CO_2 to CH_4. The nickel-containing coenzymes are important H_2 carriers in methanogens.

In the AD process, there are three principal groups of methanogens: 1) hydrogenotrophic, 2) acetotrophic (also known as aceticlastic), and 3) methylotrophic (Amani et al., 2010). The hydrogenotrophic methanogens typically use H_2 and convert CO_2 to CH_4 (Eq. 2.1) however, some use CO to produce CH_4 (Eq. 2.2). By converting CO_2 and H_2 to CH_4, these organisms help to maintain a low partial hydrogen pressure in the digester that is required for acetogenic bacteria (Amani et al., 2010).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{Eq. 2.1}$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \qquad \text{Eq. 2.2}$$

The acetotrophic methanogens "split" acetate into CH_4 and CO_2 (Eq. 2.3). This process is known as an aceticlastic reaction. The CO_2 produced from acetate may be further converted by hydrogenotrophic methanogens to methane (Eq. 2.1).

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Eq. 2.3}$$

Acetate degradation is also carried out by acetate oxidizing reactions. In contrast to the former reaction, the latter is very energetically unfavorable (Hattori, 2008). However, this reaction can occur from syntrophic interaction between certain bacteria and methanogenic archaea. The bacteria, namely syntrophic acetate-oxidizing bacteria, can oxidize acetate to produce H_2/CO_2 only when their products are subsequently utilized by the hydrogenotrophic methanogens (Hattori, 2008).

Surprisingly, some of these bacteria can also reversibly utilize H_2/CO_2 to produce acetate (Hattori, 2008).

The methylotrophic methanogens grow on substrates that contain the methyl group ($-CH_3$). Examples of these substrates include methanol (CH_3OH) (Eq. 2.4) and methylamines [$(CH_3)_3$-N] (Eq.2.5).

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O \qquad \text{Eq. 2.4}$$

$$4(CH_3)_3 - N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \qquad \text{Eq. 2.5}$$

Methanogens reproduce very slowly due to the relatively small amount of energy obtained from the use of their limited number of substrates (Gerardi, 2003). Under optimal conditions, the range of generation times varies from three days to several weeks. Therefore, if the solid retention time is too short, the population of methanogens is not able to increase accumulate.

2.1.4 Optimization of Anaerobic Digestion

Like any other microorganisms based process, successful AD operation depends on maintaining environmental conditions to optimize the microbial activity and increasing the system efficiency. Important operational parameters that must be satisfied for a stable and efficient digestion process are discussed below.

2.1.4.1 pH and Alkalinity

In general, CO_2 and VFAs tends to lower pH, while alkalinity-generating cations, like ammonium ions from protein degradation reacting with CO_2 to form ammonium biocarbonate, stabilize the pH (Bhattacharya and Parkin, 1989). The

best pH range for acetate-forming bacteria is 5.5-6.5 and for methanogens is 6.7-8.0 (Owens et al., 1979). The pH of an anaerobic digester should be maintained in a range of approximately 6.5 to 8.2 (Liu et al., 2008; Speece, 1996). A decrease in pH below 6 significantly reduces the activity of the methanogens and causes a buildup of VFAs and H_2. At higher partial pressure of H_2, acetate-forming bacteria are severely inhibited resulting in even more accumulation of VFAs and a further decrease of the pH. Further, if food waste is used for feedstocks, rapid hydrolysis of lipids can result in the accumulation of VFA and the lower methanogenic activity (Griffin et al., 1998).

2.1.4.2 Temperature

Temperature plays an important role in microbial growth and metabolism rates and the physicochemical properties of the substrate. The two optimum primary temperature ranges for AD are mesophilic (30-35°C) and thermophilic (50-55°C). AD can also occur at a psychrophilic temperate, below 20°C (Boullagui et al., 2003). The structures of the active microbial communities are dependent on the temperature range (Ward et al., 2008) and a rapid change from mesophilic to thermophilic may cause a temporary, substantial decrease in biogas yield (Ortega, 2008).

2.1.4.3 Solid Retention Times

The solid retention time (SRT), average time solids (microorganisms) spend in the AD, significantly affects digestion performance (Appels et al., 2008). For an anaerobic digester operating at 35°C, the minimum recommended SRT is 10 – 20

days so that the rate of organism growth exceeds the rate of wash out (Appels et al., 2008; Keshtkar et al., 2003).

2.1.4.4 Mixing

For optimal performance, mixing must ensure that the entire digester volume is utilized, there is extensive contact between the bacteria and the substrate, and heat is being transferred effectively (Kaparaju et al., 2008). For wastes with higher solids content, efficient mixing is a necessity to maximize biogas yields (Karim et al., 2005). However, excessive mixing can reduce biogas production, likely due to the disruption of the granule structure of acetate-forming bacteria and methanogens resulting in a reduced rate of VFA oxidation and ultimately, digester instability (McMahon et al., 2001).

2.1.4.5 Organic Loading Rate

Biogas production rate is highly dependent on the organic lading rate (OLR) (Yadvika et al., 2004). Wide and rapid variations may upset the balance between acidogenesis and methanogenesis resulting in a decrease in biogas production. The maximum OLR is determined by many factors including the mass transfer rate between substrate and microbial biomass, microbial proximity of syntrophic reactions, temperature, pH, toxicity level, design of reactor, characteristics of feedstocks, settleability, and activity of microbial biomass (Amani et al., 2007; Speece, 1996).

2.1.4.6 Macronutrients

As with any biological treatment process, nitrogen (N) and phosphorous (P) are the two macronutrients of most concern in AD. Availability to anaerobic

microorganisms is typically as soluble ammonium-nitrogen (NH_4^+–N) and orthophosphate-phosphorus (Speece, 1996).

For optimal gas production, the carbon (C) to N ratio of at least 25:1 is suggested. A high C/N ratio may limit microbial biomass growth while a lower ratio may cause ammonia accumulation resulting in pH values exceeding 8.5, which are toxic to methanogens. For the AD of fruit and vegetable waste, an optimum ratio of 100–130:4:1 was reported for the chemical oxygen demand (COD), N, and P, respectively (Bouallagui et al. 2003).

2.1.4.7 Micronutrients

Methanogens unique enzyme systems result in diverse micronutrient requirements. Included are cobalt (Co), iron (Fe), nickel (Ni), sulfide (S), selenium (Se) and tungsten (W) (Gerardi, 2003). Their incorporation is essential to ensure not only proper degradation of substrate but also efficient operation. Deficiencies of micronutrients in ADs often have been mistaken for toxicity (Speece, 1996). A more detailed literature review regarding trace metals is presented in section 2.2.

2.1.4.8 Inhibition/Toxicity

A variety of organic and inorganic matters have been reported to be inhibitory to ADs.

Propionate is the most toxic VFA and can inhibit digestion at concentrations of 3000 mg/L. Long-chain fatty acids (LCFAs) can also inhibit methanogens (Kabara et al., 1977; Zeikus, 1977) by adsorbing onto the cell membrane and interference with the transport or protective function (Rinzema et al., 1994).

Ammonia produced by the biological degradation of the nitrogenous matter, (mostly in the form of proteins and urea) may cause inhibition (Chen et al., 2008). In general, concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sung, 2002) but values from 1700 to 14000 mg/L are inhibitory (Chen et al., 2008).

Competition with sulfate reducing bacteria for available acetate, H_2, propionate, and butyrate can suppress methanogens and acetogens(McCartney and Oleszkiewicz, 1993; Colleran et al., 1995). Sulfide formed from the reduction of sulfate and the degradation of organic compounds such as sulfur-containing amino acids and proteins may inhibit the metabolic activity of anaerobic bacteria (Tursman and Cork, 1988). Hydrogen sulfide is likely the toxic form of sulfide since it can diffuse more rapidly into the cell membrane than ionized sulfide (Gerardi, 2003). Sulfide toxicity is pH dependent and increases as pH increases (McCartney and Oleszkiewicz, 1991). The inhibitory sulfide levels reported in the literature were in the range of 100–800 mg/L dissolved sulfide or approximately 50–400 mg/L dissolved H_2S (Parkin et al., 1990).

In addition, excessive concentrations of soluble metals may cause toxicity by blocking enzyme functions (Vallee and Ulner, 1972). Such toxic effect is primarily nonspecific and reversible (Nies, 1999). For example, Cr^{3+} concentration of 12 mg/L or higher can cause a 50% reduction in acetoclastic methanogenic activity. However, supplying additional Fe could revert this inhibition (Soubes et al., 1994). This type of inhibition is characterized by the reversible binding of the inhibitor with either the enzyme or the enzyme-substrate complex. Less frequently, metals act as competitive inhibitors (compete with the substrate). This type of inhibition depends

on the concentration and affinity of the metal to the enzyme (Oleszkiewicz and Sharma, 1990).

2.1.5 Anaerobic Biodegradability Assays

A brief review of anaerobic biodegradability measurements is presented in this subsection.

2.1.5.1 Overview

Anaerobic biodegradability (also called anaerobic digestibility) is defined as the fraction of a compound(s) that can be converted to biogas (or methane) under anaerobic condition (Guwy, 2004). Such assays are used to assess the quantity and rate production of biogas or methane from ADs.

Anaerobic biodegradability is typically determined based on the measurement of either substrate depletion or product formation during the digestion process. Substrate depletion can be determined either by measuring generic parameters such as VS or COD or directly by analysis of the specific substrate (Rozzi and Remigi, 2001). Determination of COD is sometimes problematic. The method for analysis of COD was developed for water and wastewater, which may not be suitable for materials with a high level of solid organic matter like food waste. Therefore, VS is usually used as primary parameters for digestibility tests for solid organic matter.

Methods based on product formation monitor the end product (biogas) and/or intermediates products such as VFAs. Because of the ease in measuring biogas, this is the most common approach. Biogas production can be determined either as volume increase under constant pressure (volumetric methods) or pressure change in constant volume (manometric methods) (Angelidaki and Sanders, 2004).

The volumetric method entails transferring the volume of biogas produced into a device that allows for its measurement to be recorded. A common approach is to collect the biogas in a lubricated syringe in which the plunger expands to balance the overpressure generated inside the reactor (Rozzi and Remigi, 2004). The syringe is inserted through a septum that is part of the reactor cap (Owen et al., 1979) or used as the reactor itself (Cohen, 1992). In a different arrangement, the biogas proceeds into an external vessel containing a barrier solution that displaces an equivalent volume of liquid, which can be manually or automatically measured. An alternative is the anaerobic respirometer equipped with a bubble courter which can measure biogas production as small as $0.1cm^3$ (Rozzi and Remigi, 2004). The biogas is transformed into small gas bubbles when passing through a liquid filled cell. A laser counter recognizes each bubble as it moves out of the cell which is then correlated to a volume (Kuss & Young1992).

2.1.5.2 Batch and Continuous System

The biodegradability of a specific substrate can be evaluated in a batch, semi-continuous or continuous system. In the batch system, the substrate remains in the reactor until the end of experimental period. While, in semi-continuous and continuous systems, substrate is withdrawn and fresh, untreated substrate is added routinely, typically daily.

The batch system is the more common method because it uses simpler equipment and requires less time to complete. However, the accumulation of byproducts in continuous system is minimal, avoiding potential inhibition that may occur in batch tests. Additionally, the continuous system is also used to simulate a

field-scale digester allowing the data to be used for design and cost purposes (Rozzi and Remigi, 2004).

2.1.5.3 Biochemical Methane Potential Assay

One example of such experimental set-ups is the BMP assay (Owen et al., 1979). The BMP is defined as the ultimate specific methane production – regardless of how long it takes to reach this level (Angelidaki and Sanders, 2004). However, in practice, degradation time is capped at a practical limit and the methane potential is estimated by extrapolation of a methane production curve. Methane potential can be expressed specifically per amount of initial mass of waste (L CH_4/kg- substrate), volume of the initial waste (L CH_4/L- substrate), mass of VS added (L CH4/kg-VS), initial COD added (L CH_4/kg-COD), or the mass of the substrate, VS or COD that was removed (requires measurement of the parameter before and a projection of the amount remaining when the ultimate biogas volume is reached). The primary purpose of BMP assay is to determine if mathematical prediction of the methane potential is reasonable and to verify the digestibility of a substrate. Methane potential determined by batch BMP assays is a preliminary estimation and is not intended to use for stimulating a real-scale digester (Speece, 1996).

Many technical issues influence the outcome of a BMP assay, including the following.

1) Inoculum. The source of inoculum greatly influences its ability to utilize the substrate being tested (Rozzi and Remigi, 2004). Wasted solids from a stable AD with a similar substrate is often the used as inoculum as the microorganisms are pre-acclimated to the expected conditions (Owen et al. 1979).

Another important factor is the amount of inoculum added. A low amount is often desired to minimize its biogas production. However, inadequate amounts can lead to an overload of the substrate resulting in accumulation of VFAs and, consequently, reducing the pH and methane production.

2) Substrate. Substrate concentration should be large enough to have good representatively and measurable biogas production but still practical. Also large quantities of substrate in a batch reactor can cause toxicity. In general, inoculum-to-substrate ratio (mass basis) should be maintained above 0.5:1 to avoid negative effect on methane production (Raposo et al., 2009).

3) Headspace Volume and Pressure. The volume of biogas produced, can be affected by variations of CO_2 solubility in the bioreactor liquor or manometric liquid. Frequent release of the headspace pressure has been shown to reduce associated errors (Johnson and Young, 1983). Maintaining a small headspace volume also improves the accuracy of biogas measurement (Rozzi and Remigi, 2004).

2.2 Trace Metals in Anaerobic Digesters

Many trace metals are essential for the growth of anaerobic microorganisms (Fermonso et al., 2008). During AD, trace metals act as: 1) microelements essential for various enzymatic reactions (Eichenberger, 1984); 2) inhibitors of sulfide toxicity (Oleszkiewicz and Sharma, 1989); 3) biomass stimulants, beyond the presumed enzymatic requirements (Takashima and Speece, 1990); 4) promoters of microbial aggregation (Oleszkiewicz, 1989). Trace metals essential for AD include nickel (Ni), iron (Fe), cobalt (Co), selenium (Se), molybdenum (Mo), tungsten (W) , Manganese (Mn), zinc (Zn) , and copper (Cu) (Oleszkiewicz and Sharma, 1989). The commonly

reported metals that have a stimulatory effect on AD are Ni, Fe, and Co (Oleszkiewicz and Sharma, 1989; Speece, 1996).

2.2.1 Functions of Nickel, Iron, and Cobalt

Trace metals including Ni, Fe, and Co are crucial components of essential enzymes that catalyze metabolic reactions during methanogenesis (Zandvoort et al., 2006). Ni is vital to the last step of methanogenesis where methyl-coenzyme M (CH_3-S-C_oM) and coenzyme B (HS-CoB) are converted to methane and CoM-S-S-CoB. This key step is catalyzed by methyl-coenzyme M reductase (MCR) complex, which includes a Ni-containing cofactor called F_{430} (Harmer et al., 2008). Ni and Fe are two critical elements for CO dehydrogenase (CODH) complex (Friedman et al., 1990). The CODH cleaves the C-C and C-S bonds in the acetyl moiety of acetyl-CoA, oxidizes the carbonyl group to CO_2, transfers the methyl group to Coenzyme M, and is involved in the formation of acetate from H_2/CO_2 and methanol (Ferry, 1999; Bainotti and Nishio, 2000). Additionally, Ni and Fe are contained in F_{420}-reducing hydrogenase that catalyzes the reduction of CO_2 to CH_4 (Michel et al., 1995). Co is required for the synthesis of the Co/corrinoid containing methyl-H_4MPT: Coenzyme M methyltransferase complex (Thauer, 1998), and Methanol: Coenzyme M methyltransferase (Sauer and Thauer, 2000).

2.2.2 Requirements of Nickel, Iron, and Cobalt

The intracellular concentrations of trace metals in unstressed condition are regarded as indicative of the essential requirement under optimal nutrient and process conditions (Oleszkiewicz and Sharma, 1989).

Various species of methanogens require different optimum or stimulatory concentrations of trace metals including Ni, Fe and Co as well as other essential metals such as Se, Mo, and W (Takashima and Speece, 1990). Zandvoort et al. (2006) provided an extensive list as shown in Table 2.2.

Trace metal requirements could be impacted by operation parameters such as temperature (mesophilic vs. thermophilic) and the experimental set-up (batch vs. continuous). It is known that the required minimum concentrations of Ni, Fe, and Co are significantly greater for thermophilic digestion than mesophilic digestion for the same substrate (Zitomer et al., 2008). Most research on trace metal requirements has been studied in batch growth modes. However, quantifying the minimum requirements using batch-scale system could be biased due to the slow response of anaerobic microorganisms, particularly methanogens (Takashima et al., 2011).

Table 2.1 Trace metal stimulation of pure cultures of methanogens

Methanogen Species	**Substrate**	**Stimulation Concentration (μM)**
Methanothrix soehngenii VNBF	Acetate	Fe (20-100) Co (2) Ni (2) Mo (2)
M. thermoautotrophicum	H_2/CO_2	Fe (> 5) Co (> 0.01) Ni (> 0.1) Mo (> 0.01) Se (1) W (10)
Methanosarcina barkeri	Methanol	Fe (35) Co (1) Ni (1) Mo (1)

Adapt from Zandvoort et al., 2006.

2.2.3 Bioavailability

The bioavailability of trace metals in AD is defined as the availability of trace metals that can be freely uptake by anaerobic microorganisms (Speece, 1990). The fact that trace metals are present in an AD process does not assure that they are readily available for uptake (Speece, 1990).

The bioavailability of trace metals is controlled by the total metal concentration in the digester and the environmental conditions affecting speciation including 1) precipitation, primarily by sulfide, carbonate, and phosphate; 2) chelation or complexing with inorganic species (ion pairs) and organic ligands, both present or synthesized by organisms to facilitate metal uptake; 3) the kinetics of precipitation and chelation reactions (Callander and Barford, 1983a).

The uptake of metals by microorganisms is generally assumed to proceed mainly via the transport of free metal ions across the cell membrane (Zandvoort et al., 2006). The precipitation and formation of inorganic and organic complexes can reduce their availabilities (Zandvoort et al., 2006). However, in some cases, specific metal complexes can be taken up directly by anaerobic microorganisms (Jansen et al., 2005).

The main species capable of precipitating metals in anaerobic digesters are sulfide (S^{2-}), carbonate (CO_3^{2-}), and, less importantly, phosphate (PO_4^{3-}) anions (Callander and Barford, 1983a). The presence of these anions poses a significant problem in trace metal bioavailability because of the reduced solubility associated with trace metal precipitates (Speece, 1996). In a typical digester with a pH of 7.3, Fe, Co, and Ni likely are precipitated as sulfides if the total concentration of metals (Fe, Co, Ni, Cu and Zn) is less than the total of sulfide (gas-liquid H_2S, HS^- and S^{2-}) level (Callander and Barford, 1983b).

Essential trace metals (Fe, Co, Ni, Se, Mo, and W) can form soluble inorganic complexes with a number of anions (HCO_3^-, CO_3^{2-}, OH^-, SO_4^{2-}, S^{2-}) and soluble organic complexes with organic complexes (such as EDTA, NTA, citric, and cysteine). The formation of these chelating complexes prevents precipitation of free metal ions. However, it is unknown if these complexes assist in trace metals uptake (Speece, 1996). Additionally, ADs often contain a high concentration of soluble microbial products (SMP) (Barker and Stuckey, 1999) that can bind metals such as nickel (Kuo and Parking, 1996).

In summary, the speciation of metals has a significant impact on their bioavailability (Fernando et al., 2009). The presence of sufficient free trace metal ions (not precipitated) and selected soluble metal complexes are a prerequisite for their uptake by anaerobic microorganisms.

2.3 The Use of Additives to Stimulate the Anaerobic Digestion Process

Various biological and chemical additives have been used in AD to increase gas production by stimulating the microbial activity (Yadvika et al., 2004). Examples include hydrolytic enzyme, trace metals, and humic substances as discussed below.

2.3.1 Hydrolytic Enzyme

Polymeric carbohydrates, lipids, and proteins present in particulate organic matter cannot be taken up by microbial cells. Therefore, microorganisms secrete hydrolytic enzymes to breakdown and solubilize these macromolecular structures into soluble monomers that can transport through the cell membrane such as simple sugars, amino acids, and fatty acids. During the hydrolysis stage, starch is converted to glucose by amylase enzymes; hydrolysis of cellulose by the cellulase

enzyme complex yields glucose; protein is converted to amino acids by proteases and fatty acids are produced by lipases degradation of lipids. The hydrolysis is typically the rate-limiting step if the substrate is in particulate form such as for lignocelluloses-rich matter.

A significant number of studies have examined the impact of supplying hydrolytic enzymes to increase the rate of hydrolysis. These enzymes are often added in a pretreatment process (in a separate reactor) before the substrate enters the reactor (Sonakya et al., 2001). The benefit of this method is that temperatures and pH can be adjusted (typically 50°C and 5-7, respectively) to optimize enzyme activity. Hydrolytic enzymes also can be directly supplied to anaerobic digesters (Romano et al., 2009) if the environment within the reactor allow for effective hydrolysis.

The effectiveness of most hydrolytic enzyme additives is strongly dependent on the characteristic of substrates. For examples, Higgins and Swartzbaugh et al. (1986) added cellulase and β-glucosidase to anaerobic digesters to treat sewage and observed an increased biogas and methane yields of 12% and 15%, respectively. Sonakya et al. (2001) pretreated wheat grains with cellulase, α-amylase, and protease prior to AD and found a 7-14% increase in methane production. In contrast, Rowena et al. (2009) evaluated the effects of the addition of enzyme products containing cellulase, hemicellulase, and β-glucosidase to AD systems using wheat grass as model substrates and found no significant effects on the biogas production and methane yield.

Unlike other hydrolytic enzymes, lipase has consistently been demonstrated to be a promising enzyme additive for the AD of high fats and grease such as

slaughterhouse wastewater (Pereira, et al., 2006; Valladao et al., 2009) and dairy wastewater (Mendes et al., 2006; Cammarota et al., 2001).

2.3.2 Trace Metal

Table 2.2 provides a partial list of reported stimulatory effects of trace metal supplementation on the AD of simple substrates and complex organic materials within various digestion systems. Noteworthy, all of the research was conducted under mesophilic condition (30-35C).

Table 2.2 Stimulation of biologic conversion in anaerobic digesters by trace metal supplementation

Substrate	System	Trace Metals and Concentration (mg/L)	Observation Compared with no Supplementation	Reference
Acetate	Batch	Fe (300-600)	Increase AUR	Hoban and Van Den, 1979
Acetate	Batch	Ni (6) Co (3) Mo (4.8)	Increase AUR	Murray and Van Den Berg, 1981
Acetate	Continuous (CSTR)	Fe (70) Ni (2.5) Co (2.5)	Increase AUR	Speece et al., 1983
Propionate	Continuous (UASB)	Fe (2.1) Ni (0.00038) Co (0.00003)	Increase PUR	Ma et al., 2009
Volatile fatty acids	Continuous (UASB)	Ni (0.05) Co (0.075) Fe (1.1)	Increase COD removal	Shen et al., 1993
Potato and pea processing wastewater	Continuous (UASB)	Fe(40) Ni (0.5) Co (0.5)	Maintain granular form	Oleszkiewicz, 1989
Cheese factory	Continuous (fixed-film)	Ni (7.4)	Increase CH_4 production	Canovas-Diaz and Howell, 1986
Distillery wastewater	Batch	Fe (10) Ni (0.5) Co(0.1)	Improve methanogenic activity	Sharma and Singh, 2001

CSTR: continuous stirred tank reactor; UASB: upflow anaerobic sludge blanket
AUR: acetate utilization rate; PUR: propionate utilization rate

Metal deficiencies can severely impact the performance of AD (Speece, 1996). Elevated concentrations of VFAs in the effluent (over 500 mg/L) of an anaerobic digester can indicate trace metal deficiency (Speece, 1996). Several researchers verified that the addition of trace metals improved the performance of AD (Kim et al., 2002; Noyola and Tinajero, 2005; Pobeheim et al., 2010) by increasing the utilization rate of specific intermediate products such as acetate, propionate, and methanol (Takashima and Speec e, 1989; Kida and et al., 2001; Osuna et al., 2003).

The chemical form of trace metals additives can impact their effectivness during the AD process. Chloride forms ($NiCl_2$, $FeCl_2$, and $CoCl_2$) are generally recommended because of their high solubility (Speece, 1996).

2.3.3 Humic Substances

Humic substances are naturally occurring, heterogeneous, high molecular weight organic compounds composed mainly of humic acids. Humic acids are a series of similar aromatic polyfunctional compounds with medium to high molecular weights (Hayes and Clapp, 2001) that are resistant to microbial degradation (Hayes and Clapp, 2001; Lovely, et al., 1996). Humic substances interact strongly with a range of trace metals and have the potential to modify their adsorption (Laxen, 1984). For example, it is known that humic acids promote the formation of chelating complexes with Fe resulting in an increase of its bioavailability for microbial (Chen and Wang, 2008) and plant cells (Mina-Garcia, 2003). Additionally, humic acids can increase the growth rate of a variety of microorganisms (Visser, 1984; Pouneva, 2005). Under anaerobic conditions, some microorganisms are able to use humic

substances as an electron acceptor for the anaerobic fermentation of organic compounds and H_2 (Lovely, 1996).

A two-year study at a municipal wastewater treatment plant demonstrated that humic substances stimulate the AD of sewage sludge by increasing methane production and improving digestion stability (Hartung, 1989). Unfortunately, the mechanism was not investigated.

2.4 Anaerobic Digestion of Food Waste

Food waste contains a high content of readily degradable organic matter and is a desirable substrate for AD that can produce a tremendous amount of energy (Zhang et al., 2011) although it is not always conducive for stable operations. The characteristic and methane potential are discussed in the following subsection.

2.4.1 Characteristic and Methane Potential of Food Waste

The characteristics are highly variable. Macronutrients are adequate for anaerobic microorganisms (Zhang et al., 2011). However, the concentrations of some trace metals are relatively low, particularly Co, Ni, and Fe (Speece, 1996). Considering the important role of these trace elements for activating and maintaining enzyme activities of anaerobic microorganisms, this deficiency may cause instability and poor efficiency.

Reported characteristics of food wastes are shown in Table 2.3.

Table 2.3 Characteristic of reported cafeteria or restaurant food wastes

Parameter	Source			
	Han and Shin, (2004)	**Zhang et al. (2007)**	**Zhang et al. (2011)**	**Banks et al. (2011)**
pH	NA	NA	6.5 ±0.2	4.7±0.1
TS (wt. %)	20.5	30.9±0.1	18.1±0.6	23.7±0.1

Table 2.3 (Cont'd)

VS (wt. %)	19.5	26.4±0.1	17.1±0.6	21.7±0.1
VS (% of TS)	95	85±0.1	94±0.1	91.4±0.4
Total COD (g/L)	NA	NA	238±4	NA
Total Carbon (% of TS)	51.4	46.8±1.2	46.7	NA
Alkalinity (g $CaCO_3$/L)	NA	NA	0.3±0.1	NA
Macro Nutrients (% of TS)				
Total Nitrogen (TN)	3.5	3.2±0.2	3.5	NA
Total Phosphorus (TP)	NA	0.5±0.1	1.5±0.1	0.5±0.1
Total Sulphur (TS)	0.1	0.8±0.1	0.33	NA
Total Calcium (Ca)	NA	2.2±0.3	NA	NA
Total Potassium (K)	NA	0.9±0.1	NA	1.4±0.1
Total Magnesium (Mg)	NA	0.14	NA	NA
Trace metals (mg/kg fresh samples)				
Cobalt (Co)	NA	NA	<0.03	<0.06
Copper(Cu)	NA	31±1	11.8	1.7±0.2
Iron (Fe)	NA	766±402	12.2	54
Manganese (Mn)	NA	60±30	3.7	20±3
Molybdenum (Mo)	NA	NA	0.1	0.1±0.1
Nickel (Ni)	NA	2±1	0.8	1.7±0.7
Selenium (Se)	NA	NA	NA	<0.07
Tungsten (W)	NA	NA	NA	<0.25
Zinc (Zn)	NA	76±22	31.9	7.8±2.6
Other parameters				
C/N ratio	14.7	14.6	13.2	NA
Ammonia-N (g/L)	NA	NA	0.2±0.1	NA

Errors= standard deviations

2.4.2 Current Development and Issues

Despite the high biochemical methane potential, using food waste as single substrate for AD is not very successful with frequent reports on elevated level of VFAs causing digester instability and even process failure (El-Mashad et al., 2008; Climenhaga and Banks, 2008; Zhang et al., 2011;Banks et al., 2012). In a single-stage digestion system, food waste could be rapidly acidified to VFAs accumulated VFAs consequently decrease the pH and inhibiting the activity of methanogenic microorganisms. Banks et al. (2012) suggested that this accumulation of VFAs

begins with an increase in the acetic acid concentration which reaches a peak around day 100 then declines followed by a longer-term accumulation of propionic acid. Previous efforts to solve this problem include using a sophisticated two-stage digestion system (Lee et al., 1999; Xu et al., 2002; Wang et al., 2005). However, the application of two-stage system is still limited and the majority of full-scale anaerobic digesters around the world remain the traditional one-stage configuration. Another alternative method is co-digestion with animal manure (Liu et al., 2009; El-Mashad and Zhang, 2010; Zhang et al., 2012). However, this strategy may not be practical for urban area where most food waste is generated. In summary, anaerobic digestion of food waste still remains as a challenge.

2.4.3 Case Studies

Prior literature investigating the AD of food waste as a sole substrate in semi-continuous and continuous single-stage digestion systems is briefly reviewed below.

Climenhaga and Banks (2008) evaluated the effect of micronutrients on the AD of cafeteria food waste containing a mix of fruits, vegetables, meats, and fried foods at the bench scale. Without micronutrients supplementation, the reactors exhibited methanogenic failure as a result of accumulation of VFAs and concluded that trace element addition (a mixture of Fe, Cu, Co, Zn, Mn, Mo, Al, and Se) was required for stable digestion.

Similarly, Zhang et al. (2011) demonstrated that when food waste was used as a sole substrate, the digester suffered from accumulation of VFAs up to 18,000 mg/L and a drop of pH from 7.2 to 4.4 which ultimately led to a process failure. The cafeteria food waste was also found to be deficient in some trace metals (likely Co, Ni, Fe, and Mo). AD of the food waste supplemented with trace element-rich piggery

wastewater or synthetic trace elements resulted in a significantly improved biogas production rate and enhanced process stability.

Banks et al. (2012) further investigated the trace element requirements for stable food waste digestion at elevated ammonia concentrations and confirmed that without supplementation VFAs accumulated. The main component was initially acetic acid which then shifted to propionate after around day 100. The authors used the fluorescent *in situ* hybridization (FISH) technique to analyze the microbial community structure and found that the dominant metabolic pathway of food waste digestion with or without trace metal additions was syntrophic acetate oxidation and hydrogenotrophic methanogenesis due to significant loss of acetoclastic methanogens under the high ammonia concentrations (above 5000 mg/L). In this case, Se was demonstrated to be vital to the proper function of this pathway and is recommended as trace metal supplementation for food waste digestion.

2.5 Vermicomposting

A brief review of vermicomposting process is presented in this subsection.

2.5.1 Principles

Vermicomposting is a simple biotechnological process in which earthworms convert the organic waste material into VC (Benitez et al., 2000). During the process microbial degrade organic matter and the earthworm acts as mechanical blenders by comminuting the organic matter, modifying its biological, physical, and chemical state, gradually reducing its C to N ratio and increasing the surface area exposed to microorganisms (Yadav and Garg, 2010).

The end-product of vermicomposting is VC which is a good structural amendment for poor soils as it provides nutrients and minimizes soil erosion.

Vermicompost can be produced from almost any kind of organic waste with suitable preprocessing and controlled processing conditions. Included are animal waste (poultry, pigs, cattle, sheep, goats, horses, and rabbits) (Edwards et al., 1985), horticultural residues from dead plants, yard wastes (Edwards, 1995), sewage sludge, and solids from wastewater (Neuhauser et al., 1988). Cattle manure is considered to be one of the easiest animal wastes to VC (Edwards, 2004).

The earthworm species most commonly used is *Eisenia Fetida* (*E. Fetida)* and the very closely related species *E. Andrei*. These species (epigeic) have a more complete enzymatic system than do endogeic species (Brown and Doube, 2004), a wide temperature tolerance ranging from 10-25˚C, and can live in organic wastes with a range of moisture contents (55-88%, Loehr et al., 1985).

