EFFECT OF TERMITE GUT MICROBE TAV5 ON METHANE PRODUCTION FROM MUNICIPAL SOLID WASTE DEGRADATION

by

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Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

Of the Requirements

For the Degree of

MASTER OF SCIENCE IN CIVIL ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

December 2015

Acknowledgements

I would like to express my sincere gratitude to my advisor, Dr. Melanie Sattler, for the motivation and support she provided me throughout this work. Dr. Sattler has been a true mentor, and her patience and constructive comments have helped me become a better researcher. The completion of this work would not have been possible without her continuous guidance, valuable suggestions and ever present encouragement.

I would like to express my gratitude to Dr. Hossain for giving me the opportunity to work in his laboratory and to Dr. Hossain for agreeing to serve on my committee.

I would further like to extend my sincere appreciation to my colleagues and friends for their constant cooperation and assistance throughout my graduate studies. Special thanks to Dr. Dipak Tiwari, Dr Rodrigues who allowed me to use their strain from termite gut and his Ph.D. student Malini Kotak who helped me learn about culturing microbes and was very supportive to get permission to use some labs in the Biology Department.

Infinite gratitude goes to my family - my parents, siblings for their endless support and encouragement.

November 16, 2015

Abstract

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The University of Texas at Arlington, 2015

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Methane from anaerobic processes is being increasingly utilized as an alternative energy source in developed countries, via large projects that extract methane from landfills or wastewater treatment plants. Anaerobic degradation of organic material (biomass) involves decomposition by bacteria under humid conditions without any oxygen. Organic carbon deposited in landfills is converted by microbes to carbon dioxide (CO_2) and methane (CH_4). If the methane is not captured, it contributes to climate change (25 times more effectively than CO_2 on a per-mass basis). If the methane is captured, it can be used as a renewable energy resource.

Lignocellulose comprises a significant portion of MSW - 40-70% in developed countries, including paper, wood, yard waste, and textiles such as cotton fibers. Cellulose, the main biodegradable plant polymer, is often shielded by lignin, as well as hemicellulose. Lignin is unfortunately resistant to microbial degradation under anaerobic conditions that normally occur in MSW landfills. Lignin destruction can make cellulose and hemicelluloses available for anaerobic microbial conversion to biogas. This could potentially increase a landfill's methane production by a factor of 2-3, depending on waste composition.

The objective of the current study was to determine the effect of a specific kind of microbe from termite gut, TAV5, in a mixed culture on accelerating MSW decomposition rate and gas generation. To accomplish this, three kinds of waste, including paper and cardboard, yard waste and wood were collected. Six laboratory scale reactors were prepared with selected MSW and operated as bioreactors by recirculating leachate. Three of these reactors were seeded with a mixture of cultures from an anaerobic digester and incubated at 38 °C, optimum temperature for these microbes. The other 3 were seeded with termite gut microbe TAV 5 added to a mixed culture from an anaerobic digester and incubated at 30 °C because the optimum temperature for TAV5 is 30 °C. The pH level was controlled in the recirculated leachate.

For both sets of reactors (through day 63 of operation of the second set), the paper reactor had the highest rate of methane generation, because paper has the largest amount of cellulose among yard waste and wood, and higher surface area in comparison with wood, as well as the largest cumulative volume of methane, followed by yard waste, and finally wood waste. The 3 reactors seeded with the TAV5 microbe reached the methanogenesis phase faster than the 3 reactors seeded with ordinary digester sludge. Through the first 63 days of reactor operation, for paper, yard waste, and wood waste, the reactors seeded with both TAV5 and digester microbes had higher rates of methane generation, as well as larger cumulative volumes of methane generated, compared to the reactors seeded with digester microbes only. Initial results thus indicate that TAV5 is increasing methane generation rate and quantity of methane generation. Reactor operation will be continued.

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Chapter 1

Introduction

1.1 Background

Landfills serve not only as waste repositories but also as significant sources of renewable energy. When microbes degrade the organic fraction of waste, methane (CH_4) is generated, along with carbon dioxide (CO_2), water, and other trace landfill gas (LFG) constituents. Methane, the primary constituent of natural gas, can be captured and used to generate electricity.

While the percentage of total municipal solid waste (MSW) being disposed of in landfills in the US is decreasing, the actual tonnage was expected to increase from 118 million tons in 1995 to 125 million tons by 2010. However, as a result of the economic boom, the tonnage had already increased to 132 million tons in 1999. It is expected that landfill disposal will continue to be the single most predominant MSW management method in future years (US EPA, 2011).

Paper is a major component of MSW, even considering that over 50% of paper products are recycled. Yard trimmings and food scraps, the other major organic wastes, combined with paper make up the largest component of MSW (EPA, 2011).

Total MSW generation in 2012 was 251 million tons. Figure 1-1, and 1-2 shows the total MSW generation in 2012 and total MSW recovery. Organic materials such as paper and paperboard, yard trimmings, and food waste continue to be the largest component of MSW. Paper and paperboard account for over 27.4 percent and yard trimmings 22.6 percent and food waste accounts 14.5 percent. Wood follows at over 6.3 percent. Total MSW recovery in 2012 was almost 87 million tons. Paper and paperboard account for over 51.2 percent and yard trimmings account for over 22.6 percent, while food waste accounts for another 2 percent, and plastic and wood about 3 percent each (US EPA, 2012).

Figure 1-1 Total MSW generation by materials before recycling in 2012

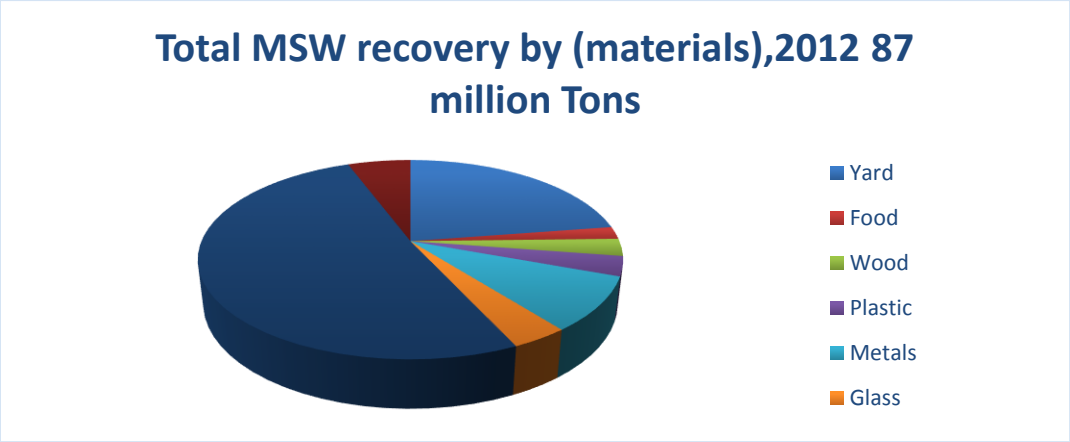


Figure 1-2 Total MSW recovery by materials in 2012

Disposal of waste by burying is one of the oldest forms of waste management. Originally landfills were designed to encapsulate and store waste. However, recent shifts have occurred and many landfills are now designed to promote active biological processes instead of as permanent storage. One of the management strategies developed and studied is a bioreactor landfill.

More than 50% of the 149 million metric tons of municipal solid waste (MSW) landfilled in the U.S. annually are derived from lignocellulosic materials (e.g. food and yard waste, wood, and pulp and paper products) (de la Cruz, 2014).As lignin is approximately 15% of residential solid

waste, understanding its behavior during anaerobic decomposition in landfills is important for a complete description of carbon decomposition and storage in landfills (de la Cruz, 2014). The overall objective of this study was to compare the decomposition of lignin during anaerobic decomposition using a mixed culture of microbes and a pure culture of microbes from termite gut which are hypothesized to accelerate the rate of decomposition in bioreactors. According to de la Cruz, “Woody tissues make up about 75% of terrestrial plant biomass, which in turn is estimated to represent 0.95 × 1018 g, or 29% of the active global organic carbon reservoir. As plant tissues are composed primarily of lignocellulosic material, the study of lignocellulose decomposition is essential to understanding carbon turnover in the environment. Plant biomass is made up primarily of three biopolymers: cellulose, hemicelluloses, and lignin. While both cellulose and hemicelluloses are readily converted to methane and carbon dioxide during anaerobic decomposition, lignin is generally considered preserved. Information on the chemical changes in lignocellulose during anaerobic decomposition is important toward understanding the fate and reactivity of lignocellulose in anaerobic environments, such as landfills, which are estimated to receive about 149 million metric tons of MSW annually in the U.S. Lignocellulose in MSW takes the form of paper products, wood, food, and yard waste. The storage of carbon in landfills due to the recalcitrance of lignocellulose has been reported. Furthermore, lignocellulosic materials from MSW represent viable feedstock for production of energy and valuable chemicals. (De la Cruz et al., 2014)

1.2 Bioreactor Landfill for Municipal Solid Waste Decomposition

A bioreactor landfill is a waste treatment landfill with technology which can accelerate decomposition of organic wastes in landfill. The increase in waste degradation and stabilization is accomplished through the addition of liquid and sometimes air to enhance microbial processes. This bioreactor concept differs from the traditional “dry tomb” municipal landfill approach. A bioreactor landfill is not just a

single design but will vary according to the operational process chosen. There are three different general types of bioreactor landfill configurations:

- Aerobic - In an aerobic bioreactor landfill, leachate is removed from the bottom layer, piped to liquids storage tanks, and re-circulated into the landfill in a controlled manner. Air is injected into the waste mass, using vertical or horizontal wells, to promote aerobic activity and accelerate waste stabilization.
- Anaerobic - In an anaerobic bioreactor landfill, moisture is added to the waste mass in the form of re-circulated leachate and other sources to obtain optimal moisture levels. Biodegradation occurs in the absence of oxygen (an aerobically) and produces landfill gas. Landfill gas, primarily methane, can be captured to minimize greenhouse gas emissions and for energy projects. Figure1-3 shows anaerobic processes in landfills.

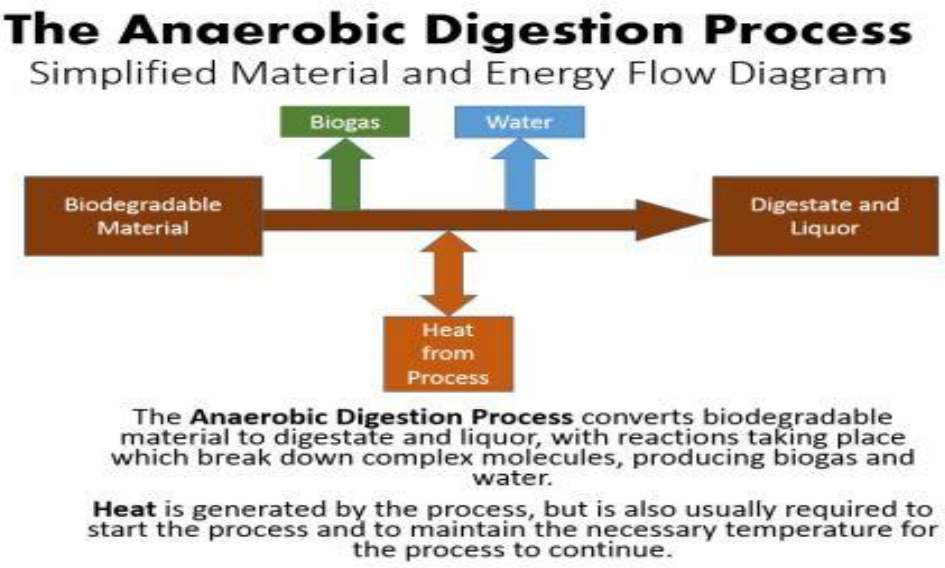


Figure 1-3 Anaerobic digestion process (US EPA, 2002)

- Hybrid (Aerobic-Anaerobic) - The hybrid bioreactor landfill accelerates waste degradation by employing a sequential aerobic-anaerobic treatment to rapidly degrade organics in the upper sections of the landfill and collect gas from lower sections. Operation as a hybrid results in the earlier onset of methanogenesis compared to aerobic landfills.(US EPA, 2003)

A bioreactor landfill is an engineered waste disposal site which has several advantages over conventional dry tomb landfills. Potential advantages of bioreactors include:

- Decomposition and biological stabilization in years vs. decades in “dry tombs”
- Lower waste toxicity and mobility due to both aerobic and anaerobic conditions
- Reduced leachate disposal costs
- A 15 to 30 percent gain in landfill space due to an increase in density of waste mass
- Significant increased LFG generation that, when captured, can be used for energy use onsite or sold
- Reduced post-closure care.

In conventional dry tomb landfills, no external moisture intrusion is allowed. As a result, the initial moisture content of the disposed waste is the only source of moisture for waste degradation. This causes a slower rate of biodegradation, taking a long time, sometimes more than 50 years. A bioreactor landfill, in contrast, is operated to enhance the microbial activity, which leads to faster degradation of waste. Moreover, bioreactor landfills have rapid settlement of waste, which leads to increased disposal capacity. Recirculation of leachate reduces the cost of wastewater treatment and increase microbial activity, which results in increased gas generation and ensuing energy conversion. The generated gas in bioreactor landfills has a high

methane flow rate, which is currently utilized in several parts in United States to produce electricity (Hettiaratchi et al., 2010).

Research has shown that municipal solid waste can be rapidly degraded and made less hazardous (due to degradation of organics and the sequestration of inorganics) by enhancing and controlling the moisture within the landfill under aerobic and/or anaerobic conditions. Leachate quality in a bioreactor rapidly improves, which leads to reduced leachate disposal costs. Landfill volume may also decrease, with the recovered airspace offering landfill operators an extended operating life for the landfill.

LFG emitted by a bioreactor landfill consists primarily of methane and carbon dioxide, plus lesser amounts of volatile organic chemicals and/or hazardous air pollutants. Research indicates that the operation of a bioreactor may generate LFG earlier in the process and at a higher rate than the traditional landfill. The bioreactor LFG is also generated over a shorter period of time because the LFG emissions decline as the accelerated decomposition process depletes the source waste faster than in a traditional landfill.

Some studies indicate that the bioreactor increases the feasibility for cost-effective LFG recovery, which in turn would reduce fugitive emissions. This presents an opportunity for beneficial reuse of bioreactor LFG in energy recovery projects. Currently, the use of LFG (in traditional and bioreactor landfills) for energy applications is only about 10 percent of its potential use. The US Department of Energy estimates that if controlled bioreactor technology were applied to 50 percent of the waste currently being landfilled, it could provide over 270 billion cubic feet of methane a year, which is equivalent to one percent of US electrical needs (US EPA MUNICIPAL LANDFTLL BIOREACTORS, 2003). Figure1-4 shows anaerobic bio reactor landfills.

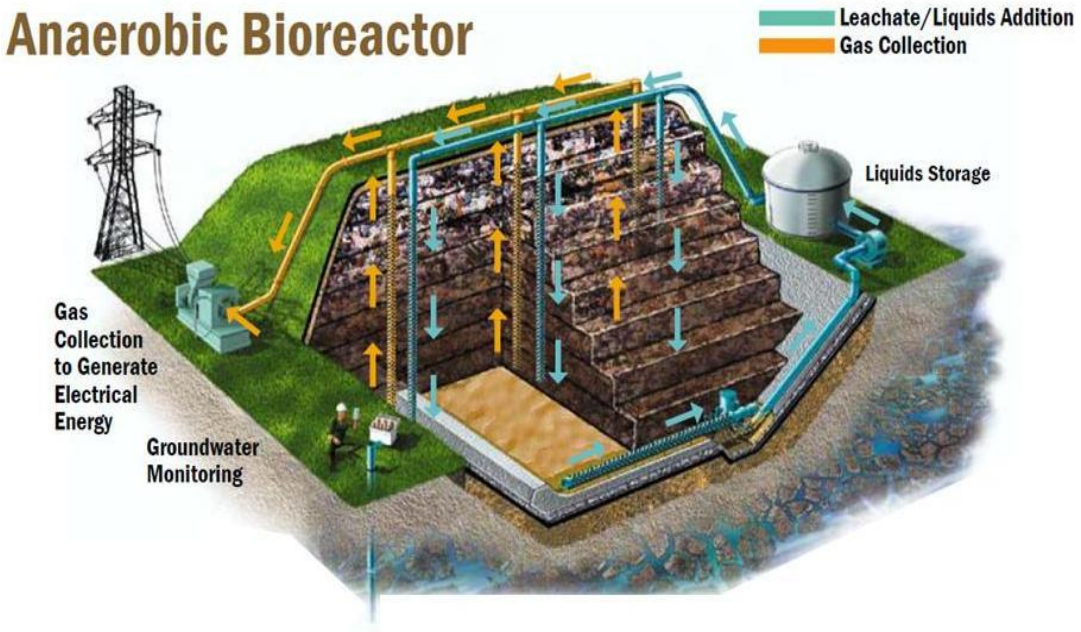


Figure 1-4 Anaerobic Bioreactor Landfills (US EPA, 2002)

1.3 Research Objectives

Paper and paperboard are major constituents found in US landfills. Typically paper consists of 79% to 98% of lignocellulose, which is considered to be the most abundant source of natural carbon on earth. Wood consists of 86% lignocellulose, grass 65 % to 74%, and leaves 47 % to 67.7%.

Lignin percent in paper is 15% to 23.9%, for grass is 17.6% to 28.4 %, for leaves 33.9 %to 43.8 %, and for branches and wood is 32.6 %.

Many microorganisms are capable of degrading and utilizing cellulose and hemi-cellulose as carbon and energy sources, while lignin is highly resistant to degradation (Higuchi, 2006). Therefore, at later stages of bioreactor operation, most of the undigested MSW could be lignin-rich waste materials. Lignin may also hinder cellulose/hemicellulose degradation in conventional

landfills, by blocking microbe access to cellulose/hemicellulose. Facilitating lignin degradation would enable more methane to be generated from landfills.

Termites are widely known to degrade wood. Accordingly, a specific kind of microbes from termite gut Verrucomicrobium TAV5 will be tested in this research to determine whether it increases methane production from solid waste containing lignin. The bacterium strain TAV5, a member of Phylum Verrucomicrobium, was isolated from the wood feeding termite hind gut. Specifically, methane generation from lignin-containing wastes (paper and card board, yard waste and wood) will be measured in lab-scale landfill reactors operated as bioreactors. One set of reactors will be seeded with a mixed culture of anaerobic microbes, and the other set with 50% microbes from termite gut (TAV5) and 50% mixed culture.

1.4 Thesis Organization

The remainder of the thesis is organized in the following manner:

The second chapter reviews the literature on municipal solid waste properties, landfilling methods and operation, and biodegradation of solid waste.

The third chapter describes the experimental setup and required laboratory test methodologies to address the research objective.

The fourth chapter presents and discusses the test results.

The fifth chapter summarizes the main conclusions of the present study and provides some recommendations for future research work.

Chapter 2

Literature Review

2.1 Introduction

This chapter includes the literature review on Municipal Solid Waste (MSW), landfills, degradation of waste in landfills, gas generation from landfills, and effects of TAV5 genome from termite gut on degradation of lignin, and gas generation of the landfills.

2.2 Municipal Solid Waste (MSW)

Municipal solid waste (MSW), commonly known as trash or garbage, consists of paper, plastic, food waste, package wrappings, glass, wood, textile, metal, etc. The heterogeneous nature of MSW is the outcome of the diverse sources of waste flow from residential, commercial, and institutional sources Wastes from industrial, hazardous, and construction sources are not categorized as MSW (US EPA, 2011).

2.2.1 Composition and properties of Municipal Solid Waste

Landfill wastes from various sources can be primarily categorized into two major categories- biodegradable and non-biodegradable. Decomposable materials like food waste, paper, wood, and textile fall into the biodegradable category, whereas non-degradable materials include plastic, glass, metals, and construction and demolition debris. Faster decomposition of materials can be ensured with a high percentage of organic contents in the waste. Food wastes decompose quickly compared to other organic components, giving a rise in landfill gas generation in the initial stage. Wood, paper, and clothes are not quickly decomposed but are degraded slowly with time.

Figure 2-1 shows the rate of degradation of waste components in a landfill.

Figure 2-1 Graphical representation of relative rate of degradation of waste components in a landfill (decomposition timeline hillside, NBCI, 2010)

According to U.S. EPA (2012), in the United States, 251 million tons of solid waste was generated in 2012. Though a recycling rate of 34.5 percent was achieved, a large amount of waste was disposed of in landfills (Table 2-1).

Table 2-1 Amount of waste disposed in landfills (EPA, 2012)

Material	Weight Generated	Weight Recovered	Recovery as Percent of Generation	Weight Discarded
Paper and paperboard	68.62	44.36	64.6%	24.26
Glass	11.57	3.20	27.7%	8.37
Metals				
Steel	16.80	5.55	33.0%	11.25
Aluminum	3.58	0.71	19.8%	2.87
Other nonferrous metals†	2.00	1.36	68.0%	0.64
Total metals	**22.38**	**7.62**	**34.0%**	**14.76**
Plastics	31.75	2.80	8.8%	28.95
Rubber and leather	7.53	1.35	17.9%	6.18
Textiles	14.33	2.25	15.7%	12.08
Wood	15.82	2.41	15.2%	13.41
Other materials	4.60	1.30	28.3%	3.30
Total materials in products	**176.60**	**65.29**	**37.0%**	**111.31**
Other wastes				
Food, other‡	36.43	1.74	4.8%	34.69
Yard trimmings	33.96	19.59	57.7%	14.37
Miscellaneous inorganic wastes	3.90	Negligible	Negligible	3.90
Total other wastes	**74.29**	**21.33**	**28.7%**	**52.96**
Total municipal solid waste	**250.89**	**86.62**	**34.5%**	**164.27**

* Includes waste from residential, commercial, and institutional sources.
† Includes lead from lead-acid batteries.
‡ Includes recovery of other MSW organics for composting.
Details might not add to totals due to rounding.
Negligible = Less than 5,000 tons or 0.05 percent.