During the vermicomposting process, total organic carbon is gradually lost due to mineralization (Edwards, 2004). Earthworm activity provided conditions that favor nitrification, resulting in the rapid conversion of ammonium into nitrates (Hartenstein, 1981). Humification of organic matter is also accelerated. This acceleration is not only due to the fragmentation and size reduction of the organic matter, but also by the significant increase in microbial activity within the intestines of the earthworms and by aeration and turnover of the organic matter that occurs as the earthworms move(Edwards, 2004).

The final physical structure of VC depends on the original organic wastes. However, VC produced from most organic wastes are usually finely divided, well-stabilized, and humified, peat-like materials with excellent structure, porosity, aeration, drainage, and a low C: N ratio (Edwards, 1983).

During the processing of organic wastes by earthworms, many of the macronutrients are changed to forms that are more readily available for uptake by

plants (Table 2.4). A comparison of the physicochemical characteristics of final cattle and pig manure VC to the initial feedstock indicates that there is an increase in the concentration of Fe, Cu, Zn, Cr, and Cd (Table 2.5). The carbon and nitrogen loss was likely due to mineralization and decompositions of organic matter (Deolalikar et al., 2005; Hartenstein, 1981; Suthar et al., 2008).

Table 2.4 Effect of earthworm activity on nutrients in organic waste

Organic Wastes	Nitrate Nitrogen (ppm)	Readily Soluble P (% dry mass)	Exchangeable (% dry mass)		
			K	Ca	Mg
Cattle waste					
without worms	8.8	0.11	0.19	0.35	0.05
with worms	259.4	0.18	0.41	0.59	0.08
Pig waste					
without worms	31.6	1.05	1.49	1.56	0.45
with worms	110.3	1.64	1.76	2.27	0.72

Adapted from Edwards, 2004

Table 2.5 Comparison of trace element content in initial cattle manure and final cattle manure vermicompost

Trace element	Initial Cattle Manure (mg/kg)	Final Cattle Manure Vermicompost (mg/kg)
Fe	1810	2280
Cu	32.4	52.6
Zn	145	193
Cd	4.29	5.84
Cr	82	194

Additionally, VC originating from animal manure and food wastes has been reported to contain high levels of humic substances (Canellas et al., 2000; Atiyeh et al., 2002). Cattle manure VC typically contains about 20% humic acids (Hervas et al., 1989; Senesi et al., 1992). Additionally, Senesi et al. (1992) analyzed the characteristic of metal-humic acid complexes of manure VC using spectroscopic and

found that humic acid –like components of vermicompost are able to bind large amounts of Fe and Cu ions in water-stable, inner-sphere complexes.

Vermicomposting systems range from very simple to complex and can be operated manually or completely automated. Reactor systems can be either batch or continuous flow. The basic principle of all successful processing system is to add the wastes at frequent intervals in small, thin layers to the surface of the system to allow earthworms movement unto the fresh, aerobic layers. The earthworms will always concentrate themselves in the upper 15 cm of waste (Edwards, 2004).

2.5.2 Vermicomposting vs. Traditional Composting

Traditional thermophilic composting involves the degradation of organic matter by microorganisms under controlled conditions. There are two stages. During the thermophilic stage, the decomposition takes place intensively at a high temperature (> 50 °C). In the maturing stage, the temperature is in the mesophilic range (20- 30°C) and the remaining organic compounds are degraded at a slower rate (Lazcano et al., 2008). Composting is well established at the industrial scale for solid organic waste treatment, although the loss of nitrogen through volatilization of NH_3 during the thermophilic stage of the process is one of the major drawbacks (Eghball et al., 1997).

A major difference between vermicomposting and traditional composting is the temperature. The temperature of traditional compost pile can exceed 70˚C compared with relatively low temperatures of typically 25˚C for vermicomposting. As a result, thermophilic microbes are the main contributors for traditional composting, while worms, mesophilic aerobic microbes, and fungi are responsible for vermicomposting.

The difference between traditional composting and vermicomposting is also reflected in the unique properties of their products. VC has a much higher nutrient concentrations that are in more available (water-soluble) forms (Subler et al., 1998; Short et al., 1999; Tognetti et al., 2005). Moreover, earthworm activity accelerates humic acids production during the humification process (Edwards, 2004). In addition, greater extra-cellular enzymatic activities including cellulose, amylase, invertase, protease, perioxidase, and urease activities are observed during vermicomposting process as compared to traditional composting process (Edwards and Bohlen, 1996). Devi et al. (2009) also found that vermicomposting achieved greatest enzyme activity by 28 day of decomposition compared to 42 days for traditional composting.

2.5.3 Vermicompost as Additive in Anaerobic Digestion

Only limited data is available on utilization VC as an additive in AD. Chen et al. (2010) reported that under mesophilic condition, anaerobic co-digestion of corn stalk with VC generated from cow manure increased biogas yield by up to 59%. Zhang et al. (2007) found that supplying VC (produced by decomposition of cow manure as well) at the concentration of 1,5, and 10% of TS to synthetic wastewater resulted in an increase in methane yield up to 25% and improved buffering. Neither study provided the mechanism of observed enhancement. Studies regarding the use of VC for enhancing anaerobic digestion of food waste have not been found.

CHAPTER 3 USE OF BIOCHEMICAL METHANE POTENTIAL ASSAYS TO EVALUATE THE EFFECTS OF MANURE VERMICOMPOST ON ANAEROBIC DIGESTIBILITY OF FOOD WASTE

The first stage of dissertation research is presented in this chapter including introduction; material and methods; results and discussion; and conclusions and implication sections.

3.1 Introduction

The objective of this research stage was to evaluate the effects of manure VC on the anaerobic biodegradability of food waste. Anaerobic biodegradability is evaluated using the BMP test, first established by Owen et al (1979). The BMP test is a simple and rapid method to evaluate the anaerobic biodegradability of a feedstock in a nutrient defined medium. From the BMP test, cumulative biogas yield, ultimate methane yield, and the kinetic rate constant can be determined.

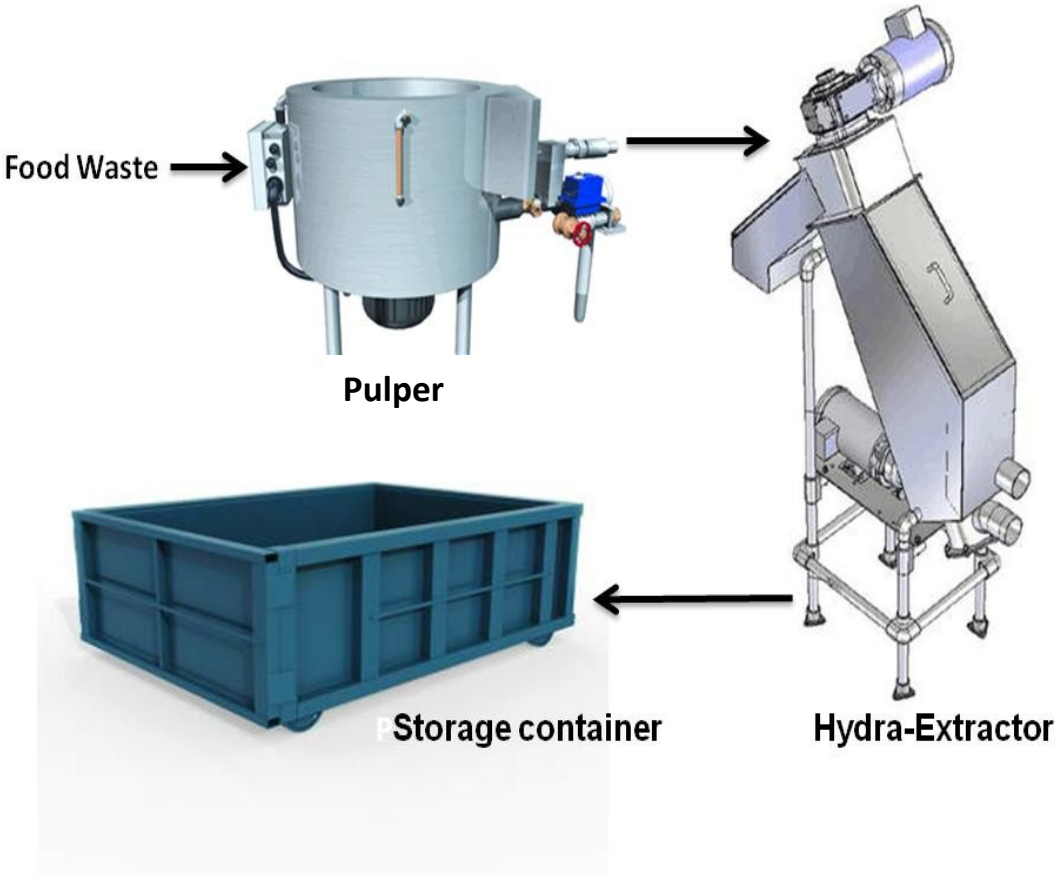
3.2 Material and Methods

Experimental material and methods are presented in this subsection.

3.2.1 Food Waste

University cafeteria food waste (FW) was utilized as sample substrate to represent the typical American food waste mixture containing fruits, vegetable, meats, and grains. Post-consumer cafeteria food waste was collected from the Brody Dining Hall at Michigan State University (MSU). An estimated 200 ton/year of food waste is generated from this cafeteria. A pulper/extractor system (Somat Remote Pulping System, Somat Company, Lancaster, PA) is used for food waste processing at the Brody Dining Hall (Figure 3.1). All FW first enters the pupler (Figure 3.2), is ground, and then mixed with water to create pulpable slurry

comprised of approximately 95% liquid and 5% solids. Next, the slurry is pumped through a pipe to a remotely located hydra-extractor (Figure 3.3). The hydra-extractor removes most of the water using specialized brushed screw augers and a cylindrical screen. Finally, the semi-dry pulp is discharged into a storage container (Figure 3.4) for disposal. FW used for this research was collected from the storage container and stored in a freezer at -18°C. Before use, the frozen food waste was thawed and stored at 4°C for no more than one week. Prior to the BMP test, three sub-samples were taken for chemical analysis to determine its initial characteristic.



For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation

Figure 3.1 Process diagram of FW pulper/extractor system (source: http://www.somatcompany.com/Products)

Figure 3.2 Pulper component of the FW processing system at Brody Dining Hall

Figure 3.3 Hydro-extractor component of the FW processing system at Brody Hall

Figure 3.4 Storage container component of the FW processing system at Brody Dining Hall

3.2.2 Dairy Manure Vermicompost

Cattle manure is one of the easiest animal wastes for VC (Edwards, 2004). Therefore it was selected as the substrate to produce manure VC. Dairy manure was collected from the Dairy Teaching and Research Center at MSU. The MSU dairy herd consists of approximately 180 Holstein milking cows ranging from 2 to 12 years of age with an average of 40 months. Most of the milking herd is housed in tie stalls and receive forage and grain mixed diet. Dairy manure used for the study was collected directly from tie stalls instead of the manure storage facility to minimize bedding materials.

The earthworm specie used for vermicomposting was *Eisenia Fetida,* and was obtained from the Student Organic Farm at MSU (Figure 3.5). These earthworms were previously cultivated in horse manure and pre-consumer food waste (fruits and vegetable scraps). The vermicomposting process for this research was carried out in plastic commercial storage bins (Figure 3.6) at a temperature range from 15-25˚C. The moisture content was checked on weekly basis and maintained at 60-70% by spraying water on the surface. Dairy manure was composted to maturity, approximately 60 days. The final product (VC) was a

dark, odourless, homogeneous, and peat like. After manually removing the earthworms, the VC was then stored at 4°C prior to use. The TS and VS of VC were 34.8% and 16.6%, respectively (Appendix Table A1.2).

Figure 3.5 Vermicomposting facilitate at the MSU Student Organic Farm

Figure 3.6 Vermicomposting bins used for this research

3.2.3 Biochemical Methane Potential Assay

3.2.3.1 Experimental Design

The experimental unit for this study was 12 identical batch-scale reactors. The completely randomized design was achieved by randomly assigning reactors to 4 treatments as shown in Table 3.1 (3 replicates per treatment).

Table 3.1 Experimental design of BMP Assay

Treatments	Inoculum (g TS) [g VS]	Food waste (g TS) [g VS]	Vermicompost (g TS) [g VS]	Initial substrate organic loading (g VS)	Con. of VC (g/L)
Control	1.30 [1.02]	0.48 [0.45]	0	0.45	0
FWVC1	1.30 [1.02]	0.47 [0.44]	0.02 [0.01]	0.45	0.4
FWVC2	1.30 [1.02]	0.43 [0.40]	0.10 [0.05]	0.45	2
FWVC3	1.30 [1.02]	0.32 [0.30]	0.31 [0.15]	0.45	6

FWVC= food waste supplemented with vermicompost; VC= vermicompost; TS= total solids; VS= organic solids; Con. =concentration

Table 3.1 also shows the constituents of each treatment. All treatments had the same amount of inoculum (1.30 g TS, 1.02 g VS) and same initial substrate organic loading of 0.45 g VS. The working volume of each reactor was 0.15 L, achieved by adding the needed amount of deionized (DI) water. Detailed methods are provided in the subsections below.

3.2.3.2 Inoculum and Vermicompost

An active inoculum was obtained from a100 L pilot-scale mesophilic (35°C) CSTR treating dairy manure for more than 6 months. The HRT and OLR of the pilot-scale reactor were 20 days and 2 g VS/L/day, respectively. The TS and VS were 3.3% and 2.6%, respectively. Three reactors containing only inoculum (1.30 g TS) were included as blanks to measure the methane production originating from the inoculum.

Significant methane production is not expected from the AD of mature VC. However, in order to confirm this assumption several additional BMP tests were conducted (Table 3.2) to indirectly estimate the biogas potential of VC.

Table 3.2 Experimental design for determination of methane potential of manure vermicompost

Test Number	Purpose	Inoculum gTS [g VS]	Food waste gTS [g VS]	Manure gTS [g VS]	VC gTS [g VS]	Conc. of VC [g/L]
1	Control	1.27 [1.00]	0.32 [0.30]	0.14 [0.10]	0	0
	Treatment	1.27 [1.00]	0.32 [0.30	0.14 [0.10]	0.10 [0.05]	2
2	Control	1.27 [1.00]	0.21 [0.20]	0.28 [0.20]	0	0
	Treatment	1.27 [1.00]	0.21 [0.20]	0.28 [0.20]	0.10 [0.05]	2
3	Control	1.27 [1.00]	0.11 [0.10]	0.41 [0.30]	0	0
	Treatment	1.27 [1.00]	0.11 [0.10]	0.41 [0.30]	0.10 [0.05]	2
4	Control	1.27 [1.00]	0	0.55 [0.40]	0	0
	Treatment	1.27 [1.00]	0	0.55 [0.40]	0.10 [0.05]	2
5	VC Control	1.30 [1.02]	0	0	0.90 [0.45]	18

These four tests represented four different nutrient conditions (such as different C/N ratios and metal concentrations) as the result of the change of substrate composition (Table 3.2). Dairy manure provided macro and micro nutrients and buffering capacity and the trace metals are assumed to be adequately supplied by the manure. The control served as blank without VC and the estimated biogas contribution from VC was calculated by subtracting the biogas production of the control from those of the treatment. Test 5 was used to directly measure the methane potential of VC. As previously, after the addition of all components the volume of each flask was brought up to 0.15 L with DI water. Consequently, the

impact of VC on biogas production for blends with different nutritional values could be surmised as without the VC.

3.2.3.3 Sample Preparation

In order to ensure representative sampling and minimize loading errors, food waste was prepared using the procedure described by Hansen et al. (2004). A large subsample was first taken to determine the dry matter content. Thereafter, water was added and the large subsample was diluted to a dry matter content of 15% and blended in a commercial high-speed mixer for 5 minutes. This resulted in homogeneous slurry that allowed for the collection of small samples could easily be drawn for further chemical analysis and the BMP test.

3.2.3.4 BMP Set Up

The reactors were 225 mL borosilicate glass serum bottles sealed with aluminium caps (manufactured by Kimble Chase). For each bottle, 30 g of rigorously stirring inoculum was transferred. Three reactors were picked randomly for each treatment. Then, the required amounts of FW and VC (as described in Table 3.1) were added to each bottle. Thereafter, additional DI water was added to bring the volume to 150 mL and each serum bottled was sealed with septa and covered with a septa cap. The headspace was flushed with pure N_2 gas at a flow rate of approximately 0.5 L/min for 5 min to ensure anaerobic conditions were present in the head-space. This was achieved using a B-D 20 gauge needle that purged the bottle septum and extended into the liquid to introduce N_2 gas and a second needle in the headspace to allow gasses to escape. The sealed reactors were then placed on a shaker (100-150 rpms) and incubated at 35°C for 30 days (Figure 3.7).

Figure 3.7 BMP assays serum bottles in a shaker being incubated in constant 35°C temperature room

3.2.3.5 Biogas Production Measurement

To measure the biogas, a glass syringe (30 mL or 100 mL capacity) with a B-D 20 gauge needle was used. DI water was applied to the inside of the glass syringe case and plunger to allow for the plunger to move freely. The serum bottles were held at a 45° angle and a needle was inserted into the headspace. The pressure from the headspace biogas caused the plunger to move upward until it reached atmospheric pressure. The volume of the biogas was then measured on the syringe scale. Initially, biogas was measured daily until the biogas production decreased and only needed to be measured every 2 – 5 days. Biogas production was recorded under room temperature (~ 22°C) and corrected to standard ambient temperature and pressure (SATP, 25°C and 1 atm) using idea gas law. Biogas production from seed (determined by blank reactors) was subtracted from total biogas production for all treatments.

Biogas composition (CH_4, CO_2, N_2, and H_2S) was analyzed weekly using SRI 8610C Gas Chromatograph (GC) and Peak simple computer software (SRI Multiple GAS Analyzer #1, SRI Instruments, Torrance, CA). The GC was equipped with a 6 inch molecular sieve column and a FID and TCD combined detector. For each measurement, a 2 mL of biogas sample was extracted from the headspace of the serum bottles using an air-tight syringe and then injected to the GC column. The concentrations of CH_4, CO_2, and N_2 gas were reported as percentage and H_2S content was reported as ppm.

3.2.3.6 Methane Production Rate Constant Calculation

The degradation of each sample was assumed to follow a first-order rate of decay, in accordance with Equation 3.1 (Chen et al., 1978).

$$B = B_0 (1 - e^{-kt}) \quad \text{Eq. 3.1}$$

B: cumulative methane yield at time t expressed in mL CH_4/g VS added.

B_0: ultimate methane yield, assumed to equal the final *B* after 30 days of digestion.

k: methane production rate constant (1/day)

The *k* was estimated by plotting Ln (1- B/B_0) versus t which yield a straight line with slope equal to negative *k*.

3.2.4 Analytical Methods

Biological triplicate samples were taken and each sample was analyzed in technical triplicate. The pH was measured immediately after sampling using a pH

meter (accumet Excel XL60, Fisher Scientific) and electrode (accuCap, Fisher Scientific). Samples for TS and VS were stored at 4°C for at most three days using EPA method 1684. Alkalinity tests were performed within 24hrs after sampling using HACH Method 8203. Samples for chemical oxygen demand (COD), ammonia-nitrogen(Ammonia-N), total Kjeldahl nitrogen (TKN) and total phosphorus (TP) measurements were collected in plastic containers and either analyzed with 24hrs, or adjusted with acids to pH< 2, stored at 4°C and analyzed within 5 days. COD was determined according to USEPA approved Hach Method 8000 (0 to 1500 mg COD/L) (Hach Company). Ammonia-nitrogen analysis was measured using Hach Method 10031(Hach Company). TKN were determined according to EPA method 351.3. Total phosphorus was analyzed according to USEPA accepted Hach Method 8190 (Hach Company).

3.2.5 Statistic Methods

The experiment contains one independent variable (VC supplementation) with 4 levels (concentrations of vermicompost at 0, 0.4, 2, and 6 g/L).The dependent variable was the digestion performance as indicated by the ultimate biogas production and ultimate methane production. There was one measure on each dependent variable for each experimental unit. The significant differences among treatments were determined by a one-way analysis of variance (ANOVA) with the Tukey-Kramer test of SAS software version 9.1(SAS Institute Inc.). Significant differences among the means were assumed to correspond to $P \leq 0.05$.

3.3 Results and Discussion

Experimental results and discussion are presented in this subsection.

3.3.1Characteristics of Cafeteria Food Waste

The characteristics of FW are shown in Table 3.3.

Table 3.3 Characteristics of food waste and comparison with literature report

Components (wet basis)	Current study [a]	Zhang et al, 2011[b]	Banks et al., 2012[b]
pH	6.6 ± 0.1	6.5 ± 0.2	4.7 ± 0.2
TS (%)	22.5 ± 0.6	18.1 ± 0.6	23.7 ± 0.1
VS (%)	20.9 ± 0.5	17.1 ± 0.6	21.7 ± 0.1
VS/TS (%)	93.0 ± 0.4	94 ± 1	91 ± 1
Total COD (g/kg)	253.6 ± 3.4	238.5 ± 3.8	NA
TKN (mg/kg)	7.7 ± 0.2	5.42 ± 0.26	8.12 ± 0.01
TP (mg/kg)	1.6 ± 0.1	1.49 ± 0.09	1.28 ± 0.08
Ammonia-Nitrogen (g/kg)	0.24 ± 0.02	NA	NA
Alkalinity (g CaCO3/kg)	0.49 ± 0.01	NA	NA

TS= total solids; VS= organic solids; COD= chemical oxygen demand; TKN = total Kjeldahl nitrogen; TP= total phosphorus;

[a] values were reported as average ± SEM (SEM is short for the standard error of the mean); the sample size was three;

[b] values were reported as average ± STD (STD is short for the standard deviation)

All values are reported on a wet weight basis. The average TS and VS of food waste used in the BMP assays were 22.5% and 20.9%, respectively. The VS was 93% of the TS, indicating that the FW contained highly digestible organic matters, as expected. Additionally, the food waste also contained 253.6 g/kg COD, 7.7 g/kg TKN, and 1.6 g/kg TP, resulting in an approximately COD:N:P ratio of 159:5:1. This is slightly lower than the optimum ratio of 350:7:1suggested by Gerardi (2003). However, these results were similar to literature reports on the characteristic of food waste originating from restaurants or source segregated domestic food waste (Zhang et al., 2011; Banks et al., 2012).

3.3.2 Estimated Biogas Production of Manure Vermicompost

As shown in Table 3.4, under different nutrient conditions, little biogas was produced from VC which suggested that the digestibility of VC (mixed with food

waste or manure) was very low and contributed to negligible amount of biogas. Additionally, when desirable conditions for digestion of food waste were achieved by adding dairy manure (Test 1, 2 and 3) (which provided buffer capacity and macro and micro nutrients), supplementation of additional VC had no significant impact on biogas production.

Table 3.4 Estimated biogas potential of manure vermicompost under various nutrient conditions[1]

Test Number	Treatment	Cumulative biogas production (mL)	Estimated biogas production from VC (mL)	Estimated biogas potential of VC (mL/g VS added)[a]
1	Control	265 ± 1	4	8
	Treatment	269 ± 7		
2	Control	248 ± 1	13	26
	Treatment	261 ± 1		
3	Control	219 ± 3	12	24
	Treatment	231 ± 2		
4	Control	182 ± 1	0	0
	Treatment	171 ± 1		

[1] VS= organic solids; VC=vermicompost; error=SEM

[a] Estimated biogas potential of VC = estimated biogas production from VC/g VS of VC added

Manure VC was also tested. The ultimate biogas and methane yields after 30 day digestion were 38 and 14 mL/g VS added, respectively. As expected, only very small amounts of biogas and methane were produced from the AD of VC (Table 3.5). This is likely due to the lack of readily digestible organic matter present after 60 days of composting.

Table 3.5 The ultimate biogas and methane productions of manure vermicompost

Parameter	Unit	Average	SEM
Biogas yield	mL/g VS added	38	5
Methane yield	mL/g VS added	14	5
Average methane content	%	36.3	3.6

[1] VS= organic solids; SEM= standard error of means

3.3.3 Volatile Solid Destruction

With the lack of biogas production associated with VC, the impact on nutritional value was studied. Consequently, the food waste was supplemented only with VC and not with manure. Table 3.6 shows the VS content of the control and treatment reactors before and after 30 days of digestion.

Table3.6 Volatile solid content before (pre-digestion) and after 30 days of digestion (post-digestion) as well as total VS destroyed

Treatment	Pre-digestion (g VS/L)	Post-digestion (g VS/L)	Total volatile solid change (g VS/L) [1]	Average total volatile solid destroyed (g VS) [2]
Control	9.57 ± 0.05	7.68 ± 0.04	2.02 ± 0.05	0.303
FWVC1	9.44 ± 0.04	7.45 ± 0.02	1.87 ± 0.03	0.299
FWVC2	9.65 ± 0.45	7.76± 0.02	1.89 ± 0.05	0.284
FWVC3	9.49 ± 0.03	7.85 ± 0.05	1.64 ± 0.05	0.246
Vermicompost	9.51 ± 0.01	9.38 ± 0.02	0.13 ± 0.01	0.020
Blank	6.82 ± 0.03	6.79 ± 0.02	0.03 ± 0.01	0.005

Values were reported as average ± SEM; FWVC= food waste supplemented with vermicompost

[1] Total volatile solid change = Pre-digestion (g VS/L) – Post-digestion (g VS/L)

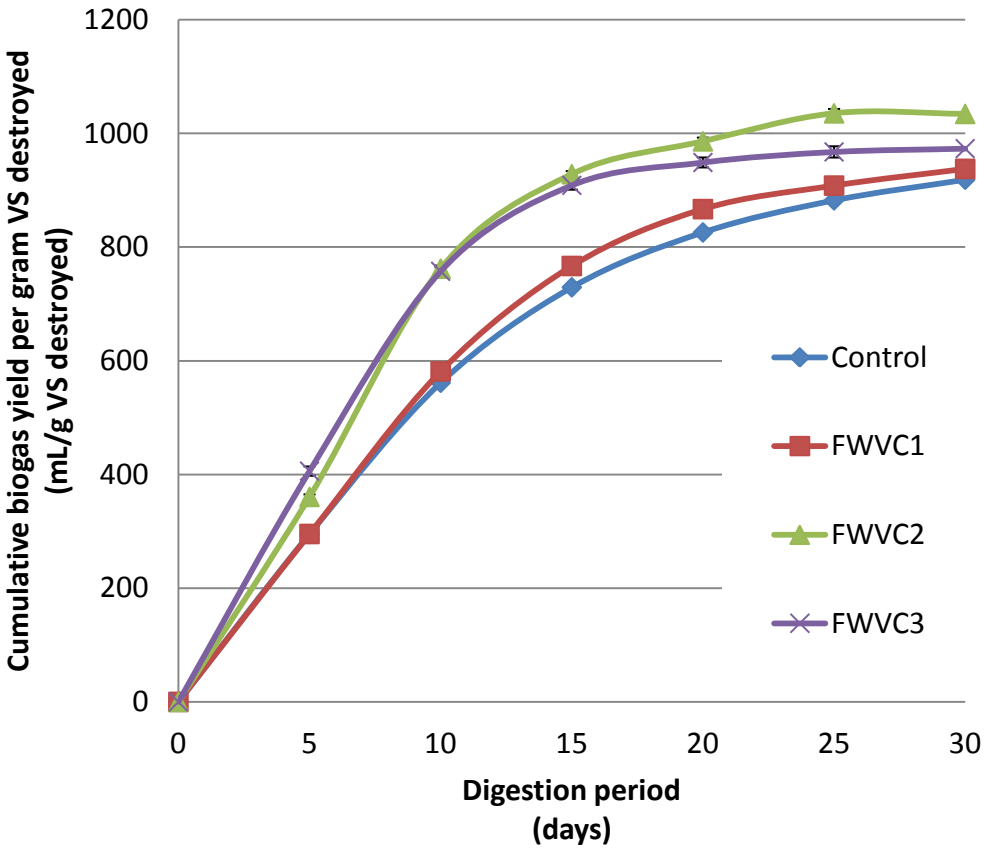
[2] Volatile solid destroyed = Total volatile solid change (g VS/L) ×Working volume of reactor (0.15L)

As previously determined, this study also confirmed that only very minimal amounts of VS of VC (0.02 g) and inoculum (0.005 g) were destroyed after 30 days of AD. The poor VS destruction of VC resulted in less VS and TS destruction for all treatments compared to the control as the VS from the VC were included in the total VS used for the calculations.

3.3.4 Biogas and Methane Production from Food Waste

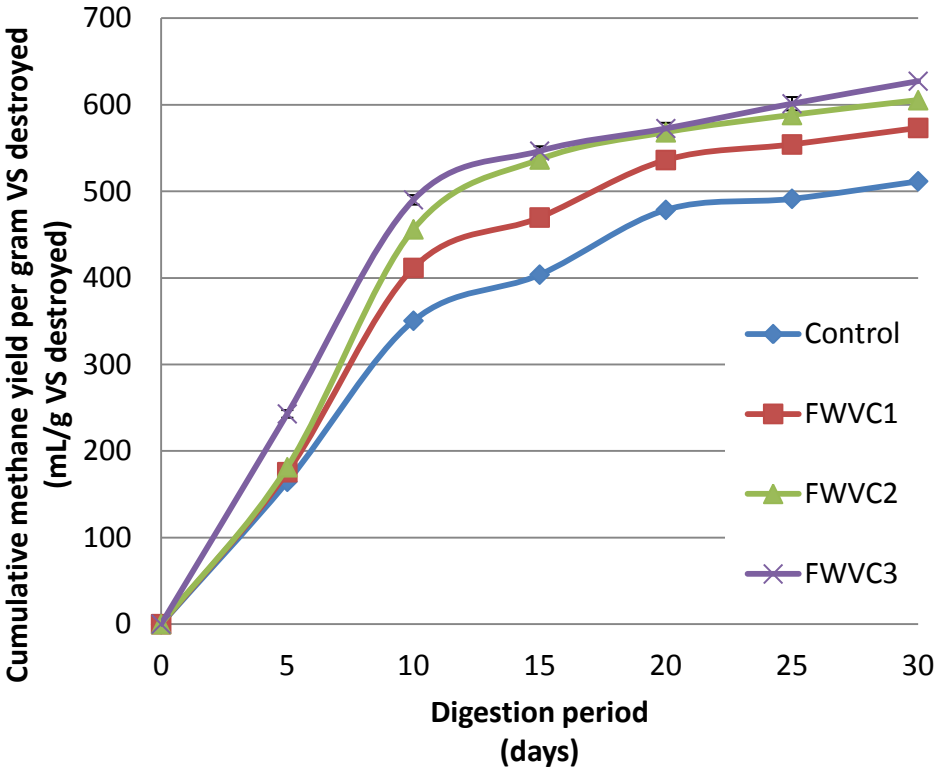
The cumulative biogas and methane production per gram VS destroyed (mL /g $VS_{destroyed}$) were calculated by dividing cumulative biogas or methane

production by the average total VS destroyed (values of the average total VS destroyed are shown in Table 3.6, column 5). The results are shown in Figure 3.8, Figure 3.9, and Table 3.7.



FWVC= food waste supplemented with vermicompost; error=SEM

Figure 3.8 Cumulative biogas yields from digestion of food waste with and without vermicompost



FWVC= food waste supplemented with vermicompost; error=SEM

Figure 3.9 Cumulative methane yields from digestion of food waste with and without vermicompost

Table 3.7 Ultimate methane yields and methane production rate

Treatment	Ultimate biogas yield (mL/g VS destroyed) [1]	Ultimate methane yield (mL/g VS destroyed)	Methane production rate constant (k, 1/day)
Control	918 ± 10^{a}	512 ± 13^{a}	0.134
FWVC1	938 ± 29^{a}	573 ± 30^{ab}	0.141
FWVC2	1034 ± 32^{c}	605 ± 35^{b}	0.149
FWVC3	973 ± 30^{b}	627± 33^{b}	0.149

Values were reported as average ± SEM; FWVC= food waste supplemented with vermicompost

^{abc} Means within a column lacking common superscript differ significantly ($P < 0.05$)

Supplementing FW with VC at the concentrations of 2 and 6 g/L (FWVC2 and FWVC3) both increased the ultimate biogas and methane yield per gram VS destroyed (determined based on 30 days of digestion; $P < 0.05$). The ultimate biogas yield of FW supplemented with VC at the concentrations of 2 and 6 g/L (FWVC2 and FWVC3) were 1034 and 973 mL/g $VS_{destroyed}$ respectively, which were both greater than the control (918 mL/g $VS_{destroyed;}$ $P < 0.05$). While, the ultimate methane yield from the FW digesters with VC at the concentrations of 2 and 6 g/L (FWVC2 and FWVC3) were 605 and 627 mL/g $VS_{destroyed}$ respectively, which were both greater than the control (512 mL/g $VS_{destroyed;}$ $P < 0.05$). There was no significant difference between the control and FWVC1.

Vermicompost addition not only increased ultimate methane yield but also improved the methane production rate as shown in Table 3.7. The methane production rate constant of food waste supplement with VC at 2 g/L (FWVC2) and 6 g/L (FWVC3) were both 0.149 1/day which is numerically greater than the *k* of the control (k=0.134 1/day).

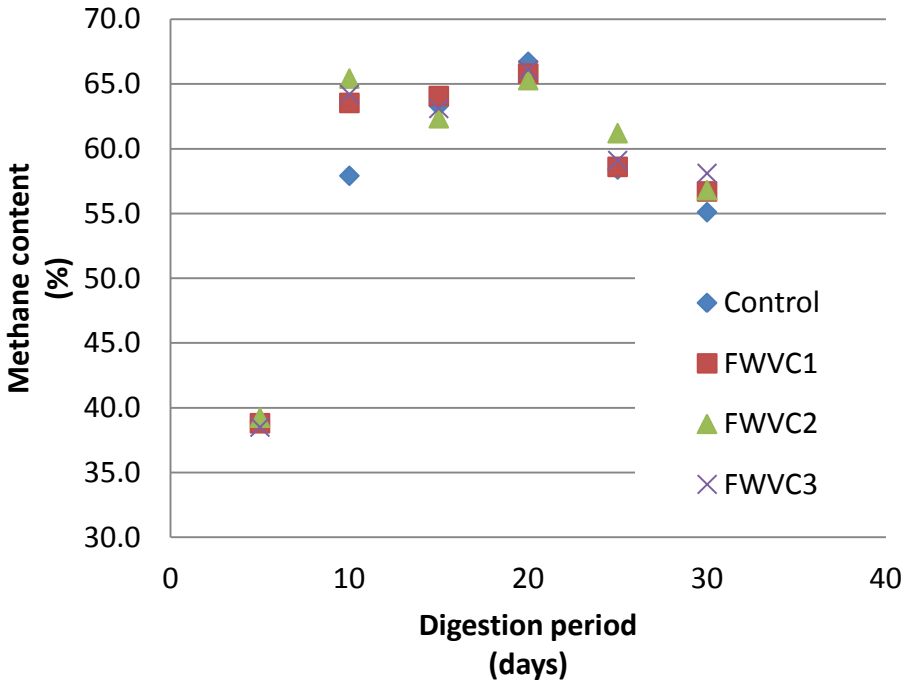
Across treatments and control, biogas and methane production increased sharply until day 15 (Figure 3.8 and 3.9) and then remained at a slow rate of production until the end of the experiments (30 days). As a result, more than 80% of biogas and methane yield was obtained within the first 15 days of digestion. This is in agreement with the result found by Zhang et al. (2007) that methane production from food waste (collected from commercial restaurants in San Francisco, CA) increased until day 16 and then remained almost constant at a low level thereafter. After 15 days of digestion, supplemented with vermicompost at the concentrations of 2 and 6 g/L (FWVC2 and FWVC3) significantly increased biogas yield by 27 and

25%, respectively, compared to the control (Figure 3.8; $P \leq 0.05$). Similarly, the average methane yield from the FW digesters with 2 and 6 g/L VC (FWVC2 and FWVC3) produced 33 and 35%, respectively, more methane than those of controls after 15 days of digestion (Figure 3.9; $P \leq 0.05$).

The methane potential of food waste without any supplementation was 351 mL/g VS added (Appendix A1.10). Previously, Cho and Park (1995) conducted BMP experiments using typical Korean food waste and obtained methane yields of 482, 294, 277, and 472 mL/g VS added for cooked meat, boiled rice, fresh cabbage, and mixed food waste (containing 73% rice, 6.4% rice, and 1.3% cabbage), respectively, after 40 days of digestion at 35°C. Heo et al. (2003) also evaluated the methane potential of Korean food waste and found similar results. Zhang et al. (2007) investigated BMP of food waste collected from American restaurants under thermophilic conditions (50°C) with the organic loading rate of 6.8 g VS/L and founded a methane potential of 425 mL /g VS added. The methane yield of food waste (control) obtained in this study was lower than the values reported by the above authors. This is likely due to the variation of food waste compositions. For instances, vegetable have much lower methane potential (in a range of 200-400 mL/g VS added; Gunaseelan, 2003) compared to fat or oil components in food waste, such as cooked meat (482 mL/g VS added; Cho and Park, 1995) or cooking oil (940 mL/g VS added; Chynoweth et al., 1993). Therefore, food wastes containing greater amounts of vegetable have a lower methane potential than those containing more fats and oils. It should also be mentioned that BMP test values are sensitive to several parameters, e.g. operating conditions (temperature, pH, and agitation intensity), the inoculum/substrate ratio, and initial organic loading (Lesteur et al., 2010). This makes it very difficult to compare BMP results among different studies.

3.3.5 Methane Content

The methane content during digestion of food waste with and without VC is shown in Figure 3.10. After 5 days of digestion, the methane content remained almost constant. No significant difference was observed among the control and different treatments (Appendix A1.9). The average methane content was measured to be approximately 60% which is similar to the value of 63% reported by Banks et al., (2011) using a CSTR to digest source segregated domestic food waste.



FWVC= food waste supplemented with vermicompost

Figure 3.10 Methane content from digestion of food waste with and without VC

3.3.6 pH Change

The pH values of all reactors before and after digestion are shown in Table 3.8. No significant difference was observed among the control and treatments before and after 30 day of digestion (P >0.05).

Table 3.8 pH change before and after digestion

Treatments	Pre-digestion	Post-digestion
Control	7.6 ± 0.1	6.9 ± 0.1
FWVC1	7.5 ± 0.1	6.9 ± 0.1
FWVC2	7.6 ± 0.1	6.9 ± 0.1
FWVC3	7.7 ± 0.1	6.8 ± 0.1
Vermicompost	7.9 ± 0.1	6.7 ± 0.1

FWVC= food waste supplemented with vermicompost; values were reported as average ± SEM

3.4 Conclusions and Implication

More than 80% of the methane yield from the AD of food waste was obtained during the first 15 days of digestion. Dairy manure VC added to the food waste reactors at concentrations of 2 g/L and 6 g/L significantly increased the ultimate methane yield and the methane production rate of food waste. The concentration of VC at 6 g/L had the most promising results with approximately 20% greater ultimate methane yield and 10% increase in methane production rate than those of controls.

In conclusion, results from BMP assays proved the hypothesis that manure VC can enhance methane production from AD of FW under batch-scale experimental setup.

Biogas and methane potential determined by batch BMP assays are preliminary estimation and are not intended for use in simulating a field-scale digester. Therefore, further research was conducted to determine if the positive effect of VC on AD of FW observed using batch experimental set-up remains in a continuous system as well as the likely cause of improvement and the results are presented in following chapters.

CHAPTER 4 USE OF SINGLE-STAGE CONTINUOUS DIGESTION SYSTEM TO EVALUATE THE EFFECTS OF MANURE VERMICOMPOST ON ANAEROBIC DIGESTIBILITY OF FOOD WASTE

The second stage of dissertation research is presented in this chapter including introduction; material and methods; results and discussion; and conclusions and implication sections.

4.1 Introduction

The previous BMP trial demonstrated that the addition of VC significantly enhanced anaerobic digestibility of FW and increased biogas and methane production. However, BMP assays provide a preliminary estimation and are not intended for use in simulating a field-scale digester. Semi-continuous systems can simulate a field-scale digester under existing or planned operating conditions (Rozzi and Remigi, 2004). Therefore, the second phase of this research was conducted to confirm the results found by the BMP trial. Additionally, this phase was designed to identify the key components of VC responsible for enhancing the digestion of food waste, including the investigation of trace minerals and humic acids.

4.1 Material and Methods

Experimental material and methods are presented in this subsection.

4.2.1 Experimental Design

A completely randomized design was used by randomly assigning the 12 identical reactors to six treatments (2 replicates per treatment) as shown in Table 4.1. The study was designed to evaluate the effects of different additives (particularly VC, trace elements, and humic acids) on the AD of FW. There was 1 independent

variable (additive) and 6 levels including no additive (control), trace elements (TE), humic acids (HA), VC, combination of trace elements and humic acids (TE+HA), and a combination of trace elements and vermicompost (TE+VC). A 15 day SRT was selected and the OLR of food waste was set at 0.6 g VS/L/day for both controls and treatments to remove this from being a variable. A relatively low OLR was selected to prevent over loading caused digester failure. The total OLR of the food waste digesters supplemented with vermicompost was 0.62 g VS/L/day (0.6 g VS/L/day of food waste and 0.02 g VS/L/day of vermicompost).

Table 4.1 Experimental design of semi-continuous study

Treatment	Additives	Concentration of additives	SRT (days)	OLR (g VS/L/d)
FW	-	-	15	0.6
FW + TE	Trace elements	0.01 mg/L Ni 0.5 mg/L Fe 0.01 mg/L Co		
FW + HA	Humic acids	0.4 g/L HA		
FW + VC	Vermicompost	2 g/L VC		
FW + TE + HA	Trace elements and humic acids	0.01 mg/L Ni 0.5 mg/L Fe 0.01 mg/L Co 0.4 g/L HA		
FW + TE + VC	Trace elements and vermicompost	0.01 mg/L Ni 0.5 mg/L Fe 0.01 mg/L Co 2 g/L VC		

-: Not applicable; FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost; SRT= solid retention time; OLR=organic loading rate

The concentration of VC was determined based on the BMP trail. The two likely stimulatory substances contained in manure VC are trace elements and humic acids. Both were also used as independent additives to provide a comparison to the VC. The trace element additives were Ni, Fe, and Co, selected because of their crucial roles for catalysing metabolic reactions during methanogenesis (Oleszkiewicz and Sharma, 1989; Speece, 1996; Shen et al., 1993; Speece, 1996; Sharma and Singh, 2001; Kim et al., 2002; Noyola and Tinajero, 2005; Ma et al., 2009; Pobeheim

et al., 2010). Concentrations of the supplemental Ni, Fe, and Co were 0.01 mg/L, 0.5 mg/L, and 0.01 mg/L, respectively, determined based on recommended values reported by previous studies (Climenhaga and Banks; 2008). The stock solution was prepared by completely dissolving laboratory grade $NiCl_2 \cdot 6H_2O$ (solubility in water: 2540 g/L, 20 °C), $FeCl_3 \cdot 4H_2O$ (solubility in water: 900 g/L, 20 °C), and $CoCl_2 \cdot 6H_2O$ (solubility in water: 529 g/L, 20 °C) in DI water. The concentration of humic acids (0.4 g/L) was estimated based on its estimated content in manure VC, approximately 20% (Hervas et al., 1989; Senesi et al., 1992) and was added as a sodium salt.

4.2.2 Food Waste and Manure Vermicompost

Three batches of FW were used for this study. A new batch was started on day 1, 25, and 56 of the digestion period. As with the BMP, the source was from the MSU Brody Dining Hall. Characteristics of each FW batch were analyzed immediately after collection and then it was stored at 4^{o}C. The same VC was used throughout the entire study (day 1-90) and was prepared using the same procedures as previously described in Chapter 3, Section 3.2. The pH, TS, VS, alkalinity, and ammonia-nitrogen of both FW and VC were determined using the same methods as described in Chapter 3, Section 3.2. For TKN and TP measurements, FW were analyzed within 24 hrs of collection using EPA method 351.3 and Hach Method 8190 (HACH Company), respectively. VC were analyzed at MSU's Soil and Plant Nutrient Laboratory for TKN, TP, and iron using the recommended chemical soil test procedures for the north central region (source: http://extension.missouri.edu/explorepdf/specialb/sb1001.pdf). Total concentrations of Ni, Fe, and Co of food waste were analyzed using HACH method 8150, 8008, and

8078, respectively. Technical triplicate samples were conducted for all analysis. Results are shown in Table 4.2 and Table 4.3.

Table 4.2 Characteristics of raw food waste

Items (wet basis)	Batch 1 (day1-30)	Batch 2 (day31-60)	Batch 3 (day61-90)
pH	6.6	6.2	6.8
TS (%)	23.4	22.1	25.2
VS (%)	21.8	20.4	23.6
VS/TS (%)	93.2	92.7	94.4
TKN (g/kg)	7.7	6.4	8.1
TP (g/kg)	1.6	1.1	1.3
Ammonia-Nitrogen (g/kg)	0.24	0.16	0.11
Alkalinity (g $CaCO_3$/kg)	0.49	0.31	0.52
Fe (mg/kg)	-	63	175
Ni (mg/kg)	-	1.2	3.3
Co (mg/kg)	-	3.0	4.6

TS= total solids; VS= organic solids;TKN = total Kjeldahl nitrogen; TP= total phosphorus; - data is not available; Fe = iron; Ni=nickel, Co= cobalt

Table 4.3 Characteristics of manure vermicompost

Items (wet basis)	Vermicompost
pH	6.98
TS (w.t. %)	35.4
VS (w.t.%)	15.0
TKN (g/kg)	7.9
TP (g/kg)	0.5
Ammonia-Nitrogen (g/kg)	0.03
Fe (mg/kg)	81

TS= total solids; VS= organic solids; TKN = total kjeldahl nitrogen; TP= total phosphorus; Fe = iron

4.2.3 Inoculum and Start-up

The inoculum was obtained from a100-L pilot-scale mesophilic CSTR that previously used for digestion of dairy manure for more than 6 months. The HRT and OLR were 20 days and 2 g VS/L/day, respectively. The TS and VS were 3.2% and 2.1%, respectively.

All reactors were started with the same amount of inoculum (0.9 L). During the initial 3 weeks, no substrate was fed and no effluent was removed until the biogas production from the inoculum ceased (less than 30 mL/day). Thereafter, FW (with or without additives) was fed and the effluent was taken daily. The first SRT of this experiment was started at this time.

4.2.4 Experimental Setup and Biogas Measurement

This experiment was carried out using anaerobic respirometers (AER-208 - Research Respirometer Aerobic/Anaerobic, Challenge Technologies Inc., Springdale, AR). Specifically, 12 identical (duplicate reactors for each treatment and control) PYREX 1L aspirator bottles with bottom outlets were used as the CSTR reactors (Figure 4.2).

Figure 4.1 Experimental setup of the semi-continuous digestion study

Each reactor contained a magnetic stir bar and was placed on a magnetic stir plate for mixing at 80 rpm. The bottles were PVC-coated to contain reagents and prevent shattering of glass if the bottle broke. The bottom outlet was used for

feeding substrates and removing of digestate. The bottle was sealed with a size 6 rubber stopper that was tightly tied by plastic cable tie. A needle attached to the gas collection line was inserted through the rubber stopper of each reactor. Biogas flowed through the needle and collection line to individual gas measuring cells (Figure 4.2). When biogas passed through the oil filled volume measurement cell, the computer equipped with the Challenge Technology AER computer software (Challenge Technologies Inc., Springdale, AR) automatically measured, calculated, and recorded cumulative biogas production (volume and rate). The compositions of biogas were measured three times a week using the GC, as described in section 3.2.3.5.

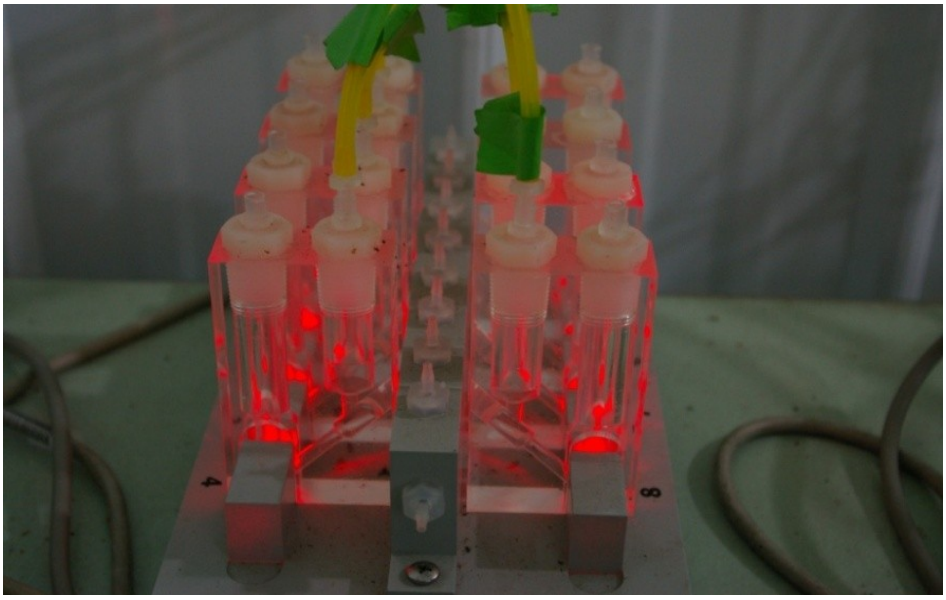


Figure 4.2 AER-208 - Research Respirometer Aerobic/Anaerobic gas measuring cells

4.2.5 Digester Operation and Monitoring

The temperature was maintained at 35ºC for the entire 90 day experiment (6 SRT, 15 days for each SRT). Digestate was removed and FW and additives (VC,

trace elements, and humic acids) were added daily to give the desired OLR of 0.6 g VS/L/day and concentrations of additives. The working volume was maintained at 0.9 L. During first SRT (days 1-14), 1 g/L of sodium bicarbonate (NaHCO3) was added to all reactors for buffering. However, no additional $NaHCO_3$ was used after that.

The pH was monitored every 2 days and TS and VS were measured every 3 days using the methods described in Chapter 3, Section 3.2. Alkalinity, TKN, and TP were analyzed once a week using the same procedures described in Chapter 3, Section 3.2.3. VFAs were determined once a week using the titration method established by O'Brien and Donlan (1977). During the fifth SRT, digester effluents were assessed for soluble trace metal concentrations to enable the estimation of metal bioavailability. Soluble metals in the AD are usually considered bioavailable (Oleszkiewicz and Sharma, 1990) which were measured as those remaining in solution (digester effluent) after centrifugation (~3500 rmp for at least 15 min) and filtration through a 0.45 μm fibreglass filter. Filtered samples were then measured for Ni, Fe, and Co using HACH method 8150, 8008, and 8078, respectively.

4.2.6 Statistic Methods

The experimental units in this study were 12 identical reactors. A completely randomized design was achieved by randomly assigning reactors to six treatments (2 replicates per treatment). There was 1 independent variable (additive) and 6 levels including no additive (control), TE, HA, VC, TE+HA, and TE+VC. The dependent variable was the digestion performance such as daily biogas and methane production, pH, and the concentration of VFAs. The dependent variable was measured repeatedly (time-series) throughout the experimental period. The

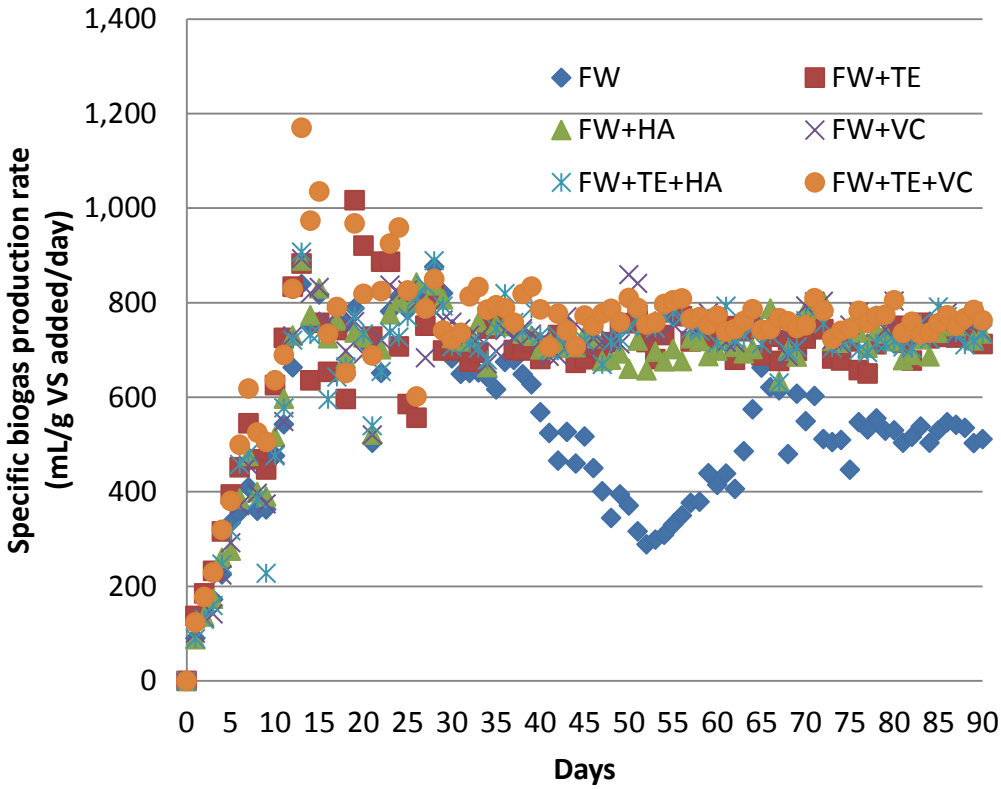
significance of the differences between treatments was determined by the PROC MIXED procedure of SAS software version 9.1 (SAS Institute Inc.). Differences were considered significant at a *P* value of ≤ 0.05.

4.3 Results and Discussion

Experimental results and discussion are presented in this subsection.

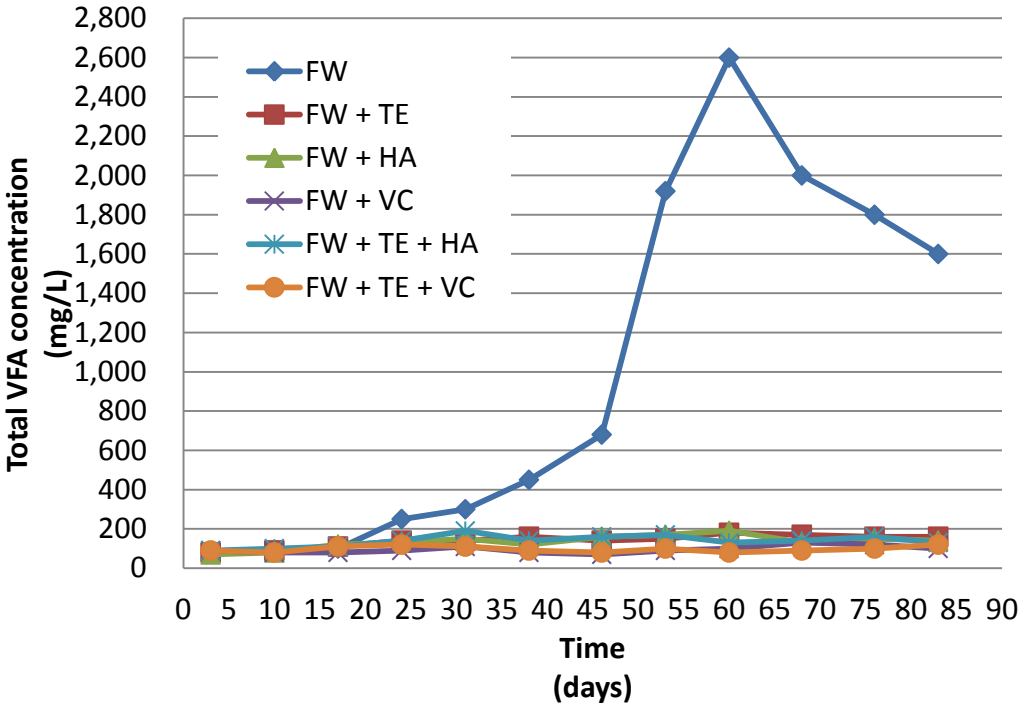
4.3.1 Biogas Production, VFA Concentration, and pH

Specific biogas production rate (mL/g VS $_{added}$), total VFA concentrations, and pH are shown in Figures 4.3, 4.4, and 4.5, respectively. For biogas production rate calculation, the total VS $_{added}$ of the digesters supplemented with VC were 0.62 g VS/L/day and all other digesters were 0.6 g VS/L/day.



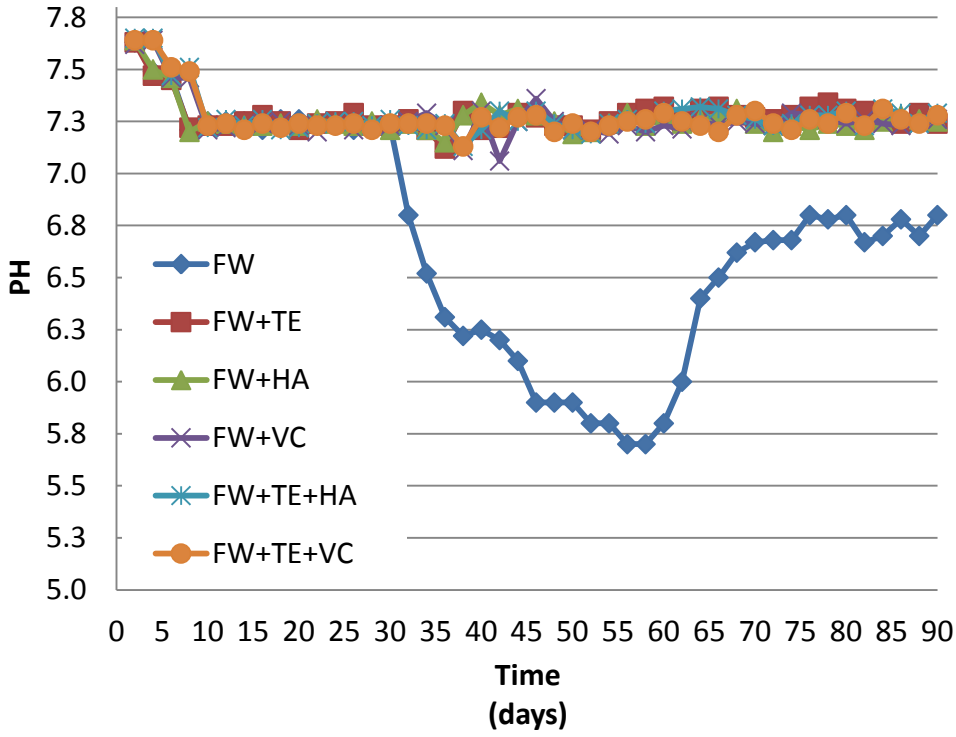
FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

Figure 4.3 Specific biogas production rates from digestion of food waste with and without additives



FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

Figure 4.4 Total VFA concentrations from digestion of food waste with andwithout additives



FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

Figure 4.5 pH change from digestion of food waste with and without additives

Table 4.4 Specific biogas production rate, total VFA concentrations, and pH during steady-state period

Items	FW	FW + TE	FW +HA	FW + VC
Biogas yield (mL/g VS $_{added}$/day)	505^{a}±73	719^{b}±21	718^{b}±24	749^{b}±24
pH value	6.4^{a}±0.3	7.3^{b}±0.1	7.2^{b}±0.1	7.2^{b}±0.1
Total VFA (mg/L)	1419^{a}±554	158^{b}±9	151^{b}±15	100^{c}±13

Values were reported as average ± SEM; FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

^{abc} Means within a row lacking common superscript differ significantly (P 0.05)

The food waste reactor without any additives (FW) were unstable and had low biogas production (Figure 4.3) due to the significant accumulation of VFA (Figure 4.4) that resulted in a decrease in pH (Figure 4.5) after the first two SRTs (corresponding to the time necessary for washout of the inoculum). In contrast, digesters with additives including VC, trace metals, and HAs all had stable biogas production, desirable pH, and low VFA concentrations (Figures 4.3, 4.4, and 4.5).

The observed unstable and low biogas production from the FW reactor without any additives (control) is consistent with results reported previously by other researchers. El-Mashad et al. (2008) found that a single-stage mesophilic AD with food waste as sole substrate was not stable at the OLR of 2 or 4 g VS/L/day as indicated by the accumulation of VFAs, low pH, and low biogas production. Similarly, Banks et al. (2008) utilized a thermophilic digester to digest source segregated domestic food waste and also observed digester instability in terms of the sudden dropping of pH and biogas production as a result of the accumulation of VFA (up to 45,000 mg/L).

The food waste reactor supplemented with trace elements maintained a stable biogas production rate of average 719 mL/g VS_{added}/day, significantly higher than the control (505 mL/g VS $_{added}$/day; Table 4.3). This result was similar to the result

found by Banks et al. (2011) who reported a specific biogas production rate of 750 mL/g VS_{added}/day from semi-continuous digestion of source-sorted food waste supplemented with additional trace elements. Moreover, the observed improvement suggests that the FW reactors operating under current experimental condition were deficient in trace elements, likely Ni, Co or Fe. However, this stage research did not identify which one of those three metals was the key factor that contributed to the enhanced performance. A further study aimed to determine which of the three metals being studied played the most crucial role is presented in Chapter 6. Several other researchers also found that unsuccessful digestions of cafeteria food waste were likely due to trace element deficiencies. For example, Zhang et al. (2011) demonstrated that when food waste is used as a sole substrate, the digester suffered from accumulation of VFAs, up to 18,000 mg/L, which dropped the pH from 7.2 to 4.4, ultimately led to a process failure. In this study, the waste was found to be deficient in Co, Ni, Fe, and Mo, all of which are required for robust and stable AD. Climenhaga and Banks (2008) evaluated the effect of micronutrients on AD of cafeteria food waste containing a varied mix of fruits, vegetables, meats, and fried foods in single-stage ADs. Without the supplement of micronutrients, the reactors exhibited methanogenic failure as a result of accumulation of VFAs and it was concluded that trace element addition (a mixture of Fe, Cu, Co, Zn, Mn, Mo, Al, and Se) was required .

The food waste reactor supplemented with humic acids (FW+HA) was also stable and had an average biogas production rate of 718 mL/g VS_{added}/day, significantly greater than the control (505 mL/g VS_{added}/day; Table 4.4). Little is known regarding the impacts of humic acids on the AD of organic waste. Hartung

(1989) conducted a two-year study using a full-scale operating plant to investigate the effect on the AD of sewage sludge and reported that supplementation of humic substances stimulated the process as evidenced by greater methane production and less sludge volume. The humic acid sodium salt (> 0.25 g/L) was also found to delay in protein hydrolysis extending the lag-phase, and ultimately, the ultimate hydrolysis rate (Brons et al., 1985). Such "inhibition" may be beneficial for the AD of food waste as it could prevent rapid hydrolysis of protein caused VFA accumulation.

The food waste reactor supplemented with VC had the greatest biogas production of 749 mL/g VS_{added}/day. Such improvement could be a combination effect of trace elements and humic acids that naturally presented in manure vermicompost.