In 2012, approximately 30.31 million tons of waste was landfilled in Texas according to the Texas Commission on Environmental Quality (TCEQ). Figure 2-2 shows Texas total and per capita waste disposal.

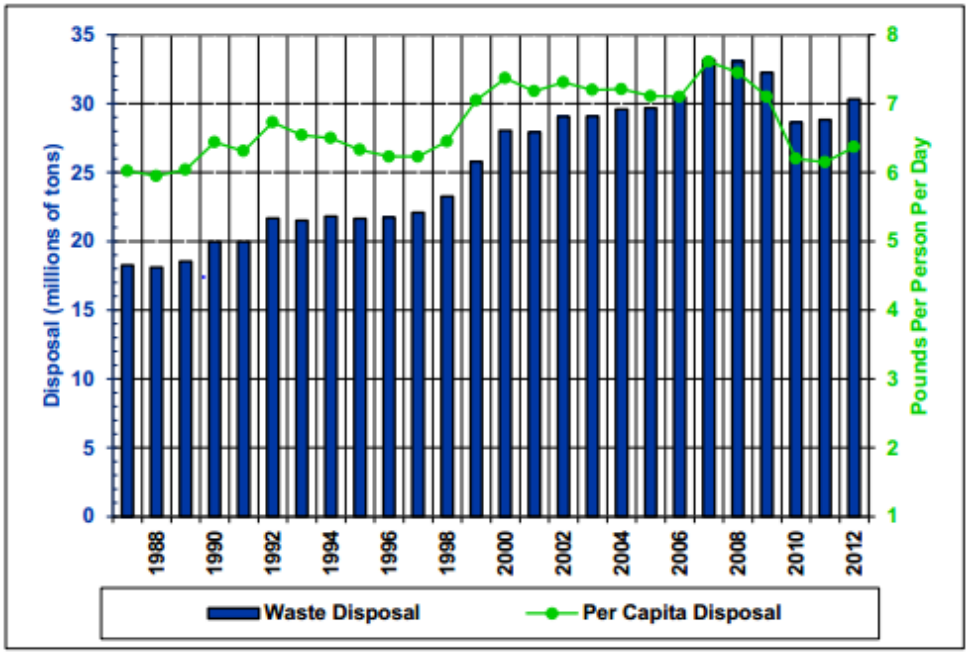


Figure 2-2 Texas total and per capita waste disposal (TCEQ Report, 2013)

2.2.2 Lignocellulosic Waste

Termites are widely known to degrade wood. Accordingly, a specific kind of microbes from termite gut Verrucomicrobium TAV5 will be tested in this research to determine whether it increases methane production from solid waste containing lignin. The bacterium strain TAV5, a member of Phylum Verrucomicrobium, was isolated from the wood feeding termite hind gut. Specifically, methane generation from lignin-containing wastes (paper and card board, yard waste and wood) will be measured in lab-scale landfill reactors operated as bioreactors. One set of reactors will be seeded with a mixed culture of anaerobic microbes, and the other set with 50% microbes from termite gut (TAV5) and 50% mixed culture.

, understanding its behavior during anaerobic decomposition in landfills is important for a complete description of carbon decomposition and storage in landfills.

2.3 Landfills

Landfills have been considered as one of the most economic options for solid waste disposal. Two types of landfills can be found around the US – conventional landfills and more recently bioreactor landfills. These two types of landfills are discussed in the following sections.

2.3.1 Conventional Landfills (Dry Tomb Landfills)

The design parameters and operational procedures of conventional landfills are based on the principles described in Subtitle D of the Resource Conservation and Recovery Act (Federal Register, 1991). They are also known as 'dry tomb landfills'. In conventional landfills, the decomposition rate of waste is low because of the absence of auspicious surroundings that are needed to enhance microbial activity. Thus it takes a long time, sometimes as long as 100 years, to complete total decomposition for landfilled waste. According to the regulations, a post-closure monitoring period of 30 years is specified, which adds to the long life span of conventional landfills (Barlaz et al., 2002). To enhance microbial decomposition and minimize the long-term monitoring requirements, a novel approach to landfill design was proposed by Pohland in the 1970s (Pohland, 1970), which is known as bioreactor landfills, or ELR (i.e. Enhanced Leachate Recirculation) landfills.

2.3.2 Bioreactor or ELR Landfills

Bioreactor or ELR landfills introduced the concept of adding additional water to the landfilled waste to increase microbial activity and recirculation of generated leachate afterwards. Research conducted by Barlaz showed that additional moisture will enhance microbial activity by providing better interactions among insoluble substrates, soluble nutrients, and microorganisms (Barlaz et al., 1990). In bioreactor landfills, decomposition of degradable fractions occurs rapidly and within 5-10 years the landfills are stabilized, which is less than the time required for post-closure of the RCRA Subtitle D landfills.

Advantages of bioreactor landfills over conventional landfills are:

- Rapid decomposition of organic waste leads to increased gas generation rate in the initial years of landfill operation, which makes landfill gas recovery and utilization more economical;
- Leachate recirculation reduces environmental impact on ground and surface water as well as the surrounding environment;
- Landfilling costs can be minimized as cells of bioreactor landfills can be reused in the future;
- The decomposed end-product of a bioreactor landfill can be reused as compost;
- Generated landfill gas can be used and converted into renewable energy;
- Post closure care can be reduced.

2.4 Biodegradation of MSW and Gas Generation from Landfills

The conversion of organic content of MSW into methane can be divided into two stages – aerobic stage and anaerobic stage.

2.4.1 Stages of Biodegradation of MSW in Landfills

2.4.1.1 Aerobic Stage

As soon as waste is disposed of, the biodegradable fraction starts reacting with oxygen from inter-waste void spaces. Organic contents are oxidized in the presence of aerobic bacteria, producing carbon dioxide and water vapor. As time passes, oxygen is depleted and gradually the whole aerobic process starts shifting to the anaerobic stage. The transition time depends on availability of oxygen, which is dependent on the composition of the waste and permeability of the cover soil. The more permeable the cover soil, the more oxygen can intrude through the soil.

2.4.1.2 Anaerobic Stage

Hydrolysis, acidogenesis, acetogenesis, and methanogenesis – these four subsequent steps constitute the methane fermentation phenomenon, as shown in Fig. 2-3. At first

fermentative bacteria hydrolyze lipids, proteins, and polysachharides. This produces acetate, fatty acids, carbon dioxide, and hydrogen. Then methanogenic bacteria take control and convert complex organic compounds into simple structured gaseous end products like methane (Christensen et al., 1996). One of the most important facts in this methanogenesis process is that the methane molecule retains about 90% of the substrate energy. The entire process can be summarized and expressed by the following equations 2.1, 2.2 and 2.3 (Perez et al., 2002).

$$Equation\ 2.1: Acetogenesis: C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

$$Equation\ 2.2: Methanogenesis: CH_3COOH \rightarrow CH_4 + CO2$$

$$Equation\ 2.3: CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

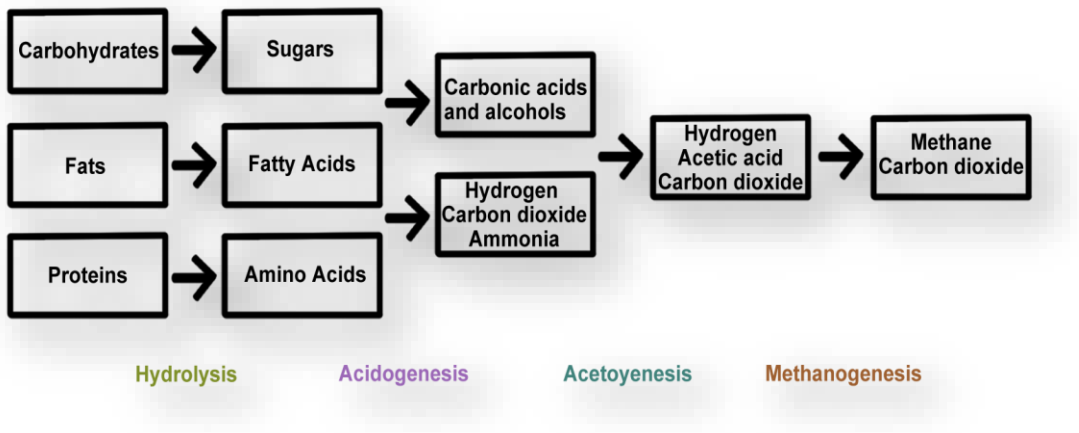


Figure 2-3 Stages of anaerobic digestion of organic matter

The methanogenic bacteria, i.e. methantrophs, are highly pH susceptible and cannot survive at pH values much less than 6. Low redox potential and moderate hydrogen concentration are needed to maintain the ambient surroundings for methanogenic bacteria.

2.4.2 Stages of Biodegradation of MSW in Landfill

Several studies on phases of biodegradation of MSW in landfills have been conducted and reported by Barlaz et al. (1989), Warith (2003), Warith et al. (2005), White et al. (2005), Zacharof et al. (2004), Al-Kaabi (2007), Kjeldsen et al. (2002), and Christensen et al. (1989). According to these studies, there are five distinct phases of biodegradation of MSW, indicated by leachate quality and emitted gas composition. The phases reported in the previous literatures are described as follows.

- Phase I : Initial adjustment phase (Aerobic phase or lag phase)

  In this phase, the entrapped oxygen in waste is used and consumed by microbes, which eventually are responsible for the oxygen depletion at the end of this phase.

- Phase II: Transition phase

  Bacteria via enzymes cause water to break down long chain carbohydrates, lipids, and proteins into short soluble monomers (sugars, fatty acids, and amino acids, respectively). During this phase an initial lag time is observed due to the absence of sufficient moisture needed to ensure proper microbial activity.

  The continued oxygen depletion causes the whole degradation process to shift from aerobic to anaerobic phase. At the end of this phase, BOD and COD concentration of the leachate increases and organic fatty acids like acetic acid can be found in the leachate.

- Phase III: Acid formation phase (Acidogenesis)

  Acidogenic bacteria convert soluble monomers to volatile fatty acids (lactic, propionic, and butyric acids). Acetic Acid Production (Acetogenesis): Acetogenic bacteria convert volatile fatty acids to acetic acid (CH_3COOH).

  The pH levels of leachate drop significantly in this phase due to acid formation. BOD and COD reach their peak in this phase.

- Phase IV: Methane production phase

  Methanogenic bacteria (strict anaerobes) convert acetic acid to methane (CH_4).Carbon dioxide and hydrogen also converted to methane. In this phase, methanogenic bacteria vigorously transform the accumulated acids, carbon dioxide, and hydrogen of Phase II into methane gas. The pH value increases to 7 or more and then stabilizes for the rest of the biodegradation process.

- Stage V: Maturation phase

  In this last phase, methane concentration gradually decreases until eventually the microbial activity ceases.

Figure 2-4 shows the 4 phases.

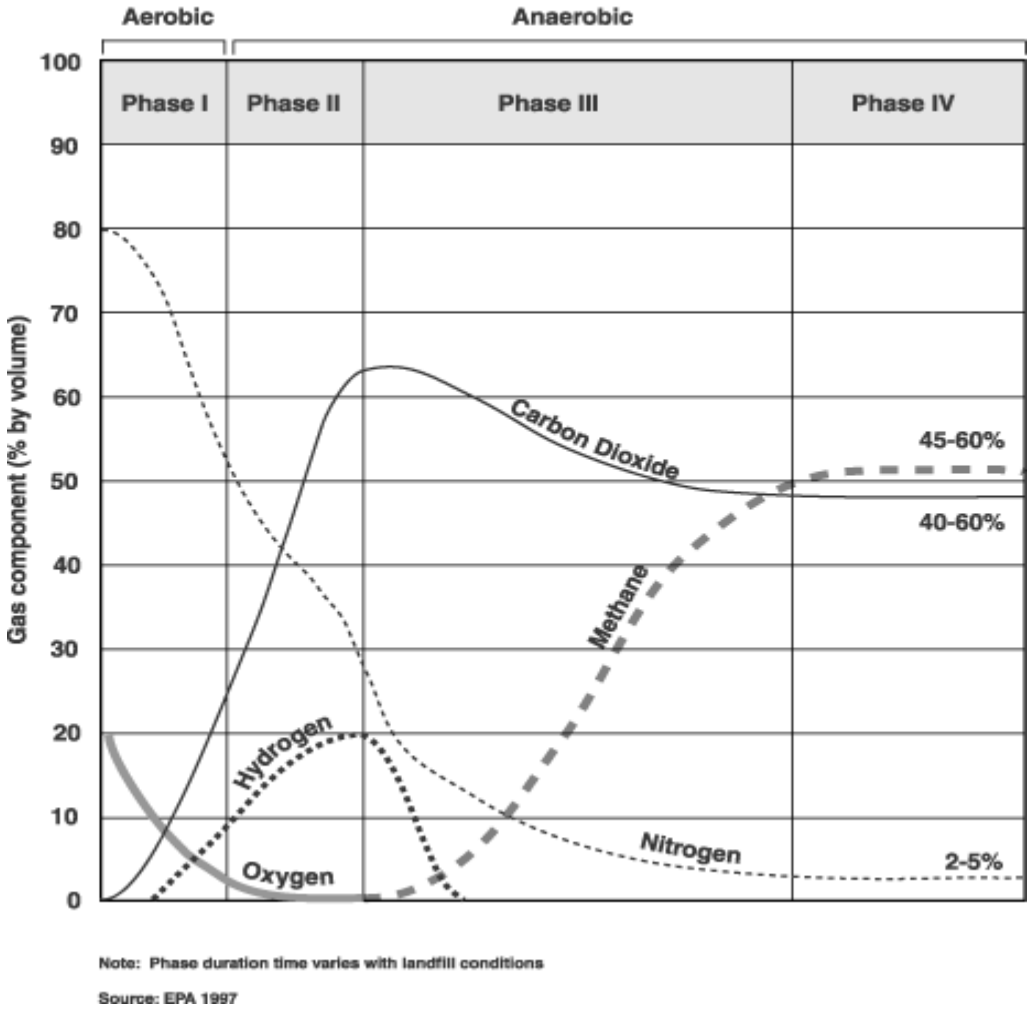


Figure 2-4 Degradation phases in landfills (EPA, 1997)

2.4.3 Factors affecting biodegradation in landfills

Moisture content, pH, alkalinity, temperature, and available nutrients significantly affect the biodegradation process in landfills. Details of these factors are given below.

2.4.3.1 Moisture content

A number of studies have confirmed that methane generation rate increases with an increase in waste moisture content (Barlaz et al., 1990; Mehta et al., 2002; Wreford et al., 2000; Alvarez and Martinez-Viturtia, 1986; Chan et al., 2002; Lay et al., 1998). This may be due to

increased contact between microbes and waste, as well as mobilization of nutrients, buffer and dilution of inhibitors. The biodegradation process accelerates with the increase in moisture content of the landfilled waste, up to a point. Maximum methane production has been reported at moisture contents of 60% to 80% on wet weight basis in Figure 2-5 (Rees,1980). Rees (1980) plotted the methane generation and moisture content data published in research papers and found that the log of methane generation rate produced from landfills is directly proportional to the moisture. This is a fundamental and governing concept for the effective operation of bioreactor landfills. Pohland (1986) and Rees (1980) observed that for rapid waste decomposition and increased gas generation, moisture content of 60% can be considered optimum.

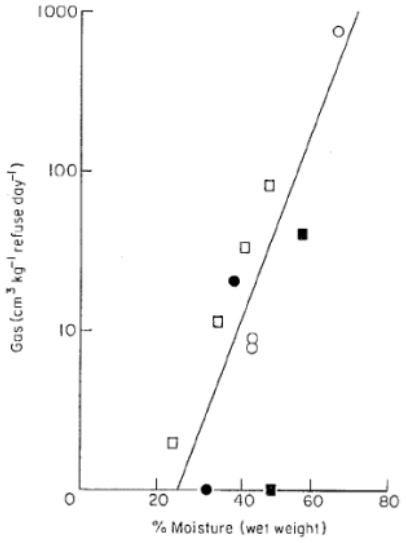


Figure 2-5 Effects of moisture content on gas generation rate (Rees, 1980)

2.4.3.2 pH

PH range from 6 – 8 is considered ideal for methane generation from the landfilled waste. PH level lower than 5 creates acidic conditions which cause inhibition of microbial activities and thus affects methane generation. (Barlaz et al., 2009)

2.4.3.3 Alkalinity

An alkaline environment is necessary for optimum methane generation. Studies conducted by Farquhar and Rovers (1973) reported an optimum alkalinity value of 2000 mg/L (Barlaz et al., 2009).

2.4.3.4 Temperature

Typically, bacterial activity drops off dramatically below 50° Fahrenheit (F). Weather changes have a great effect on gas generation, maximizing gas production. Bacterial activity releases heat, stabilizing the temperature of a landfill between 77° F and 113° F, although temperatures up to 158° F have been noted. Temperature increases also promote volatilization and chemical reactions. As a general rule, emissions of NMOCs double with every 18° F increase in temperature (ATSDR, 2001; EPA, 1993). According to Hartz et al. (1982), the optimum temperature for methanogenesis is 41°C, although the phases of decomposition are well observed in between 37°C and 41°C temperature range.

In this study a temperature of 100°F (around 38°C) was maintained for mixed culture and 30°C for microbes from termite gut (TAV5 genome) in a mixed culture.

2.4.3.5 Nutrients

The presence of a certain amount of water in a landfill increases gas production because moisture encourages bacterial growth and transports nutrients and bacteria to all areas within a landfill. A moisture content of 40% or higher, based on wet weight of waste, promotes maximum gas production (e.g., in a capped landfill). Waste compaction slows gas production because it increases the density of the landfill contents, decreasing the rate at which water can infiltrate the

waste. The rate of gas production is higher if heavy rainfall and/or permeable landfill covers introduce additional water into a landfill (ATSDR, 2001).

According to Christensen and Kjeldsen (1989), all sorts of nutrients are available in the landfill waste. If any kind of depreciation of nutrients occurs, degradation ceases, which results in low methane generation.

2.4.4 Landfill gas generation with biodegradation of waste

Landfill gases are the byproducts of methanogenesis in the anaerobic degradation phase. In the first aerobic phase, the amount of carbon dioxide is greater due to the oxidation of organic compounds. In Phase II, carbon dioxide along with hydrogen is produced. In Phase III, oxygen gets depleted, which gives rise to the anaerobic phase. From this phase, methane generation starts and carbon dioxide and hydrogen decrease because of the absence of oxygen. In Phase IV, the amount of methane exceeds the amount of carbon dioxide as methane: carbon dioxide becomes more than 1. In the final phase, overall gas production drops suddenly. Methane production decreases and stabilizes with time in the maturation phase. The following simplified reaction can explain the overall process of decomposition of cellulose content of solid waste.

$$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3\,CO_2$$

Typically a landfill can generate gases for 10-80 years or more. Aerobic degradation phase remains for first 6 months and can continue up to 18 months. According to EMCON (1998), a summary of landfill gas generation is presented in the following Table 2-2.

Table 2-2 Landfill gas generation phases and time duration

Phase No.	Phase name	Activities	Phase duration
I	Aerobic	No oxygen	Several hours to 1 week
II	Acid formation	Formation of fatty acids, methane generation begins	1-6 months
III	Transition	Methane and carbon dioxide stabilization, no nitrogen	3 months to 3 years
IV	Anaerobic	Methane and carbon dioxide concentrations decrease, a small amount of nitrogen	8 to 40 years
V	Maturation	Final stabilization of methane and carbon dioxide, all anaerobic decomposition ends	1-40 or more years

2.4.5 Composition of landfill gas

Landfill gases can be divided into two groups – principal gases and trace gases. Principal gases include methane, carbon dioxide, and oxygen, whereas trace gases are toxic gases such as hydrogen sulfide. The principal gases are mainly the dominant kind of gases in total gas composition. (ATSDR, 2001)

2.4.5.1 Methane (CH_4)

Methane is a byproduct of the anaerobic degradation of solid waste. It is one of the greenhouse gases, highly explosive when present in high concentration and generally colorless and tasteless. (ATSDR, 2001)

2.4.5.2 Carbon Dioxide (CO_2)

Carbon Dioxide is also colorless and odorless in nature. It is the byproduct of both aerobic and anaerobic decomposition phases and present in relatively high concentrations in the initial phases, which lowers the pH level of leachate. As the decomposition level shifts from aerobic to anaerobic, its concentration decreases and is stabilized in the final maturation phase. (ATSDR, 2001)

2.4.5.3 Oxygen (O_2)

The concentration of oxygen depletes as the decomposition phases move to aerobic to anaerobic. The typical amount of oxygen in landfills is less than 5 percent. Increased volume of oxygen is an indication of air leak in the gas collection system. (ATSD, 2001)

2.5.5.4 Hydrogen (H_2)

Hydrogen is produced in low concentration in the aerobic decomposition phase and also can be found in the anaerobic phase.

2.4.5.4 Trace gases

A total of 100 gases were identified as trace gases in landfill according to US EPA (2008). These gases are toxic and harmful for living things. There are some other constituents of landfill trace gases such as Non Methane Organic Compounds (NMOCs) and volatile organic compounds. These components exist in landfill gas in unpredictable quantity.

A study conducted by Tchobanoglous et al. (1993) reported landfill gases and their percentages, as shown in table 2-3.

Table 2-3 Landfill gas percentages (Tchobanoglous et al., 1993)

Landfill Gases	Percentage (on the basis of dry volume)
Methane	45-60
Carbon Dioxide	40-60
Oxygen	2-5
Sulfides, disulfides, mercaptans, etc.	0.1-1.0
Ammonia	0.1-1.0
Hydrogen	0-0.2
Carbon monoxide	0-0.2
Other trace constituents	0.01-0.6

2.5 Landfill leachate

Landfill leachate quantity largely depends on the field moisture capacity. If the field moisture capacity is exceeded, leachate is produced. Studies conducted by Reinhart (1996), Rees (1980), Kjeldsen et al. (2002), and El-Fadel et al. (1997) reported that generation of leachate largely depends on initial moisture content, amount of recirculated leachate into landfill, climate, and density of waste.

2.6.1 Leachate composition

Factors that affect leachate composition are, waste composition, waste age, and phase of degradation are some of the A study conducted by Kjeldsen et al. (2002) revealed that major components of leachate are dissolved organic matter, macro nutrients such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ammonium (NH^{4+}), iron (Fe^{2+}), manganese (Mn^{2+}), chloride (Cl^{-}), and sulfate (SO_4^{2-}). There are some heavy metals that can be present in

leachate like cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}), and zinc (Zn^{2+}). That study also reported the leachate composition with different biodegradation phases, as shown in Table 2-4.

Table 2-4 Leachate composition for different biodegradation phases (Kjeldsen et al., 2002)

Parameter	Acid phase		Methanogenic phase		Average
	Average	Range	Average	Range	
pH	6.1	4.5-7.5	8	7.5-9	
Biological Oxygen Demand (BOD_5)	13000	4000-40000	180	20-550	
Chemical Oxygen Demand (COD)	22000	6000-60000	3000	500-4500	
BOD_5/COD (ratio)	0.58		0.06		
Sulfate	500	70-1750	80	10-420	
Calcium	1200	10-2500	60	20-600	
Magnesium	470	50-1150	180	40-350	
Iron	780	20-2100	15	3-280	
Manganese	25	0.3-65	0.7	0.03-45	
Ammonia-N					740
Chloride					2120
Potassium					1085
Sodium					1340
Total phosphorus					6
Cadmium					0.005
Chromium					0.28
Cobalt					0.05
Copper					0.065
Lead					0.09
Nickel					0.17
Zinc	5	0.1-120	0.6	0.03-4	

Hazardous waste components could be found in landfill leachate if it is more than 30 years old, as there were fewer restrictions on landfilling of hazardous waste. This hazardous waste content can include mono aromatic hydrocarbons like benzene, toluene, ethylbenzene, and xylenes and halogenated hydrocarbons like tetrachloroethylene and trichloroethylene.

2.5.1 pH

PH of the leachate affects the methanogenesis process in landfills. Optimum pH range is considered in between (6-8). PH level less than 6 would hamper the methanogenesis process as acute acidic condition has a deterrent effect on microbial activity. This results in a low methane yield. PH level greater than 8 may sometimes inhibit methane production.

2.6 Effects on Degradation and Gas Generation

There are several factors that affect landfill waste degradation and gas generation. Some of the factors are discussed in the following subsections.

2.6.1 Effects of Composition of Municipal Solid Waste

The biodegradability of landfilled waste largely depends on the composition of waste. If the waste is comprised of high organic substances such as – food or paper waste, the landfill is expected to yield high amounts of methane. Study conducted by Eleazer et al. (1997) found that composition of waste highly affects waste degradation rate and methane generation. Paper and paperboard are major constituents found in US landfills. Typically paper consists of 79% to 98% of lignocellulose, which is considered to be the most abundant source of natural carbon on earth. . Based on Eleazer et al 1997 lignin percent in mixed paper is 15.9, in grasses is 17.63 % and 33.8% in leaves and 32.6% in branches.

The extents of decomposition were measured by dividing generated methane volume with methane yield and carbon-dioxide. The results are shown in the followingTable2-5.

Table 2-5 Methane Yield and Extent of Decomposition Data (Eleazer et al., 1997)

Reactor Series	Methane Yield, (mL of CH_4/dry g)	Extent of Decomposition
Grass	144.4	94.3
Leaves	30.6	28.3
Branch	62.6	27.8
Food	300.7	84.1
Old Newsprint	74.33	31.1
Office Paper	217.3	54.6
MSW	92	58.4

The changing extent of decomposition of different waste components signifies varying potential of wastes for the conversion of cellulose and hemicellulose into methane and carbon-dioxide.

2.6.2 Effects of Leachate Recirculation

Bioreactor landfills are operated and maintained by recirculating generated leachate periodically to enhance the microbial growth for acceleration of waste degradation. Numerous researches have been conducted to date to determine the effects of leachate recirculation on landfilled waste degradation. According to Reinhart et al. (1996), leachate recirculation has significant impacts on leachate composition, gas production, leachate stabilization rate, and waste volume reduction. San and Onay (2001) studied the effects of leachate recirculation on municipal solid waste degradation by building two reactors – with and without leachate recirculation operation. They found that in the leachate recycled reactor waste stabilized more quickly than the other one. Also the removal of chemical oxygen was faster in case of leachate recirculation.

A study conducted by Chan et al. (1998) proved that leachate recirculation can accelerate methane generation from landfills. According to Morris et al. (2003), leachate recirculation has tremendous impact on subsequent waste stabilization due to degradation in landfills.

Temperature variation in landfills can affect the biodegradation and subsequent gas production process profoundly. Two types of bacteria, mesophilic and thermophilic, which are responsible for waste degradation, are largely dependent on temperature for microbial growth within the landfill.

Optimum temperature range for mesophilic bacteria is 30 to 35°C, whereas thermophilic bacteria can survive in higher temperature range such as 45 to 65°C. Although thermophilic bacteria can produce higher gas yield from landfills, the temperature in most landfills remains in mesophilic range. Research by McBean et al. (1995) reported that optimum temperature for accelerated microbial growth and degradation lies in between 30 to 40°C. Temperature under 15°C may inhibit bacterial growth within landfill, which will affect biodegradation and further gas generation.

2.6.3 Effects of Aerobic and Anaerobic Conditions

A study conducted by Erses et al. (2007) on comparison of aerobic and anaerobic degradation of municipal solid waste reported that aerobic conditions have high efficiency in removal of organic, nitrogen, alkali, and metals from landfill leachate than anaerobic conditions. Two laboratory scale reactors were operated in an insulated room at a constant temperature of 32 ˚ C. Aerobic conditions were simulated with an air compressor.

2.7 Lignin and its chemical structure

2.7.1 An overview of cellulose, hemicellulose, and lignin

Utilization of lignocelluloses materials to generate bioenergy is attracting much attention because of their abundance and sustainability in nature. Plant biomass derived from crop waste

or dedicated feedstock (e.g., perennial plants) has the potential for biofuel production if a robust, efficient and economic system is established to utilize these substrates. Moreover, conversion of biomass to bio energy is a sustainable approach in terms of reducing environmental pollutants, especially greenhouse gas through traditional combustion processes (Charles, 2009; Feng et al., 2011). However, one of the major barriers to the lingo cellulosic bio fuel engineering is the presence of lignin, which blocks the enzymatic hydrolysis on the internal cellulose and hemi cellulose so as to limit their bioavailability (Wu and He, 2013).

Lignocelluloses in nature derive from wood, grass agricultural residues, forestry wastes and municipal solid wastes. The major component of lignocellulose materials is cellulose, along with lignin and hemicellulose. Cellulose and hemicellulose are macromolecules from different sugars; whereas lignin is an aromatic polymer synthesized from phenyl propanoid precursors, as discussed above. The composition and percentages of these polymers vary from one plant species to another. Moreover, the composition within a single plant varies with age, stage of growth, and other conditions.

2.7.2 Cellulose

Cellulose makes up about 45% of the dry weight of wood. In this conformation, cellulose is more susceptible to enzymatic degradation. Cellulose appears in nature associated with other plantsubstances and this association may affect its biodegradation.

2.7.3 Hemicelluloses

Hemicellulose is a complex carbohydrate polymer and makes up 25–30% of total wood dry weight. It is a polysaccharide with a lower molecular weight than cellulose.

2.7.4 Lignin

Lignin is the second most abundant natural polymer in the world, surpassed only by cellulose, and is most commonly derived from wood and 15 -25 % of total dry weight is made of lignin. It is an integral part of the secondary cell walls of plants, conferring structural support, impermeability, and resistance against microbial attack and oxidative stress. Lignin functions as

the connection with hemi cellulose and cellulose structure, as shown in Figure 2-6 (DeAngelis et al., 2011).

Of the polymers found in plant cell walls, lignin is the only one that is not composed of carbohydrate (sugar) monomers. Lignin is a complex polymer of aromatic alcohols known as mono lignols. Lignin is the only large-scale biomass source of an aromatic functionality.

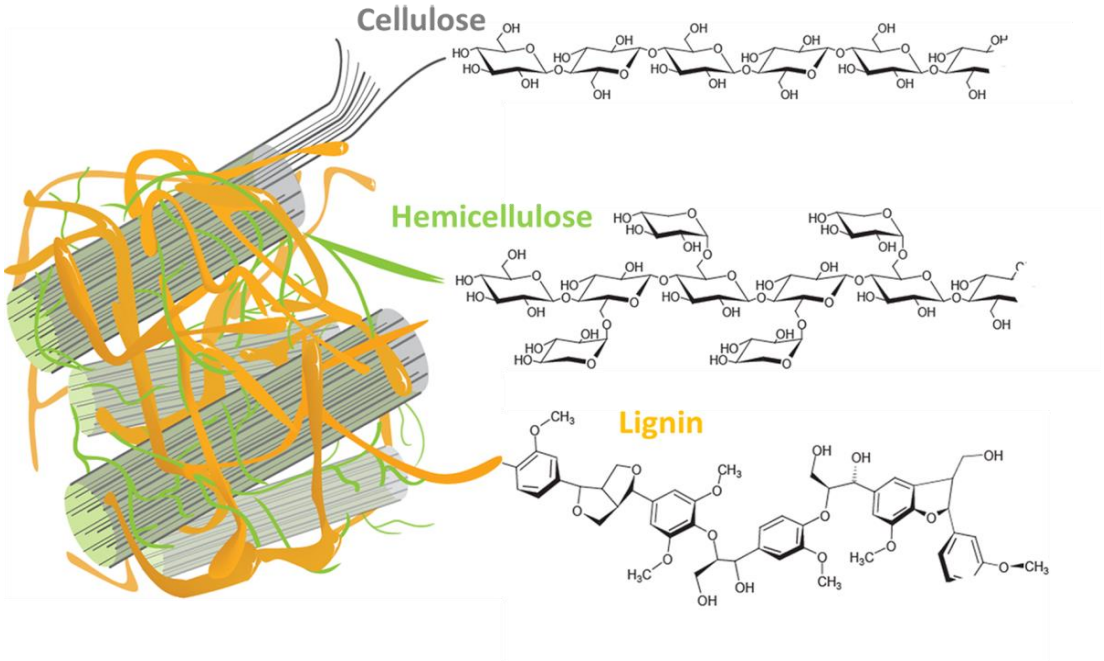


Figure 2-6 Structure of Lignocelluloses (DeAngelis et al., 2011)

Lignin represents a complex and non-repeating three-dimensioned polymer connected by both ether and carbon–carbon linkage with the basic repeating units of phenolic monomer, shown in Figure 2-7. In other words, lignin is an amorphous heteropolymer composed of three phenylpropane subunits (coniferyl, sinapyl, and p-coumaryl alcohols) linked by a variety of carbon-carbon and ether bonds (p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) (Bugg et al., 2010; Vanholme et al., 2008). Figure 2-8 shows three different phenyl propane monomers of lignin, depending on the species. Coniferyl alcohol occurs in all species and is the dominant monomer in conifers (softwoods). Hardwood species contain up to 40% sinapyl

alcohol units, while grasses and agricultural crops may also contain coumaryl alcohol units (Singh et al., 2013)

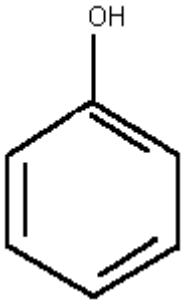


Figure 2-7 Phenol monomer

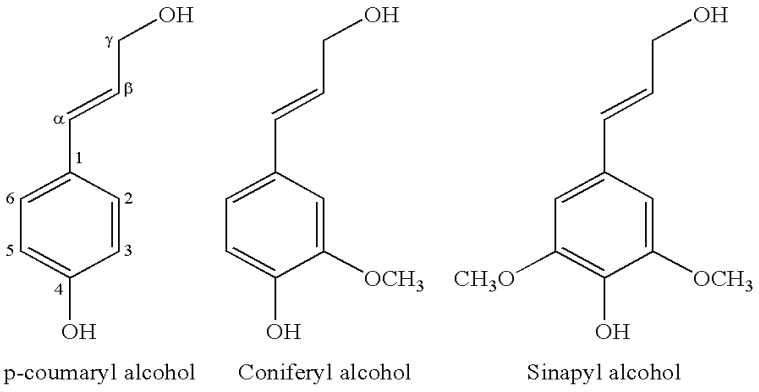


Figure 2-8 Three phenyl monomers in lignin (American Chemical Society, 2011)

An additional complexity of lignin is that there are many possible bonding patterns between individual units. Thus our knowledge of lignin chemical structure is less precise than our knowledge of other natural and synthetic polymers. Figure 2-9 shows a representative lignin fragment containing the most important bonding patterns.

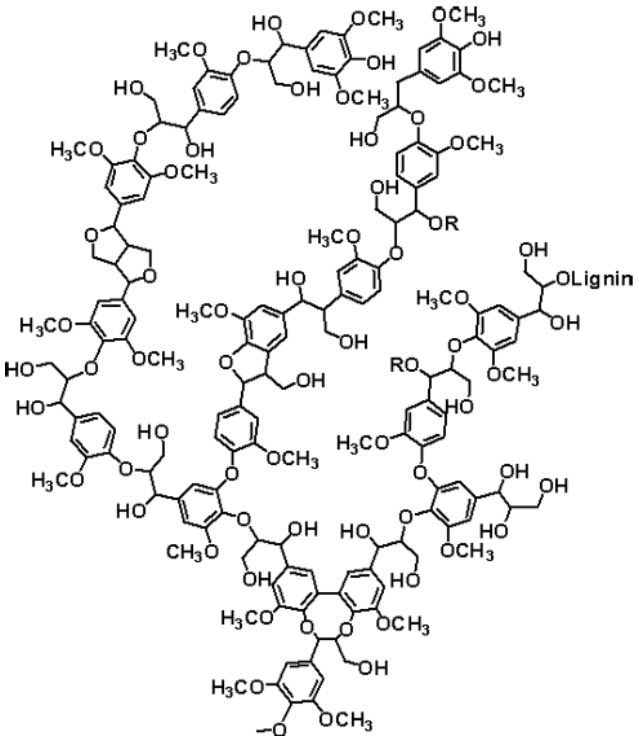


Figure 2-9 Complex structure of lignin (American Chemical Society, 2011).

Lignin contains several acid resistant C–C linkages. Lignin could be only partly degraded to monomeric compounds by hydrolysis and is mostly degraded by oxidative attack on the C–C bonds (Martinez et al., 2005; Higuchi, 2006).

2.7.4.1 Determination of Cellulose, Hemicellulose and Lignin (C, H, L)

Several researchers have attempted to find the ultimate methane potential of waste by finding the cellulose, hemicellulose and lignin content of the waste (Barlaz et al. 1990; Rees 1980; Eleazer et al. 1997; Komilis and Ham 2003; Rao et al. 2000; Brenda et al. 1998; Jones et al. 1983; Rhew and Barlaz 1995). Typical cellulose, hemicellulose and lignin content found in municipal solid waste components are shown below in Table 2-6. While lignin is assumed to be poorly degradable and is unaffected during biological degradation, cellulose and hemicellulose are easily degraded under anaerobic conditions.

Hence, the ratio of cellulose and hemicelluloses to lignin ((C+H)/L) is considered as an indicator of waste decomposition in landfills. It has been reported that the (C+H)/L ratio decreases as the waste age increases (Mehta et al. 2002; Barlaz 2006; Bookter and Ham 1982). The methane generated due to cellulose and hemicelluloses decomposition can be calculated using Equations 2-15 and 2-16 (Barlaz, 2006).

$$Equation\ 2-15: (C_6H_{10}O_5)_n + nH_2O \rightarrow 3nCO_2 + 3nCH_4$$

$$Equation\ 2-16: (C_55H_8O_4)_n + nH_2O \rightarrow 2.5\ nCO_2 + 2.5\ nCH_4$$

Table 2-6 Cellulose, Hemicelluloses, and Lignin percent Content of Waste Components Reported in the

Literature

Waste	Reference	Cellulose	Hemicellulose	Lignin	Extent of decomposition
Grass	Eleazer et al(1997)	26.5	10.2	28.4	94.3
	Komilis and Ham(2003)	39.67	16.89	17.63	
Leaves	Eleazer et al(1997)	15.3	10.5	43.8	28.3
	Komilis and Ham(2003)	9.48	3.24	33.88	
Branches	Eleazer et al(1997)	35.4	18.4	32.6	27.8
Food Waste	Eleazer et al(1997)	55.4	7.2	11.4	84.1
	Komilis and Ham(2003)	46.09	0	12.03	
Coated Paper	Eleazer et al(1997)	42.3	9.4	15	39.2
Old Newspaper	Eleazer et al(1997)	48.5	9	23.9	31.1
Office paper	Eleazer et al(1997)	87.4	8.4	2.3	54.6
Mixed Paper	Eleazer et al(1997)	69.66	7.79	15.9	

2.8 Methods for lignin degradation

There are different ways to degrade lignin based on past researches; an overview of some of them is provided below.

2.8.1 Physical and chemical process for degradation of lignin

Traditional pretreatment of the lignocellulosic materials is through chemical or physical process to destroy lignin structure in order to release polysaccharides. These processes are

expensive, and most importantly they usually generate a variety of toxic contaminants that will affect the efficiency of following hydrolysis and fermentation processes (Mishra and Thakur, 2010). Therefore, conversion of raw lignocellulosic materials via biological methods could become a more direct, economic, and favorable process.

2.8.2 Degradation of lignin by fungal species and bacteria

The diversity of cellulosic and lignocellulosic substrates has contributed to the difficulties found in enzymatic studies. Fungi are the best-known microorganisms capable of degrading these three polymers. Because the substrates are insoluble, both bacterial and fungal degradation have to occur exocellularly, either in association with the outer cell envelope layer or extracellularly. Microorganisms have two types of extracellular enzymatic systems: the hydrolytic system, which produces hydrolysis and is responsible for cellulose and hemicellulose degradation; and a unique oxidative and extracellular ligninolytic system, which de-polymerizes lignin.

Currently, lignin has been reported to be degraded by fungal species as their powerful lignin-degrading enzymatic systems (Vanholme et al., 2008). However, the stability of fungi is not attractive in practical treatment when they are exposed to certain environmental conditions, such as higher pH (>7) or anaerobic conditions. Bacteria may be a potential candidate possessing ligninolytic activity because of their immense environmental adaptation and biochemical versatility (Bugg et al., 2010; Chandra et al., 2007). Bacteria with phenolic compounds degrading capability may degrade lignin as well (Peng et al., 2008). Actually, in natural environments, lignocellulosic substances are found to be aerobically or an aerobically transformed and degraded by different bacteria in syntrophic association (DeAngelis et al., 2011). However, reports on lignin degradation by anaerobic bacteria are still limited; thus investigations of anaerobic bacteria capable of lignin-degradation would be beneficial to the industrial production of the next-generation biofuels, with merits of environmental friendly

treatment of substrates as well as the economical and compatible application to downstream hydrolysis and fermentation at anaerobic conditions (Bugg et al., 2010; DeAngelis et al., 2010).

Studies have shown that bacteria also have the capacity to catabolize non-phenolic compounds; however, the relationship of these activities with respect to lignin degradation remains unclear. Many soil bacteria, especially actinomycetes, have been reported to react with lignin to both solubilize it and produce a high molecular weight metabolite termed acid-precipitable polymeric lignin.

2.8.2.1 Effect of peroxide enzymes made from white rot fungi on lignin degradation

White rot fungi have the unique ability of degrading lignin by oxidation (Higuchi, 2006; Sanchez, 2009). A special category of commercially available enzymes made from white rot fungi are peroxides. Peroxides could potentially catalyze the lignin degradation process. There is considerable interest in using peroxides in contaminated site remediation (Husain et al., 2009) and sludge dewatering (Neyens and Baeyens, 2003). Recent studies have shown that the use of peroxides in waste management processes could be effective in breaking down many organic pollutants. Enhancing degradation of lignin-rich waste materials by the addition of peroxides enzymes has also been studied previously.