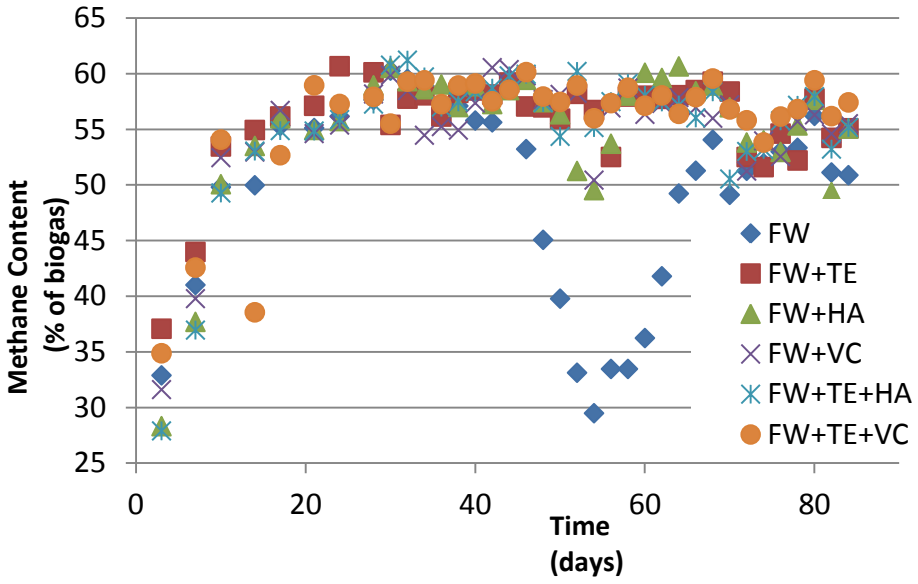
A recovery of digestion performance was observed for the control reactor during the fifth and sixth SRT. For example, daily biogas production jumped from the average of 202 mL for the fourth SRT up to the average of 289 and 284 mL for the fifth and sixth SRT (Appendix A2.24). The pH increased from below 6 during fourth SRT up to 6.8 during the fifth and sixth SRT (Appendix A2.4). However, the VFAs were remained high for the control (Appendix A2.29). The cause was not clear but it is worth noting that there was a change of food waste on fifth and sixth SRT when batch 3 was used as shown in Table 4.2. Although the TS and VS of this new batch were similar to the previous batches, it had higher concentrations of trace elements, particularly Fe. The recovery of biogas production may be also due to the acclimation of methanogens. Methanogens are known for their exceptional acclimation capability to some inhibitors (Speece, 1996). In this alternative scenario, during the first SRT, the inoculum and additional $NaHCO_3$ provided adequate buffering capacity and neutralized excessive free VFAs which helped to maintain the

balance between anaerobic bacteria and methanogens. This also allowed methanogens to adapt to food waste. After washing out inoculum and stopping the supplementation of $NaHCO_3$, free VFAs produced by anaerobic bacteria were not utilized by methanogens resulting in the accumulation of VFAs that partially inhibited methanogenesis. Later, methanogens acclimated to the environment and started to produce biogas again.

Regardless, supplementation of trace elements appeared to resulted in no significant reduction in biogas production for the food waste digester. Similarly, no significant drops of biogas production were observed for the food waste digesters supplemented with humic acids and VC. In these cases, the buffering capacities of humic acids and VC were likely also assistant the acclimation process.

4.3.2 Biogas Composition and Methane Production Rate

The methane content of biogas as a function of time during the entire trial period is shown in Figure 4.6 and the statistic results were shown in Table 4.5.



FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

Figure 4.6 Methane content from digestion of food waste with and without additives

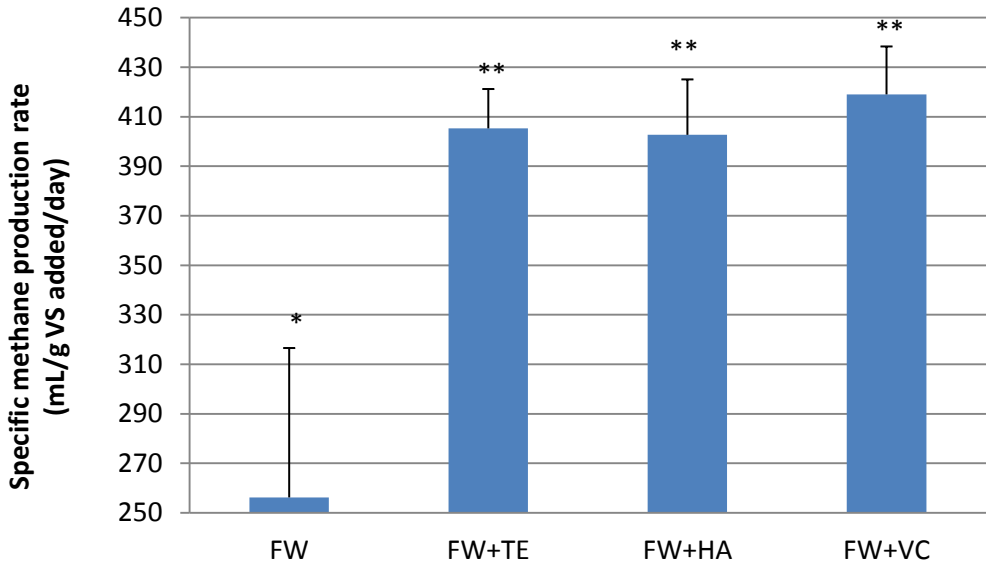
Table 4.5 Average biogas compositions during the steady-state period

Items	FW	FW + TE	FW +HA	FW + VC
Methane (% of biogas)	49^{a}±6	57^{b}±1	56^{b}±2	56^{b}±1
Carbon dioxide (% of biogas)	39^{a}±4	33^{b}±1	33^{b}±1	33^{b}±1
Nitrogen (% of biogas)	9±4	7±2	8±2	8±2
Hydrogen sulfide (ppm of biogas)	711±445	394±98	164±104	88±44

Values were reported as average ± SEM; FW= food waste; TE=trace elements; HA= humic acids; VC= vermicompost
^{ab} Means within a row lacking common superscript differ significantly ($P < 0.05$)

Except for the control, all treatments had similar methane content ranging from 50% to 60% with an average of approximately 57% after first SRT. This value is within the range reported for AD of food waste (52-63% CH_4; Banks et al., 2011; Zhang et al., 2011).

The calculated average specific methane production rate (mL/g VS added) during the steady-state period is shown in Figure 4.7. The non-supplemented control (FW) had an average specific methane production rate of 254 mL/g VS $_{added}$/day which were significantly lower than the food waste reactor supplemented with trace elements (FW+TE), humic acids (FW+HA) and vermicompost (Figure 4.7, $P< 0.05$). The observed greater specific methane production rates (more than 400 mL/g VS $_{added}$/day) for the digesters supplemented with trace elements, humic acids, and VC were similar to the results found by Zhang et al. (2011) who reported the specific methane yield up to 450 mL/g VS $_{added}$/day) from long-term anaerobic digestion of cafeteria food waste supplemented with trace elements (Co, Fe, Mo and Ni) in semi-continuous single-stage reactors.



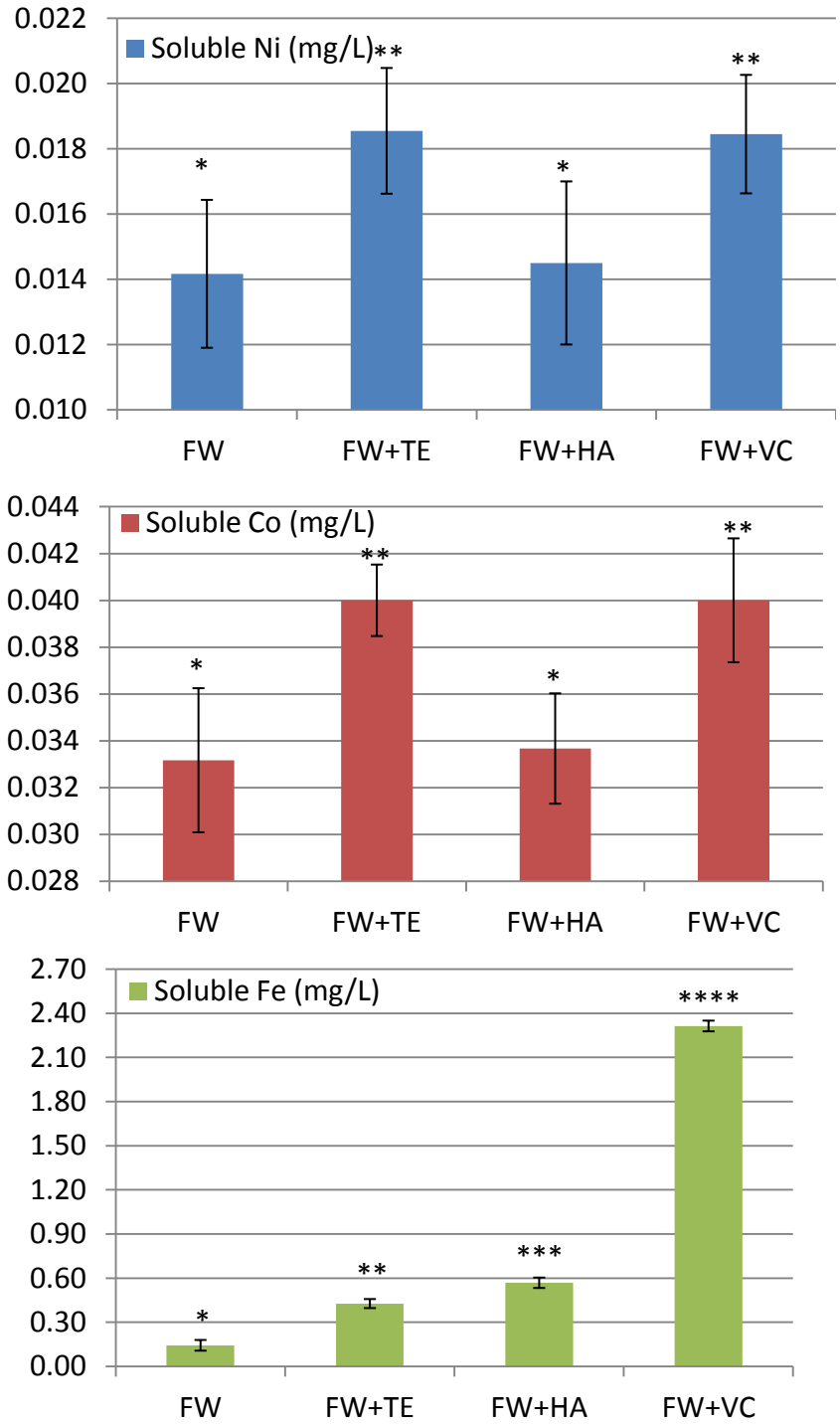
FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

* Data with different superscript differ significantly (P <0.05); error = SEM

Figure 4.7 Specific methane production rates (per gram VS added) of food waste digesters with and without additives

4.3.3 Trace Metal Analysis

Since only soluble metal ions are considered to be available for uptake by anaerobic microorganisms (Callander and Barford, 1983; Zandvoort et al., 2006); the concentrations of soluble Ni, Fe, and Co of digester effluents were analyzed to estimate bioavailability (Figure 4.8). As shown in Figure 4.8, the concentrations of soluble Ni and Co of the food waste digester supplemented with trace elements and vermicompost were significantly greater than the control (FW; $P < 0.05$). No differences in the concentration of soluble Ni and Co were observed between the food waste digester supplemented with or without humic acid. This suggested that enhanced methane production by supplementation of humic acid (Figure 4.7) was not related to change in the concentrations of Ni or Co.



FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost

* Data with different superscript differ significantly (P <0.05); error = SEM

Figure 4.8 Soluble metal concentrations of food waste digesters with and without additives

In the other hand, the soluble Fe concentrations of the food waste reactor supplemented with additives (trace elements, humic acid, and VC) were all

significantly higher than the control (Figure 4.8, $P<0.05$). This suggested that the control reactor had less available Fe for microorganism to uptake which may be related to its unstable and lower biogas production rate compared to other treatments.

Because humic acids contains negligible amount of Fe (an estimated 0.01 mg/L of Fe in the reactors originated from humic acids), it was surprising to discover that the soluble Fe concentration of the FW+HA reactor was significantly greater than the control. One possible explanation is that the humic acids increased the solubility of Fe as its presence could prevent the formation of insoluble Fe salts (Rashid and Leonard, 1973). Fe (II) can chelate with humic acids to form soluble humic acid-Fe complexes (Chen et al., 2004). In this study, it is possible that humic acids and Fe (II) (ferrous iron originating from food waste) formed soluble humic acid-Fe complexes that decreased the precipitation of Fe (II) and, consequently, increased its solubility.

4.3.4 Digester Effluent Measurement

The alkalinity, total VS reduction, and ammonia-N concentration of digester effluents during the steady-state period were analyzed and reported in the Table 4.6.

Compared to the control, the food waste digester supplemented with trace elements, humic acids, and VC had greater alkalinity (Table 4.6). The reactor supplemented with VC had highest alkalinity suggested that supplementation of VC likely increased the capacity of the food waste reactor to buffer the pH in the presence of additional acids.

The total VS reduction of the food waste digester supplemented with vermicompost, trace elements and humic acids were greater than the control. The

less total VS destruction of the control was likely attributed to the accumulation of VFAs and drop of the digester pH.

Table 4.6 Digester effluent measurement during steady-state period

Items	FW	FW + TE	FW +HA	FW + VC
Alkalinity (mg/L as $CaCO_3$)	943^{a}±110	1419^{b} ±30	1345^{b} ±49	1717^{c} ±15
Total VS reduction (%)	54^{a}±5	70^{b}±2	68^{b}±4	70^{b}±3
Ammonia-N (mg/L)	223±6	219±7	220±8	227±8
COD: N: P	176:21:1	168:22:1	139:18:1	120:10:1

Values were reported as average ± SEM; FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost; COD= chemical oxygen demand; N= nitrogen; P= phosphorus

^{abc} Means within a row lacking common superscript differ significantly ($P< 0.05$)

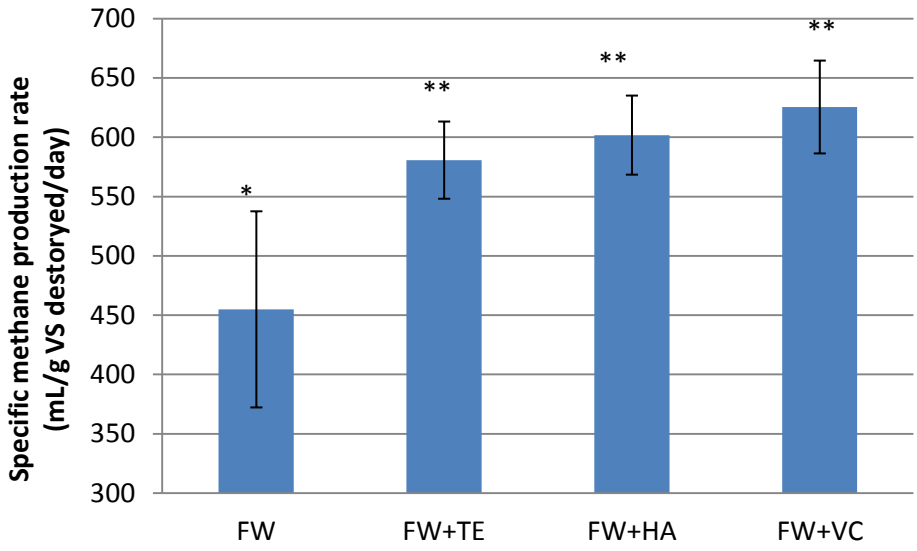
There was no significant difference in the concentrations of ammonia-N between the control and other treatments (Table 4.6) which indicated that ammonia-N inhibition is not the cause of poor performance. In fact, the digester ammonia-N concentrations were much lower than the toxic level of 1,700 to 14,000 mg/l reported in the literature (Chen et al., 2008).

The COD/N/P ratios of FW digesters with or without additives were not in the recommended range of 100-130:4:1 (Bouallagui et al., 2003) for optimal gas production from fruit and vegetable waste. This suggested that the COD/N ratio was not effectively adjusted by adding manure VC.

4.3.5 Specific Methane Production

The specific methane production per gram VS destroyed (mL/g $VS_{destroyed}$/day) was calculated by dividing daily biogas or methane production by total

VS destroyed (VS of food waste and vermicompost) during steady-state are shown in Figure 4.9



FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost
* Data with different superscript differ significantly (P <0.05); error = SEM

Figure 4.9 Specific methane production rates (per gram VS destroyed) of food waste digesters with and without additives

The non-supplemented control (FW) had an average specific methane production rate of 455 mL/g VS $_{destroyed}$/day, which was significantly lower than the food waste reactors supplemented with trace elements, humic acids, and vermicompost. There was no significant difference between the food waste reactors supplemented with different additives. Numerically, the food waste digester receiving vermicompost (FW+VC) as the supplement had the greatest specific methane production rate of 625 mL/g VS $_{destroyed}$/day.

4.4 Conclusions and Implication

The FW used in the study (particularly second batch) appeared to be deficient in trace elements which caused the failure of single-stage AD in terms of unstable

and low biogas productions, decreased pH, and the significant accumulation of VFA. With the supplementations of manure VC, trace metals (Ni, Fe, and Co), or humic acids, biogas production and the pH were maintained at desirable ranges while the concentration of VFAs in the reactors remained low. Additionally, it was also found that humic acids naturally presented in mature manure vermicompost were able to increase the solubility of Fe (II).

CHAPTER 5 EFFECTS OF VERMICOMPOST ON METHANOGENIC ACTIVITY DURING ANAEROBIC DIGESTION OF FOOD WASTE

The third stage of dissertation research is presented in this chapter including introduction; material and methods; results and discussion; and conclusions and implication sections.

5.1 Introduction

The semi-continuous study (Chapter 4) indicated that the single-stage food waste digester without supplements experienced a significant accumulation of VFA. VFA accumulation reflects a kinetic uncoupling between acid producers and acid consumers which is typical for stress situations (Ahring et al., 1995). Digesters supplemented with VC, on the other hand, maintained stable biogas productions with no significant VFA accumulation. To better understand the cause, methanogenic activities were assessed.

The specific methanogenic activity test enables the measurement of activity for the various physiological groups of microorganisms involved in the terminal processes of methanogenesis (Sorensen and Ahring, 1993). Activity is estimated by supplying sufficient substrate (such as acetate and propionate) to saturate the catabolic systems of the various physiological groups and then measuring the specific methane production rate or the substrate utilization rate (Sorensen and Ahring, 1993; Switzenbaum et al., 1990).

Although there are three principal groups of methanogens, including acetotrophic (also known as acetoclastic), hydrogenotrophic, and methlotrophic methanogens, involved in utilizing substrates to produce CH_4. A 70% of CH_4 is typically derived from acetate through the activity of acetoclastic methanogens

(Gujer and Zehnder, 1983). Also, CH_4 is produced by the syntrophic activity of acetate-oxidizing bacteria and hydrogenotrophic methanogens under certain environmental conditions (Hattori, 2008). Therefore, the maximum acetate utilization rate (MAUR) is a simple and good indicator of methanogenic activities (Dolfing and Bloemen, 1985; James et al., 1990; Nopharatana et al., 1997) and was used in this study to evaluate methanogenic activities. In addition to acetate, propionate is another key intermediate in AD. It has been reported that its accumulation was the primary cause for the food waste digester failure (Banks et al. 2012). Therefore, the maximum propionate utilization rate (MPUR) also was used for assessment of methanogenic activities.

In summary, the objective of this study is to determine the effects of VC on specific methanogenic activities in terms of MAUR and MPUR during the AD of food waste.

5.2 Material and Methods

Experimental material and methods are presented in this subsection.

5.2.1 Sampling and Experimental Design

During the sixth SRT of semi-continuous study (Chapter 4), digester effluents from the food waste reactor without any additive (Control), the food waste reactor with trace elements (FW+TE), the food waste reactor with humic acids (FW+HA), and the food waste reactor with vermicompost (FW+VC) were collected to represent the digester contents. The methanogenic activity test followed immediately and was repeated three times (on three different days) to ensure repeatability. The experimental design is shown in Table 5.1.

Table 5.1 Experimental design of the methanogenic activity test

Treatments	Sample (inoculum) source	MAUR test substrate	MPUR test substrate
Control	FW only reactors	7500 mg/L Acetate	3000 mg/L Propionate
FW+TE	FW+TE reactors		
FW+HA	FW+HA reactors		
FW+VC	FW+VC reactors		

FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost; MAUR= the maximum acetate utilization rate; MPUR= the maximum propionate utilization rate

5.2.2 Experimental Setup

The MAUR and MPUR tests were performed using the assays described by Speece (1988) with slight modifications. A 120 mL representative sample of the digestate was placed in a 225 mL liquid capacity serum bottle (same as those used in the BMP trial; Chapter 3, section 3.2.3). An additional 100 mL of DI water was added to bring the total volume to 220 mL and the bottles were covered tightly with septa caps. The DI water was added to minimize the volume of head space which improves accuracy as discussed in Chapter 2 section 2.1.5. The headspace was flushed with pure N_2 gas at a flow rate of approximately 0.5 L/min for 5 min to ensure anaerobic conditions. All bottles were placed on a shaker (100-150 rpms) and incubated at 35°C. After reaching temperature equilibration (about 2 hours), each bottle was injected with a small volume (5 mL) of sodium acetate (the MAUR test) or sodium propionate (the MPUR test) stock solution. The target concentration of acetate and propionate were 7500 mg/L and 3000 mg/L, respectively. These levels are sufficient to allow the methanogens to function at their maximum rate. A needle attached to the gas collection line was inserted through the caps of each bottle (Figure 5.1).

Figure 5.1 Methanogenic activity test experimental set-up

Biogas first flowed through the needle to a carbon dioxide adsorption unit (a bottle containing 220 mL sodium hydroxide solution with a blue color indicator) and then into the individual automatic gas measuring cells manufactured by Challenge Technologies (AER-208 - Research Respirometer Aerobic/Anaerobic, Challenge Technologies Inc., Springdale, AR) (Figure 5.1). Attachment of a carbon dioxide adsorption unit in the gas line allowed for the direct determination of the specific methane production. Methane production was continuously monitored for 24 hrs using computer software as described in Chapter4, section 4.2.

5.2.3 Data Processing

The 24-hr cumulative methane production was divided by the volume of effluent sample (120 mL) to normalize the data as volumes of methane per volume of effluent sample per day (L/L/day).

The experimental units of the study were 12 identical reactors. There was 1 independent variable (additive) and 4 levels including no additive (control), TE, HA, and VC. Each was run in triplicate. The dependent variable in this experiment was

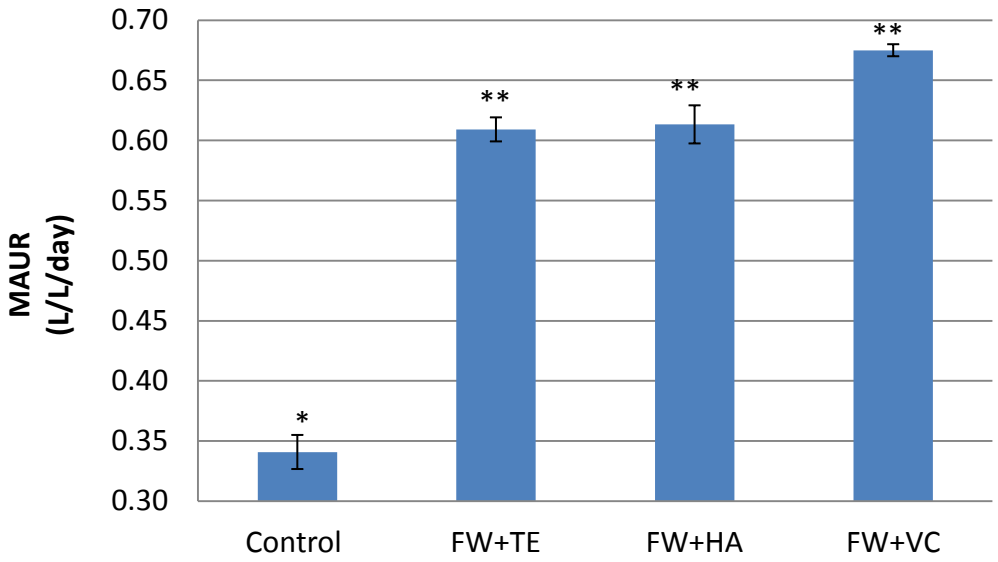
the MAUR and the MPUR. Statistical analysis was performed using SAS software version 9.1(SAS Institute Inc.). Significant differences among treatments were determined by one-way ANOVA with the Tukey-Kramer multiple-comparison test. Differences were considered significant at a *P* value of ≤ 0.05.

5.3 Results and Discussion

Experimental results and discussion are presented in this subsection.

5.3.1 Maximum Acetate Utilization Rate

The maximum acetate utilization rate is shown in Figure 5.2.



*Data with different superscript differed significantly, P<0.05;
FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost;
MAUR= the maximum acetate utilization rate; error bars represents SEM

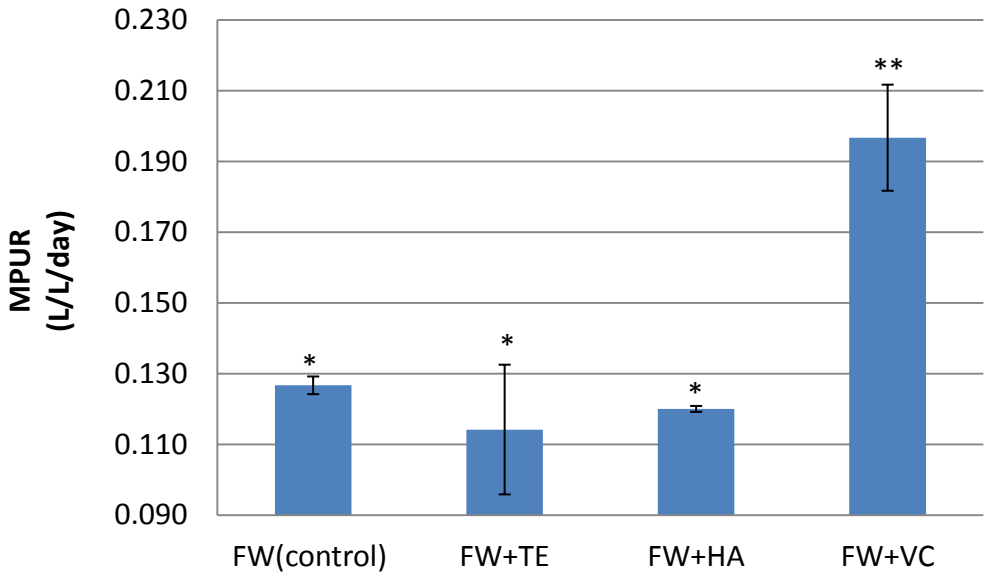
Figure 5.2 Maximum acetate utilization rates of food waste digesters with and without additives

The food waste reactor supplemented with vermicompost had the greatest maximum acetate utilization rate, 0.68 L/L/day, nearly double that of the control (0.34 L/L/day) (Figure 5.2). However, the difference among the reactors supplemented

with trace elements, humic acids, and VC were not significant. Results showed that food waste reactors with supplements all contained more soluble Fe than the control reactors (Chapter 4, Figure 4.8) which may be the cause of the observed MAUR improvement. Iron is essential for enzymes that catalyze metabolic reactions during methanogenesis (Zandvoort et al., 2006). Hoban and Berg (1979) indicated that addition of Fe to methanogenic cultures (obtained from AD treated food processing waste) significantly increased the conversion of acetate to methane.

5.3.2 Maximum Propionate Utilization Rate

The maximum propionate utilization rate is shown in Figure 5.3.



*Data with different superscript differed significantly, P<0.05
FW= food waste; TE= trace elements; HA= humic acids; VC= vermicompost; MPUR= the maximum propionate utilization rate; error bars represents SEM

Figure 5.3 Maximum propionate utilization rates of food waste reactors with and without additives

The food waste reactor supplemented with vermicompost had a much greater maximum propionate utilization rate (0.197 L/L/day) than the control and other

treatments (Figure 5.3). There was no difference among the control and the reactors supplemented with trace elements or humic acids (average 0.12 L/L/day). Consequently, Ni, Fe, Co, and humic acids do not appear to have an impact. However, some previously undetermined factors of VC significantly enhanced the MPUR of food waste digesters. This enhancement in corresponded to the increased overall biogas and methane production (reported in Chapter 4) from the food waste reactor supplemented with vermicompost compared to those from the reactors supplemented with trace elements and humic acids

Propionate is a key intermediate in the conversion of complex organic matter under methanogenic conditions (De Bok et al., 2004). Propionate oxidation can only proceed if the products, H_2 and formate are removed by methanogens or H_2 or formate utilizing bacteria (Stams, 1994). Mo, W, and Se are crucial components of essential enzymes catalyzing formate dehydrogenase (FDH) (Dong et al., 1994). In defined cultures of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* with propionate as the sole substrate, limitation of Mo and W lowered the methane production rate and the FDH activity (Jiang, 2006). Therefore, insufficient amounts of Mo and W could result in a slow rate of propionate utilization (Fernando et al., 2009). This could be the case for current findings. Unfortunately, Mo, W, and Se were not measured in this study but the literature shows that manure VC is abundant with these trace metals (Hervas et al., 1989).

Finally, the MAUR and MPUR were analyzed during the last SRT. Therefore, results only reflected the MAUR and MPUR of reactors operated during the sixth SRT. It is expected that the MAUR and MPUR of the food waste reactors supplemented with trace elements (FW+TE), humic acids (FW+HA), and vermicompost (FW+VC) were relatively consistent as evidenced by stable biogas

and methane production. In contrast, the MAUR and MPUR of the control reactor during the third and fourth SRT would have been likely lower than the values reported in this chapter, if they would have been measured, as the result of depressed methanogens activities.

5.4 Conclusions and Implication

Manure VC significantly improved methanogenic activities of FW digesters as evidenced by a nearly doubled MAUR and a 60% increase in MPUR compared to FW digesters with no supplements. Improvement in acetate and propionate utilization by VC is likely the cause of the overall enhancement in digestion performance described in earlier chapters.

CHAPTER 6 ASSESSMENT OF BIOAVAILABILITY AND STIMULATION EFFECTS OF NICKEL, IRON AND COBALT ON ANAEROBIC DIGESTION OF FOOD WASTE

The fourth stage of dissertation research is presented in this chapter including introduction; material and methods; results and discussion; and conclusions and implication sections.

6.1 Introduction

To further quantify the stimulatory effect of trace metals (Ni, Co, and Fe) (individually instead of as a mixture) on the methanogenesis of FW digestion, the bioavailability assay procedure described by Speece (1987) was used in this phase of the research.

6.2 Material and Methods

Experimental material and methods are presented in this subsection.

6.2.1 Experimental Design and Setup

The experimental design is shown in Table 6.1.

Table 6.1 Experimental design of trace metal bioavailability trial

Treatments	Trace metal concentrations	Inoculum source	Substrate
Control	0 mg/L	Food waste digesters (control digesters in chapter 4)	Acetate (7500 mg/L)
T1	Ni (0.01 mg/L)		
T2	Ni (1 mg/L)		
T3	Ni (10 mg/L)		
T4	Fe (0.5 mg/L)		
T5	Fe (5 mg/L)		
T6	Fe (100 mg/L)		
T7	Co (0.01 mg/L)		
T8	Co (1 mg/L)		
T9	Co (10 mg/L)		

Effluents from the semi-continuous FW reactors (control reactors) during the sixth SRT were used for sample evaluation in the current study. The experimental design consisted of one control and 9 treatments (Table 6.1) with each run in triplicate (total of 30 reactors).

Due to the limitation of equipment, the study was completed in series. For each trace metal, three dosages were evaluated; low, medium, and high. The low dosage was set equal to the concentrations used during the semi-continuous study. These concentrations were 0.01, 0.5, and 0.01 mg/L for Ni, Fe, and Co, respectively (Chapter 4, Table 4.2). The medium dosages of Ni, Fe, and Co were 1, 5, and 1 mg/L, respectively. The purpose of this dosage was to evaluate if further improvement is possible at an elevated concentration. The high dosages of Ni, Fe, and Co were 10, 100, and 10 mg/L, respectively. The objective of this group is to determine if these levels are toxic.

The experimental setup was similar to the MAUR and MPUR assays described in Chapter 5, Section 5.2. A 120 mL sample (inoculum) was placed in each 225 mL serum bottles, diluted with additional DI water, flushed with N_2 gas, and sealed for incubation at 35ºC. After initial temperature equilibration (2 hours), all reactors were then injected with 5 mL of a sodium acetate stock solution to bring the acetate concentrations to 7500 mg/L. Then additional trace metal solutions with the desired Ni, Fe, and Co concentrations (Table 6.1) were injected. The methane production was monitored using the same procedure as described in Chapter 5, Section 5.2.

6.2.2 Data Process and Interpretation

The experimental units were 30 identical batch-scale reactors. A completely randomized design was achieved by randomly assigning reactors to 10 treatments (3

replicate per treatment). However, the statistical analysis was conducted separately to study the effects of each individual metal on digestion of FW. For each metal, there was one independent variable with 4 levels (concentrations of metal at 0, low, medium, and high). The dependent variable was the daily methane production. Significant differences were determined by one-way ANOVA with the Tukey-Kramer multiple-comparison test. Differences were considered significant at a $P \leq 0.05$.

In addition, any treatment that produced more methane ($P < 0.05$) than the control was considered to stimulate methane production.

6.3 Results and Discussion

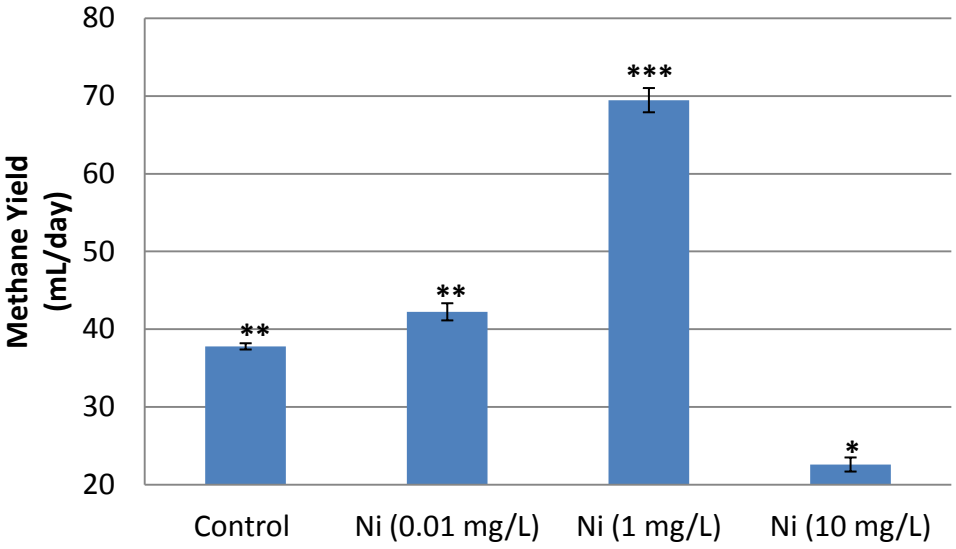
Experimental results and discussion are presented in this subsection.

6.3.1 Nickel Addition

As shown in Figure 6.1, there was no difference between the control and 0.01 mg/L Ni treatment, suggesting that the daily methane yield was not stimulated by Ni at this concentration. Therefore, the observed improvement in earlier studies (semi-continuous study and methanogenic activity assays) was not likely due to the existence of additional Ni at 0.01 mg/L.

However, nickel stimulated the methane production rate significantly at the concentration of 1 mg/L ($P < 0.05$). Nickel is essential for the methyl-coenzyme M reductase (Harmer et al., 2008) and carbon monoxide dehydrogenase (Friedman et al., 1990). This indicated that food waste as sole substrate for AD could be deficient in Ni and supplementation of additional Ni may result in greater methanogenic activity and consequently increase the methane production. However, inhibition

could occur at the concentration of 10 mg/L under the current experimental condition (Figure 6.1).

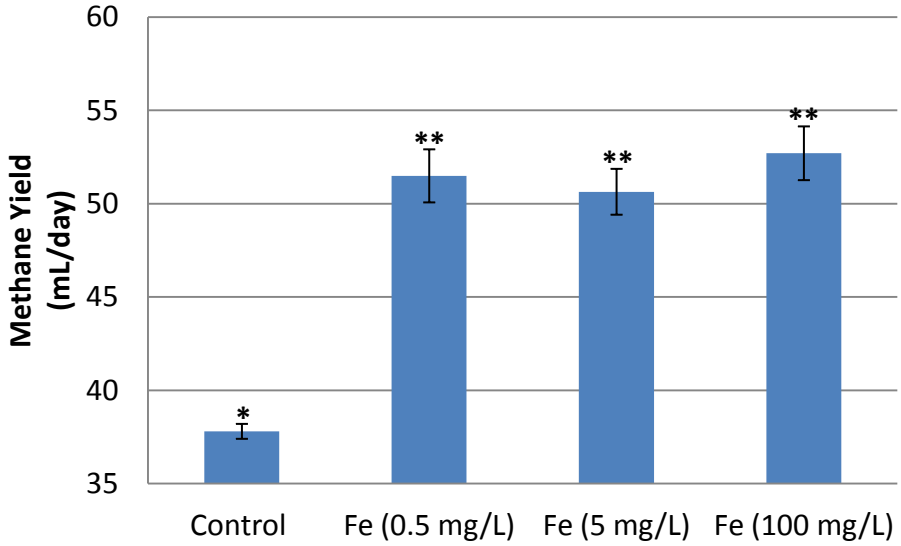


*Data with different superscript differ significantly, P < 0.05; error =SEM

Figure 6.1 Effects of nickel on daily methane yield from the food waste digester

6.3.2 Iron Addition

Effects of iron on daily methane yield are shown in Figure 6.2.



*Data with different superscript differ significantly, P < 0.05; error =SEM

Figure 6.2 Effects of iron on daily methane yield from the food waste digester

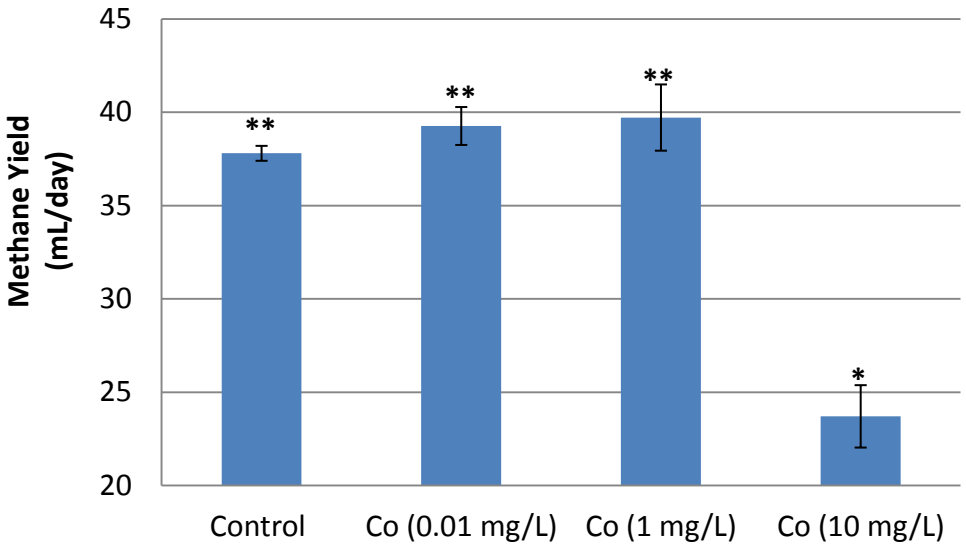
Iron supplementation at 0.5, 5 or 100 mg/L all significantly improved daily methane yields (Figure 6.2). Combined with the results found in the earlier studies, the FW (especially batches 2) used for current study was deficient in Fe which resulted in a slow rate of acetate conversion and suppressed methane production. This low acetate utilization rate resulted in the accumulation of acetate which could explain the higher concentration of VFA and pH drop observed in the semi-continuous study.

Iron is a critical element for carbon monoxide dehydrogenase complex (Friedman et al., 1990), an enzyme complex involved in the formation of acetate and methanol (Ferry, 1999; Bainotti and Nishio, 2000). Iron is also needed for F_{420}-reducing hydrogenase that catalyzes the reduction of CO_2 to CH_4 (Michel et al., 1995). Due to its essential role in metabolizing enzymes, it has been frequently reported that supplementation of Fe enhances AD (Hoban and van den, 1979; Ma et al., 2009; Oleszkiewicz, 1989; Sharma and Singh, 2001; Shen et al., 1993).

6.3.3 Cobalt Addition

The effect of cobalt on the acetate conversion rate in food waste digester is shown in Figure 6.3.

No significant improvements were observed at any concentrations of Co supplementation and methane production was inhibited at the concentration of 10 mg/L. Consequently, Co was not likely impacting AD performance.



*Data with different superscript differ significantly; error =SEM

Figure 6.3 Effects of cobalt on daily methane yield from the food waste digester

6.4 Conclusions

Additional nickel and iron could stimulate the AD of FW. In contrast, cobalt had no significant effects on the methane yield under current experimental conditions.

CHAPTER 7 GENERAL CONCLUSIONS

In summary, this research proved its central hypothesis that the supplementation of manure VC to a single-stage AD system using food waste as the substrate stimulated methane production and enhance process stability.

A brief summary of this research is shown in Figure 7.1.

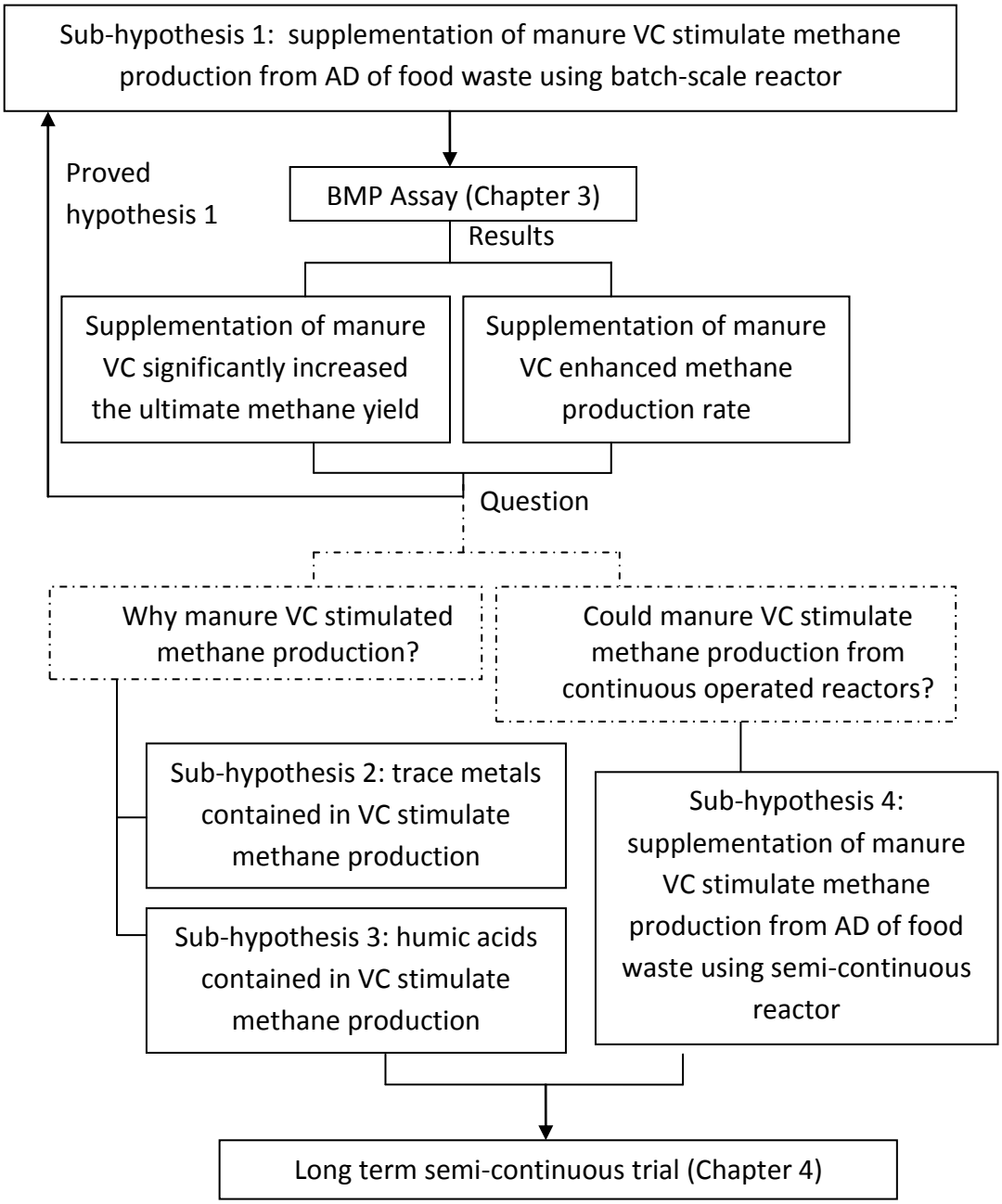
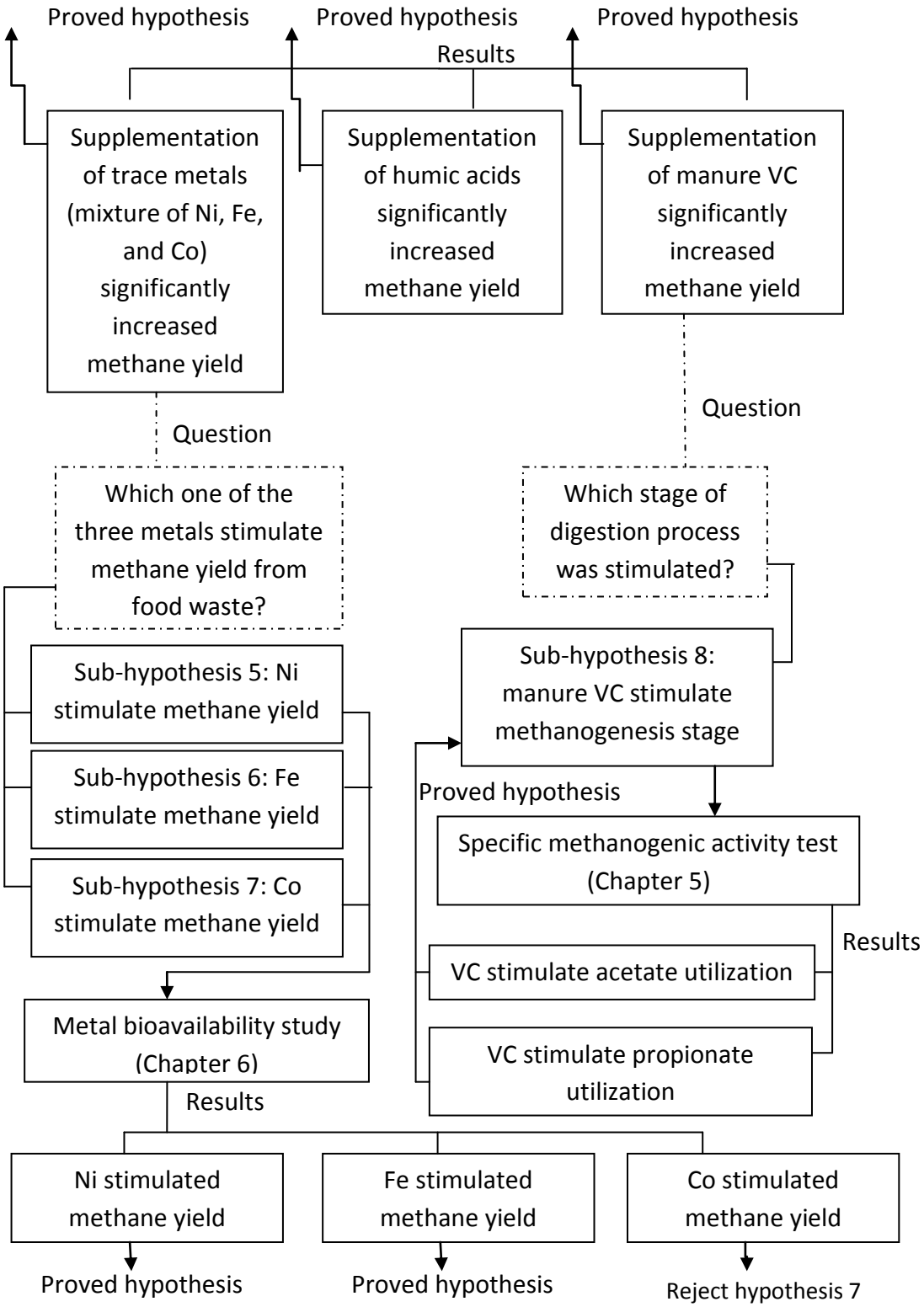


Figure 7.1 Summary of research

Figure 7.1 (cont'd)



This study demonstrated that the AD of cafeteria FW without supplementation experienced unstable and low methane production resulting from a dramatic pH drop

and the accumulation of VFAs. Supplementation of manure VC to the food waste digester was an effective strategy to improve digestion performance. In this research, supplementation of VC increased biogas and methane production from food waste by 53% and 70%, respectively, nearly doubled the acetate utilization rate, and enhanced the propionate utilization rate by 60%. Such enhancements were likely due to the trace metals (particularly iron and nickel) and humic acids naturally presented in manure VC. The likely mechanism is illustrated in Figure 7.2.

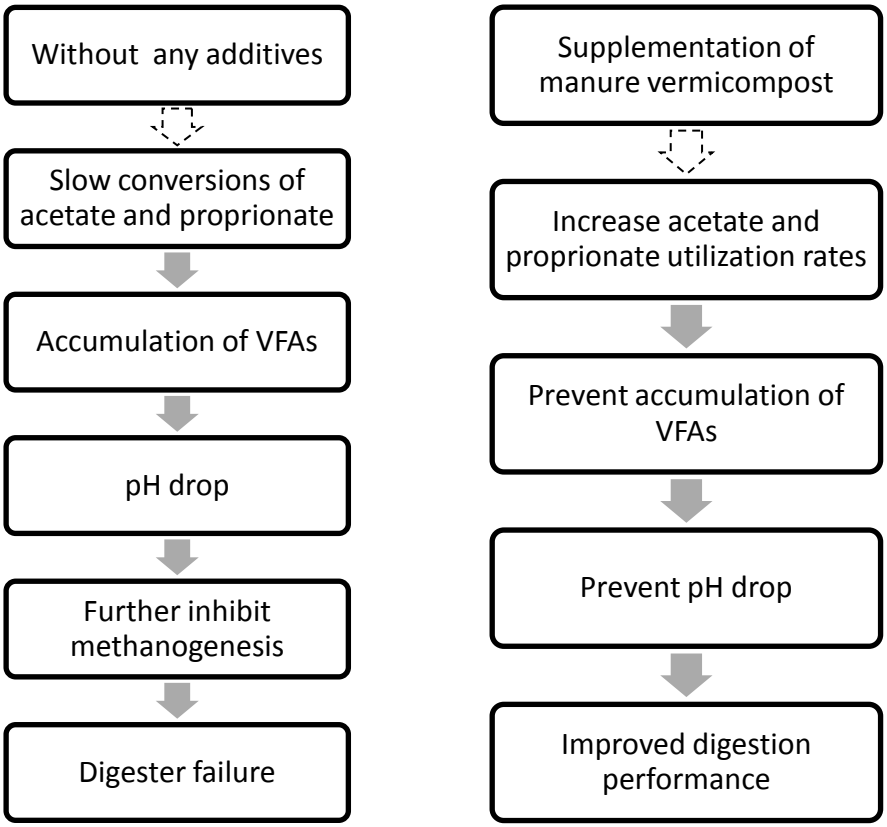


Figure 7.2 Mechanisms associated with enhanced digestion performance of the food waste digester supplemented with vermicompost

Many trace metals that are essential for AD of food waste, such as Se, Mo, W, and Mn, were not investigated in this study but are likely contained in VC. Banks et al. (2012) found that additional Se is critical for stabile digestion of high OLR food

waste (5 g VS/L/day) with elevated ammonia concentrations (5000 mg/L). Similarly, a trace element supplementation experiment conducted by Feng et al. (2010) using food industry waste showed that addition of Se and W increased methane yield as well as maintained low VFA concentrations.

Interestingly, the third batch of food waste (containing similar VS as the other batches but with greater trace metals) resulted in the slow recovery of methane production yet still a reduced level of methane production. This may further supports that a variety of bioavailable trace metals in imperative need for the AD of food waste and the benefits of supplementation with VC.

Although the metal bioavailability studies conducted in this research allowed for the efficient realization of the hypothesis, a more in-depth understanding of the impact of vermicompost on methanogenic activity and optimization of dosage is possible using microbial community analyses tools. A number of analyses targeting rRNA or protein-coding genes have been used for the purpose of studying the microbial communities' composition of anaerobic process (Feng et al., 2010). For example, Fermoso et al. (2008) used fluorescence *in situ* hybridization to quantify the abundance of key microorganisms in a mesophilic anaerobic reactor (fed with methanol) under cobalt limiting conditions. It was suggested by Talbot et al. (2008) that microbial community fingerprinting techniques using small subunit rRNA gene may be the most suitable molecular method for detecting changes in community composition or metabolic activities during AD process. Examples of such fingerprinting techniques include denaturing gradient gel electrophoresis, ribosomal RNA intergenic spacer analysis, and terminal restriction fragment length polymorphism.

Compared to commercial mineral nutrient products, which are primarily produced from nonrenewable resources, VC is a more eco-friendly additive. The main production requirements for the vermicomposting process are land, shelter, labors and mechanical energy to move materials on site. A future study should entail conducting a life cycle analyses to fully quantify both options.

Supplementation of VC in the food waste digester should also consider some possible disadvantages such as the additional volume requirements within the digester, skill to manage the vermicomposting system, and the difficulty in controlling the concentration of specific trace metals.

This study demonstrated that trace metals and humic acids are the major stimulatory factors contained in manure VC. However, from a system design standpoint, using food waste itself as the feedstock for the vermicomposting process is desirable as the addition of manure is not required. However, carbon is lost during vermicomposting process, reducing valuable energy output from the digester. Therefore, an alternative to consider is the use of digestate from a food waste digester as the substrate for the vermicomposting procss as nutrients and metals are still present. This allows for an integrated vermicomposting and AD system as shown in Figure 7.3.

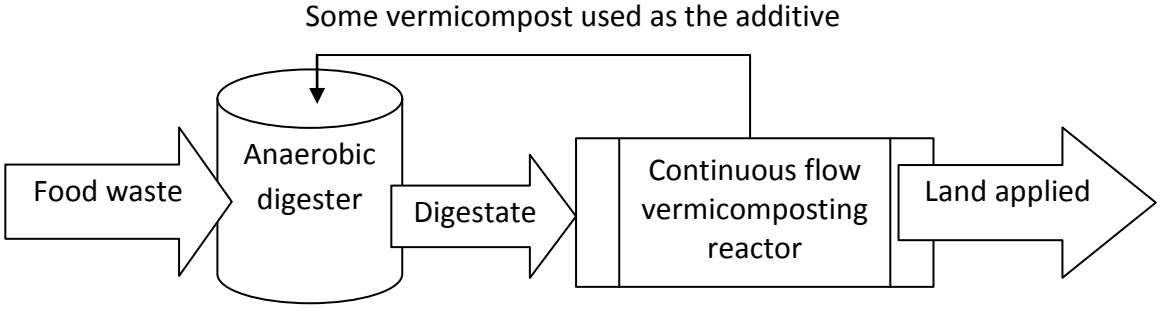


Figure 7.3 Integrated vermicomposting and anaerobic digestion system for food waste management

Food waste is fed to the anaerobic digester for energy production. The digestate is then added to the continuous flow vermicomposting reactor, in thin layers to the surface from mobile gantries at 1 to 2 day intervals, and the VC is collected mechanically at the bottom of the reactor. Portion of the VC is added back to the anaerobic digester for stimulation of digestion process. The remaining VC can be used as soil amendment. Such an integrated system should be tested as it allows for more stable and efficient digestion of food waste for energy recovery, produces higher value fertilizer, and produces worms that could be used for baits and fish food.

APPENDICES

APPENDIX A

Biochemical Methane Potential Assays Data Summary

Table A1.1 Characteristic of raw food waste

Parameter	Subsamples			AVG	STD
	1	2	3		
pH	6.5	6.6	6.6	6.6	0.1
TS (w.t. %)	22.6	21.2	23.8	22.5	1.3
VS (w.t.%)	21.1	19.8	21.9	20.9	1.0
VS/TS (%)	93.4	93.5	92.0	93.0	0.8
Total COD (g/kg)	246.0	260.5	254.3	253.6	7.3
TKN (g/kg)	7.7	7.2	8.1	7.7	0.5
TP (g/kg)	1.6	1.4	1.7	1.6	0.2
Ammonia-N (g/kg)	0.23	0.20	0.29	0.24	0.05
Alkalinity (g CaCO3/kg)	0.46	0.50	0.52	0.49	0.03

Table A1.2 Characteristic of dairy manure vermicomposts

Parameter	Subsamples			AVG	STD
	1	2	3		
pH	6.9	6.8	6.9	6.9	0.1
TS (w.t. %)	15.4	15.8	15.2	15.5	0.3
VS (w.t.%)	6.3	6.8	6.4	6.5	0.3

Table A1.3 pH change during the BMP assay

Treatments	Pre-digestion		Post-digestion	
	AVG	STD	AVG	STD
Control (C)	7.6	0.1	6.9	0.1
FWVC1	7.5	0.1	6.9	0.1
FWVC2	7.6	0.1	6.9	0.1
FWVC3	7.7	0.1	6.8	0.1
VC	7.9	0.1	6.7	0.1

Table A1.4 Ammonia-N change during the BMP assay (mg/kg)

Treatments	Pre-digestion		Post-digestion	
	AVG	STD	AVG	STD
Control (C)	111	2	186	2
FWVC1	112	3	201	6
FWVC2	111	4	204	4
FWVC3	114	2	195	4
VC	109	1	144	1

Table A1.5 COD change during digestion (g/kg)

Treatments	Pre-digestion		Post-digestion	
	AVG	SEM	AVG	SEM
Control (C)	16.0	0.1	11.9	0.1
FWVC1	16.2	0.3	12.0	0.1
FWVC2	16.4	0.2	11.6	0.2
FWVC3	16.9	0.2	11.8	0.1
VC	15.5	0.1	14.9	0.1
Blank	9.6	0.1	9.2	0.1

Table A1.6 Average weekly cumulative biogas yield (mL)

Day	Control		FWVC1		FWVC2		FWVC3		VC	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
5	89	4	88	5	102	5	100	2	3	1
10	170	4	174	5	216	8	186	4	11	1
15	221	6	229	6	264	7	224	6	13	1
20	250	6	259	6	280	8	233	6	15	2
25	267	5	272	7	294	9	238	7	16	2
30	278	4	280	9	294	10	239	8	17	2

Table A1.7 Average specific biogas production rate (mL/g FW VS added)

Day	Control		FWVC1		FWVC2		FWVC3		VC	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
5	199	9	201	12	256	14	333	7	6	2
10	378	9	395	12	541	20	621	15	24	2
15	491	13	521	14	659	18	745	18	28	3
20	556	13	589	14	700	19	778	18	33	4
25	594	11	617	16	735	22	793	22	36	5
30	618	10	637	19	734	25	798	26	38	5

Table A1.8 Average methane content (%)

Day	Control		FWVC1		FWVC2		FWVC3		VC	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
5	39.0	0.4	38.8	0.4	39.2	0.4	38.5	0.4	30.7	0.3
10	57.9	0.6	63.5	0.6	65.4	0.7	64.2	0.6	38.4	0.4
15	63.3	0.6	64.1	0.6	62.4	0.6	63.1	0.6	40.7	0.4
20	66.7	0.7	65.8	0.7	65.3	0.7	66.1	0.7	38.0	0.4
25	58.4	0.6	58.6	0.6	61.2	0.6	59.1	0.6	36.0	0.4
30	55.1	0.6	56.7	0.6	56.8	0.6	58.1	0.6	34.0	0.3
Overall Mean	60.3		61.7		62.2		62.1		37.4	

Table A1.9 Average cumulative methane yield (mL)

Day	Control		FWVC1		FWVC2		FWVC3		VC	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD	AVG	STD
5	50	4	52	5	54	5	60	2	1	1
10	106	4	123	5	137	8	121	4	4	1
15	122	6	140	6	161	7	134	6	5	1
20	145	6	160	6	170	8	141	6	5	2
25	149	5	166	7	176	9	148	7	6	2
30	155	4	171	9	182	10	154	8	6	2

Table A1.10 Average specific methane production rate (mL/g FW VS added)

Day	Control		FWVC1		FWVC2		FWVC3	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
5	113	9	116	12	145	14	194	5
10	240	9	267	12	326	20	381	10
15	289	13	315	14	384	18	435	12
20	328	13	347	14	405	19	451	12
25	337	11	357	16	418	22	462	15
30	351	10	369	19	429	25	475	29

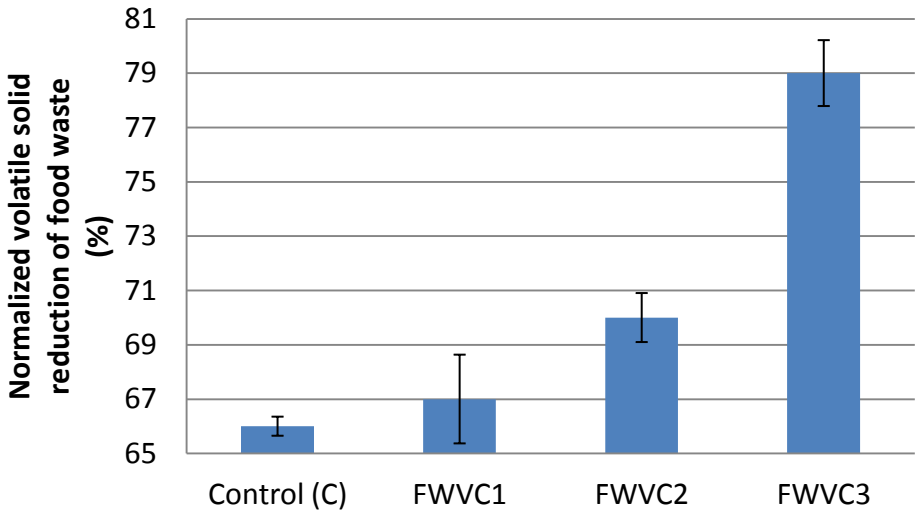


Figure 8.1 Normalized volatile solid (VS) destruction of food waste after 30 days of AD*

* The normalized VS destruction of food waste was calculated by subtracting volatile solid destruction of vermicompost and inoculum); total VS destructions of control and treatments were shown in Table 3.5, column 5; The VS destruction of inoculum was 0.005 g; The VS destruction of vermicompost = 0.02 g-0.005 g = 0.015 g (with a 0.45 g initial VS). The calculated vermicompost destruction rate (%) =0.015 g/0.45 g = 3.3%; for each treatment, vermicompost destruction = initial VS of supplemented vermicompost (g) ×vermicompost destruction rate (%). The calculation is shown in the following table

Table A1.11 Normalized volatile solid reduction of food waste

Item	Average total VS destroyed (g)	Vermicompost and inoculum VS destruction (g)	Normalized destroyed VS of food waste (g)	Initial VS of food waste (g)	Normalized VS destruction of food waste (%)
Control	0.303	0.005	0.298	0.45	66
FWVC1	0.299	0.005	0.294	0.44	67
FWVC2	0.284	0.007	0.277	0.4	70
FWVC3	0.246	0.01	0.236	0.3	79

APPENDIX B

Semi-continuous Study Data Summary

Table A2.1 Characteristics of raw food waste

Items	Batch			AVG	STD
	1	2	3		
pH	6.6	6.2	6.8	6.5	0.2
TS (w.t. %)	23.4	22	25	23.5	1.2
VS (w.t.%)	21.8	20.4	23.6	21.9	1.3
VS/TS (%)	93.2	92.7	94.4	93.4	0.7
TKN (g/kg)	7.7	6.4	8.1	7.4	0.7
TP (g/kg)	1.6	1.1	1.3	1.3	0.2
Ammonia-N (g/kg)	0.24	0.16	0.11	0.2	0.1
Alkalinity (g CaCO3/kg)	0.49	0.31	0.52	0.4	0.1

Table A2.2 Characteristics of dairy manure vermicompost

Items	Samples			AVG	STD
	1	2	3		
pH	6.9	6.8	6.9	6.9	0.1
TS (w.t. %)	35.7	35.0	35.6	35.4	0.4
VS (w.t.%)	15.0	15.0	14.9	15.0	0.1
Total COD (g/kg)	-	-	-	-	0.000
TKN (g/kg)	7.9	-	-	7.9	-
TP (g/kg)	0.47	-	-	0.5	-
Ammonia-N (g/kg)	0.03	0.028	0.038	0.032	0.005
Alkalinity (g CaCO3/kg)	3.6	3.2	2.8	3.2	0.4