There are some studies about the feasibility of augmenting leachate with different peroxide enzymes to increase the rate of waste degradation during later stages of anaerobic landfill bioreactor operation. In one of the past research programs, laboratory batch experiments were conducted to determine the effectiveness of some enzymes to enhance methane production, determine the factors affecting the enzyme supported degradation process, and identify the enzyme type most suitable for enhancing methane production. Types of enzymes which have been used in studies are commercially available peroxidases such as lignin peroxidase (Lip). Lignin peroxidase (Lip) was the first lignolytic enzyme to be isolated from Phanerochaete chrysosporium and was found to contain a heme cofactor that is competent to oxidize unusually high potential sites, such as aromatic rings. In addition to LiP, fungi also utilize other secreted

metalloenzymes to break down lignin, including the heme-containing manganese peroxidases (MnP) and versatile per- oxidases (VP), as well as multicopper-dependent laccases, manganese peroxidase (MnP), soybean peroxidase (SbP), horseradish peroxidase (HRP), and laccases. Of these peroxidases, LiP and MnP are described as true lignin degraders because of their high potential redox value (Martinez et al., 2005). The three types of peroxidase enzymes, LiP, MnP, and SbP, were selected to evaluate their ability to further degrade partly degraded MSW. These peroxidases had been activated by mixing with hydrogen peroxide (H_2O_2). MnP shows the best performance in methane yield (Jayasinghe et al., 2011).

2.8.3 Types of bacteria which can degrade lignin

Many soil bacteria, especially actinomycetes, have been reported to react with lignin to both solubilize it and produce a high molecular weight metabolite termed acid- precipitable polymeric lignin (APPL). Although the metabolism of lignin is not as complete compared to fungal systems, it is clear that bacteria can react with lignin and possibly produce smaller aromatics that can be imported into the cell for aromatic catabolism, which is also widespread in soil bacteria .The first molecular information on bacterial APPL formation was reported with the isolation of a secreted bacterial heme peroxidase from a gram-positive bacterium, Streptomyces viridosporus T7A ,which indicated that bacteria also likely possess a set of extracellular oxidative enzymes involved in lignin metabolism. Initial studies showed that the T7A peroxides was also biochemically competent for the degradation of non-phenolics, but was not as oxidizing as fungal peroxides (Brown and Chang, 2013). Brown and Chang write: “Microbial systems can provide molecular information on lignin depolymerization as they have evolved to break lignin down using metalloenzyme-dependent radical pathways. Both fungi and bacteria have been observed to metabolize lignin; however, their differential reactivity with this substrate indicates that they may utilize different chemical strategies for its breakdown.”

According to Breznak and Brune in 1994 “Termites play an important role in the turnover and mineralization of complex biopolymers, such as wood and other cellulose- and

hemicellulose-containing materials. It seems that the microbe has a significant impact on cellulose degradation (cf. Breznak and Brune 1994; Varma et al. 1994).

The fungus-growing termites consume the fungal celluloses together with the fungus nodules (Rouland et al., 1988). Celluloses were found in the gut of some termites (Veivers et al. 1982). In Nasutitermes takasagoensis the endo-b-1,4-glucanase was mainly present in the mid gut (Tokuda et al. 1997). Recently, the first cellulose gene from a termite was sequenced (Watanabe et al. 1998). A few cellulose-degrading bacteria have been isolated and identified from some termite species". (Clostridium termitidis, Hethener et al., 1992; Micromonospora propionici and Clostridium sp., Hungate, 1946; Streptomyces sp. and Micromonospora sp., Pasti and Belli, 1985; Staphylococcus saprophyticus,
Paul et al., 1986; Micrococcus luteus and Mc. roseus, Saxena et al., 1993; Mm acetiformici)

2.8.3.1 Discovery of new lignin-reactive bacteria

According to Chang in 2013 "certainly the exploration of greater microbial biodiversity in new environments will help to extend our understanding of the scope of lignin metabolism in microbes. Scaled-up screening studies have identified many new bacterial species that appear to be much more active than previously characterized strains. Indeed, the study of these new microbes may reveal the presence of new mechanisms for lignin reactivity especially when they originate from unusual environments where active biomass degradation occurs. For instance, greater availability of microbial metagenomes from sources where biomass is rapidly degraded — such as wood-feeding insects, the rumen of cows, or active soils — will allow us to assess greater diversity at the genetic level .In particular, environments where lignin degradation occurs under anaerobic or micro aerobic conditions".

"Indeed, insufficient knowledge of the suite of genes involved in lignin modification creates roadblocks in functional annotation of metagenomes by sequence homology alone. However, continuing physiological and structural studies could help to identify new chemical transformations on lignin as a result of microbial metabolism".

"By exploring greater diversity of the organisms that can react with and metabolize lignin, we may be able to gain new insight into strategies for biomass deconstruction. Bacterial systems have been found to be less oxidatively powerful compared to lignolytic fungal systems to date but may provide a rich source for elucidating new accessory enzymes that could act synergistically with the major oxidative enzymes to activate and uncap various sites, similar to what has been involved in cellulose degradation. For example, hydroxylation or de methylation of various sites could serve to generate new chemical handles for further degradation. In addition, new studies show the existence of oxidative enzymes that are capable of associating with lignin. This could indicate that the process is not completely mediated through secondary small molecule mediators. In this regard, the existence of direct interactions between the substrate and lignin processing enzymes may also begin to explain differential reactivity profiles that are observed. Taken together, work in this area will continue to help bring more molecular detail into understanding how lignin degradation occurs in the environment". (Chang, 2013)

2.8.4 Cellulose biodegradation

Most of the cellulolytic microorganisms belong to bacteria and fungi, even though some anaerobic protozoa and slime molds able to degrade cellulose have also been described. Cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions.

2.8.5 Termite; microaerophilic, Verrucomicrobia

Termites have long been recognized for their ability to consume lignocellulosic plant material and soil (humus), converting it into substrates (primarily acetate) on which the termite depends for carbon and energy. These social insects are not only important for the global carbon cycling, but also for their biotechnological potential as efficient lignocellulose degraders (Brune, 1998). The success of termite feeding behavior is intimately associated with the

presence of a diverse and abundant gut microbial community (Ohkuma and Brune, 2011). In the lower termite, R. flavipes, the complexity of symbiosis spans three domains of life: methane producing Archaea, cellulolytic Eukarya (protozoa) and bacteria—all acting cooperatively to degrade lignocellulose, fix/recycle nitrogen and remove oxygen, suggesting a division of metabolic activities among members of the community.(Isanapong and Rodrigues, 2013)

2.8.5.1 Microbes from termite gut TAV2

Termite hindguts are populated by a dense and diverse community of microbial symbionts working to transform lignocellulosic plant material and derived residues into acetate, to recycle and fix nitrogen, and to remove oxygen. Although much has been learned about the breadth of microbial diversity in the hindgut, the eco physiological roles of its members is less understood. In Diplosphaera colotermitum strain TAV2, an autochthonous member of the Reticulitermes flavipes gut community was studied by Isanapong and Rodrigues (2013). .TAV2 is involved hemicellulose degradation and consumption of O_2 in the termite hindgut. (Isanapong and Rodrigues, 2013)

2.8.5.2 Microbes used for this research TAV5

2.8.5.3 Colotermitum TAV5 and isolate of the phylum Verrucomicrobia

The genome of the *Opitutaceae* bacterium strain TAV5, a mesophilic Verrucomicrobium isolated from the hindgut of the wood-feeding termite Reticulitermes flavipes, contains genes associated with methylotrophic competency.

The TAV5 genome contains a number of glycoside hydrolysis involved in the degradation of cellulose and hemicellulose, as observed for the TAV1 and TAV2 genomes. The genome has genes for the enzymes 3-carboxymuconate cyclase and 4-carboxymuconolactone decarboxylase, which are involved in the degradation of protocatechuate that is derived from lignin, as well as genes coding for dioxygenases and dienelactone hydrolase, known for ring cleavage of aromatic compounds. These enzymes structurally modify lignin, improving the accessibility of polysaccharides to glycoside hydrolyses and increasing the efficiency of

degradation. The genomic analysis reveals genes for methylotrophic, lignocellulose degradation, and ammonia and sulfate assimilation (Kotak, 2015).

Chapter 3

Methodology

3.1 Introduction

The objective of the study was to determine the effects of pure culture of microbe from termite gut *(*colotermitum TAV5, an isolate of the phylum Verrucomicrobia) microbe and mix culture of microbes on degradation of 3 kinds of waste which contain, cellulose, hemicellulose and lignin in bioreactor landfills. This required a series of extensive laboratory tests and a solid experimental setup. This chapter is focused on the methods of these laboratory tests and instrumentation of laboratory scale reactors.

Laboratory scale reactors were built with a separation of waste, including paper, yard waste, and wood. The lignin percent in paper, yard and wood are different: 15.9% for mixed paper (Eleazer et al., 1997), 17.6% for grass and 33.9% for leaves (Komilis and Ham, 2003), and 32.6% for branches (Eleazer et al., 1997). Mixed paper has the largest amount of cellulose 69.66%, wood has the largest amount of hemicellulose 25-30%, and yard waste largest amount of lignin 28-43.8 %.

Three of the reactors were seeded with a mixture of cultures from an anaerobic digester and incubated at 38°C, optimum temperature for these microbes. The other 3 were seeded with termite gut microbe TAV 5 added to a mixed culture from an anaerobic digester and incubated at 30 °C because the optimum temperature for TAV5 is 30°C. %. Table 3-1 summarizes the experimental design.

Table 3-1 Summary of experiments

Summary of Experiments		
Reactor	**Temperature**	**Microbe type**
Paper1	100 °F (38°C)	Digester microbe
Paper2	86°F (30°C)	50% Digester microbes, 50% Termite gut TAV5
Yard1	100 °F (38°C)	Digester microbe
Yard2	86°F (30°C)	50% Digester microbe, 50% Termite gut TAV5
Wood1	100 °F (38°C)	Digester microbe
Wood2	86°F (30°C)	50% Digester microbe, 50% Termite gut TAV5

The experiments were conducted in multiple steps, described in the following sections.

3.2 Building reactors

Following the method used by Karanjekar (2012), experiments were conducted in 6 gallon HDPE wide-mouth plastic buckets (United States Plastic Corporation, OH) modified for gas and leachate collection and for water addition. A hole was made at the bottom of 6 gallon bucket and 4 holes at the top of gamma seal. Figure 3-1 shows gamma seal for top of reactors and Figure 3-2 shows 6 gal plastic bucket which were used as bioreactors.

Figure 3-1 Gamma seal

Figure 3-2 6 gallon HDPE plastic bucket

The threaded adapters at the bottom and at the top of gamma seal were screwed. Figure 3-3 shows threaded adapter which was used for gamma seal and bottom of reactors. Figures 3-4 – 3-6 show the steps of using these adapters for gamma seal and the way they are taped and sealed to avoid leakage. Figure 3-7 Shows Tygon tubes and 2-3 way valves, clamps and tee on the gamma seal.

Figure 3-3 Threaded adapter

Figure 3-4 Using emery on gamma seal for making a rough surface for better application of sealant

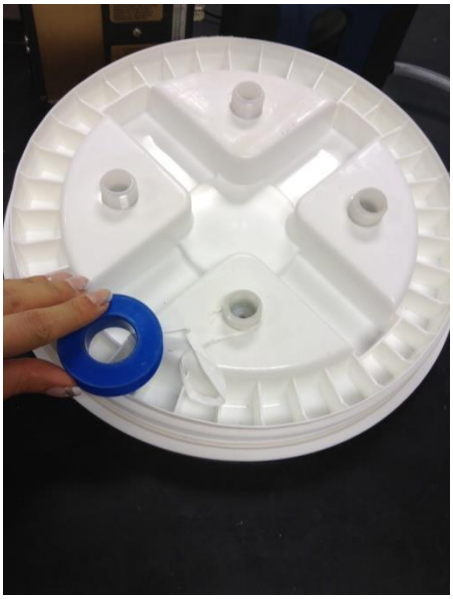
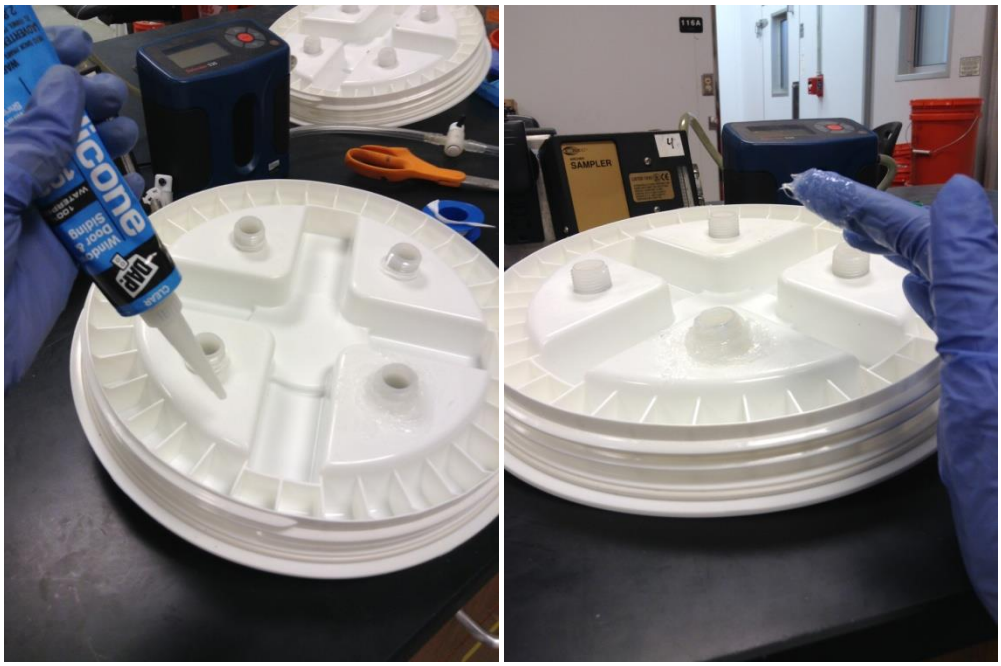


Figure 3-5 Using plastic tape around threaded adapter to ensure there is not any leakage



(a) (b)

Figure 3-6 (a) Applying sealant around threaded adapter (b) after taping around it

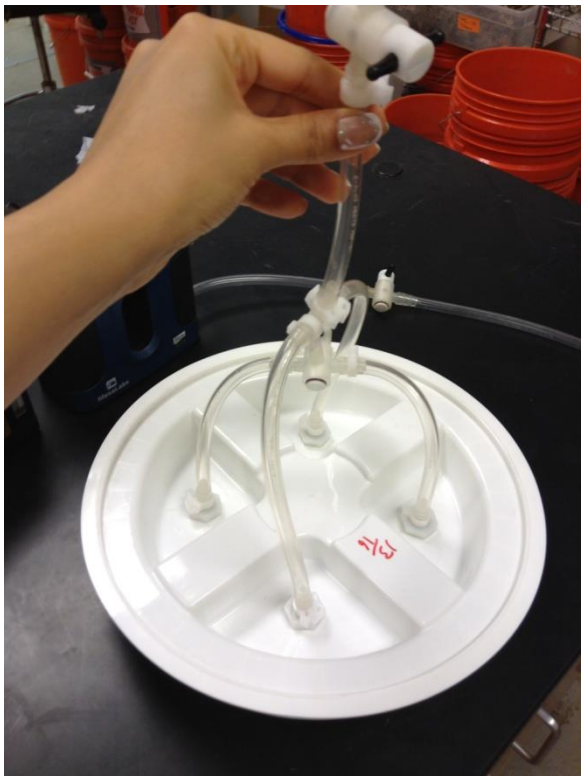


Figure 3-7 Tygon tubes, 2-3 way valves, clams, and tee on the gamma seal

Transparent sealant was applied around the 5 threaded adapters (4 adapters of gamma seal and 1 of the bucket) and was left for 24 hours to dry completely. As shown in Fig. 3-8, the sealant was also applied at the bottom of the reactor in a thick layer to ensure no leakage and left for 12 to 24 hours to dry.

Figure 3-8 Transparent sealant was applied at the bottom of reactor

Gas collection bags were installed to collect the generated gas from the reactors. Six- layer aluminized gas sampling bags with storage volume of 10L (Calibrated Instruments, Inc.), as shown in Figure 3-9, were used to collected reactor produced gas.

Figure 3-9 Gas collection bag

Leachate collection bags were installed at the bottom of the reactors to collect the leachate generated from reactors. For this purpose, medical drainage bags (Kendall Ken guard Economy Drainage Bags, capacity 2L) were installed at the bottom of the reactors, as shown in Figure 3-10.

Figure 3-10 Leachate drainage bag

The complete reactor setup is shown schematically in Figure 3-11.

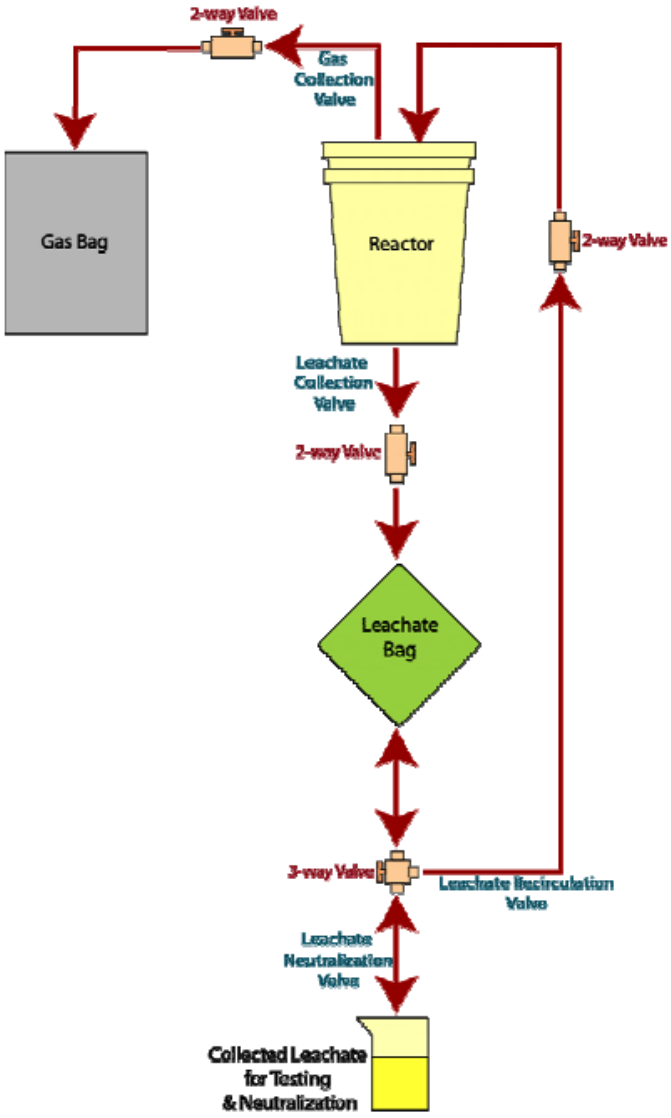


Figure 3-11 Schematic of bioreactor

Before filling the reactors with waste, all reactors were leak-checked. Leak tests were conducted using a simple U-tube manometer (Dwyer Instruments Inc., Michigan City, IN), after

proper sealing of reactors. To verify that there was no significant leakage, reactors were monitored for 1-2 days. The head difference at 12 and 48 hours was recorded to confirm that it was within permissible limits of 0.5 in, and 3 in. of water column, respectively (Haque, 2007). Once the reactors were leak-tested, their empty weight was measured.

3.3 Waste Collection

Paper waste was obtained from the university's recycling bins (office paper), and faculty and student's personal recycling bins (newspapers, mail, magazines, office papers). Large pieces of waste were cut in order to fit into the reactors. The average paper size in the reactor was 4" x 4". Paper was not cut into finer pieces or shredded because it has been reported to become bio-available due to shredding, which can lead to faster degradation and larger *k* values (Buivid et al., 1981). Hence, the coarse structure of waste was maintained in the reactors as much as possible to try to replicate the actual conditions in the landfills.

A mixture of grass, leaves, and tree/bush trimmings was obtained from the university's Environmental Health and Safety Office (Summit St.) and is representative of the variety particularly found in Texas. The species of trees found in Texas are mostly Live Oak, Post Oak, Red Oak, American Elm, Pecan, Bald Cypress, and Creepy Myrtle.

Wood waste was obtained from the wood chips located at Civil Engineering Lab Building and some thick branches of oak trees in the vicinity of Arbor Oak in UTA.

As mentioned earlier, wood, paper and yard were the major biodegradable waste components considered in this study. Waste components were collected from individual sources instead of the waste transfer station or landfill in order to obtain pure waste .Pure waste is commonly used term, which indicates that the waste components are not mixed.

Figure 3-12 (a, b, c, d) shows 3 types of waste which is used for this experiments (a) wood chips,(b) mix of office paper, card board and newspaper and magazine, (c) yard waste like dried leaves ,grasses and branches of trees.

Figure 3-12 a) Wood chips, b) Mixed paper c, d) Yard waste, grass and leaves

Table 3-2 shows the percent of Lignocellulose (cellulose, hemicellulose, and lignin) in these waste and yellow colored shows the highest amount of lignocellulose in each type of waste.

Table 3-2 Percent amount of Lignocellulose in mixed paper, wood, and yard waste

Lignocellulose Percent	Wood	Mixed Paper	Grass	Leaves
Cellulose	45%-50 %	69.66%	26.5%-39.67%	9.84%-15.3%
Hemicellulose	25%-30 %	7.79%	10.2%-16.89%	3.2%-10.5%
Lignin	15%-25%	15.90%	17.63%-28.4%	33.8%-43.8%

3.4 Reactor Setup

Tubing at the top of gamma seal and at the bottom of the bucket was added for leachate collection and recirculation, and generated gas collection, under the bottom geocomposite layer, a gravel layer was provided to ensure better drainage of leachate. The geocomposite layer acts like a strainer and will distribute leachate to all parts of reactor (Figure 3-13). At the top and bottom of the reactors, geocomposite layers were attached to simulate the landfill liner system (Figure 3-14).