- Data is not available

Table A2.3 Average influent pH of all reactors

SRT	FW only	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
1	6.5	6.5	6.5	6.5	6.5	6.5
2	6.6	6.6	6.6	6.6	6.6	6.6
3	6.6	6.7	6.6	6.6	6.6	6.7
4	6.6	6.6	6.6	6.6	6.6	6.6
5	6.6	6.6	6.5	6.5	6.5	6.5
6	6.6	6.6	6.7	6.7	6.6	6.6
AVG	6.6	6.6	6.6	6.6	6.6	6.6
STD	0.1	0.1	0.1	0.1	0.1	0.1

Table A2.4 Average effluent pH of all reactors

SRT	Days	FW Only	FW+ TE	FW+ HA	FW+ VC	FW+ TE+HA	FW+ TE+VC
1	2	7.6	7.6	7.6	7.6	7.7	7.6
	4	7.5	7.5	7.5	7.6	7.7	7.6
	6	7.5	7.5	7.5	7.5	7.5	7.5
	8	7.2	7.2	7.2	7.5	7.5	7.5
	10	7.3	7.2	7.3	7.2	7.2	7.2
	12	7.3	7.2	7.2	7.2	7.3	7.2
	14	7.3	7.3	7.2	7.2	7.2	7.2
2	16	7.2	7.3	7.2	7.2	7.3	7.2
	18	7.3	7.3	7.2	7.3	7.2	7.2
	20	7.3	7.2	7.2	7.2	7.2	7.2
	22	7.2	7.2	7.3	7.2	7.2	7.2
	24	7.2	7.3	7.2	7.3	7.3	7.2
	26	7.2	7.3	7.2	7.2	7.3	7.2
	28	7.2	7.2	7.3	7.2	7.2	7.2
	30	7.2	7.2	7.2	7.2	7.3	7.2
3	32	6.8	7.3	7.2	7.3	7.2	7.2
	34	6.5	7.2	7.2	7.3	7.2	7.2
	36	6.3	7.1	7.2	7.2	7.2	7.2
	38	6.2	7.3	7.3	7.1	7.1	7.1
	40	6.3	7.2	7.3	7.3	7.2	7.3
	42	6.4	7.2	7.3	7.1	7.3	7.2
	44	6.6	7.3	7.3	7.3	7.3	7.3
4	46	6.1	7.3	7.3	7.4	7.3	7.3
	48	5.8	7.2	7.3	7.3	7.2	7.2
	50	5.9	7.2	7.2	7.2	7.2	7.2
	52	5.8	7.2	7.2	7.2	7.2	7.2
	54	5.8	7.3	7.2	7.2	7.2	7.2
	56	5.7	7.3	7.3	7.2	7.3	7.3
	58	5.7	7.3	7.2	7.2	7.2	7.3
	60	5.8	7.3	7.3	7.2	7.3	7.3
5	62	6.0	7.3	7.2	7.2	7.3	7.3
	64	6.4	7.3	7.3	7.3	7.3	7.2
	66	6.5	7.3	7.3	7.2	7.3	7.2
	68	6.6	7.3	7.3	7.3	7.3	7.3
	70	6.7	7.3	7.2	7.2	7.3	7.3
	72	6.7	7.3	7.2	7.2	7.2	7.2
	74	6.7	7.3	7.2	7.3	7.2	7.2
6	76	6.8	7.3	7.2	7.3	7.3	7.3
	78	6.8	7.3	7.2	7.3	7.3	7.2
	80	6.8	7.3	7.2	7.2	7.2	7.3
	82	6.7	7.3	7.2	7.3	7.2	7.2
	84	6.7	7.3	7.3	7.2	7.3	7.3

Table A2.4 (cont'd)

6	86	6.8	7.2	7.3	7.2	7.3	7.3
	88	6.7	7.3	7.3	7.2	7.2	7.2
	90	6.8	7.2	7.3	7.3	7.3	7.3
Steady-state AVG		6.4	7.3	7.2	7.2	7.3	7.2
Steady-state STD.		0.4	0.1	0.1	0.1	0.2	0.01
Overall AVG		6.7	7.3	7.3	7.3	7.3	7.3
Overall STD		0.5	0.1	0.1	0.1	0.2	0.1

Table A2.5 Average influent alkalinity of all reactors (mg/L as $CaCO_3$)

SRT	FW only	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
1	208	209	211	221	208	221
2	205	206	204	211	207	213
3	209	207	205	216	208	214
4	205	204	207	214	206	217
5	187	189	186	201	187	204
6	186	184	185	204	187	206
AVG	200	200	200	211	201	213
STD	10	10	10	7	10	6

Table A2.6 Average effluent alkalinity of all reactors (mg/L as $CaCO_3$)

SRT	FW only	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
1	1,487	1,521	1,495	1,758	1,513	1,698
	1,425	1,534	1,492	1,737	1,547	1,742
2	1,253	1,472	1,459	1,690	1,456	1,703
	1,105	1,511	1,472	1,682	1,448	1,721
3	1,116	1,514	1,478	1,691	1,452	1,723
	1,009	1,414	1,287	1,708	1,427	1,690
4	863	1,440	1,264	1,703	1,448	1,706
	823	1,432	1,271	1,712	1,436	1,711
5	721	1,402	1,314	1,723	1,418	1,726
	775	1,383	1,375	1,745	1,404	1,713
6	1,123	1,375	1,383	1,755	1,404	1,729
	1,113	1,391	1,388	1,698	1,427	1,719
Steady-state AVG	943	1,419	1,345	1,717	1,427	1,715
Steady-state STD.	156	42	69	21	17	12
Overall AVG	1,068	1,449	1,390	1,717	1,448	1,715
Overall STD	245	59	89	26	43	15

Table A2.7 Average influent total solid content of all reactors (g/L)

SRT	Days	FW	FW+TE	FW+HA	FW+VC	FW+TE+HA	FW+TE+VC
1	3	11.0	11.3	12.8	11.7	10.9	12.4
2	17	10.7	11.2	11.7	11.6	11.5	11.1
3	31	10.9	11.8	10.6	12.8	10.9	10.3
3	38	10.7	11.1	11.0	11.4	10.5	10.8
4	46	10.8	11.5	12.3	12.3	10.9	11.5
4	53	10.5	10.8	12.4	11.1	10.9	11.5
5	61	10.8	11.3	11.5	11.9	10.9	11.3
5	68	10.5	10.8	11.9	11.1	10.9	12.1
6	76	10.7	11.0	11.8	11.2	11.7	11.9
6	83	10.1	10.8	12.1	11.6	11.3	11.5

Table A2.8 Average influent volatile solid content of all reactors (g/L)

SRT	Days	FW	FW+TE	FW+HA	FW+VC	FW+TE+HA	FW+TE+VC
1	3	9.3	10.1	8.9	9.4	8.9	10.4
2	17	9.1	9.3	9.2	9.7	9.2	9.5
3	31	9.3	9.1	8.9	9.2	8.9	9.8
3	38	9.1	9.0	9.0	9.4	9.0	9.7
4	46	9.3	9.8	9.2	9.7	9.2	10.0
4	53	9.0	9.4	8.9	9.3	8.9	9.2
5	61	9.3	9.6	9.2	10.0	9.2	9.5
5	68	9.0	9.3	8.9	9.1	8.9	9.8
6	76	9.0	9.5	9.2	9.8	9.2	10.0
6	83	8.9	9.3	9.3	9.6	9.3	9.5

Table A2.9 Average effluent total solid content of all reactors (g/L)

Days	FW	FW+TE	FW+HA	FW+VC	FW+TE+HA	FW+TE+VC
3	15.3	15.7	15.5	16.2	15.8	16.3
7	13.3	13.2	14.3	13.7	13.6	14.1
10	9.2	9.6	10.2	10.4	10.0	10.7
14	9.2	9.3	10.1	9.6	9.5	9.8
17	7.3	7.2	8.2	7.7	8.7	9.4
21	6.4	6.8	7.8	7.0	8.0	8.3
24	5.6	6.6	7.6	6.9	7.6	8.0
28	4.9	5.6	7.4	5.8	6.5	6.7
31	4.6	4.6	6.0	4.7	5.9	6.0
35	4.9	4.5	4.9	4.8	5.0	5.4
38	4.8	4.5	5.0	4.8	5.0	5.4
42	4.7	4.4	5.1	4.5	4.6	4.7
46	5.4	4.6	5.2	4.5	4.7	4.6
50	6.3	4.5	5.3	4.4	4.4	4.4
53	5.1	4.5	5.6	4.7	5.6	5.9
57	6.8	4.7	6.1	5.0	4.9	5.1
61	6.7	4.4	4.9	4.5	4.8	5.0
65	6.6	4.4	5.1	4.6	4.7	4.9
68	6.6	4.2	4.7	4.4	4.9	5.1
72	5.7	4.3	4.3	4.5	4.6	4.8
76	5.4	4.6	5.1	5.0	5.1	5.5
80	5.6	4.7	5.4	4.8	4.9	5.0
83	6.1	4.4	4.9	4.7	5.1	5.5
87	6.8	4.5	4.5	4.7	4.8	5.0

Table A2.10 Average effluent volatile solid content of all reactors (g/L)

Days	FW	FW+TE	FW+HA	FW+VC	FW+TE+HA	FW+TE+VC
3	10.0	10.1	10.3	10.4	10.1	10.4
7	9.7	10.0	10.0	10.4	9.7	10.1
10	8.2	8.2	8.8	8.9	8.6	9.3
14	8.3	8.1	9.0	8.3	9.0	9.2
17	5.9	5.8	6.4	6.2	6.6	7.0
21	5.6	5.8	5.9	6.0	6.2	6.4
24	4.5	4.0	4.9	4.2	5.0	5.2
28	4.4	4.3	4.9	4.4	4.9	5.1
31	3.2	3.8	2.4	3.9	3.6	3.6
35	3.3	3.2	2.6	3.4	3.5	3.8
38	3.5	2.5	3.0	2.7	2.8	3.1
42	3.5	2.7	3.6	2.8	2.9	3.0
46	4.7	2.9	3.2	2.8	3.0	2.9
50	4.9	2.6	3.7	2.6	3.1	3.1
53	4.6	2.6	3.4	2.8	2.6	2.8
57	4.3	2.5	3.5	2.7	2.6	2.7
61	5.1	2.8	2.6	2.9	2.6	2.7
65	5.1	2.5	2.6	2.6	2.6	2.7
68	4.8	2.6	2.6	2.8	2.7	2.8
72	3.9	2.7	2.7	2.8	2.5	2.6
76	3.6	2.9	2.8	3.1	2.4	2.6
80	3.8	2.7	2.9	2.7	2.6	2.7
83	4.3	2.7	2.5	2.9	2.5	2.6
87	4.1	2.6	2.7	2.7	2.6	2.7

Table A2.11 Total volatile solid reduction (%)

SRT	Days	FW	FW+TE	FW+HA	FW+VC	FW+TE+HA	FW+TE+VC
	31	66	58	73	58	60	63
	35	65	65	71	63	60	61
	38	62	72	67	71	68	68
3	42	62	70	60	71	68	70
	46	49	70	65	71	68	71
	50	48	73	59	73	67	69
	53	49	72	62	70	70	70
4	57	52	73	61	71	71	70
	61	46	71	72	71	72	72
	65	46	74	71	74	72	71
	68	46	72	71	70	70	71
5	72	56	71	70	69	72	74
	76	60	69	70	68	74	74
	80	58	72	68	72	72	73
	83	51	71	73	70	73	72
6	87	54	72	71	72	72	72
AVG		54	70	68	70	69	70
SEM		5	2	3	3	3	2

Table A2.12 Average influent COD of all reactors (g/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
1	13.3	13.4	13.2	18.3	13.3	18.1
2	13.3	13.1	13.0	17.8	14.6	19.5
3	13.1	13.2	13.1	18.1	13.1	17.9
4	13.3	13.0	12.8	17.6	14.4	19.3
5	13.5	13.6	13.5	18.7	13.5	18.4
6	13.7	13.4	13.2	18.2	14.9	19.9
AVG	13.4	13.3	13.1	18.1	14.0	18.9
STD	0.2	0.2	0.2	0.4	0.7	0.8

Table A2.13 Average effluent COD of all reactors (g/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
1	5.8	5.1	5.4	8.7	5.4	8.0
	5.9	5.1	5.5	8.7	5.4	8.0
2	4.7	3.8	4.5	7.0	4.6	7.6
	5.0	3.8	4.3	7.1	4.5	7.6
3	4.9	4.1	4.1	7.4	4.3	7.7
	4.6	4.2	4.4	7.4	4.2	7.9
4	4.8	4.0	4.2	6.8	4.2	6.3
	4.7	4.1	4.4	7.0	4.3	6.5
5	4.6	3.6	4.3	6.8	4.4	7.4
	4.8	3.8	4.2	6.9	4.4	7.4
6	4.7	4.0	4.0	7.3	4.2	7.5
	4.9	4.3	4.6	7.7	4.3	8.2
Steady-state AVG	4.7	4.0	4.3	7.1	4.3	7.3
Steady-state STD.	0.1	0.2	0.2	0.3	0.1	0.6
Overall AVG	4.6	4.2	4.5	7.4	4.5	7.5
Overall STD	1.3	0.5	0.5	0.7	0.4	0.6

Table A2.14 Average effluent ammonia-N of all reactors (mg/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	213	232	224	223	245	242
4	226	216	236	212	236	230
5	215	224	205	245	226	238
6	236	204	215	229	205	212
AVG	223	219	220	227	228	231
STD	9	10	11	12	15	12

Table A2.15 Average influent TKN of all reactors (mg/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	612	610	614	756	618	750
4	615	612	621	740	620	743
5	624	623	600	735	603	740
6	625	636	612	770	623	732
AVG	619	620	612	750	616	741
STD	6	10	8	14	8	6

Table A2.16 Average effluent TKN of all reactors (mg/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	590	535	550	600	560	595
	587	565	575	620	585	610
4	578	540	555	635	570	630
	574	560	545	625	560	655
5	580	550	575	620	540	625
	596	540	585	615	555	645
6	583	570	565	635	540	630
	592	565	585	640	580	650
AVG	585	553	567	624	561	630
STD.	7	13	15	12	16	19

Table A2.17 Average Influent total phosphorus of all reactors (mg/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	25	26	32	97	30	96
4	27	27	34	96	33	97
5	29	28	30	92	30	92
6	23	23	35	99	35	98
AVG	26	26	33	96	32	96
STD	2	2	2	3	2	2

Table A2.18 Average effluent total phosphorus of all reactors (mg/L)

SRT	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	30	26	28	54	29	57
	29	28	30	51	28	53
4	27	25	31	74	39	64
	25	24	32	66	41	64
5	31	24	34	62	31	70
	25	24	34	70	32	67
6	28	24	33	55	34	58
	24	25	36	59	37	61
AVG	27	25	32	61	34	62
STD.	2	1	2	8	4	5

Table A2.19 Average volatile fatty acids of all reactors (mg/L)

Days	FW	FW + TE	FW + HA	FW + VC	FW + TE + HA	FW + TE + VC
3	80	80	70	90	90	90
10	90	90	80	80	100	80
17	100	110	120	80	110	110
24	250	140	130	90	140	120
31	300	140	150	110	190	110
38	450	160	120	80	140	90
46	680	140	160	70	160	80
53	1,920	150	170	90	170	100
61	2,600	180	190	100	130	80
68	2,000	170	130	130	140	90
76	1,800	160	150	120	160	100
83	1,600	160	140	100	130	120
Steady-state AVG	1,419	158	151	100	153	96
Steady-state STD.	783	13	21	19	20	13

Table A2.20 Soluble Ni concentrations of food waste digesters with different additives

Days	FW	FW+TE	FW+HA	FW+VC
69	0.013	0.019	0.012	0.018
70	0.010	0.018	0.015	0.020
71	0.016	0.020	0.016	0.019
72	0.017	0.022	0.013	0.017
73	0.014	0.015	0.019	0.021
74	0.015	0.018	0.012	0.015
AVG	0.014	0.019	0.015	0.018
STD	0.002	0.002	0.003	0.002

Table A2.21 Soluble Co concentrations of food waste digesters with different additives

Days	FW	FW+TE	FW+HA	FW+VC
69	0.030	0.039	0.032	0.042
70	0.034	0.038	0.032	0.041
71	0.038	0.039	0.035	0.039
72	0.036	0.042	0.038	0.036
73	0.031	0.040	0.034	0.038
74	0.030	0.042	0.031	0.044
AVG	0.033	0.040	0.034	0.040
STD	0.003	0.002	0.002	0.003

Table A2.22 Soluble Fe concentration of food waste digesters with different additives

Days	FW	FW+TE	FW+HA	FW+VC
69	0.17	0.45	0.57	2.34
70	0.15	0.42	0.61	2.37
71	0.10	0.47	0.54	2.31
72	0.19	0.40	0.62	2.28
73	0.09	0.44	0.54	2.26
74	0.16	0.39	0.53	2.32
AVG	0.14	0.43	0.57	2.31
STD	0.04	0.03	0.04	0.04

Table A2.23 Comparison of soluble metals concentration of food waste digesters with different additives

Trace Metals	FW	FW+TE	FW+HA	FW+VC
Ni (mg/L)	0.014^{a}±0.002	0.019^{b}±0.002	0.015^{a}±0.003	0.018^{b}±0.002
Fe (mg/L)	0.14^{a}±0.04	0.43^{b}±0.03	0.57^{c}±0.04	2.31^{d}±0.04
Co (mg/L)	0.033^{a}±0.003	0.040^{b}±0.002	0.034^{a}±0.002	0.040^{b}±0.003

Table A2.24 Daily biogas productions from food waste reactors

Days	FW reactors (mL/day)			
	Duplicate 1	Duplicate 2	AVG	STD
1	46	51	49	3
2	71	90	80	10
3	93	93	93	0
4	131	112	122	10
5	163	199	181	18
6	201	184	193	9
7	240	203	221	19
8	197	191	194	3
9	186	206	196	10
10	253	262	257	5
11	273	313	293	20
12	341	375	358	17
13	433	473	453	20
14	386	423	405	19
15	432	451	441	9
16	383	410	396	13
17	435	415	425	10
18	425	391	408	17
19	419	432	426	7
20	397	378	388	10
21	279	265	272	7
22	335	369	352	17
23	382	462	422	40
24	401	477	439	38
25	378	469	424	45
26	411	481	446	35
27	398	465	432	34
28	461	485	473	12
29	447	439	443	4
30	373	364	368	5
31	353	349	351	2
32	349	354	352	2
33	357	347	352	5
34	339	355	347	8
35	344	321	333	11
36	360	369	364	4
37	365	368	367	1
38	366	335	350	16
39	343	335	339	4
40	319	294	307	13
41	283	283	283	0
42	247	256	252	5

Table A2.24 (cont'd)

43	279	289	284	5
44	291	205	248	43
45	292	266	279	13
46	248	238	243	5
47	229	204	217	12
48	191	181	186	5
49	222	204	213	9
50	191	210	200	9
51	176	166	171	5
52	155	157	156	1
53	157	165	161	4
54	185	148	167	18
55	208	149	178	30
56	214	164	189	25
57	225	182	203	22
58	210	200	205	5
59	276	198	237	39
60	227	221	224	3
61	258	216	237	21
62	203	236	219	16
63	280	244	262	18
64	321	300	310	10
65	359	356	358	2
66	327	344	335	8
67	315	349	332	17
68	265	254	259	5
69	318	338	328	10
70	330	263	297	33
71	343	308	325	18
72	281	271	276	5
73	294	252	273	21
74	306	244	275	31
75	262	220	241	21
76	292	299	296	4
77	294	281	287	7
78	301	299	300	1
79	279	293	286	7
80	286	.	286	.
81	272	.	272	.
82	279	.	279	.
83	291	.	291	.
84	272	.	272	.
85	286	.	286	.
86	295	.	295	.

Table A2.24 (cont'd)

87	292	.	292	.
88	289	.	289	.
89	272	.	272	.
90	276	.	276	.

. Data is not available

Table A2.25 Daily biogas productions from trace elements supplemented reactors

Days	FW+TE rectors			
	Duplicate 1	Duplicate 2	AVG	STD
1	77	72	74	3
2	97	103	100	3
3	126	125	126	0
4	168	173	171	2
5	215	211	213	2
6	249	239	244	5
7	304	285	294	10
8	267	239	253	14
9	251	232	241	10
10	345	331	338	7
11	393	390	392	1
12	450	451	450	0
13	467	487	477	10
14	339	347	343	4
15	406	412	409	3
16	378	328	353	25
17	405	397	401	4
18	312	332	322	10
19	563	535	549	14
20	503	491	497	6
21	395	392	393	1
22	476	482	479	3
23	461	496	479	18
24	393	371	382	11
25	323	309	316	7
26	297	305	301	4
27	405	406	406	1
28	444	438	441	3
29	381	374	377	3
30	375	380	377	3
31	376	398	387	11
32	370	359	365	5
33	379	371	375	4

Table A2.25 (cont'd)

34	406	398	402	4
35	404	410	407	3
36	400	412	406	6
37	377	379	378	1
38	377	378	377	1
39	399	374	386	12
40	365	371	368	3
41	387	379	383	4
42	396	394	395	1
43	392	374	383	9
44	369	358	363	5
45	377	358	367	10
46	371	392	381	11
47	356	391	374	17
48	377	396	387	9
49	395	422	409	13
50	424	393	409	16
51	427	409	418	9
52	396	378	387	9
53	373	362	368	5
54	401	389	395	6
55	423	419	421	2
56	410	423	417	7
57	391	384	388	3
58	384	395	390	6
59	391	392	391	1
60	418	405	412	7
61	409	401	405	4
62	358	376	367	9
63	389	409	399	10
64	406	404	405	1
65	368	376	372	4
66	371	415	393	22
67	359	372	365	7
68	386	392	389	3
69	383	371	377	6
70	391	391	391	0
71	404	407	406	2
72	395	408	402	7
73	352	385	368	16
74	374	358	366	8
75	393	383	388	5
76	351	359	355	4
77	348	354	351	3

Table A2.25 (cont'd)

78	386	383	384	1
79	386	396	391	5
80	406	.	406	.
81	386	.	386	.
82	366	.	366	.
83	409	.	409	.
84	391	.	391	.
85	393	.	393	.
86	402	.	402	.
87	392	.	392	.
88	398	.	398	.
89	396	.	396	.
90	385	.	385	.

. Data is not available

Table A2.26 Daily biogas productions from humic acids supplemented reactors

Days	FW+HA			
	Duplicate 1	Duplicate 2	AVG	STD
1	49	46	48	1
2	74	73	74	0
3	94	93	94	0
4	144	137	141	4
5	157	141	149	8
6	240	177	208	32
7	291	223	257	34
8	242	189	216	26
9	200	221	211	10
10	276	282	279	3
11	327	318	322	5
12	417	372	395	22
13	519	441	480	39
14	443	392	417	26
15	441	455	448	7
16	357	426	391	35
17	394	429	412	17
18	349	386	368	18
19	372	423	398	26
20	390	394	392	2
21	292	270	281	11
22	402	355	379	23
23	447	390	419	28
24	470	398	434	36

Table A2.26 (cont'd)

25	459	405	432	27
26	490	420	455	35
27	439	425	432	7
28	440	464	452	12
29	406	466	436	30
30	393	390	392	2
31	395	380	388	8
32	387	399	393	6
33	408	411	410	1
34	357	360	358	2
35	415	398	407	9
36	407	406	406	1
37	403	408	405	3
38	402	391	397	6
39	402	386	394	8
40	396	362	379	17
41	369	408	389	19
42	378	383	380	3
43	394	414	404	10
44	365	397	381	16
45	396	393	395	2
46	403	379	391	12
47	372	359	365	7
48	376	358	367	9
49	367	381	374	7
50	358	356	357	1
51	386	392	389	3
52	367	342	355	13
53	378	374	376	2
54	367	363	365	2
55	390	368	379	11
56	378	353	365	13
57	388	395	391	4
58	377	399	388	11
59	376	366	371	5
60	387	367	377	10
61	392	387	389	3
62	384	380	382	2
63	372	376	374	2
64	394	364	379	15
65	408	399	403	4
66	424	426	425	1
67	360	325	342	17

Table A2.26 (cont'd)

68	388	367	377	11
69	372	369	370	2
70	422	425	423	2
71	411	440	426	15
72	417	450	434	16
73	390	387	388	1
74	375	398	386	11
75	417	391	404	13
76	391	405	398	7
77	370	391	381	10
78	394	406	400	6
79	392	402	397	5
80	388	.	388	.
81	366	.	366	.
82	372	.	372	.
83	390	.	390	.
84	371	.	371	.
85	398	.	398	.
86	407	.	407	.
87	405	.	405	.
88	413	.	413	.
89	389	.	389	.
90	397	.	397	.

. Data is not available

Table A2.27 Daily biogas productions from vermicompost supplemented reactors

Days	FW+VC			
	Duplicate 1	Duplicate 2	AVG	STD
1	63	50	56	6
2	73	75	74	1
3	67	91	79	12
4	119	131	125	6
5	159	167	163	4
6	203	211	207	4
7	267	242	254	13
8	226	217	221	4
9	224	193	209	16
10	274	260	267	7
11	286	326	306	20
12	422	393	407	14
13	489	507	498	9
14	435	480	457	22

Table A2.27 (cont'd)

15	446	483	464	19
16	423	426	424	1
17	439	430	434	4
18	436	343	389	47
19	428	421	424	4
20	363	409	386	23
21	248	332	290	42
22	361	444	403	42
23	398	537	467	70
24	391	525	458	67
25	394	529	461	67
26	395	497	446	51
27	393	370	381	11
28	438	437	437	1
29	438	414	426	12
30	436	411	423	13
31	409	422	415	6
32	404	412	408	4
33	409	401	405	4
34	366	383	374	8
35	391	387	389	2
36	409	419	414	5
37	413	398	406	7
38	422	410	416	6
39	402	416	409	7
40	402	399	401	1
41	374	392	383	9
42	375	392	384	8
43	425	434	430	5
44	410	432	421	11
45	398	392	395	3
46	400	409	404	5
47	393	407	400	7
48	398	406	402	4
49	391	404	398	6
50	499	460	479	20
51	468	470	469	1
52	420	429	425	5
53	404	415	410	5
54	445	422	434	11
55	434	408	421	13
56	420	436	428	8
57	414	427	420	7
58	413	424	418	5

Table A2.27 (cont'd)

59	441	428	434	7
60	421	417	419	2
61	397	403	400	3
62	378	420	399	21
63	415	426	421	6
64	411	439	425	14
65	409	429	419	10
66	409	423	416	7
67	416	414	415	1
68	388	379	384	5
69	394	393	394	1
70	424	461	443	18
71	445	450	447	2
72	439	457	448	9
73	394	411	403	8
74	392	403	398	6
75	402	438	420	18
76	410	461	435	26
77	406	457	432	26
78	424	435	429	5
79	413	416	415	1
80	448	.	448	.
81	409	.	409	.
82	416	.	416	.
83	407	.	407	.
84	417	.	417	.
85	424	.	424	.
86	434	.	434	.
87	406	.	406	.
88	425	.	425	.
89	421	.	421	.
90	411	.	411	.

. Data is not available

Table A2.28 Daily biogas productions from trace elements and humic acids supplemented reactors

Days	FW+TE+HA			
	Duplicate 1	Duplicate 2	AVG	STD
1	48	48	48	0
2	68	72	70	2
3	86	86	86	0
4	127	139	133	6
5	152	191	172	20
6	181	314	247	67
7	239	280	260	21
8	186	229	208	22
9	196	50	123	73
10	253	262	257	5
11	284	340	312	28
12	364	415	389	25
13	469	511	490	21
14	403	388	395	8
15	437	356	397	41
16	305	338	321	16
17	372	322	347	25
18	371	346	359	13
19	419	415	417	2
20	389	404	396	7
21	288	295	291	3
22	327	380	353	26
23	373	423	398	25
24	392	394	393	1
25	413	419	416	3
26	435	441	438	3
27	454	449	452	2
28	507	453	480	27
29	521	335	428	93
30	376	387	382	6
31	386	398	392	6
32	373	398	385	13
33	372	389	380	8
34	377	356	367	10
35	401	403	402	1
36	441	443	442	1
37	416	402	409	7
38	406	392	399	7
39	431	418	425	7
40	379	410	394	15
41	361	393	377	16
42	379	408	394	14

Table A2.28 (cont'd)

43	383	378	381	3
44	393	371	382	11
45	384	394	389	5
46	399	401	400	1
47	361	361	361	0
48	386	389	387	2
49	387	395	391	4
50	401	408	405	4
51	407	415	411	4
52	402	410	406	4
53	410	395	403	7
54	428	404	416	12
55	417	424	420	4
56	421	409	415	6
57	391	413	402	11
58	402	397	399	3
59	411	418	414	3
60	379	392	386	6
61	427	429	428	1
62	384	397	390	6
63	385	405	395	10
64	398	408	403	5
65	393	376	385	9
66	404	386	395	9
67	338	343	340	3
68	383	369	376	7
69	394	369	381	13
70	409	414	411	3
71	436	428	432	4
72	414	402	408	6
73	387	373	380	7
74	396	378	387	9
75	394	385	390	4
76	370	386	378	8
77	365	385	375	10
78	381	391	386	5
79	378	390	384	6
80	388	.	388	.
81	381	.	381	.
82	380	.	380	.
83	408	.	408	.
84	391	.	391	.
85	427	.	427	.
86	416	.	416	.
87	398	.	398	.

Table A2.28 (cont'd)

88	384	.	384	.
89	388	.	388	.
90	396	.	396	.

. Data is not available

Table A2.29 Daily biogas productions from trace elements and vermicompost supplemented reactors

Days	FW+TE+VC			
	Duplicate 1	Duplicate 2	AVG	STD
1	72	66	69	3
2	104	94	99	5
3	138	118	128	10
4	192	164	178	14
5	206	219	212	6
6	302	256	279	23
7	384	306	345	39
8	334	253	293	41
9	282	282	282	0
10	355	355	355	0
11	385	385	385	0
12	463	463	463	0
13	653	653	653	0
14	543	543	543	0
15	578	578	578	0
16	439	380	410	30
17	446	437	441	5
18	359	368	363	4
19	527	553	540	13
20	519	395	457	62
21	463	306	384	78
22	546	374	460	86
23	560	472	516	44
24	579	491	535	44
25	488	434	461	27
26	367	304	336	32
27	458	421	439	19
28	471	478	474	4
29	386	441	414	27
30	404	402	403	1
31	411	411	411	0
32	447	461	454	7
33	462	468	465	3
34	422	455	438	17
35	435	451	443	8
36	423	457	440	17
37	406	439	423	17

Table A2.29 (cont'd)

38	450	463	457	6
39	455	476	465	10
40	449	428	438	10
41	406	382	394	12
42	443	424	433	9
43	419	408	413	5
44	404	385	395	9
45	426	436	431	5
46	423	416	419	4
47	430	437	434	4
48	428	450	439	11
49	410	437	423	13
50	447	456	452	4
51	423	458	440	18
52	411	431	421	10
53	415	431	423	8
54	431	459	445	14
55	445	453	449	4
56	450	453	451	2
57	425	429	427	2
58	434	425	429	5
59	423	418	421	2
60	420	442	431	11
61	409	416	412	3
62	409	422	415	7
63	421	428	425	4
64	430	447	439	8
65	414	414	414	0
66	418	413	415	2
67	424	432	428	4
68	433	416	424	8
69	428	406	417	11
70	428	411	420	8
71	455	448	452	4
72	436	438	437	1
73	406	401	404	2
74	407	419	413	6
75	421	411	416	5
76	431	442	437	5
77	425	420	422	2
78	434	427	430	4
79	436	428	432	4
80	449	.	449	.
81	411	.	411	.
82	424	.	424	.
83	410	.	410	.
84	411	.	411	.

Table A2.29 (cont'd)

85	422	.	422	.
86	431	.	431	.
87	419	.	419	.
88	428	.	428	.
89	438	.	438	.
90	426	.	426	.