One reactor was filled with each type of waste. The paper and yard waste were highly compacted using a compaction too, as shown in Figure 3-15, but wood waste was not, due to the space between wood chips.

Figure 3-13 Bottom gravel drainage layer

(a) (b)

Figure 3-14 The geotextile used for the bottom (a) and top (b) of bioreactors

Figure 3-15 Compacting of waste in reactors by a compacting tool

Seed was obtained from a continuously-stirred anaerobic sludge digester operated at a hydraulic residence time of 19 days at 20^{o}C and added to each reactor to achieve 15 -20 % by weight. Sludge, as shown in Figure 3-16, was obtained from the Village Creek Wastewater Treatment Plant, Fort Worth, TX.

Figure 3-16 Adding mixed culture of microbes to bioreactors

In addition, tap water was added to the waste to make sure that the waste was near saturation limit. Leachate is produced only when the waste has moisture exceeding its saturation limit. Each reactor was then weighed and placed in a temperature controlled room of the Civil Engineering Lab Building at 100°F for the first three sets of reactor Paper1, Yard1, and Wood1. The mixed culture of microbes from the digester can grow at this temperature well. The reactors were then connected to a leachate collection bag (2-L Kendall-Ken Guard Drainage Bag) and gas collection bag (10-L Cali 5-Bond Bag, Calibrated Instruments, Inc.). Figure 3-17 shows the gas collection bags in top of reactor and also the leachate recalculating glass.

Figure 3-17 Gas collection bag at top of white reactor (with my name Hoda on it), and leachate recirculating glass at top

These reactors were kept in the environmental growth hot chamber for enhanced microbial activities, as shown in Figure 3-18.

Figure 3-18 Reactors in environmental growth chamber (hot room)

3.5 Reactor operation and reactor monitoring

Reactors were operated and monitored in a routine way, which included several activities such as collection of generated leachate, recirculation of leachate, leachate quality monitoring, collection of gases produced, and measurement of gas quantity and composition. These activities are discussed in the following subsections.

3.5.1 Leachate collection and recirculation

A rainfall rate of 42 mm in a week was simulated (equivalent to 6 mm/day, or 21 mm twice per week). Tap water was added to the reactors to simulate rainfall. Based on the reactor cross-sectional area, a rainfall volume of 1050 ml was found to produce a rainfall height of 21 mm twice a week .Based on previous research on the leachate circulation, adding the rainfall twice a week generated more gas, compared to once a week or daily. Water addition was done using the three-way valve attachment in the Gamma seal of the reactor. Potassium hydroxide (KOH) addition was required in the initial period to avoid excessive acid accumulation. The pH was maintained above 6, as lower pH values are toxic to methanogens.

In the initial days, water was added to each reactor to increase the moisture content to ensure higher decomposition. Reactor-generated leachate was collected on a weekly basis and leachate pH was measured. The volume of the generated leachate was also measured using a graduated cylinder and then 1L of leachate was recirculated in respective reactors. This 1 L recirculated leachate was used for 1050 mL and was done twice a week, it means that generated leachate from reactors was recirculated in all reactors twice a week and each time 1L leachate recirculated, if the generated leachate was less than 1L, water was added to ensure 1L of recirculated leachate. In the initial phases, the pH of leachate was as low as 5. Therefore, before recirculating in the reactors, KOH was added to the leachate to ensure a basic condition to keep the methanogens alive.

3.5.2 Leachate quality monitoring

3.5.2.1 pH

The pH of the generated leachate was measured using a bench top Oakton pH meter as shown in Figure 3-18. The pH meter was calibrated before, and was rinsed 5 times after using for measuring the pH of leachates.

Figure 3-19 pH meter for measuring pH of Leachate

3.5.3 Gas collection and measurement

3.5.3.1 Composition of gases

Generated gas was collected and volume and composition of gases were measured on a regular basis, normally once a week, but in first month generated gas for Paper2 was measured twice a week because the gas collection bag was totally full. Six layer aluminized gas sampling bags with storage volume of 10L (Calibrated instruments, Inc. Cali 5 Bond) were used to collect the gas. Composition of the collected gases was measured using Landtec GEM 2000 with infrared analyzer. This instrument measured the concentration of methane (CH_4), carbon-dioxide (CO_2), oxygen (O_2), and trace gases in the gas bags (Figure 3-20).

Figure 3-20 Gas generated from reactor; gas composition measured by using Landtec

3.5.3.2 Volume of collected gas

Volume of collected gas was measured using an air sampling pump (Universal XR Pump Model 44XR) and Defender 330. The fixed rate of flow of gas was measured at the beginning of the sampling and time was recorded with a stopwatch until the gas bags were completely empty. The process of volume measurement is shown in Figure 3-21.

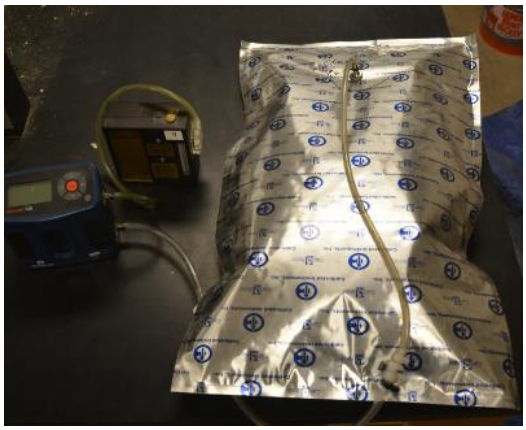


Figure 3-21 Volume measurement tool

3.6 Growing microbes from termite gut TAV5 genome

3.6.1 Culture Equipment

When microbiologists want to identify microbes in a sample or study microbes in-depth, they often try to culture, or grow, the microbial cells in their labs. The scientists can then manipulate the cells or their environments to see what effects these changes have on the organisms. In this research TAV5 genome from termite gut was grown.

3.6.1.1 Petri Plates and Flasks

These are the standard vessels in which microbial cells are grown. Petri plates are clear glass or acrylic dishes with lids that fit together like the two halves of a pillbox. Nutrients in either solid or liquid form can be put in them. Flasks are glass or acrylic bottle-like containers that can hold nutrients in liquid form.

Figure 3-22 shows flasks and Figure 3-23 shows Petri dishes used.

Figure 3-22 Flasks

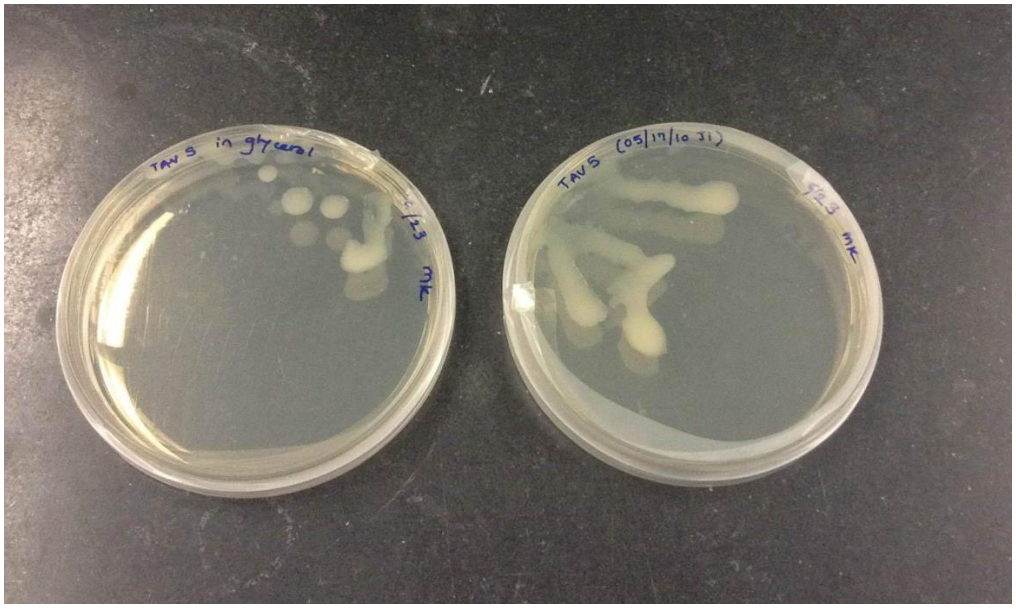


Figure 3-23 Petri Dishes

3.6.1.2 Hypoxia chamber

A hypoxia chamber is a system that is hermetically sealed and allows continuous control of oxygen. There is a catalyst box inside the chamber which can produce an environment free of oxygen, and by attaching the regulator to a nitrogen gas (N_2) cylinder, an anaerobic environment can be created inside the chamber. For culturing TAV 5 genome, 2 percent oxygen was used because these microbes can be cultured in 2 % oxygen not zero percent oxygen, nitrogen cylinder was used for purging nitrogen in to chamber to make 2 % oxygen environment inside the chamber. Figure 3-24 shows the hypoxia chamber used for culturing microbes.(the manufacturer of this chamber is Coy)

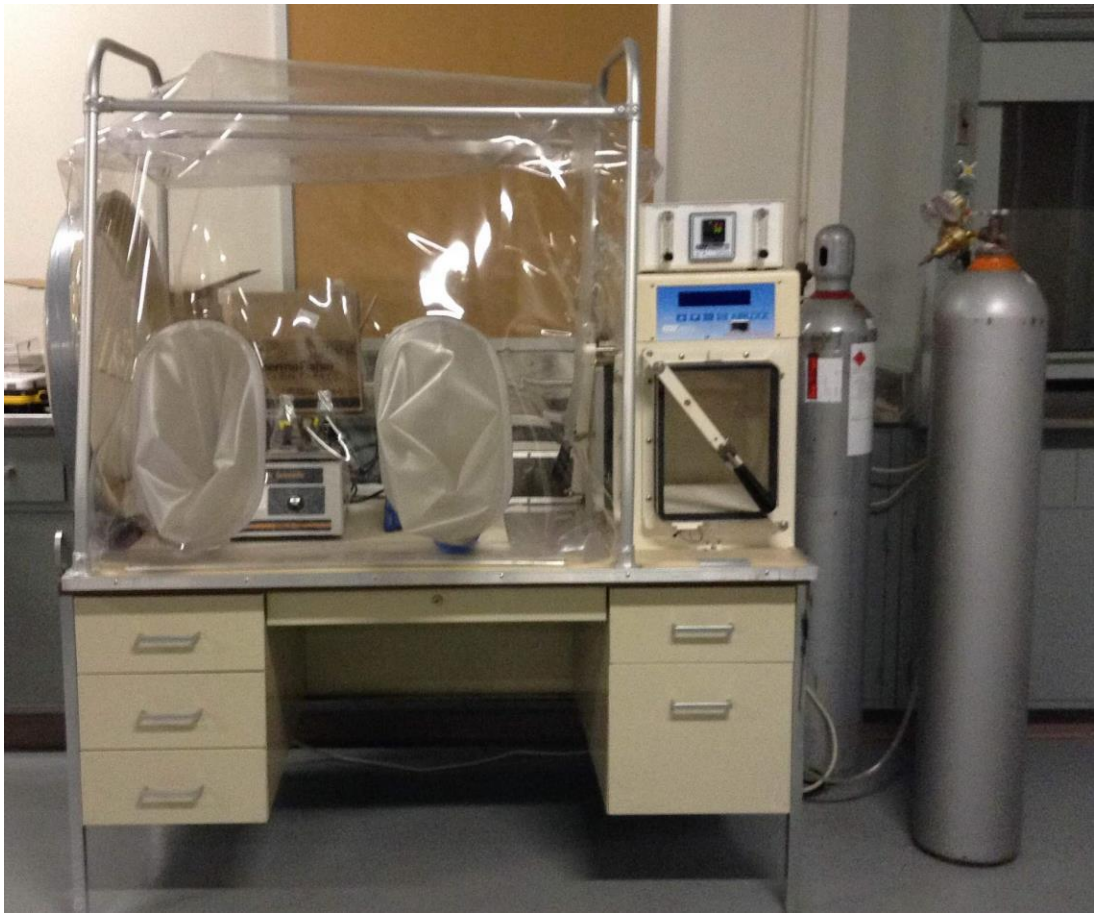


Figure 3-24 Hypoxia chamber

3.6.2 Preparing Media for Culturing Microorganisms

Microbes require nutrients to grow. These are supplied by either solid or liquid culture media. The standard solid medium is nutrient agar, a gelatinous substance derived from seaweed. The basic liquid medium is nutrient broth, typically a mix of water with Agar.

Some microorganisms are more finicky than others and require media enriched with growth-promoting ingredients such as animal blood, glucose or egg. Examples of commonly used enriched media are blood agar, chocolate agar, and Loeffler medium (Microbe World, 2010).

For TAV5 genome, R2A agar was mixed with distilled water in a large flask and autoclaved at 120°C for about 45 minutes for sterilizing. Figure 3-25 shows the flask containing distilled water and Agar R2A, ready to autoclave, and Figure 3-26 shows the oven using for autoclaving.

(a) (b)

Figure 3-25 a) Flask containing media ready to autoclave at 120°C,b) flask inside autoclave oven

Figure 3-26 Oven used for autoclaving media

R2A Agar was developed by Reasoner and Geldreich for bacterial plate counts of treated potable water. R2A Agar is a low nutrient medium, and in combination with a lower incubation temperature and longer incubation time, stimulates the growth of stressed and chlorine-tolerant bacteria. Nutritionally-rich media support the growth of fast-growing bacteria, and may suppress slow- growing or stressed bacteria found in treated water. When compared with Tryptone Glucose Yeast Extract Agar or Standard Methods Agar, R2A Agar improved recovery of stress and chlorine-tolerant bacteria from drinking water systems. (R2A AGAR 7390 by Acumedia)

After 45 minutes autoclaving at 120°C, media was poured into Petri dishes and given about 4 to 5 days to solidify. Figure 3-27 shows prepared media which is ready to pour into petri dishes. Next, TAV5 strain which had been stored at -80°C was inoculated into the petri dishes. Since these microbes are slow growing and 1.5 liters of microbes in liquid media was needed, after inoculating in petri dishes the microbes were inoculated into three 5 ml flasks and put those flasks into the hypoxia chamber with 2 percent oxygen on an orbital shaker. After growing microbes in

5 ml flasks, the microbes were inoculated into 50 ml flasks and after that again in 500 ml flasks to obtain 1.5 liters of TAV5 microbe.

Figure 3-27 Prepared media ready to put into petri dishes

3.6.3 Inoculating Microbes

Inoculating the microbes required a sterilized air flow, the air flow bench generates UV to kill other microbes and bacteria to prevent a contaminated environment when inoculating microbe. First, the bench environment was sterilized using 70 percent ethanol, and then a flame was used to sterilize the loop to be used for inoculating. The TAV5 microbes were obtained from the freezer, sterilized loop was used and put in to the tube of TAV5 microbes to get some of those, and then

petri dishes opened, and the loop was moved through the media in a zigzag pattern. The microbes were allowed 7 days to grow in the petri dishes, before, inoculating in flask. Figure 3-28 shows the petri dishes containing of a colony of microbes.

Figure 3-28 Colony of microbes in petri dishes

3.6.3.1 Inoculating microbes from petri dishes to flasks

A sterilized loop was used to inoculate microbes from petri dishes to flasks. The flasks were then transferred to the hypoxia chamber which was set to 2 percent oxygen, and put in the shaker at 35 RPM. The oxygen percentage and the color of media were checked every day. If the color of media changed to cloudy, it has been contaminated and microbes will not grow. Figure 3-29 shows petri dishes and 5 ml media in flasks and Figure 3-30 shows the clean bench and flame and the environment for inoculation. Figure 3-31 shows inoculating from petri dishes to 5 ml flasks.

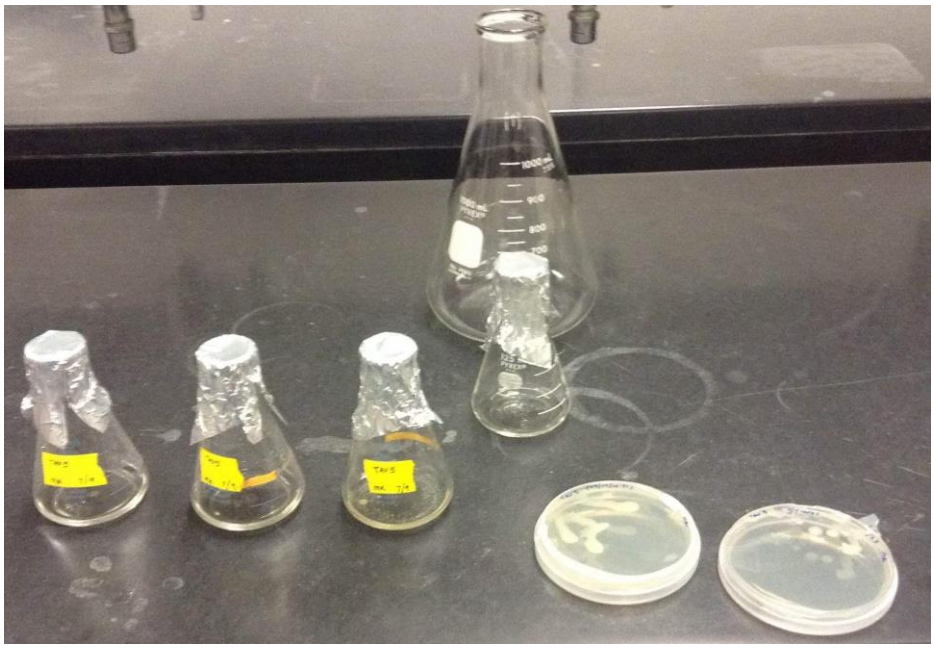


Figure 3-29 Petri dishes and flask used for inoculating from petri dishes to 5ml flasks containing media

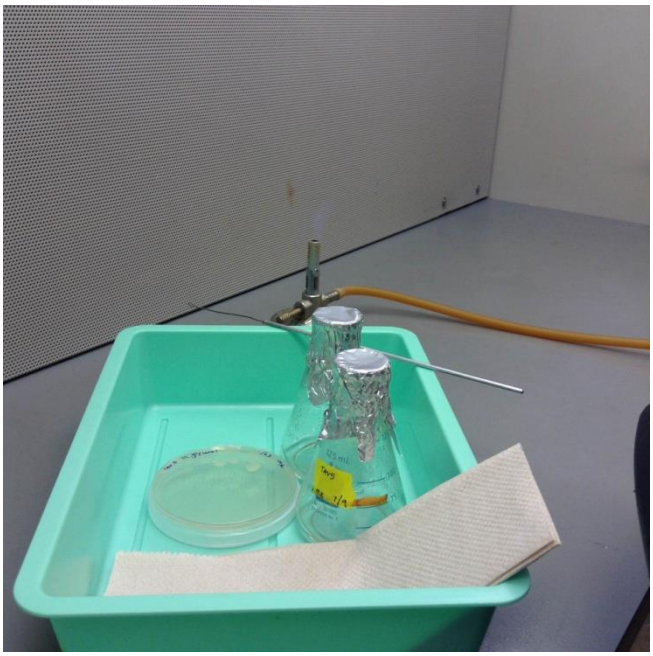
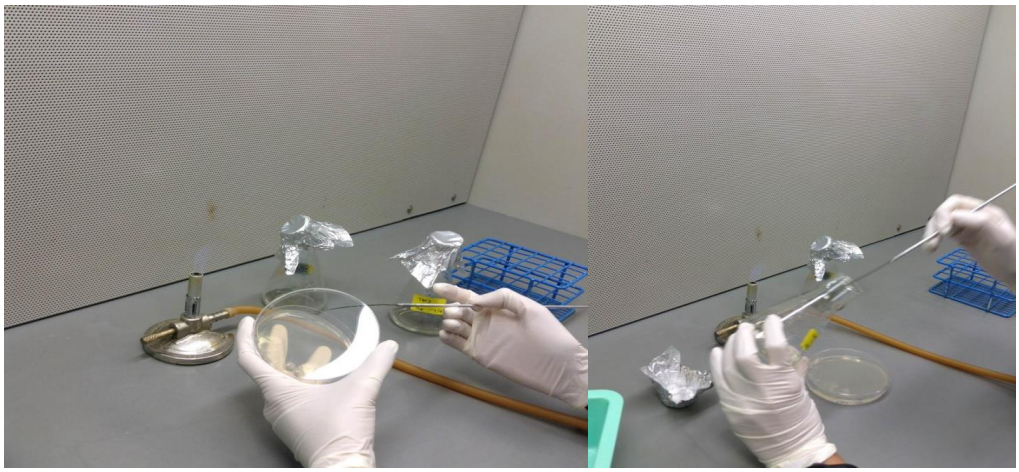


Figure 3-30 Clean bench and flame, flasks, petri dish and sterile loop for inoculating

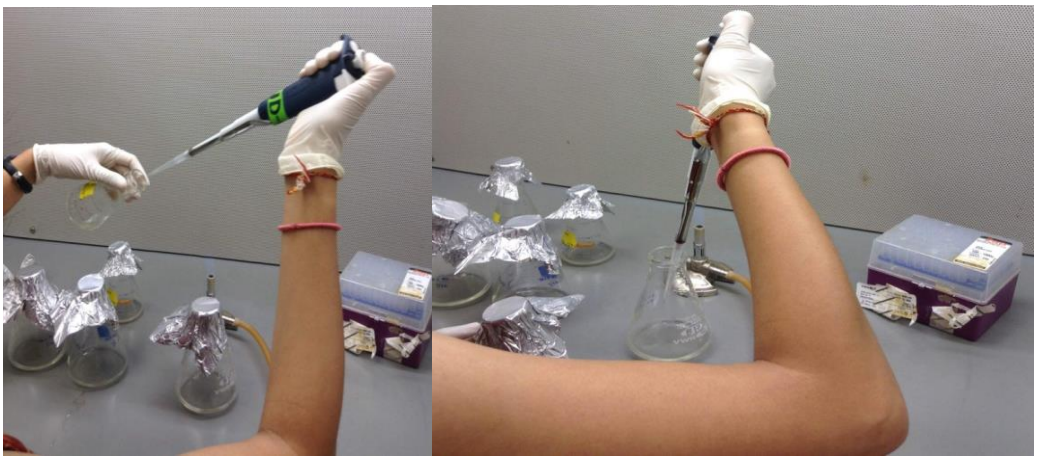


(a) (b)

Figure 3-31 a) Inoculating microbe from petri dish to flask b) flask contains 5 ml of media

3.6.3.2 Inoculating microbe from 5ml flask to 50 ml flask

After passing 7 days, the color of 5 ml flasks turned to cloudy, which means that microbes are growing in the Hypoxia chamber. These flasks were then inoculated into larger flasks (50 ml), as shown in Figure 3-32. All steps for inoculating using clean bench air flow and flame and sterile loop in sterilized environment were repeated.