. Data is not available

Table A2.30 Biogas composition of FW only reactors

Days	FW							
	N_2 (%)		CH_4 (%)		CO_2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	23	5	33	2	40	2	4800	106
7	9	1	41	1	43	0	4964	127
10	9	1	50	0	38	1	1613	214
14	5	1	50	1	38	1	1925	255
17	5	1	55	0	35	1	1501	233
21	5	1	55	1	35	0	1501	0
24	5	0	56	0	34	0	431	0
28	8	2	58	2	33	0	641	99
30	5	0	60	1	32	1	497	58
32	5	1	60	1	32	0	391	22
34	5	1	58	2	34	2	269	11
36	5	2	57	2	34	1	292	100
38	5	1	57	2	35	1	413	71
40	6	1	56	3	36	2	298	99
42	4	0	56	3	36	2	431	49
44	6	1	59	1	31	1	509	34
46	5	0	53	2	39	2	665	87
48	9	3	45	6	42	3	331	211
50	14	5	40	8	44	4	153	134
52	15	6	33	9	50	3	83	30
54	24	8	29	9	47	2	58	9
56	13	7	33	14	50	7	166	51
58	13	7	33	14	50	7	166	51
60	14	9	36	17	47	9	439	299
62	10	3	42	9	46	7	1198	957
64	6	1	49	2	41	3	1189	965
66	7	1	51	1	38	2	2808	2046
68	10	2	54	4	32	3	1174	780
70	12	1	49	4	36	3	1950	143
72	9	1	51	6	37	5	1586	923
74	9	0	52	6	34	6	1267	891
76	8	1	53	6	36	5	1183	787
78	8	0	53	6	36	6	832	449
80	1	0	56	6	38	6	883	484
82	9	4	51	6	35	3	234	34
84	6	2	51	4	39	2	453	361

Table A2.31 Biogas composition of trace elements supplemented reactors

Days	FW+TE							
	N2 (%)		CH4 (%)		CO2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	36	15	33	4	29	8	2740	2269
7	22	13	42	2	31	8	2729	2222
10	14	7	53	1	29	5	1225	805
14	7	2	56	1	29	3	1394	769
17	8	3	58	1	29	3	1041	543
21	11	7	55	2	30	4	740	282
24	7	2	61	0	27	2	550	89
28	6	0	63	2	29	3	509	58
30	9	4	57	1	29	5	560	67
32	6	0	61	4	28	4	396	38
34	7	2	60	2	29	4	399	103
36	5	1	59	3	31	3	600	14
38	7	2	59	1	30	3	623	68
40	6	0	62	3	30	4	577	49
42	5	0	61	3	28	4	511	37
44	5	0	62	2	28	3	681	95
46	9	4	61	6	27	2	485	35
48	6	0	62	3	27	4	407	10
50	8	1	61	4	28	4	407	10
52	8	2	62	2	28	4	313	96
54	8	3	61	2	29	5	267	34
56	12	1	57	3	27	3	252	74
58	7	2	61	4	29	2	391	125
60	7	0	60	2	31	2	266	81
62	6	0	62	4	30	4	275	44
64	8	1	61	3	29	4	132	118
66	6	1	62	3	28	3	329	30
68	4	1	63	3	28	4	421	190
70	7	0	63	4	27	4	252	55
72	14	1	55	5	28	6	223	58
74	11	0	56	5	28	5	471	307
76	12	3	57	2	28	5	444	105
78	14	2	57	5	26	4	470	84
80	7	4	59	1	29	6	432	95
82	5	2	61	3	29	5	239	30
84	4	2	61	5	30	7	203	111

Table A2.32 Biogas composition of humic acids supplemented reactors

Days	FW+HA							
	N_2 (%)		CH_4 (%)		CO_2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	32	1	28	1	37	0	3745	27
7	14	0	38	1	42	1	4419	297
10	9	1	50	1	38	0	1665	32
14	5	0	53	0	36	1	1685	113
17	6	2	56	2	33	0	1151	57
21	7	1	55	1	34	0	542	123
24	7	0	56	0	33	0	382	8
28	7	1	59	1	32	0	279	1
30	6	1	61	2	31	1	167	67
32	6	0	59	0	32	0	154	81
34	6	0	59	0	32	0	132	38
36	4	1	59	1	33	0	163	49
38	7	0	57	0	33	0	97	15
40	6	1	59	0	33	0	94	38
42	7	0	57	0	32	0	121	26
44	7	0	59	0	31	0	139	35
46	5	0	59	0	33	0	140	16
48	6	1	57	0	32	1	165	1
50	8	1	56	1	33	0	62	24
52	9	1	51	2	37	0	105	66
54	14	3	50	3	35	0	72	33
56	9	1	54	2	34	1	67	5
58	7	2	58	4	33	2	76	43
60	5	0	60	1	32	1	82	32
62	6	1	60	1	31	0	53	12
64	6	0	61	0	31	0	68	19
66	5	0	58	0	34	0	197	66
68	8	1	59	2	29	1	91	6
70	8	0	57	1	32	0	111	44
72	9	2	54	1	34	1	107	9
74	8	1	54	0	33	1	374	27
76	11	2	53	1	33	1	573	203
78	8	1	55	0	33	0	424	260
80	3	1	58	1	36	0	623	482
82	16	10	49	6	31	3	94	29
84	4	2	55	2	36	1	37	3

Table A2.33 Biogas composition of vermicompost supplemented reactors

Days	FW+ VC							
	N_2 (%)		CH_4 (%)		CO_2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	28	2	32	0	37	1	4709	19
7	11	1	40	1	42	0	4425	232
10	7	2	52	2	37	0	1466	28
14	5	0	53	1	36	0	1462	71
17	5	0	57	0	33	0	851	3
21	6	1	55	0	34	0	851	0
24	7	2	55	1	34	0	349	9
28	7	2	58	3	32	1	282	9
30	5	1	60	3	32	2	152	57
32	6	1	58	1	33	0	145	71
34	9	0	54	2	34	2	84	24
36	5	1	55	4	36	3	154	29
38	7	0	55	3	36	3	197	22
40	5	1	57	0	36	1	198	80
42	4	0	61	3	31	3	158	1
44	6	1	60	2	30	2	201	4
46	4	0	60	0	32	0	165	7
48	6	0	58	0	32	0	107	0
50	8	1	58	1	32	0	83	31
52	8	2	58	1	32	1	103	37
54	20	1	50	0	29	1	43	6
56	7	2	57	2	32	0	30	6
58	7	1	59	0	32	0	37	3
60	8	2	56	1	33	1	35	16
62	6	0	57	1	34	0	39	5
64	7	1	58	1	32	0	10	2
66	6	0	57	0	34	0	167	62
68	12	0	56	0	29	1	18	8
70	6	0	57	0	34	1	62	3
72	13	3	51	2	34	1	45	22
74	9	0	54	1	33	0	58	8
76	12	2	53	1	33	1	53	13
78	8	1	56	1	33	0	42	4
80	5	0	56	0	35	1	29	29
82	7	2	55	2	34	0	29	12
84	3	0	55	0	35	0	22	1

Table A2.34 Biogas composition of trace elements and humic acids supplemented reactors

Days	FW+ TE+HA							
	N_2 (%)		CH_4 (%)		CO_2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	32	1	28	1	37	0	3874	27
7	15	1	37	2	42	1	3870	206
10	11	3	49	2	37	1	1152	25
14	5	1	53	2	35	0	1412	95
17	8	1	55	1	33	0	528	20
21	7	2	55	2	34	0	528	0
24	5	1	56	1	35	0	240	28
28	8	3	57	2	33	0	169	1
30	5	1	61	2	31	1	96	39
32	5	1	61	1	31	0	97	21
34	5	0	60	2	32	2	83	6
36	7	2	57	1	33	1	61	7
38	7	0	57	0	33	0	131	57
40	6	0	58	0	34	0	87	18
42	5	1	59	0	32	1	115	1
44	5	0	60	0	31	0	134	6
46	4	1	60	0	33	0	120	5
48	6	0	57	0	32	0	48	0
50	10	1	54	0	34	1	22	12
52	6	0	60	1	31	1	40	10
54	14	4	55	4	29	0	27	17
56	7	3	57	2	31	1	21	14
58	6	1	59	0	32	0	44	6
60	5	0	58	0	34	0	25	1
62	6	1	57	1	34	1	25	15
64	7	1	57	1	34	0	29	6
66	7	2	56	1	34	1	99	36
68	6	1	58	1	32	0	33	8
70	20	6	51	4	28	2	25	3
72	9	0	53	0	35	0	37	2
74	8	2	53	1	34	1	203	28
76	7	0	56	0	35	0	281	41
78	5	2	57	2	35	1	415	137
80	4	0	58	0	35	0	299	143
82	11	5	53	4	32	1	71	56
84	5	3	55	3	35	1	44	34

Table A2.35 Biogas composition of trace elements and vermicomposts supplemented reactors

Days	FW+TE+VC							
	N_2 (%)		CH_4 (%)		CO_2 (%)		H2S (ppm)	
	AVG	STD	AVG	STD	AVG	STD	AVG	STD
3	23	0	35	0	38	0	4824	34
7	9	1	43	2	41	3	4465	6
10	7	0	54	0	35	0	1447	0
14	24	20	39	13	32	4	1268	295
17	10	4	53	5	33	2	730	119
21	3	0	59	1	33	1	344	96
24	8	2	57	2	31	0	182	138
28	9	2	58	2	31	1	106	66
30	11	6	56	3	30	2	193	84
32	6	0	59	1	31	1	171	52
34	4	2	59	0	33	2	107	20
36	7	2	57	0	32	2	127	33
38	6	0	59	0	32	0	162	61
40	6	0	59	0	33	0	141	32
42	6	0	58	0	32	0	103	0
44	6	2	59	1	31	0	262	139
46	5	0	60	0	32	0	194	86
48	6	0	58	0	32	0	143	45
50	8	0	57	0	32	0	135	53
52	6	2	59	1	32	1	152	55
54	11	1	56	1	32	0	108	38
56	7	1	57	1	32	1	132	53
58	6	1	59	0	32	0	98	46
60	7	0	57	0	33	0	95	59
62	6	0	58	0	33	0	80	55
64	10	2	56	1	31	1	76	59
66	7	0	58	0	32	0	97	68
68	4	1	60	0	32	1	122	112
70	10	1	57	0	31	0	215	0
72	6	0	56	0	35	0	37	0
74	8	0	54	0	33	1	79	0
76	7	0	56	0	33	0	49	0
78	9	1	57	1	31	0	21	21
80	1	0	59	0	35	0	12	6
82	6	1	56	1	33	0	0	0
84	3	1	57	1	35	0	21	8

APPENDIX C

Methanogenic Activities Study Data Summary

Table A3.1 Acetate utilization rates of food waste only (control) digesters

Hours	FW			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	0.7	0.9	0.7	0.8	0.1
1.0	1.0	1.4	1.6	1.3	0.3
1.5	1.1	1.7	2.2	1.7	0.5
2.0	1.3	1.9	2.5	1.9	0.5
2.5	1.8	2.1	2.8	2.2	0.4
3.0	2.1	2.4	2.9	2.5	0.3
3.5	2.5	2.7	3.1	2.8	0.3
4.0	3.0	3.1	4.3	3.5	0.6
4.5	3.7	3.5	4.4	3.9	0.4
5.0	4.3	4.3	4.4	4.3	0.1
5.5	5.0	5.0	4.4	4.8	0.3
6.0	5.7	5.6	4.4	5.3	0.6
6.5	6.5	6.2	5.4	6.1	0.4
7.0	7.2	6.8	7.1	7.1	0.2
7.5	8.2	7.4	8.2	7.9	0.3
8.0	9.0	8.3	9.1	8.8	0.3
8.5	9.8	9.1	9.8	9.6	0.3
9.0	10.7	9.9	10.8	10.5	0.4
9.5	11.6	10.6	12.0	11.4	0.6
10.0	12.5	11.4	12.9	12.3	0.6
10.5	13.4	12.1	13.8	13.1	0.7
11.0	14.6	12.9	14.6	14.0	0.8
11.5	15.4	13.6	15.5	14.9	0.9
12.0	16.4	14.7	16.8	16.0	0.9
12.5	17.5	15.6	17.7	17.0	0.9
13.0	18.5	16.6	18.6	17.9	1.0
13.5	19.5	17.4	19.4	18.8	1.0
14.0	20.7	18.4	20.5	19.8	1.1
14.5	21.8	19.2	21.5	20.8	1.2
15.0	22.8	20.1	22.4	21.8	1.2
15.5	23.9	21.2	23.2	22.8	1.2
16.0	25.0	22.3	23.9	23.8	1.1
16.5	26.1	23.4	25.1	24.9	1.1
17.0	27.1	24.4	26.1	25.9	1.1
17.5	28.4	25.4	26.9	26.9	1.2
18.0	29.4	26.4	27.8	27.9	1.2
18.5	30.5	27.4	29.1	29.0	1.3
19.0	31.6	28.6	30.1	30.1	1.2
19.5	32.8	29.8	30.9	31.2	1.2
20.0	33.8	30.9	32.1	32.3	1.2
20.5	35.1	31.9	33.2	33.4	1.3

Table A3.1 (cont'd)

21.0	36.2	33.0	34.1	34.5	1.3
21.5	37.4	34.4	35.1	35.6	1.3
22.0	38.5	35.6	36.0	36.7	1.3
22.5	39.6	36.8	36.7	37.7	1.4
23.0	40.7	37.9	37.4	38.7	1.4
23.5	42.0	39.3	38.2	39.8	1.6
24.0	43.2	40.6	39.0	40.9	1.7

Table A3.2 Acetate utilization rates of trace elements supplemented food waste digesters

Hours	FW+TE			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.2	1.7	1.2	1.4	0.2
1.0	1.7	2.3	2.5	2.2	0.4
1.5	2.0	2.7	3.5	2.7	0.6
2.0	2.3	3.0	4.6	3.3	0.9
2.5	3.1	3.4	5.5	4.0	1.1
3.0	3.7	4.0	6.4	4.7	1.2
3.5	4.3	4.8	7.6	5.6	1.4
4.0	5.2	5.6	9.1	6.7	1.8
4.5	6.3	6.6	10.4	7.7	1.8
5.0	7.4	7.9	11.6	9.0	1.9
5.5	8.7	9.2	12.9	10.2	1.9
6.0	9.9	10.4	14.1	11.5	1.9
6.5	11.2	11.7	15.5	12.8	1.9
7.0	12.5	12.9	17.1	14.2	2.1
7.5	14.1	14.1	18.5	15.6	2.1
8.0	15.5	15.6	19.9	17.0	2.1
8.5	16.8	17.2	21.2	18.4	2.0
9.0	18.5	18.6	22.7	19.9	1.9
9.5	20.0	20.0	24.5	21.5	2.1
10.0	21.6	21.5	25.9	23.0	2.1
10.5	23.2	22.9	27.4	24.5	2.1
11.0	25.1	24.3	28.8	26.1	2.0
11.5	26.6	25.7	30.3	27.5	2.0
12.0	28.3	27.5	32.1	29.3	2.0
12.5	30.1	29.1	33.6	31.0	1.9
13.0	31.9	30.8	35.1	32.6	1.8
13.5	33.7	32.4	36.6	34.2	1.8
14.0	35.6	34.0	38.4	36.0	1.8
14.5	37.5	35.5	40.1	37.7	1.9
15.0	39.3	37.1	41.7	39.4	1.9

Table A3.2 (cont'd)

15.5	41.2	38.9	43.3	41.1	1.8
16.0	43.1	40.7	44.8	42.9	1.7
16.5	45.0	42.5	46.9	44.8	1.8
17.0	46.8	44.3	48.6	46.6	1.8
17.5	48.9	46.0	50.2	48.4	1.7
18.0	50.7	47.7	51.9	50.1	1.8
18.5	52.6	49.4	54.0	52.0	1.9
19.0	54.6	51.4	55.6	53.9	1.8
19.5	56.5	53.3	57.4	55.7	1.8
20.0	58.4	55.1	59.6	57.7	1.9
20.5	60.6	57.0	61.5	59.7	1.9
21.0	62.4	58.9	63.3	61.5	1.9
21.5	64.5	61.0	65.2	63.6	1.8
22.0	66.4	63.0	67.0	65.5	1.7
22.5	68.3	65.1	68.6	67.3	1.6
23.0	70.2	67.0	70.3	69.2	1.5
23.5	72.5	69.3	71.9	71.3	1.4
24.0	74.5	71.5	73.4	73.1	1.2

Table A3.3 Acetate utilization rates of humic acids supplemented food waste digesters

Hours	FW+HA			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.3	1.6	1.6	1.5	0.2
1.0	1.7	2.4	2.3	2.1	0.3
1.5	2.0	3.2	3.5	2.9	0.6
2.0	2.4	4.1	4.4	3.6	0.9
2.5	3.2	5.0	5.2	4.4	0.9
3.0	3.8	5.9	5.9	5.2	1.0
3.5	4.4	6.9	6.8	6.0	1.1
4.0	5.3	7.9	7.9	7.0	1.2
4.5	6.4	8.9	9.5	8.3	1.3
5.0	7.6	10.2	10.7	9.5	1.4
5.5	8.9	11.3	12.0	10.7	1.4
6.0	10.1	12.6	13.3	12.0	1.4
6.5	11.4	13.8	14.6	13.3	1.3
7.0	12.8	15.0	16.2	14.7	1.4
7.5	14.4	16.3	17.5	16.1	1.3
8.0	15.9	17.7	19.0	17.5	1.3
8.5	17.2	19.2	20.2	18.9	1.2
9.0	18.9	20.7	21.7	20.4	1.2
9.5	20.5	22.1	23.6	22.1	1.2

Table A3.3 (cont'd)

10.0	22.1	23.6	25.1	23.6	1.2
10.5	23.7	25.0	26.6	25.1	1.2
11.0	25.7	26.4	28.0	26.7	0.9
11.5	27.3	27.8	29.7	28.2	1.0
12.0	29.0	29.5	31.5	30.0	1.1
12.5	30.9	31.1	33.1	31.7	1.0
13.0	32.7	32.8	34.5	33.3	0.8
13.5	34.5	34.3	36.0	34.9	0.7
14.0	36.5	35.9	38.0	36.8	0.9
14.5	38.4	37.3	39.8	38.5	1.0
15.0	40.2	38.8	41.4	40.2	1.1
15.5	42.2	40.5	43.0	41.9	1.1
16.0	44.2	42.3	45.0	43.8	1.2
16.5	46.1	44.0	46.9	45.7	1.2
17.0	47.9	45.7	48.6	47.4	1.2
17.5	50.1	47.3	50.8	49.4	1.5
18.0	51.9	48.8	52.6	51.1	1.6
18.5	53.8	50.4	54.4	52.9	1.8
19.0	55.9	52.3	56.1	54.8	1.8
19.5	57.8	54.1	57.7	56.5	1.7
20.0	59.8	55.8	59.3	58.3	1.8
20.5	62.0	57.5	60.8	60.1	1.9
21.0	63.9	59.2	62.3	61.8	2.0
21.5	66.0	61.2	63.7	63.6	2.0
22.0	68.0	63.0	65.8	65.6	2.0
22.5	70.0	64.9	67.6	67.5	2.1
23.0	71.9	66.7	69.3	69.3	2.1
23.5	74.2	68.7	70.9	71.3	2.3
24.0	76.2	72.0	72.5	73.6	1.9

Table A3.4 Acetate utilization rates of vermicompost supplemented food waste digesters

Hours	FW+VC			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.3	1.7	2.0	1.7	0.3
1.0	1.8	2.7	3.2	2.6	0.6
1.5	2.1	3.6	4.2	3.3	0.9
2.0	2.5	4.5	5.2	4.1	1.1
2.5	3.4	5.6	6.1	5.0	1.2
3.0	4.0	6.5	7.1	5.9	1.3

Table A3.4 (cont'd)

3.5	4.7	7.6	8.4	6.9	1.6
4.0	5.6	8.7	9.8	8.0	1.8
4.5	6.8	9.8	11.1	9.2	1.8
5.0	8.1	11.2	12.5	10.6	1.9
5.5	9.4	12.6	13.8	11.9	1.9
6.0	10.7	14.0	15.2	13.3	1.9
6.5	12.1	15.4	17.0	14.8	2.0
7.0	13.5	16.8	18.5	16.2	2.1
7.5	15.3	18.1	20.0	17.8	1.9
8.0	16.8	19.7	21.4	19.3	1.9
8.5	18.2	21.3	22.8	20.8	1.9
9.0	20.0	23.1	24.8	22.6	2.0
9.5	21.7	24.7	26.6	24.3	2.0
10.0	23.4	26.3	28.3	26.0	2.0
10.5	25.1	27.9	29.9	27.6	2.0
11.0	27.2	29.5	31.6	29.5	1.8
11.5	28.8	31.2	33.7	31.2	2.0
12.0	30.7	33.2	35.5	33.1	2.0
12.5	32.6	35.2	37.2	35.0	1.9
13.0	34.6	37.1	38.9	36.8	1.8
13.5	36.5	38.9	40.8	38.7	1.8
14.0	38.6	40.8	42.8	40.7	1.7
14.5	40.6	42.6	44.6	42.6	1.6
15.0	42.5	44.3	46.4	44.4	1.6
15.5	44.7	46.3	48.0	46.3	1.4
16.0	46.7	48.4	50.2	48.4	1.4
16.5	48.7	50.5	52.2	50.4	1.4
17.0	50.7	52.5	54.0	52.4	1.4
17.5	52.9	54.4	55.7	54.4	1.1
18.0	54.9	56.3	58.0	56.4	1.3
18.5	56.9	58.2	59.8	58.3	1.2
19.0	59.1	60.4	61.6	60.4	1.0
19.5	61.1	62.5	64.0	62.5	1.2
20.0	63.2	64.6	66.0	64.6	1.2
20.5	65.6	66.6	68.0	66.7	1.0
21.0	67.6	68.6	70.0	68.7	1.0
21.5	69.8	70.9	71.8	70.8	0.8
22.0	71.9	73.1	73.5	72.8	0.7
22.5	74.0	75.2	75.1	74.7	0.5
23.0	76.0	77.3	76.8	76.7	0.5
23.5	78.5	79.6	78.3	78.8	0.6
24.0	80.6	81.9	80.5	81.0	0.6

Table A3.5 Propionate utilization rates of food waste only digesters

Hours	FW only			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.4	1.9	1.9	1.7	0.2
1.0	2.1	2.5	2.3	2.3	0.2
1.5	2.5	2.6	2.5	2.5	0.1
2.0	2.8	2.9	2.6	2.8	0.1
2.5	3.0	3.2	3.3	3.2	0.1
3.0	3.2	3.4	3.4	3.3	0.1
3.5	3.4	4.1	3.6	3.7	0.3
4.0	3.6	4.5	4.1	4.0	0.4
4.5	4.1	4.7	4.4	4.4	0.3
5.0	4.4	5.0	4.5	4.6	0.3
5.5	4.8	5.3	4.8	4.9	0.2
6.0	5.0	5.8	5.3	5.4	0.3
6.5	5.2	6.0	5.5	5.6	0.3
7.0	5.4	6.3	5.7	5.8	0.4
7.5	5.9	6.4	6.2	6.2	0.2
8.0	6.3	7.1	6.5	6.6	0.3
8.5	6.6	7.4	6.6	6.9	0.4
9.0	6.8	7.5	7.1	7.2	0.3
9.5	7.1	7.6	7.4	7.4	0.2
10.0	7.3	8.3	7.6	7.7	0.4
10.5	7.8	8.5	7.9	8.0	0.3
11.0	8.1	8.7	8.3	8.4	0.2
11.5	8.3	8.9	8.6	8.6	0.2
12.0	8.5	9.4	8.7	8.8	0.4
12.5	9.0	9.5	9.1	9.2	0.2
13.0	9.3	9.7	9.4	9.5	0.2
13.5	9.5	10.2	9.5	9.7	0.3
14.0	9.6	10.4	9.7	9.9	0.3
14.5	10.1	10.7	10.2	10.3	0.3
15.0	10.3	10.7	10.5	10.5	0.1
15.5	10.5	11.2	10.6	10.8	0.3
16.0	10.7	11.5	11.2	11.1	0.3
16.5	11.1	11.6	11.4	11.4	0.2
17.0	11.3	12.2	11.6	11.7	0.4
17.5	11.7	12.4	11.7	11.9	0.3
18.0	12.0	12.8	12.3	12.4	0.3
18.5	12.2	13.1	12.5	12.6	0.4
19.0	12.4	13.3	12.7	12.8	0.4
19.5	12.5	13.5	12.8	12.9	0.4
20.0	12.6	13.9	13.3	13.3	0.5
20.5	13.1	14.1	13.6	13.6	0.4

Table A3.5 (cont'd)

21.0	13.3	14.1	13.8	13.7	0.3
21.5	13.5	14.5	13.8	13.9	0.4
22.0	13.6	14.8	14.3	14.2	0.5
22.5	13.7	15.0	14.6	14.4	0.5
23.0	14.0	15.0	14.7	14.6	0.4
23.5	14.3	15.6	14.8	14.9	0.5
24.0	15.0	15.6	14.9	15.2	0.3

Table A3.6 Propionate utilization rates of trace elements supplemented food waste digesters

Hours	FW+TE			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.2	1.4	1.3	1.3	0.1
1.0	1.9	1.9	1.6	1.8	0.1
1.5	2.2	2.2	1.9	2.1	0.1
2.0	2.5	2.5	2.2	2.4	0.1
2.5	2.6	2.8	2.7	2.7	0.1
3.0	2.9	3.1	2.9	3.0	0.1
3.5	3.0	3.9	3.1	3.3	0.4
4.0	3.2	4.4	3.6	3.7	0.5
4.5	3.6	4.8	3.9	4.1	0.5
5.0	3.9	5.1	4.0	4.4	0.5
5.5	4.1	5.5	4.3	4.6	0.6
6.0	4.2	6.2	4.7	5.0	0.9
6.5	4.3	6.5	4.9	5.2	1.0
7.0	4.3	6.9	5.0	5.4	1.1
7.5	4.7	7.2	5.4	5.8	1.0
8.0	5.0	7.8	5.6	6.2	1.2
8.5	5.3	8.2	5.8	6.4	1.3
9.0	5.4	8.4	6.1	6.7	1.3
9.5	5.4	8.7	6.4	6.8	1.3
10.0	5.6	9.2	6.5	7.1	1.5
10.5	6.0	9.6	6.7	7.4	1.5
11.0	6.3	9.8	7.0	7.7	1.5
11.5	6.4	10.1	7.2	7.9	1.6
12.0	6.5	10.6	7.3	8.1	1.8
12.5	7.0	10.8	7.7	8.5	1.7
13.0	7.1	11.0	7.8	8.6	1.7
13.5	7.4	11.4	7.9	8.9	1.8
14.0	7.5	11.7	8.1	9.1	1.9
14.5	7.9	12.0	8.4	9.4	1.8
15.0	8.1	12.1	8.6	9.6	1.8

Table A3.6 (cont'd)

15.5	8.2	12.6	8.7	9.8	2.0
16.0	8.4	12.8	9.1	10.1	1.9
16.5	8.7	13.0	9.2	10.3	1.9
17.0	8.9	13.4	9.4	10.6	2.0
17.5	9.3	13.6	9.5	10.8	2.0
18.0	9.6	14.0	9.9	11.2	2.0
18.5	9.8	14.4	10.1	11.4	2.1
19.0	10.0	14.5	10.2	11.5	2.1
19.5	10.1	14.7	10.2	11.7	2.1
20.0	10.1	15.1	10.7	12.0	2.2
20.5	10.6	15.3	10.8	12.2	2.2
21.0	10.9	15.5	11.0	12.4	2.1
21.5	11.0	15.8	11.1	12.6	2.3
22.0	11.1	16.1	11.4	12.9	2.3
22.5	11.2	16.3	11.6	13.0	2.3
23.0	11.5	16.4	11.7	13.2	2.3
23.5	11.8	16.8	11.8	13.5	2.4
24.0	12.4	16.9	11.9	13.7	2.2

Table A3.7 Propionate utilization rates of humic acids supplemented food waste digesters

Hours	PW+HA			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	2.4	2.7	2.0	2.4	0.3
1.0	3.2	3.4	2.4	3.0	0.4
1.5	3.7	3.6	2.5	3.3	0.6
2.0	3.9	3.7	2.7	3.5	0.5
2.5	4.1	3.8	3.3	3.7	0.3
3.0	4.2	3.8	3.5	3.8	0.3
3.5	4.4	4.5	3.6	4.1	0.4
4.0	4.5	4.8	4.0	4.4	0.3
4.5	5.2	4.9	4.4	4.8	0.3
5.0	5.5	4.9	4.5	5.0	0.4
5.5	5.7	5.2	4.8	5.2	0.4
6.0	5.8	5.8	5.3	5.6	0.2
6.5	5.8	6.0	5.4	5.7	0.2
7.0	5.8	6.2	5.5	5.8	0.3
7.5	6.4	6.2	6.2	6.3	0.1
8.0	6.7	6.8	6.4	6.7	0.2
8.5	7.0	7.1	6.5	6.9	0.2

Table A3.7 (cont'd)

9.0	7.1	7.2	7.0	7.1	0.1
9.5	7.2	7.2	7.3	7.2	0.1
10.0	7.4	7.9	7.5	7.6	0.2
10.5	7.9	8.1	7.7	7.9	0.2
11.0	8.1	8.1	8.2	8.1	0.1
11.5	8.2	8.3	8.4	8.3	0.1
12.0	8.2	8.9	8.4	8.5	0.3
12.5	8.8	9.1	8.9	8.9	0.1
13.0	9.1	9.1	9.2	9.1	0.1
13.5	9.2	9.6	9.3	9.4	0.2
14.0	9.2	9.9	9.5	9.5	0.3
14.5	9.8	10.0	10.0	9.9	0.1
15.0	10.0	10.1	10.2	10.1	0.1
15.5	10.1	10.6	10.2	10.3	0.2
16.0	10.3	10.8	10.9	10.7	0.3
16.5	10.7	10.9	11.2	10.9	0.2
17.0	10.9	11.5	11.3	11.2	0.3
17.5	11.2	11.7	11.4	11.4	0.2
18.0	11.7	12.2	12.0	12.0	0.2
18.5	11.9	12.5	12.3	12.2	0.2
19.0	12.0	12.6	12.4	12.3	0.2
19.5	12.0	12.6	12.4	12.4	0.3
20.0	12.0	13.0	13.0	12.7	0.5
20.5	12.5	13.2	13.3	13.0	0.3
21.0	12.8	13.2	13.4	13.1	0.3
21.5	12.9	13.7	13.4	13.3	0.3
22.0	12.9	13.9	13.9	13.6	0.5
22.5	12.9	13.9	14.2	13.7	0.5
23.0	13.3	14.0	14.3	13.9	0.4
23.5	13.6	14.6	14.4	14.2	0.4
24.0	14.2	14.6	14.4	14.4	0.1

Table A3.8 Propionate utilization rates of vermicompost supplemented food waste digesters

Hours	FW+VC			AVG	STD
	1	2	3		
0.0	0.0	0.0	0.0	0.0	0.0
0.5	1.7	2.1	2.3	2.0	0.2
1.0	2.5	2.9	2.9	2.8	0.2
1.5	3.2	3.4	3.2	3.2	0.1
2.0	3.5	3.7	3.6	3.6	0.1
2.5	3.7	4.0	4.2	4.0	0.2
3.0	3.9	4.3	4.5	4.3	0.3

Table A3.8 (cont'd)

3.5	4.0	5.2	4.8	4.7	0.5
4.0	4.1	5.7	5.4	5.1	0.7
4.5	4.8	6.1	5.9	5.6	0.5
5.0	5.2	6.4	6.2	6.0	0.5
5.5	5.6	6.8	6.7	6.3	0.6
6.0	5.7	7.7	7.3	6.9	0.9
6.5	5.9	8.2	7.7	7.2	1.0
7.0	6.0	8.5	8.0	7.5	1.1
7.5	6.8	8.8	8.7	8.1	1.0
8.0	7.2	9.7	9.1	8.7	1.1
8.5	7.7	10.3	9.4	9.1	1.1
9.0	8.0	10.7	10.0	9.5	1.2
9.5	8.2	10.9	10.6	9.9	1.2
10.0	8.6	11.8	10.9	10.4	1.4
10.5	9.3	12.3	11.2	11.0	1.2
11.0	9.8	12.7	11.9	11.5	1.2
11.5	10.1	13.1	12.3	11.8	1.3
12.0	10.3	13.9	12.5	12.3	1.5
12.5	11.1	14.4	13.2	12.9	1.3
13.0	11.6	14.7	13.6	13.3	1.3
13.5	11.9	15.4	13.9	13.7	1.4
14.0	12.2	16.0	14.3	14.2	1.6
14.5	13.0	16.4	14.9	14.8	1.4
15.0	13.5	16.7	15.2	15.1	1.3
15.5	13.8	17.5	15.5	15.6	1.5
16.0	14.1	18.0	16.3	16.1	1.6
16.5	14.8	18.4	16.7	16.7	1.5
17.0	15.2	19.3	17.0	17.2	1.7
17.5	15.9	19.7	17.2	17.6	1.6
18.0	16.5	20.5	17.9	18.3	1.7
18.5	17.0	21.1	18.4	18.8	1.7
19.0	17.4	21.5	18.7	19.2	1.7
19.5	17.6	21.8	19.0	19.5	1.8
20.0	17.9	22.6	19.7	20.1	2.0
20.5	18.6	23.0	20.1	20.6	1.8
21.0	19.1	23.3	20.4	20.9	1.8
21.5	19.5	24.0	20.7	21.4	1.9
22.0	19.7	24.6	21.2	21.8	2.0
22.5	19.9	25.0	21.7	22.2	2.1
23.0	20.5	25.2	22.0	22.6	2.0
23.5	21.1	26.0	22.3	23.1	2.1
24.0	22.1	26.1	22.5	23.6	1.8