(a) (b)

Figure 3-32 a) Inoculating microbe from 5ml media flask to b) 50 ml media flask

3.6.3.3 Inoculating from 50 ml media to 500 ml media

After passing 14 days, by the color of 50ml flasks turned to cloudy which means that microbes were growing in the Hypoxia chamber. The 50 ml flasks were inoculated into larger flasks (500 ml), as shown in Figure 3-33. All steps for inoculating using clean bench air flow and flame and sterile loop in sterilized environment were repeated.

Figure 3-33 Inoculating microbes from 50 ml flasks to 500 ml flasks

3.7 Last Step

After 8 days, TAV5 microbes in three 500ml flasks were ready to mix with sludge from the wastewater treatment plant in a 50 % /50% ratio by volume. Three additional reactors, one each of paper, yard waste, and wood, were assembled. Figure 3-34 shows the 1.5 liters of TAV5 microbes ready to add to the bioreactors.

(a) (b)

Figure 3-34 a),b) 1.5 ml of microbes from termite gut TAV5 ready for using for bioreactors

Figure 3-35 shows the hot room in lab 116 at CELB lab after operation of other 3 reactors with termite gut microbes.

Figure 3-35 Hot room operation of another 3 reactors with termite gut microbes

Chapter 4

Results and Discussions

In this chapter the results obtained from laboratory instrumented reactors and tests are presented and analyzed to evaluate the effect of a specific strain from termite gut TAV5 on amount of methane generation.

4.1 pH of leachate

In the initial days of the reactors, the frequency of pH measurement was higher as base needed to be added if pH dropped too low. Therefore for the first 30 days, pH was measured more frequently than the rest of the active time of the reactors. After the first 30 days, pH was measured once in a week. The pH variation over time in all reactors is shown in Figure 4-1.

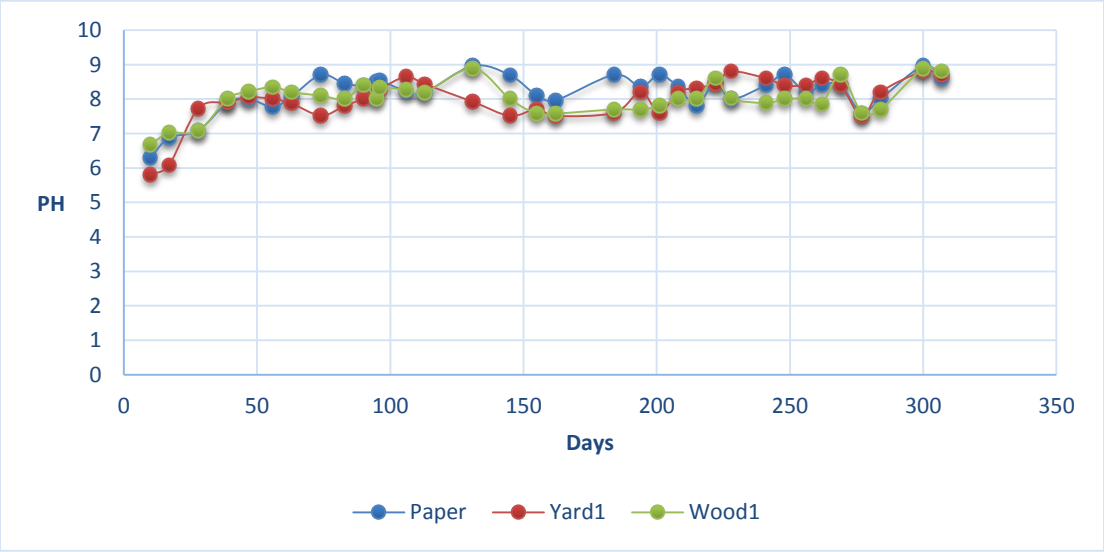


Figure 4-1 pH variation vs. time in laboratory reactors Paper1, Yard1, and Wood1

In the initial days, the pH level of the reactors was less than 7. This acidic phase existed because of the ongoing acid accumulation state in waste degradation. The initial pH levels of reactor Paper1, Yard1, and Wood1, were 6, 5.6, 6.4, respectively. The low pH level continued up to 27 days for Paper1, 24 days for Yard1, and 17 days for Wood1. A gradual rise in pH level was observed afterwards in these reactors, so that values fluctuated between 7 and 8. This was due to the conversion of carboxylic acid into methane and carbon dioxide, which is an indication of the fourth phase of biodegradation. The maximum pH values were 8.96, 8.8, and 8.89 for Paper 1, Yard1 Wood 1, respectively.

Degradation phases in reactors Paper1, Yard1 and Wood1 in relation to the pH level variation are summarized in the following Table 4-1.

Table 4-1 Phases of degradation with change in pH levels

Degradation Phase	**REACTOR-Paper1**		**REACTOR-Yard1**		**REACTOR-Wood1**	
	pH	**Time (Days)**	**pH**	**Time (Days)**	**pH**	**Time (Days)**
I	6-6.3	0-10	5.6-5.9	0-10	6-6.68	0-10
II	6.3-6.87	10-17	5.9-6.8	10-17	6.68-6.9	11-15
III	6.87-7.08	17-28	6.8-7	18-21	6.9-7	16-17
IV	>7	28-315	>7	22-315	>7	17-315

According to the degradation phases based on pH results, Wood1 reached the methanogenesis phase quicker than other reactors. It took only 16 days, whereas reactors Yard1 and Paper1 took 24 and 27 days, respectively.

Warith et al. (2002) recorded change in pH with time (Figure 4-2), which is in accordance with the obtained results from the reactors operated this study. PH was initially low, and then increased to between 7 and 8.

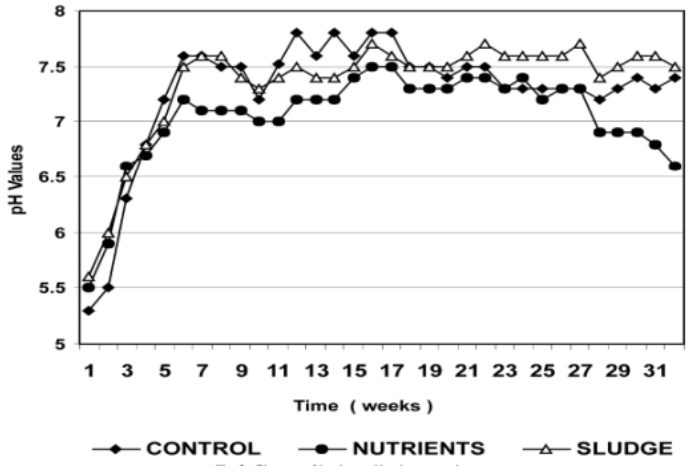


Figure 4-2 Changes in pH of leachate with time (Warith et al., 2002)

4.2 Gas data for reactors, seeded with digester sludge alone

4.2.1 Gas composition

Figures 4-3, 4-4, 4-5 shows composition of gases generated from the reactors Paper1, Yard1, and Wood1respectively. In the initial days, oxygen depleted severely leading to anaerobic conditions inside the reactors and the amount of carbon dioxide increased over time. This increase in percent of carbon dioxide was due to the acetogenesis phase, which produces volatile fatty acids but also carbon dioxide and water vapor. Carbon dioxide for Paper1 peaked at 24 and 96 days, and for Wood1 at 76 days, and then gradually decreased with a mild slope. The carbon dioxide for the Yard 1 reactor decreased gradually from a maximum percentage of 57.9 at day 10 and to 38 % at day 56 and then gradually 28.5% at day 300. For all 3 reactors, initially the percentage of carbon dioxide is greater than the methane percentage but as methanogenesis continues, the methane percent increases and surpasses carbon dioxide.

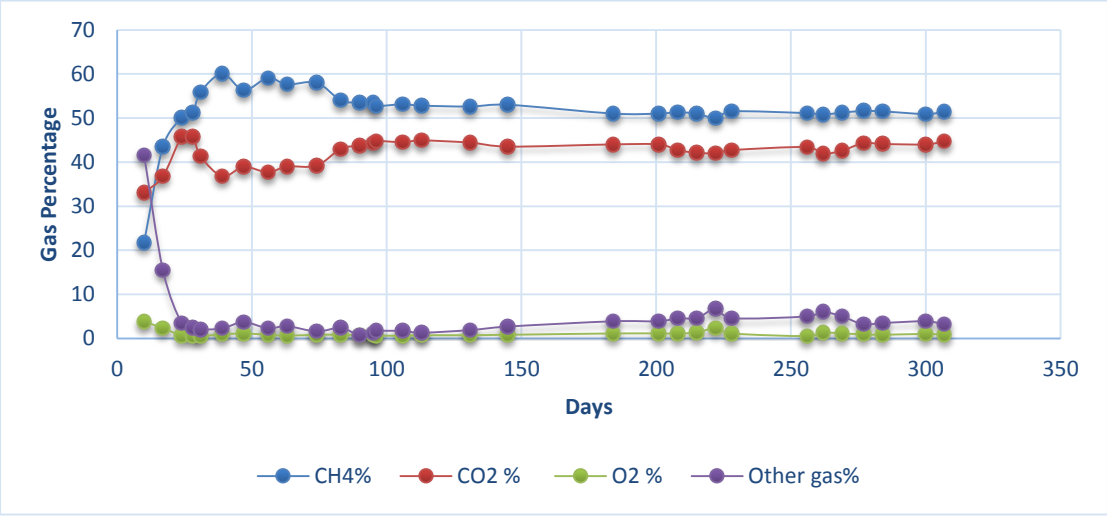


Figure 4-3 Gas composition percent vs. time for Reactor Paper1

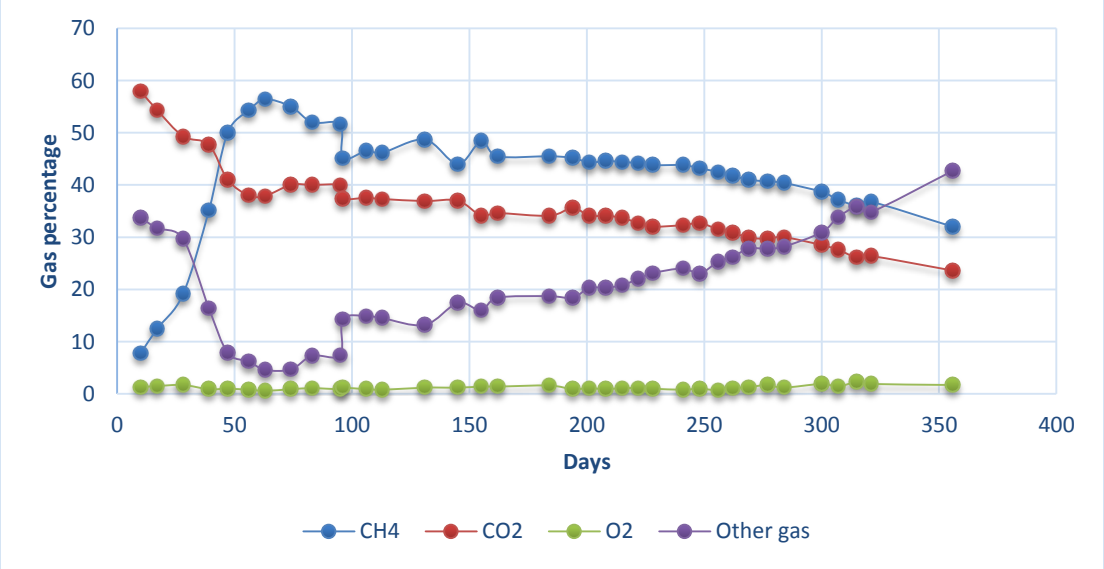


Figure 4-4 Gas composition percent vs. time for Reactor Yard 1

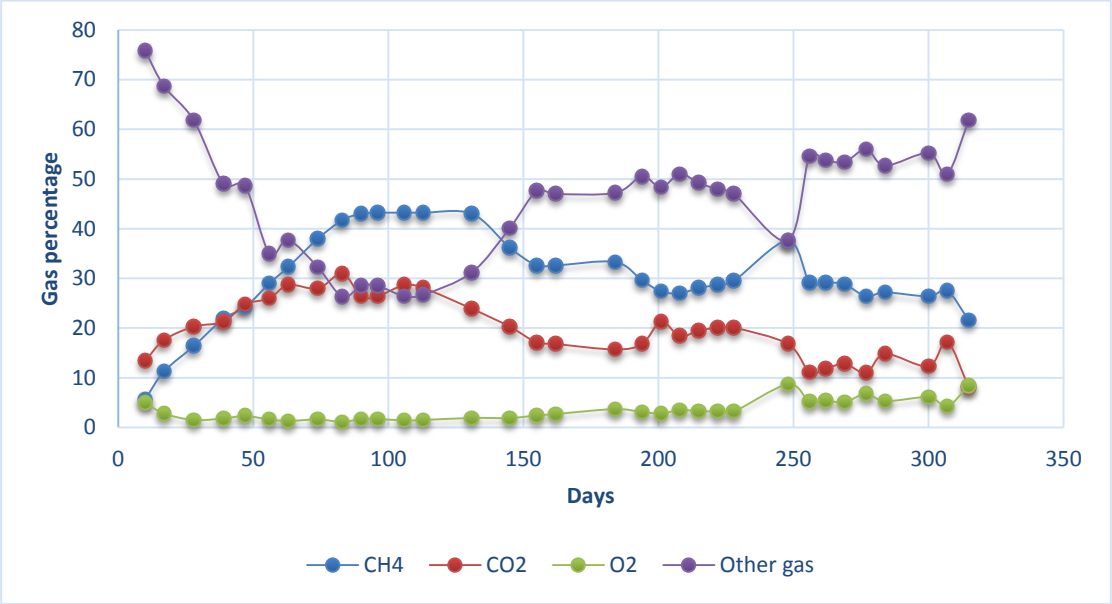


Figure 4-5 Gas composition percent vs. time for Reactor Wood 1

In Figures 4-3, 4-4, 4-5 the “other gases” initially represents molecular nitrogen present in air, which decreases over time. However, for Yard1 and Wood1, the percent of “other gases” increases again after day 100, likely due to production of hydrogen and water vapor during the acidogenesis and methanogenesis phases, respectively. “Other gases” can also include sulfides, disulfides, mercaptans, and ammonia generated from organic compounds containing sulfur and nitrogen.

Figure 4-4 shows methane percent over time for all reactors Paper1, Yard1 and, Wood1. At the very beginning, it took 10 days for all reactors to generate some gas. For all reactors methane percent increases to a peak: 60% on day 39 for Paper1, 56.4% on day 63 for Yard1, and 43.2% on day 96 for Wood1. Methane then decreases and levels off at a fairly constant value: around 50% for Paper1, 40% for Yard1, and 30% for Wood1. Paper1 thus has the highest percent methane, followed by Yard1. Wood1 has the lowest methane percent. Paper has the highest percent methane, likely because it has the largest amount of cellulose, and also

has larger surface area per unit volume of waste for the microbes to access, compared to yard waste and particularly wood waste.

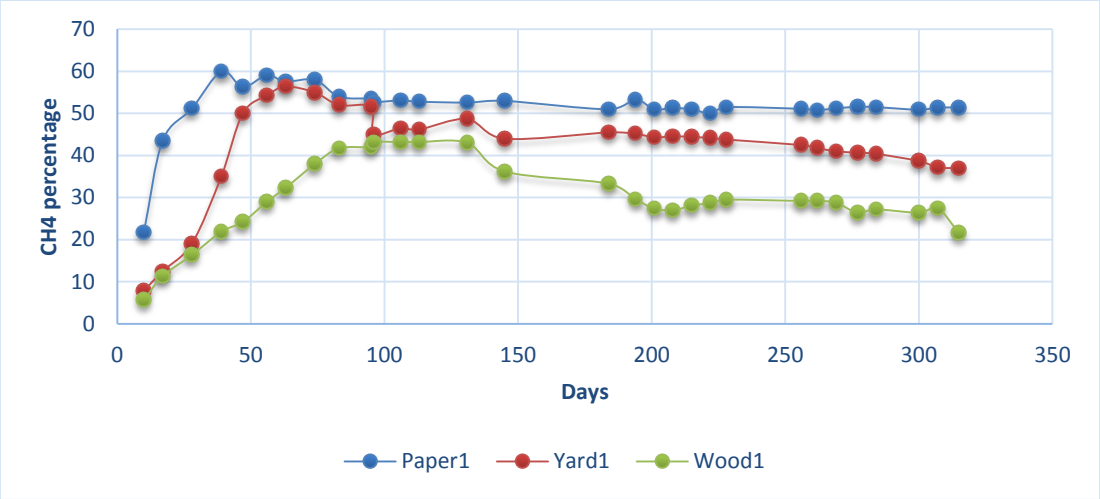


Figure 4-6 Methane percent vs. time for Paper1, Yard1, and Wood1

Figure 4-7 shows trends of CH_4: CO_2 with time. The ratio of methane to carbon dioxide increased gradually for all reactors. The methane to carbon dioxide ratio peaked at values of 1.6 (day 39) and 1.5 (day 56) for Paper1 and Yard1, respectively. After that, the ratio for Paper1 and Yard 1decreases and follows a fairly flat line around 1.2 and 1.4, respectively. For reactor Wood1 the methane to carbon dioxide ratio increases to 2.1 on day 184, decreases to around 1.5, and then increases to over 2 on day 256, with some fluctuation observed to day 315.

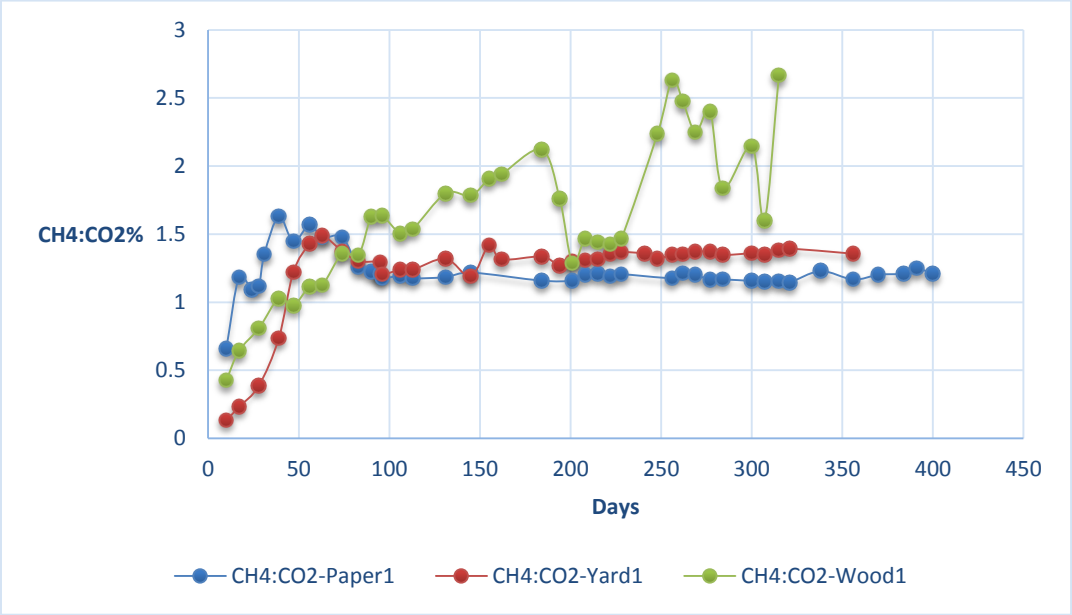


Figure 4-7 Methane to carbon dioxide ratio vs. time for Paper1, Yard1 and, Wood1

4.2.1.1 Cumulative volume and rate of methane generation

Figure 4-8 compares cumulative methane generation (liter/kg) over time for reactors Paper1, Yard1 and Wood1. At the very beginning, it took 10 days for all reactors to generate some gas. Yard1 and Wood1 reach asymptotic values around day 250, indicating that methane generation is complete. Methane generation for Paper1, however, continued to increase.

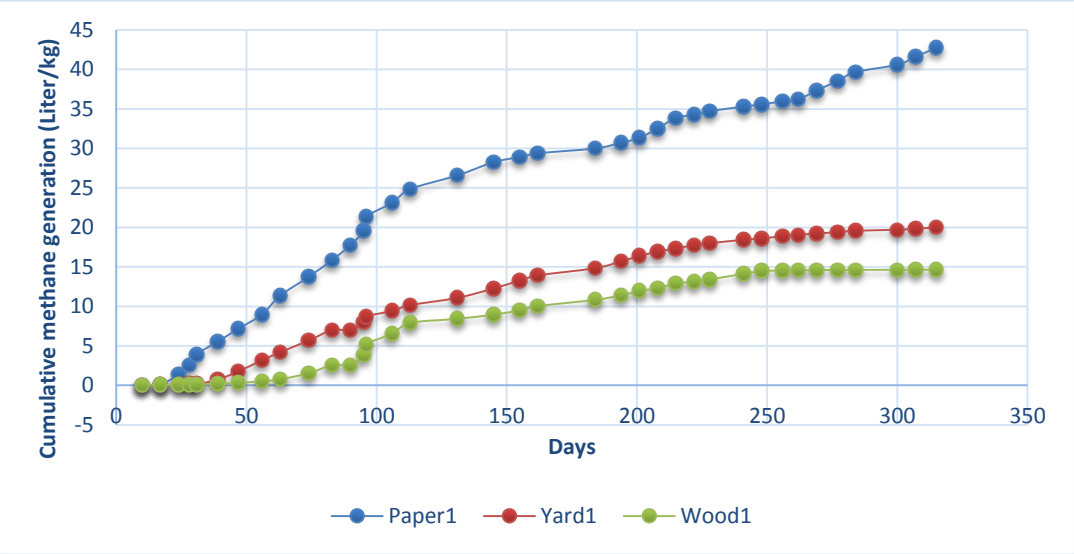


Figure 4-8 Cumulative methane generation (Liter/kg) vs. time for Paper1, Yard1, and Wood1

Table 4-2 shows cumulative methane generation from reactors Paper1, Yard1, and Wood1 at 315 days, and Table 4-3 shows cumulative methane generation at the end of each reactor's operation. Yard1 and Wood1 had ceased generating methane. Paper1 was still generating methane, but the reactors Yard1, and Wood1 was taken out of operation at 356 and 315 days respectively.

Table 4-2 Comparison of cumulative methane generated for reactors at 315 days

Reactor	Number of days	Cumulative CH_4 (Liters)	Cumulative CH_4 (Liters/kg)
Paper1	315	308.3	42.66
Yard1	315	163.6	19.77
Wood1	315	62.62	14.59

Table 4-3 Comparison of cumulative methane generated at end of reactor operation

Reactor	Number of days	Cumulative CH_4 (Liters)	Cumulative CH_4 (Liters/kg)
Paper1	391	350.24	48.46
Yard 1	356	166.7	20.14
Wood1	315	62.62	14.59

Figure 4-9 shows the rate of methane generation vs. time for all reactors. Paper1 has

Highest rate of methane generation due to largest amount of cellulose in mixed paper, and Wood 1 has the lowest. The maximum methane generation rate for Paper1, Yard1, and Wood1 occurs on day 28, day 56, and day 96, respectively. Study conducted by Barlaz et al. (2006) showed that maximum methane generation rate was found within the first 100 days of reactor operation, as shown in Figure 4-10, which is in good agreement with the results obtained in this study.

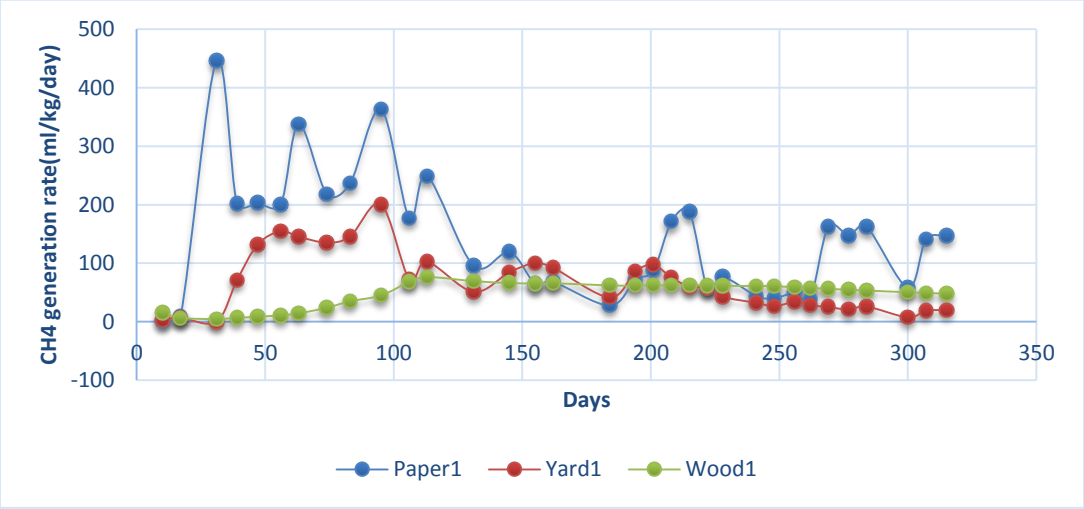


Figure 4-9 Methane generation rate (ml/kg/day) vs. time

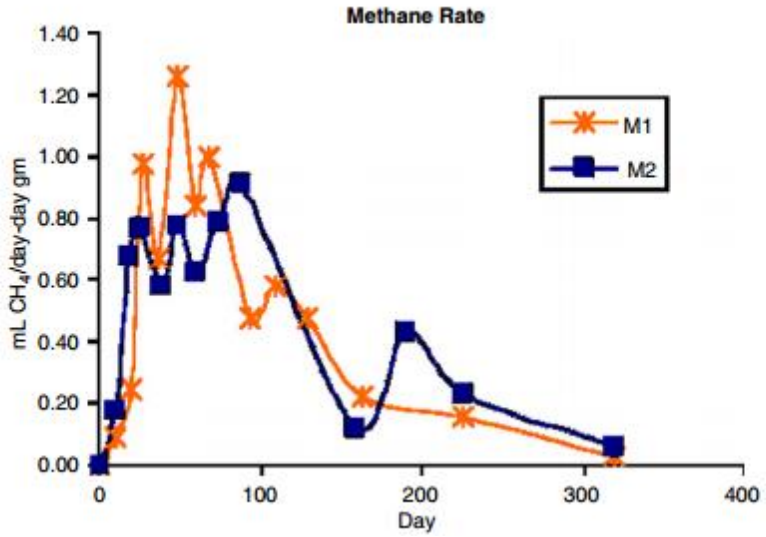


Figure 4-10 Generation of methane in experimental reactors (Barlaz et al. 2006)

4.3 Leachate pH for reactors seeded with a mixture of digester and termite gut TAV5 microbes, and comparison to reactors seeded with digester microbes

Figure 4-11 shows pH variation over time in reactors seeded with mixture of digester and termite gut TAV5 microbes. In the initial days, pH level of the reactors was less than 7. This acidic phase existed because of the ongoing acid accumulation state in waste degradation. Decreased pH level continued up to 17 days for Reactor Paper2, 12 days for Yard2, and 5 days for Wood2. A gradual rise in pH level was observed afterwards in these reactors, stabilizing in the range between 7 and 8, but fluctuating between these values. The rise in pH was due to the conversion of carboxylic acid into methane and carbon dioxide, which is an indication of the fourth phase of biodegradation.

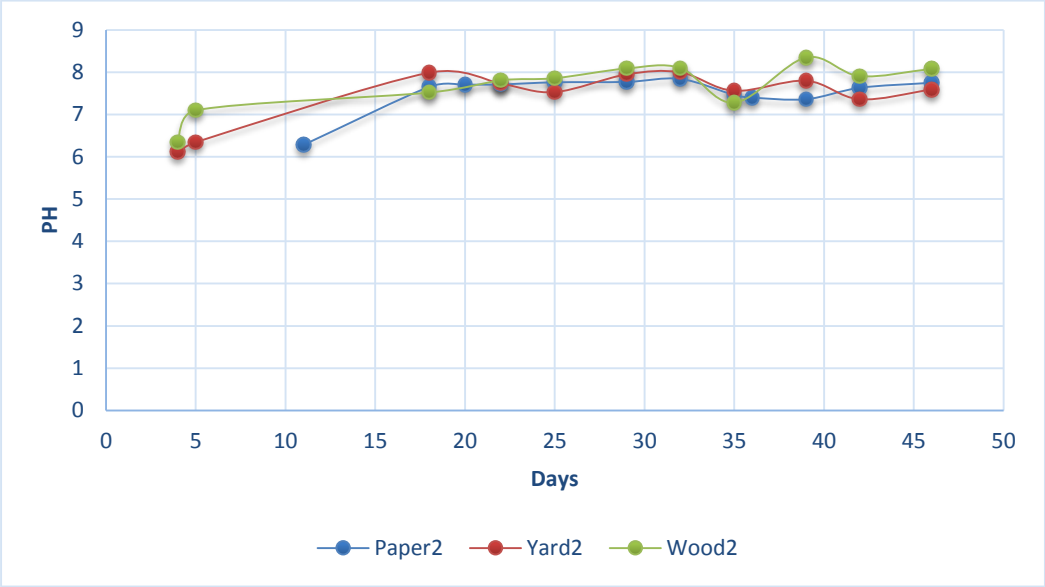


Figure 4-11 pH variation vs. time for Reactors Paper2, Yard2, and Wood2

Figures 4-12, 4-13and 4-14 compare pH variations with time for reactors Paper1 & Paper2, Yard1 & Yard2, and Wood1, & Wood2, respectively. For all reactors, the pH level was initially less than 7. A gradual rise in pH was observed, stabilizing in the range between 7 and 8, but fluctuating between these values. The addition of the TAV5 microbes did not noticeably impact reactor PH.

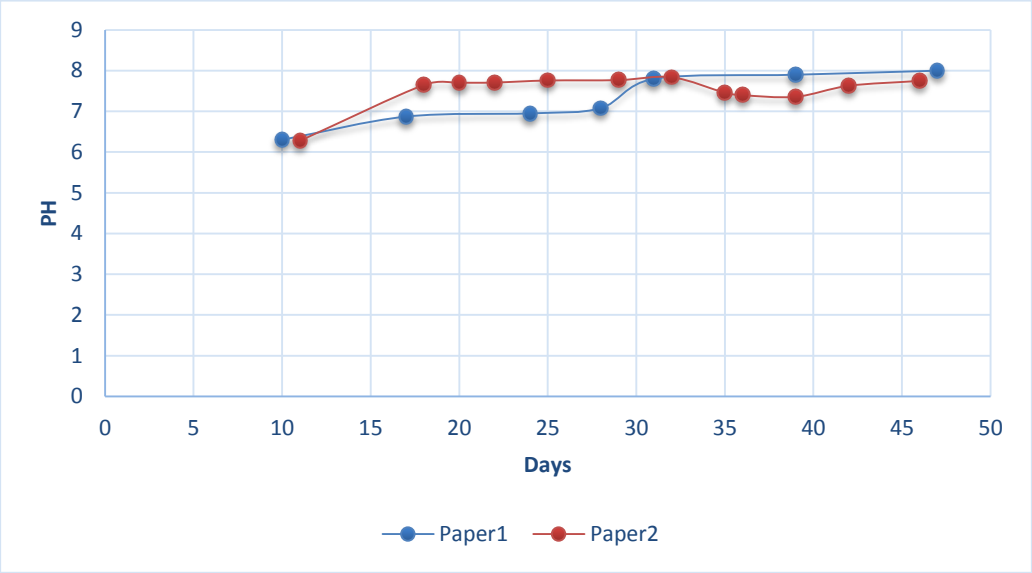


Figure 4-12 pH variation vs. time for Reactors Paper1 and Paper2

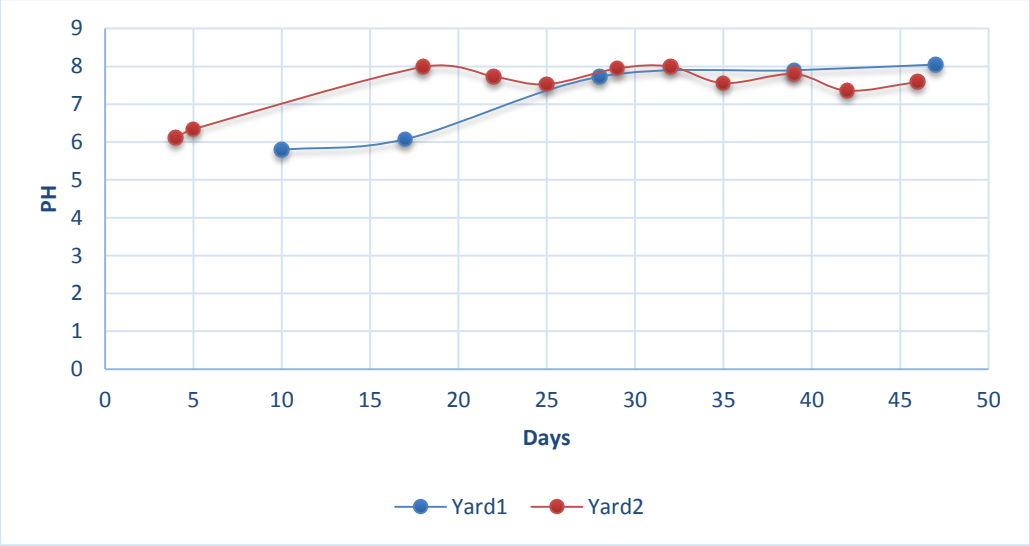


Figure 4-13 pH variation vs. time for Reactors Yard1 and Yard2

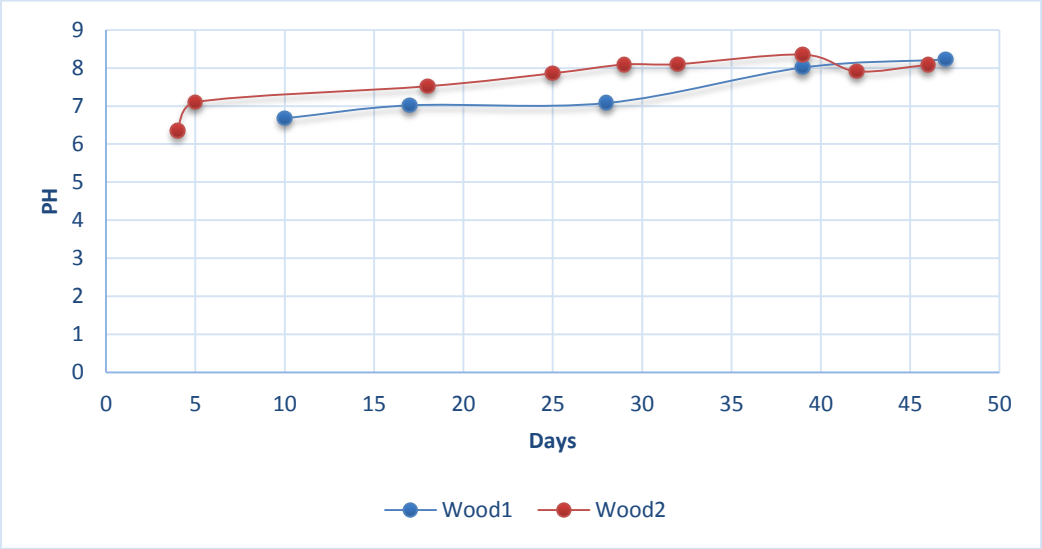


Figure 4-14 pH variation vs. time for Reactors Wood1 and Wood2

4.4 Gas data for reactors seeded with a mixture of digester and termite gut TAV5 microbes, and comparison to reactors with digester microbes only

4.4.1 Gas composition

Figures 4-15, 4-16, and 4-17 shows composition of gases generated from the reactors Paper2, Yard2, and Wood2. Oxygen remains low with time, indicating anaerobic conditions. The “other gases” represents molecular nitrogen present in air, which decreases over time.

The carbon dioxide percent is initially higher than methane percentage for Paper2 and Yard2, as expected due to the acetogenesis phase which produces carbon dioxide. For Paper 2 and Yard 2, the percent of carbon dioxide decreases over time and methane increases over time, as the methanogenesis phase produces methane. For Paper1, percent of CO_2 and CH_4 both stabilize around 50%.

For reactor Wood2, carbon dioxide and methane simultaneously increase with time. The reason that carbon dioxide increases rather than decreasing in Wood2 is unknown.

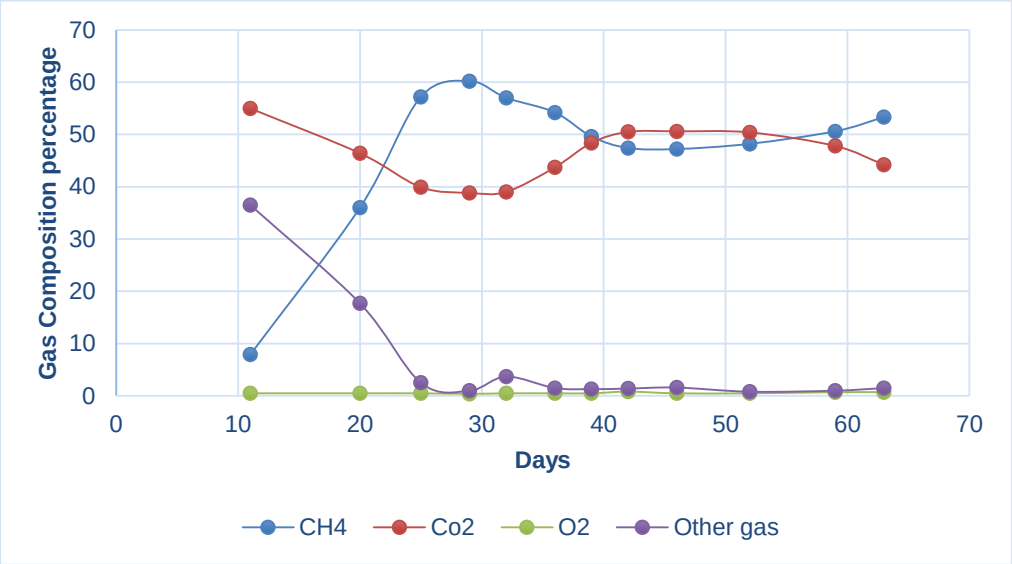


Figure 4-15 Gas composition percent vs. time for Reactor Paper2

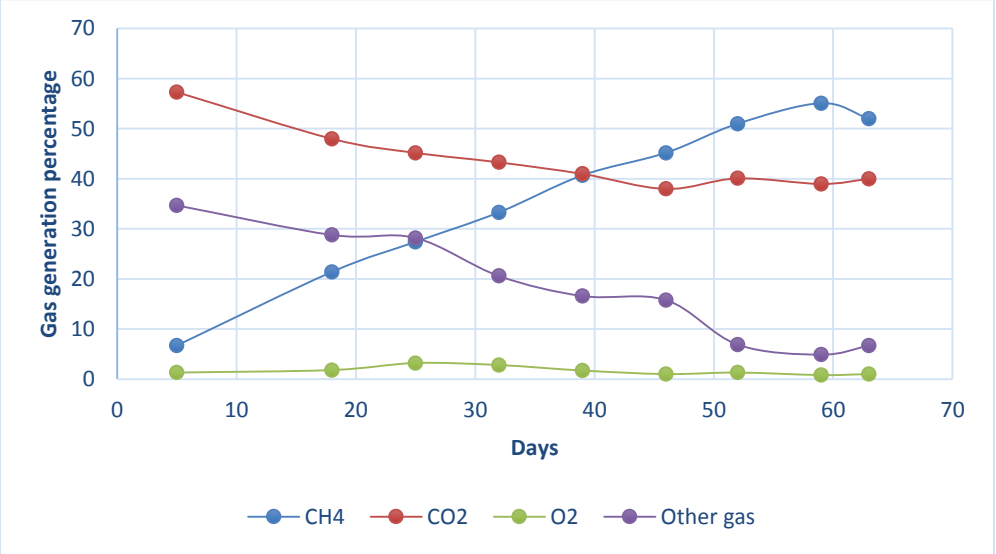


Figure 4-16 Gas composition percent vs. time for Reactor Yard2

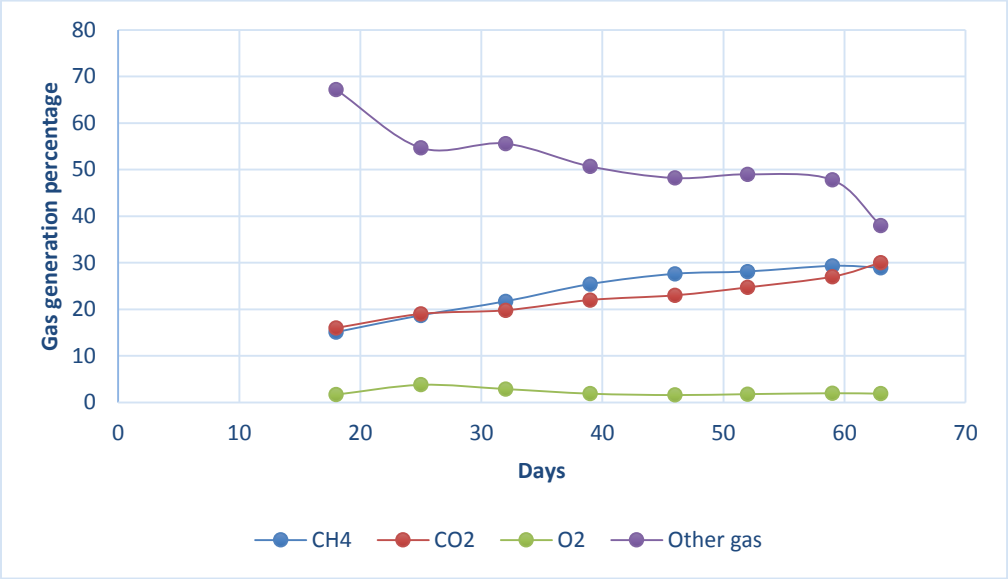


Figure 4-17 Gas composition percent vs. time for Reactor Wood2

Figure 4-18 shows methane percentage over time for reactors Paper2, Yard2, and Wood2. For Paper2, methane peaks at around 60% on day 28, and then decreases, leveling off at about 48% after day 40. Methane percent for Yard2 and Wood2 is continuing to increase; these reactors are still being operated.