APPENDIX D

Metal Bioavailability Study Data Summary

Table A4.1 Cumulative methane production from acetate oxidation in the food waste digester

Hours	FW only							
	1	2	3	4	5	6	AVG	STD
0.5	1.3	1.2	1.3	1.2	0.8	0.77	1.1	0.2
1.0	2.5	2.2	2.3	2.2	1.8	1.69	2.1	0.3
1.5	3.4	2.9	2.9	2.9	2.4	2.23	2.8	0.4
2.0	4.3	3.6	3.7	3.6	3.0	2.76	3.5	0.5
2.5	5.5	4.3	4.4	4.3	3.7	3.43	4.3	0.7
3.0	6.2	4.9	5.0	4.9	4.3	3.92	4.9	0.8
3.5	7.0	5.5	5.6	5.5	5.3	4.84	5.6	0.7
4.0	7.7	5.9	6.1	5.9	6.4	5.85	6.3	0.7
4.5	8.5	6.6	6.7	6.6	7.0	6.48	7.0	0.8
5.0	9.2	7.1	7.3	7.1	7.6	7.01	7.6	0.8
5.5	9.8	7.9	8.0	7.9	8.3	7.60	8.2	0.8
6.0	10.5	8.6	8.8	8.6	9.1	8.37	9.0	0.8
6.5	11.6	9.4	9.6	9.4	10.4	9.59	10.0	0.9
7.0	12.2	10.0	10.2	10.0	10.9	10.06	10.6	0.9
7.5	12.9	10.7	10.9	10.7	11.6	10.65	11.2	0.9
8.0	13.6	11.2	11.4	11.2	12.2	11.23	11.8	1.0
8.5	14.3	12.3	12.5	12.3	12.8	11.81	12.7	0.9
9.0	14.9	13.1	13.3	13.1	14.3	13.17	13.6	0.8
9.5	15.7	13.8	14.1	13.8	15.0	13.84	14.4	0.8
10.0	16.7	14.7	15.0	14.7	15.6	14.33	15.1	0.9
10.5	17.3	15.4	15.7	15.4	16.3	14.96	15.8	0.8
11.0	17.9	16.0	16.4	16.0	16.8	15.49	16.4	0.9
11.5	18.6	16.8	17.2	16.8	17.5	16.07	17.2	0.9
12.0	19.3	17.8	18.2	17.8	19.0	17.48	18.3	0.7
12.5	20.0	18.7	19.1	18.7	19.6	18.01	19.0	0.7
13.0	21.2	19.5	19.9	19.5	20.2	18.59	19.8	0.9
13.5	21.8	20.2	20.7	20.2	20.9	19.22	20.5	0.9
14.0	22.4	21.0	21.4	21.0	22.6	20.77	21.5	0.8
14.5	23.1	21.6	22.0	21.6	23.3	21.44	22.2	0.8
15.0	23.8	22.7	23.2	22.7	24.0	22.07	23.1	0.7
15.5	24.8	23.6	24.1	23.6	24.7	22.70	23.9	0.8
16.0	25.6	24.4	24.9	24.4	26.0	23.96	24.9	0.8
16.5	26.3	25.1	25.6	25.1	26.9	24.74	25.6	0.8
17.0	27.0	25.8	26.4	25.8	27.4	25.22	26.3	0.8
17.5	27.8	26.9	27.5	26.9	28.2	25.90	27.2	0.8
18.0	28.5	27.8	28.4	27.8	29.7	27.30	28.2	0.8
18.5	29.4	28.6	29.2	28.6	30.3	27.83	29.0	0.8
19.0	30.0	29.4	30.0	29.4	31.1	28.62	29.7	0.8
19.5	30.7	30.0	30.6	30.0	31.8	29.25	30.4	0.9
20.0	31.4	31.3	31.9	31.3	33.3	30.65	31.6	0.9
20.5	32.1	32.1	32.8	32.1	34.0	31.28	32.4	0.9

Table A4.1 (cont'd)

21.0	33.1	32.8	33.5	32.8	34.7	31.95	33.1	0.9
21.5	33.8	33.5	34.2	33.5	35.7	32.83	33.9	1.0
22.0	34.5	34.5	35.2	34.5	36.7	33.79	34.9	1.0
22.5	35.3	35.3	36.1	35.3	37.9	34.90	35.8	1.1
23.0	36.1	36.1	36.9	36.1	39.2	36.02	36.7	1.2
23.5	36.8	36.9	37.6	36.9	39.9	36.75	37.5	1.2
24.0	37.4	37.6	38.4	37.6	40.8	37.52	38.2	1.3

Table A4.2 Effect of 0.01mg/L Co on acetate utilization rate in the food waste digester

Hours	Co (0.01 mg/L)			AVG	STD
	1	2	3		
0.5	1.3	1.2	1.2	1.2	0.0
1.0	2.2	2.2	2.2	2.2	0.0
1.5	2.9	3.0	2.9	2.9	0.0
2.0	3.6	3.6	3.5	3.6	0.0
2.5	4.1	4.3	4.2	4.2	0.1
3.0	4.9	5.0	4.9	4.9	0.1
3.5	5.4	5.6	5.5	5.5	0.1
4.0	6.0	6.2	6.0	6.1	0.1
4.5	6.6	6.8	6.6	6.6	0.1
5.0	7.2	7.4	7.2	7.3	0.1
5.5	7.9	8.1	7.8	7.9	0.1
6.0	8.5	8.7	8.4	8.5	0.1
6.5	9.2	9.4	9.1	9.2	0.1
7.0	9.8	9.9	9.6	9.8	0.1
7.5	10.6	10.6	10.3	10.5	0.1
8.0	11.2	11.3	11.0	11.2	0.1
8.5	12.4	12.6	12.2	12.4	0.2
9.0	13.3	13.5	13.1	13.3	0.2
9.5	14.1	14.4	14.0	14.2	0.2
10.0	15.0	15.3	14.8	15.0	0.2
10.5	15.5	16.1	15.6	15.7	0.3
11.0	16.2	16.7	16.2	16.4	0.2
11.5	17.0	17.6	17.0	17.2	0.3
12.0	18.1	18.7	18.1	18.3	0.3
12.5	19.0	19.6	19.0	19.2	0.3
13.0	19.8	20.5	19.9	20.1	0.3
13.5	20.6	21.2	20.6	20.8	0.3
14.0	21.2	21.9	21.3	21.4	0.3
14.5	21.8	22.5	21.9	22.1	0.3
15.0	22.9	23.8	23.1	23.3	0.4
15.5	23.8	24.8	24.0	24.2	0.4
16.0	24.6	25.7	24.9	25.0	0.5

Table A4.2 (cont'd)

16.5	25.2	26.4	25.6	25.7	0.5
17.0	25.9	27.1	26.3	26.5	0.5
17.5	27.1	28.3	27.5	27.6	0.5
18.0	28.0	29.3	28.4	28.6	0.5
18.5	28.9	30.2	29.3	29.5	0.6
19.0	29.6	31.0	30.1	30.2	0.6
19.5	30.3	31.8	30.9	31.0	0.6
20.0	31.4	33.2	32.2	32.3	0.7
20.5	32.3	34.1	33.1	33.2	0.7
21.0	33.1	35.0	34.0	34.0	0.8
21.5	33.7	35.8	34.7	34.7	0.9
22.0	34.8	37.0	35.9	35.9	0.9
22.5	35.8	38.0	36.8	36.8	0.9
23.0	36.6	38.9	37.7	37.7	0.9
23.5	37.4	39.7	38.5	38.5	0.9
24.0	38.0	40.5	39.3	39.3	1.0

Table A4.3 Effect of 1 mg/L Co on acetate utilization rate in the food waste digester

Hours	Co (1 mg/L)			AVG	STD
	1	2	3		
0.5	0.9	0.8	0.8	0.8	0.0
1.0	1.7	1.7	1.6	1.7	0.1
1.5	2.5	2.5	2.4	2.5	0.1
2.0	3.3	3.2	3.0	3.2	0.1
2.5	4.5	4.3	4.1	4.3	0.2
3.0	5.1	5.0	4.7	4.9	0.2
3.5	5.9	5.8	5.4	5.7	0.2
4.0	6.7	6.6	6.2	6.5	0.2
4.5	7.4	7.4	7.0	7.3	0.2
5.0	8.1	8.2	7.7	8.0	0.2
5.5	8.7	8.9	8.4	8.7	0.2
6.0	9.4	9.6	9.0	9.3	0.2
6.5	10.3	10.6	9.9	10.3	0.3
7.0	11.1	11.2	10.5	10.9	0.3
7.5	11.9	11.9	11.2	11.7	0.3
8.0	12.6	12.7	11.9	12.4	0.3
8.5	13.3	13.4	12.6	13.1	0.3
9.0	13.9	14.1	13.3	13.8	0.4
9.5	14.6	14.9	14.0	14.5	0.4
10.0	15.5	15.8	14.9	15.4	0.4
10.5	16.2	16.5	15.5	16.1	0.4
11.0	17.0	17.3	16.3	16.9	0.4

Table A4.3 (cont'd)

11.5	17.8	18.1	17.0	17.6	0.5
12.0	18.5	18.8	17.7	18.3	0.5
12.5	19.1	19.6	18.4	19.0	0.5
13.0	20.3	20.8	19.6	20.2	0.5
13.5	21.0	21.5	20.2	20.9	0.5
14.0	21.8	22.3	21.0	21.7	0.5
14.5	22.5	23.1	21.7	22.4	0.6
15.0	23.2	24.1	22.6	23.3	0.6
15.5	24.1	25.4	23.8	24.4	0.7
16.0	25.0	26.4	24.8	25.4	0.7
16.5	25.7	27.4	25.7	26.3	0.8
17.0	26.6	28.5	26.8	27.3	0.8
17.5	27.3	29.5	27.8	28.2	1.0
18.0	28.1	30.6	28.8	29.2	1.1
18.5	29.1	32.0	30.1	30.4	1.2
19.0	29.7	33.0	31.0	31.2	1.3
19.5	30.6	33.9	31.9	32.1	1.4
20.0	31.3	34.8	32.7	32.9	1.4
20.5	32.1	35.6	33.4	33.7	1.4
21.0	33.2	36.8	34.6	34.8	1.5
21.5	34.0	37.5	35.3	35.6	1.5
22.0	34.9	38.5	36.1	36.5	1.5
22.5	35.6	39.5	37.1	37.4	1.6
23.0	36.3	40.4	37.9	38.2	1.7
23.5	37.1	41.3	38.8	39.0	1.7
24.0	37.7	42.0	39.5	39.7	1.8

Table A4.4 Effect of 10 mg/L Co on acetate utilization rate in the food waste digester

Hours	Co (10 mg/L)			AVG	STD
	1	2	3		
0.5	0.7	0.6	0.7	0.7	0.0
1.0	1.9	1.3	2.0	1.7	0.3
1.5	2.1	1.6	2.3	2.0	0.3
2.0	2.5	2.0	2.6	2.4	0.2
2.5	2.8	2.4	2.9	2.7	0.2
3.0	3.0	2.8	3.2	3.0	0.2
3.5	3.6	3.5	3.7	3.6	0.1
4.0	4.3	4.3	4.5	4.4	0.1
4.5	4.6	4.7	4.9	4.7	0.1
5.0	4.9	5.1	5.2	5.1	0.1
5.5	5.3	5.5	5.5	5.4	0.1
6.0	5.9	6.0	6.1	6.0	0.1

Table A4.4 (cont'd)

6.5	6.8	7.0	7.2	7.0	0.1
7.0	7.1	7.3	7.5	7.3	0.1
7.5	7.4	7.8	7.8	7.6	0.2
8.0	7.8	8.1	8.2	8.0	0.2
8.5	8.1	8.4	8.5	8.4	0.2
9.0	9.1	9.4	9.5	9.3	0.2
9.5	9.5	9.9	9.9	9.8	0.2
10.0	9.8	10.3	10.3	10.1	0.2
10.5	10.1	10.6	10.7	10.5	0.2
11.0	10.5	11.0	11.0	10.8	0.2
11.5	10.8	11.4	11.4	11.2	0.3
12.0	11.8	12.5	12.4	12.2	0.3
12.5	12.1	12.9	12.7	12.6	0.3
13.0	12.4	13.2	13.0	12.8	0.4
13.5	12.6	13.6	13.2	13.1	0.4
14.0	13.4	14.8	14.0	14.1	0.6
14.5	13.6	15.2	14.3	14.3	0.7
15.0	13.8	15.6	14.5	14.6	0.7
15.5	14.1	16.0	14.8	15.0	0.8
16.0	15.0	17.1	15.7	16.0	0.9
16.5	15.4	17.5	16.1	16.3	0.9
17.0	15.6	17.8	16.4	16.6	0.9
17.5	16.0	18.2	16.8	17.0	0.9
18.0	16.6	19.3	17.5	17.8	1.1
18.5	16.9	19.6	17.8	18.1	1.1
19.0	17.2	20.0	18.0	18.4	1.2
19.5	17.5	20.3	18.4	18.7	1.2
20.0	18.3	21.4	19.2	19.7	1.3
20.5	18.5	21.7	19.5	19.9	1.4
21.0	18.7	22.2	19.7	20.2	1.4
21.5	19.4	23.0	20.4	20.9	1.5
22.0	20.0	23.6	21.0	21.6	1.5
22.5	20.6	24.3	21.7	22.2	1.6
23.0	21.3	25.1	22.4	22.9	1.6
23.5	21.7	25.6	22.8	23.4	1.6
24.0	22.0	26.0	23.1	23.7	1.7

Table A4.5 Effect of 0.01 mg/L Ni on acetate utilization rate in the food waste digester

Hours	Ni (0.01 mg/L)			AVG	STD
	1	2	3		
0.5	1.0	1.0	1.1	1.0	0.0
1.0	1.9	2.0	2.1	2.0	0.1
1.5	2.7	2.8	2.8	2.8	0.1
2.0	3.3	3.4	3.5	3.4	0.1
2.5	3.9	4.0	4.1	4.0	0.1
3.0	4.5	4.6	4.7	4.6	0.1
3.5	5.0	5.2	5.3	5.1	0.1
4.0	5.5	5.7	5.9	5.7	0.1
4.5	6.1	6.3	6.4	6.3	0.1
5.0	6.6	6.7	7.0	6.8	0.2
5.5	7.2	7.3	7.6	7.4	0.2
6.0	7.8	8.0	8.3	8.0	0.2
6.5	8.5	8.6	9.0	8.7	0.2
7.0	9.1	9.3	9.7	9.4	0.2
7.5	9.8	9.9	10.4	10.0	0.3
8.0	10.4	10.5	11.0	10.7	0.3
8.5	11.7	11.9	12.4	12.0	0.3
9.0	12.7	12.9	13.4	13.0	0.3
9.5	13.6	13.9	14.4	14.0	0.3
10.0	14.4	14.8	15.3	14.8	0.4
10.5	15.1	15.5	16.0	15.5	0.4
11.0	15.8	16.2	16.7	16.2	0.4
11.5	16.6	17.1	17.5	17.1	0.4
12.0	17.8	18.3	18.8	18.3	0.4
12.5	18.7	19.3	19.8	19.3	0.5
13.0	19.5	20.2	20.7	20.1	0.5
13.5	20.3	20.9	21.5	20.9	0.5
14.0	20.9	21.6	22.2	21.6	0.5
14.5	21.5	22.3	22.8	22.2	0.5
15.0	22.9	23.8	24.2	23.6	0.6
15.5	23.9	24.8	25.3	24.6	0.6
16.0	24.7	25.5	26.2	25.5	0.6
16.5	25.4	26.4	27.0	26.3	0.6
17.0	26.2	27.1	27.7	27.0	0.6
17.5	27.3	28.3	29.0	28.2	0.7
18.0	28.3	29.5	30.0	29.3	0.7
18.5	29.3	30.5	31.0	30.3	0.7
19.0	30.1	31.4	31.9	31.1	0.8
19.5	30.8	32.0	32.6	31.8	0.8
20.0	32.1	33.5	34.0	33.2	0.8

Table A4.5 (cont'd)

20.5	33.1	34.6	35.1	34.3	0.8
21.0	33.9	35.5	36.0	35.1	0.9
21.5	34.7	36.4	36.8	36.0	0.9
22.0	35.8	37.7	38.0	37.2	1.0
22.5	37.0	38.9	39.2	38.3	1.0
23.0	37.9	39.9	40.2	39.3	1.0
23.5	38.7	40.8	41.1	40.2	1.0
24.0	39.5	41.6	41.8	41.0	1.1

Table A4.6 Effect of 1 mg/L Ni on acetate utilization rate in the food waste digester

Hours	Ni (1 mg/L)			AVG	STD
	1	2	3		
0.5	1.4	1.3	1.3	1.3	0.0
1.0	2.7	2.6	2.6	2.6	0.1
1.5	4.0	3.8	3.8	3.9	0.1
2.0	5.1	4.9	4.9	5.0	0.1
2.5	6.9	6.6	6.6	6.7	0.2
3.0	8.2	7.8	7.9	8.0	0.2
3.5	9.7	9.2	9.3	9.4	0.2
4.0	11.0	10.5	10.6	10.7	0.2
4.5	12.3	11.7	11.8	12.0	0.3
5.0	13.6	12.9	13.1	13.2	0.3
5.5	14.8	14.1	14.2	14.4	0.3
6.0	16.0	15.2	15.3	15.5	0.3
6.5	17.8	16.9	17.1	17.3	0.4
7.0	19.0	18.1	18.3	18.5	0.4
7.5	20.5	19.5	19.7	19.9	0.4
8.0	22.0	20.9	21.1	21.3	0.5
8.5	23.3	22.1	22.4	22.6	0.5
9.0	24.6	23.4	23.6	23.9	0.5
9.5	26.1	24.8	25.1	25.3	0.6
10.0	27.9	26.5	26.7	27.0	0.6
10.5	29.3	27.8	28.1	28.4	0.6
11.0	30.8	29.2	29.6	29.9	0.7
11.5	32.3	30.6	31.0	31.3	0.7
12.0	33.7	32.0	32.3	32.7	0.7
12.5	35.0	33.3	33.6	34.0	0.8
13.0	37.0	35.2	35.5	35.9	0.8
13.5	38.4	36.5	36.9	37.3	0.8
14.0	40.0	38.0	38.4	38.8	0.9
14.5	41.5	39.4	39.8	40.3	0.9
15.0	42.9	40.8	41.2	41.6	0.9

Table A4.6 (cont'd)

15.5	44.7	42.5	42.9	43.4	1.0
16.0	46.3	44.0	44.5	44.9	1.0
16.5	47.8	45.4	45.9	46.4	1.0
17.0	49.4	46.9	47.5	47.9	1.1
17.5	50.9	48.3	48.9	49.4	1.1
18.0	52.5	49.9	50.4	50.9	1.1
18.5	54.4	51.7	52.3	52.8	1.2
19.0	55.9	53.1	53.7	54.2	1.2
19.5	57.5	54.6	55.2	55.8	1.3
20.0	59.0	56.0	56.7	57.2	1.3
20.5	60.5	57.4	58.0	58.6	1.3
21.0	62.4	59.3	59.9	60.5	1.4
21.5	63.9	60.7	61.4	62.0	1.4
22.0	65.6	62.3	63.0	63.6	1.4
22.5	67.2	63.8	64.5	65.1	1.5
23.0	68.7	65.2	66.0	66.6	1.5
23.5	70.2	66.7	67.4	68.1	1.5
24.0	71.6	68.0	68.8	69.5	1.6

Table A4.7 Effect of 10 mg/L Ni on acetate utilization rate in the food waste digester

Hours	Ni (10 mg/L)			AVG	STD
	1	2	3		
0.5	0.4	0.5	0.5	0.5	0.0
1.0	1.8	2.0	1.9	1.9	0.1
1.5	1.9	2.1	2.0	2.0	0.1
2.0	1.9	2.1	2.1	2.0	0.1
2.5	2.0	2.2	2.1	2.1	0.1
3.0	2.0	2.2	2.2	2.1	0.1
3.5	2.3	2.5	2.4	2.4	0.1
4.0	3.1	3.4	3.3	3.3	0.1
4.5	3.2	3.5	3.4	3.4	0.1
5.0	3.4	3.7	3.6	3.6	0.1
5.5	3.6	3.9	3.8	3.8	0.2
6.0	3.7	4.0	3.9	3.9	0.2
6.5	4.6	5.1	5.0	4.9	0.2
7.0	4.8	5.3	5.1	5.1	0.2
7.5	5.0	5.5	5.4	5.3	0.2
8.0	5.2	5.7	5.6	5.5	0.2
8.5	5.3	5.9	5.8	5.7	0.2
9.0	6.5	7.2	7.0	6.9	0.3
9.5	6.8	7.5	7.3	7.2	0.3
10.0	7.0	7.7	7.5	7.4	0.3

Table A4.7 (cont'd)

10.5	7.2	8.0	7.8	7.7	0.3
11.0	7.5	8.2	8.0	7.9	0.3
11.5	7.7	8.4	8.3	8.1	0.3
12.0	9.0	9.9	9.7	9.5	0.4
12.5	9.2	10.1	9.9	9.7	0.4
13.0	9.4	10.3	10.1	9.9	0.4
13.5	9.6	10.5	10.3	10.2	0.4
14.0	10.9	12.0	11.8	11.6	0.5
14.5	11.2	12.3	12.0	11.8	0.5
15.0	11.4	12.5	12.3	12.0	0.5
15.5	11.7	12.8	12.6	12.4	0.5
16.0	12.9	14.2	13.9	13.7	0.5
16.5	13.3	14.6	14.3	14.0	0.6
17.0	13.5	14.8	14.5	14.2	0.6
17.5	13.7	15.1	14.8	14.6	0.6
18.0	15.0	16.5	16.2	15.9	0.6
18.5	15.3	16.8	16.5	16.2	0.6
19.0	15.5	17.1	16.7	16.4	0.7
19.5	15.8	17.3	17.0	16.7	0.7
20.0	17.1	18.8	18.4	18.1	0.7
20.5	17.3	19.0	18.6	18.3	0.7
21.0	17.6	19.3	19.0	18.6	0.7
21.5	18.4	20.2	19.8	19.5	0.8
22.0	19.1	21.0	20.6	20.3	0.8
22.5	19.9	21.8	21.4	21.0	0.8
23.0	20.7	22.7	22.3	21.9	0.9
23.5	21.0	23.1	22.6	22.3	0.9
24.0	21.4	23.5	23.0	22.6	0.9

Table A4.8 Effect of 0.5 mg/L Fe on acetate utilization rate in the food waste digester

Hours	Fe (0.5 mg/L)			AVG	STD
	1	2	3		
0.5	2.9	3.3	3.1	3.1	0.1
1.0	3.8	4.1	4.0	4.0	0.1
1.5	5.2	5.7	5.5	5.5	0.2
2.0	6.1	6.7	6.4	6.4	0.2
2.5	7.0	7.7	7.4	7.3	0.3
3.0	8.0	8.7	8.4	8.4	0.3
3.5	8.9	9.6	9.4	9.3	0.3
4.0	9.8	10.5	10.4	10.2	0.3
4.5	10.6	11.3	11.3	11.1	0.3
5.0	11.4	11.8	12.1	11.8	0.3

Table A4.8 (cont'd)

5.5	12.8	13.3	13.6	13.2	0.3
6.0	13.6	14.3	14.4	14.1	0.4
6.5	14.5	15.4	15.3	15.1	0.4
7.0	15.4	16.4	16.3	16.1	0.5
7.5	16.3	17.4	17.3	17.0	0.5
8.0	17.2	18.5	18.2	17.9	0.6
8.5	18.2	19.6	19.2	19.0	0.6
9.0	19.3	20.9	20.5	20.2	0.7
9.5	20.1	21.5	21.3	20.9	0.6
10.0	21.1	22.4	22.3	21.9	0.6
10.5	22.0	23.4	23.3	22.9	0.6
11.0	22.9	24.5	24.3	23.9	0.7
11.5	23.7	25.3	25.2	24.7	0.7
12.0	25.2	26.6	26.7	26.1	0.7
12.5	26.0	27.7	27.5	27.1	0.8
13.0	26.9	29.0	28.5	28.1	0.9
13.5	27.9	30.2	29.5	29.2	1.0
14.0	28.7	31.1	30.5	30.1	1.0
14.5	30.1	32.4	31.9	31.5	1.0
15.0	31.1	33.4	32.9	32.5	1.0
15.5	31.9	34.6	33.8	33.4	1.1
16.0	32.9	35.9	34.9	34.6	1.2
16.5	33.9	37.0	35.9	35.6	1.3
17.0	35.0	38.2	37.0	36.7	1.3
17.5	36.3	39.9	38.4	38.2	1.5
18.0	37.1	41.0	39.3	39.2	1.6
18.5	38.1	42.3	40.4	40.2	1.7
19.0	39.0	43.2	41.4	41.2	1.7
19.5	40.0	44.0	42.4	42.1	1.7
20.0	41.4	45.2	43.9	43.5	1.6
20.5	42.3	45.8	44.8	44.3	1.5
21.0	43.3	46.7	45.9	45.3	1.5
21.5	44.3	47.5	47.0	46.3	1.4
22.0	45.3	48.3	48.0	47.2	1.3
22.5	46.3	49.0	49.0	48.1	1.3
23.0	47.2	49.9	50.0	49.0	1.3
23.5	48.4	51.1	51.3	50.3	1.3
24.0	49.5	52.5	52.4	51.5	1.4

Table A4.9 Effect of 5 mg/L Fe on acetate utilization rate in the food waste digester

Hours	Fe (5 mg/L)			AVG	STD
	1	2	3		
0.5	3.1	3.2	3.3	3.2	0.1
1.0	3.9	4.0	4.2	4.0	0.1
1.5	4.6	4.7	4.9	4.8	0.1
2.0	5.4	5.5	5.7	5.5	0.1
2.5	6.1	6.2	6.4	6.2	0.2
3.0	6.8	6.9	7.2	7.0	0.2
3.5	7.4	7.6	7.8	7.6	0.2
4.0	8.2	8.3	8.6	8.4	0.2
4.5	9.0	9.1	9.5	9.2	0.2
5.0	9.7	9.9	10.3	9.9	0.2
5.5	10.6	10.8	11.2	10.8	0.3
6.0	11.4	11.6	12.1	11.7	0.3
6.5	12.1	12.4	12.8	12.4	0.3
7.0	12.8	13.1	13.6	13.1	0.3
7.5	14.2	14.5	15.1	14.6	0.4
8.0	15.3	15.6	16.2	15.7	0.4
8.5	16.4	16.7	17.3	16.8	0.4
9.0	17.3	17.7	18.3	17.8	0.4
9.5	18.2	18.6	19.3	18.7	0.5
10.0	18.9	19.3	20.1	19.5	0.5
10.5	19.9	20.3	21.1	20.5	0.5
11.0	21.2	21.7	22.5	21.8	0.5
11.5	22.3	22.8	23.7	22.9	0.6
12.0	23.4	23.8	24.8	24.0	0.6
12.5	24.3	24.8	25.7	24.9	0.6
13.0	25.1	25.6	26.6	25.7	0.6
13.5	25.8	26.3	27.3	26.5	0.6
14.0	27.2	27.8	28.8	27.9	0.7
14.5	28.3	28.9	30.0	29.1	0.7
15.0	29.4	30.0	31.1	30.2	0.7
15.5	30.3	30.9	32.1	31.1	0.8
16.0	31.1	31.8	33.0	32.0	0.8
16.5	32.4	33.1	34.4	33.3	0.8
17.0	33.6	34.3	35.6	34.5	0.8
17.5	34.6	35.4	36.7	35.6	0.9
18.0	35.6	36.3	37.7	36.5	0.9
18.5	36.5	37.2	38.7	37.5	0.9
19.0	38.0	38.8	40.3	39.0	0.9
19.5	39.2	40.0	41.5	40.2	1.0
20.0	40.2	41.0	42.6	41.2	1.0
20.5	41.1	42.0	43.6	42.2	1.0

Table A4.9 (cont'd)

21.0	42.4	43.3	44.9	43.5	1.1
21.5	43.7	44.6	46.3	44.9	1.1
22.0	44.8	45.7	47.5	46.0	1.1
22.5	45.8	46.8	48.6	47.1	1.1
23.0	46.7	47.7	49.5	48.0	1.2
23.5	48.2	49.1	51.0	49.4	1.2
24.0	49.3	50.3	52.3	50.6	1.2

A4.10 Effect of 100 mg/L Fe on acetate utilization rate in the food waste digester

Hours	Fe (100 mg/L)			AVG	STD
	1	2	3		
0.5	1.6	1.6	1.8	1.7	0.1
1.0	4.0	3.5	4.6	4.1	0.5
1.5	4.5	4.3	5.2	4.7	0.4
2.0	4.9	5.1	5.7	5.2	0.3
2.5	5.4	6.0	6.2	5.9	0.3
3.0	6.1	6.8	7.1	6.7	0.4
3.5	7.7	8.4	8.8	8.3	0.5
4.0	9.0	10.1	10.3	9.8	0.6
4.5	9.7	11.0	11.1	10.6	0.7
5.0	10.4	12.0	11.9	11.4	0.8
5.5	10.8	12.9	12.4	12.0	0.9
6.0	11.5	14.2	13.3	13.0	1.1
6.5	12.8	16.0	14.8	14.5	1.3
7.0	13.3	16.9	15.3	15.2	1.5
7.5	14.0	17.6	16.1	15.9	1.5
8.0	14.5	18.5	16.7	16.6	1.6
8.5	15.1	19.3	17.4	17.2	1.7
9.0	16.8	21.1	19.3	19.1	1.8
9.5	17.4	22.1	20.1	19.9	1.9
10.0	17.9	22.9	20.6	20.5	2.0
10.5	18.8	23.7	21.6	21.4	2.0
11.0	19.3	24.5	22.3	22.0	2.1
11.5	19.9	25.1	22.9	22.6	2.2
12.0	21.4	26.8	24.7	24.3	2.2
12.5	21.9	27.4	25.2	24.8	2.3
13.0	22.4	28.2	25.8	25.5	2.4
13.5	23.3	29.1	26.8	26.4	2.4
14.0	24.9	31.1	28.7	28.3	2.6
14.5	25.6	32.2	29.5	29.1	2.7
15.0	26.4	33.3	30.4	30.0	2.8

Table A4.10 (cont'd)

15.5	27.1	34.1	31.2	30.8	2.9
16.0	28.7	35.6	33.1	32.5	2.9
16.5	29.6	36.5	34.1	33.4	2.8
17.0	30.2	37.3	34.8	34.1	2.9
17.5	30.8	38.1	35.5	34.8	3.0
18.0	33.4	39.5	38.5	37.2	2.7
18.5	34.9	40.5	40.2	38.5	2.6
19.0	36.2	41.2	41.7	39.7	2.5
19.5	37.6	42.0	43.3	41.0	2.4
20.0	40.4	43.6	46.5	43.5	2.5
20.5	41.3	44.4	47.6	44.4	2.6
21.0	42.2	45.0	48.6	45.3	2.6
21.5	43.5	46.2	50.1	46.6	2.7
22.0	44.8	47.2	51.6	47.9	2.8
22.5	46.1	48.3	53.2	49.2	2.9
23.0	47.5	50.1	54.8	50.8	3.0
23.5	48.4	51.0	55.8	51.7	3.1
24.0	49.4	52.0	56.9	52.7	3.1

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