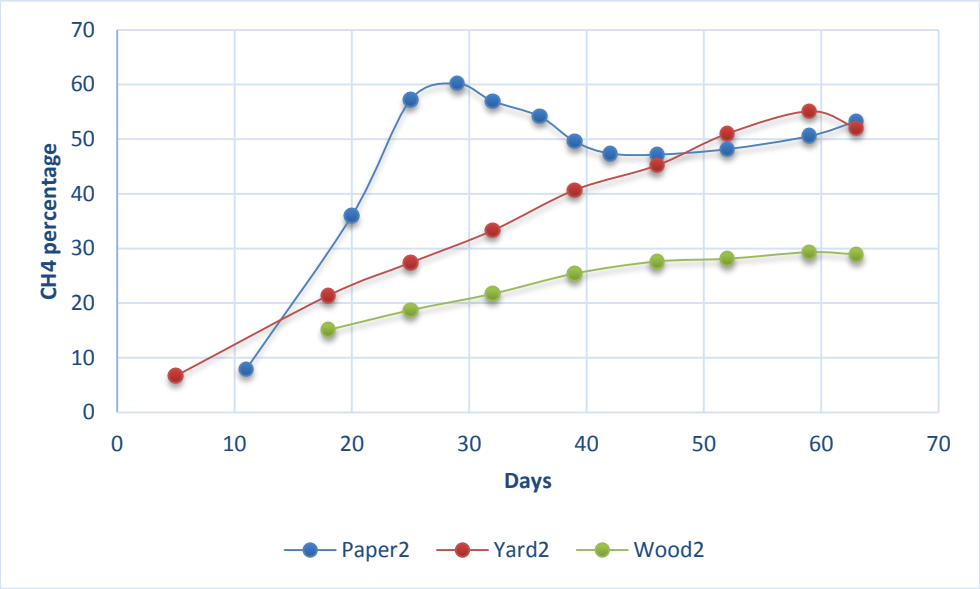


Figure 4-18 Methane percent vs. time for Reactors Paper2, Yard2, and Wood2

Figure 4-19 shows methane to carbon dioxide ratio for Paper2, Yard2, and Wood2. We can see that in all 3 reactors we have increase in methane to carbon dioxide ratio. For reactor Paper 2 the methane to carbon dioxide ratio peaked at 1.55 (day 29) and then decreased, leveling off at around 0.9. Methane to carbon dioxide ratio for Yard2 and Wood2 is continuing to increase; these reactors are still being operated.

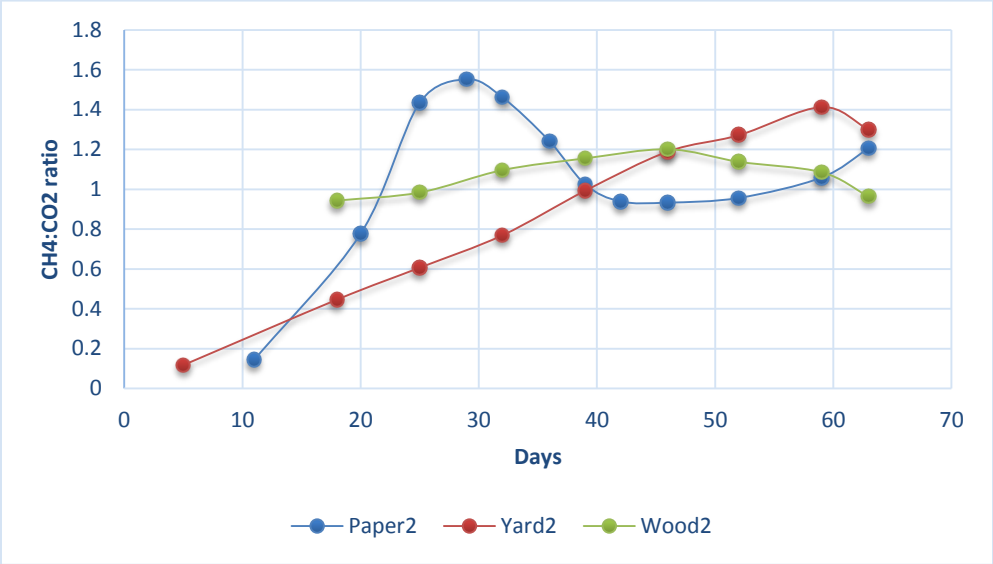


Figure 4-19 Methane to carbon dioxide ratio for Paper2, Yard2, and Wood2

Figure 4-20 compares methane percentage in reactors Paper1 and Paper 2, for the first 63 days of operation. Methane percent for Paper 1 is initially higher than for Paper2, but we have a sudden rise in methane percentage for Paper 2 at day 29. The peak for both reactors is around 60%, but occurs on day 29 for Paper2 and day 39 for Paper1. The TAV5 microbe could be causing the peak to occur sooner.

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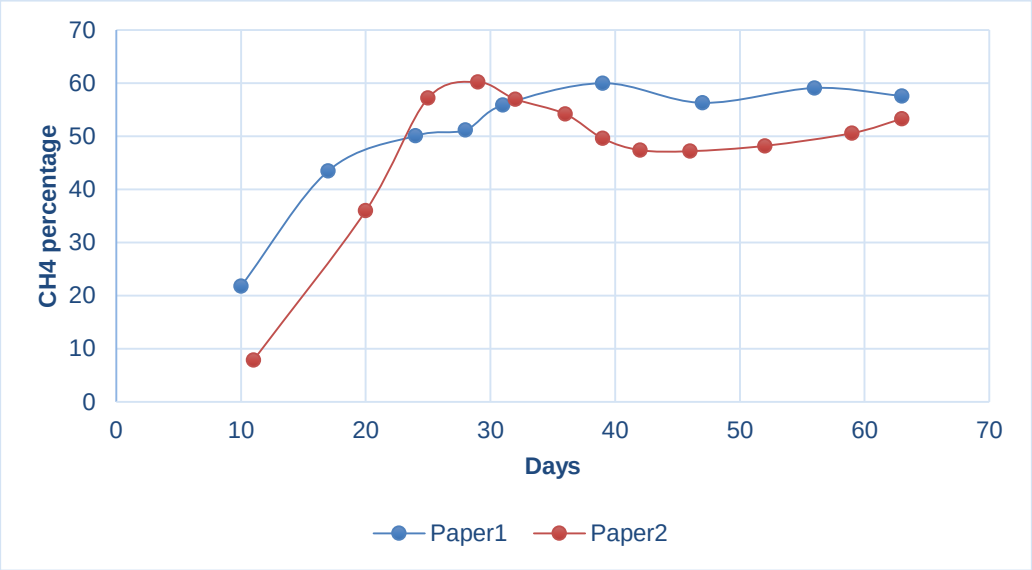

Figure 4-20 Methane percent vs. time for Paper1 and Paper2

Figure 4-21 compares methane to carbon dioxide ratio for reactors Paper1 and Paper2 for the first 63 days of operation. The initial methane to carbon dioxide ratio for Paper2 is less than Paper1, but for Paper2 we can see a sudden rise in methane to carbon ratio from day 10 to day 29. The peak for both reactors is around 1.6, but occurs on day 29 for Paper2 and day 39 for Paper1.

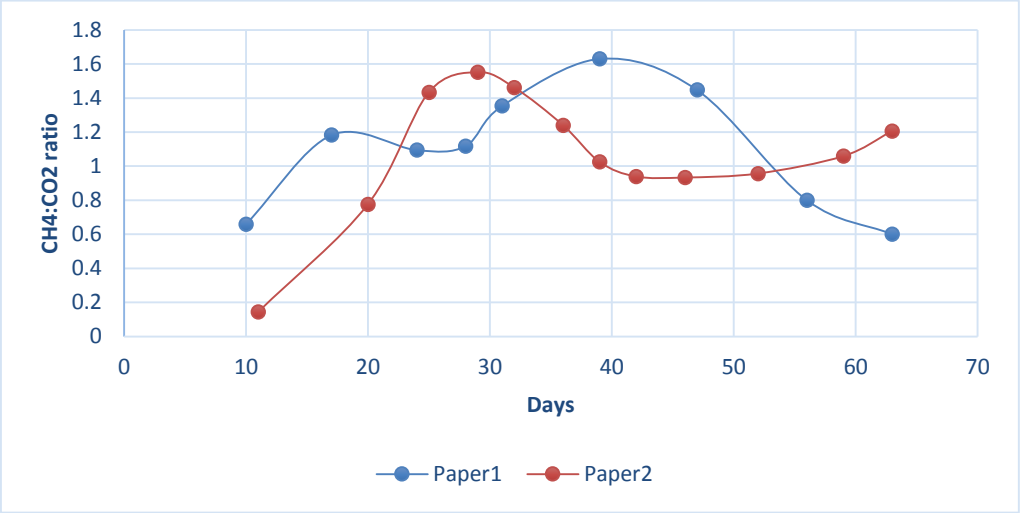

Figure 4-21 Methane to carbon dioxide ratio vs. time for Paper1 and Paper2

Figure 4-22 compares methane percent vs. time for Yard1 and Yard2 for the first 63 days of operation. Methane generation started in Yard2 sooner than Yard1, and methane percent for Yard2 is higher than Yard1. Methane to carbon dioxide ratio, shown in Figure 4-23, shows a similar trend as methane percentage. The TAV5 microbe may be causing the percent to be higher for Yard2 compared to Yard1. Reactor operation will be continued, to determine how the methane percent changes with time.

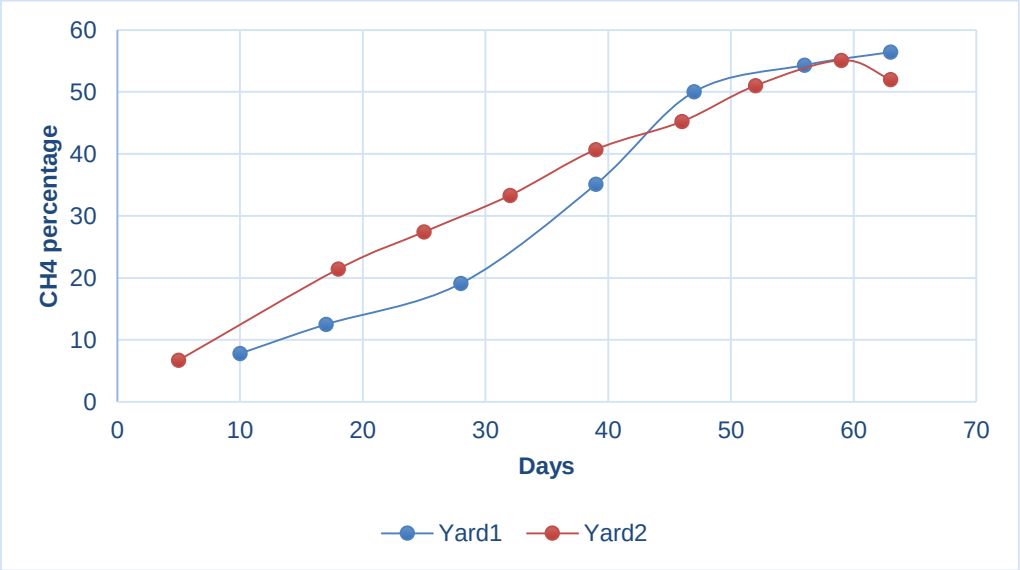

Figure 4-22 Methane percent vs. time for Yard1 and Yard2

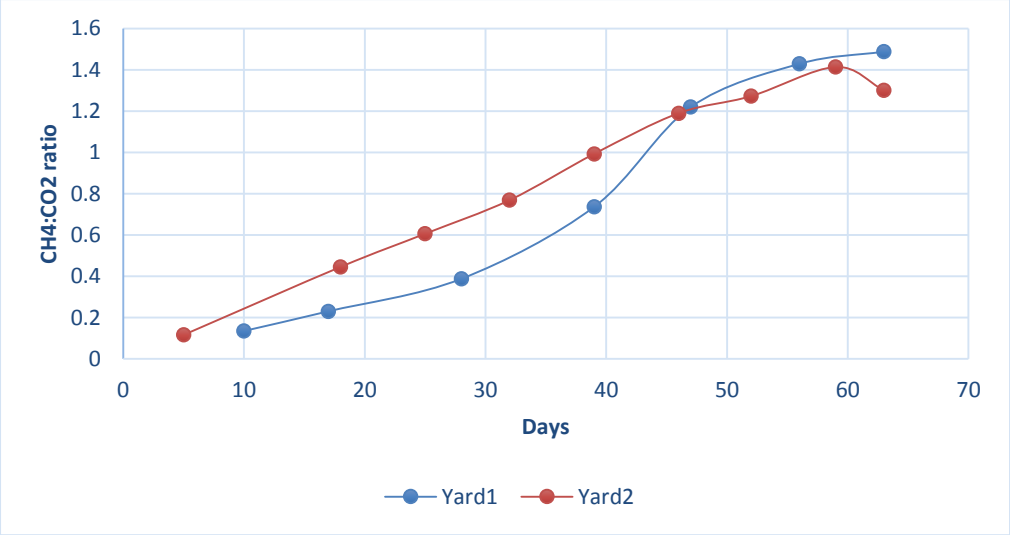

Figure 4-23 Methane to carbon dioxide ratio vs. time for Yard1 and Yard2

Figures 4-24 and 4-25 compare Wood1 and Wood2 for methane percentage and methane to carbon-di-oxide ratio vs. time. Methane generation for Wood1 started faster than Wood2, but the methane percent for Wood2 is greater than Wood1. Methane to carbon dioxide ratio shows

a similar trend as methane percentage. The TAV5 microbe may be causing the percent to be higher for Wood2 compared to Wood1. Reactor operation will be continued, to determine how the methane percent changes with time.

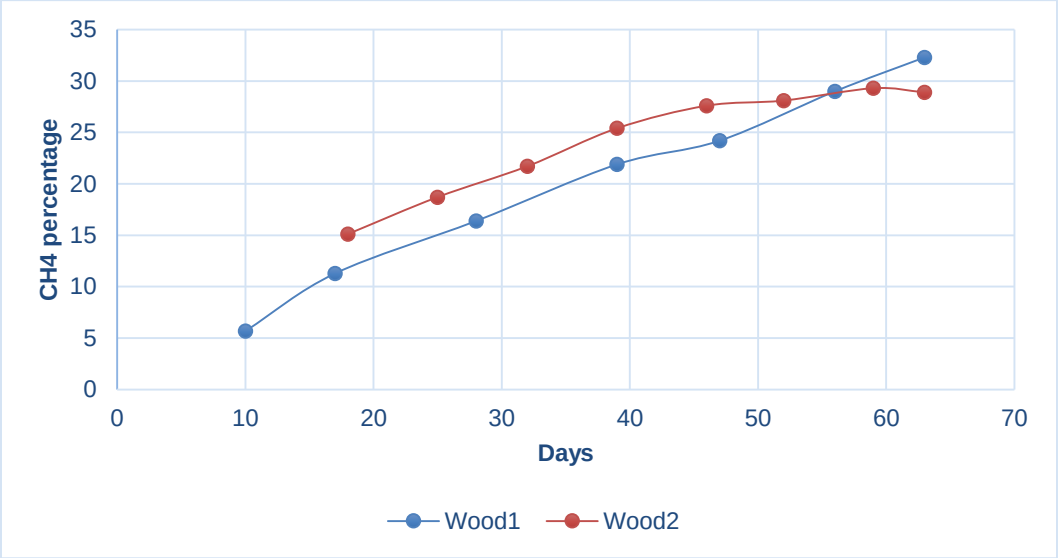

Figure 4-24 Methane percent vs. time for Wood1 and Wood2

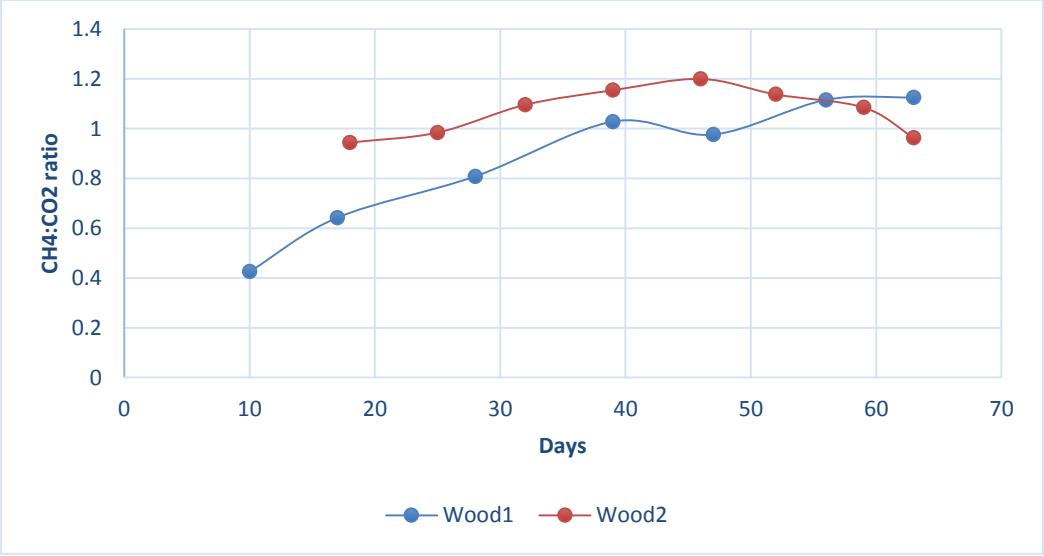

Figure 4-25 Methane to carbon dioxide ratio vs. time for Wood1 and Wood2

4.4.1.1 Cumulative volume and rate of methane generation

Cumulative methane generated from reactors Paper2, Yard2, Wood2 with time is shown in Figure 4-26. It was observed that reactor containing Paper2 generated the highest amount of methane compared to the others. Wood2 generated the least amount of methane.

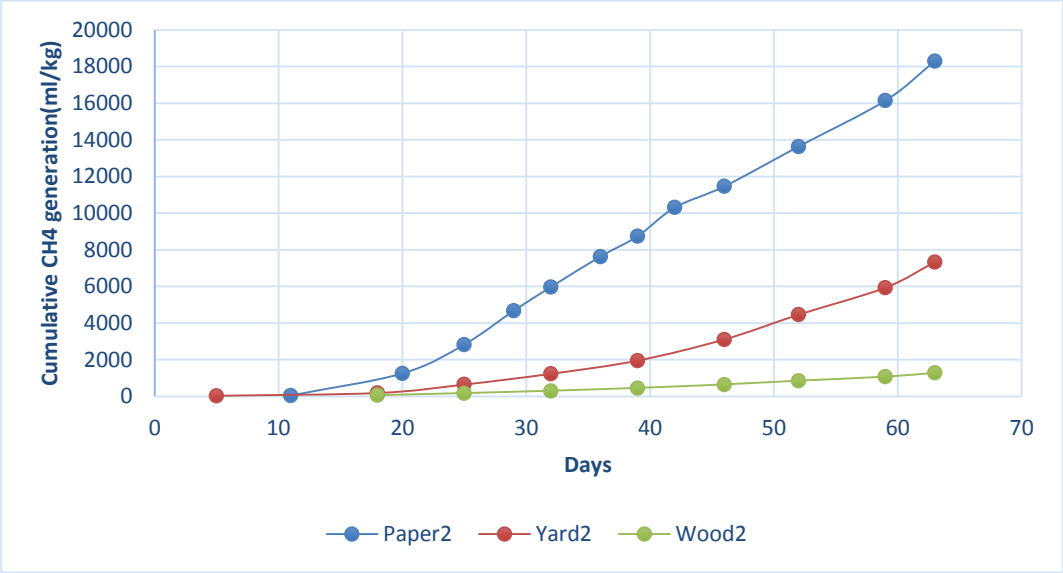

Figure 4-26 Cumulative methane generation in reactors Paper2, Yard2 and Wood2 to 63 days

Figure 4-27 shows methane generation rate (Liter/kg/day) for Paper2, Yard2, and Wood2. In all three reactors methane generation rate increases over time. Methane generation rate for Paper2 is higher than Yard2, which is higher than Wood2. For Paper 2 rate of methane generation peaks between 25 and 29 days and then decreases. For Yard2 methane generation rate is generally increasing with time.

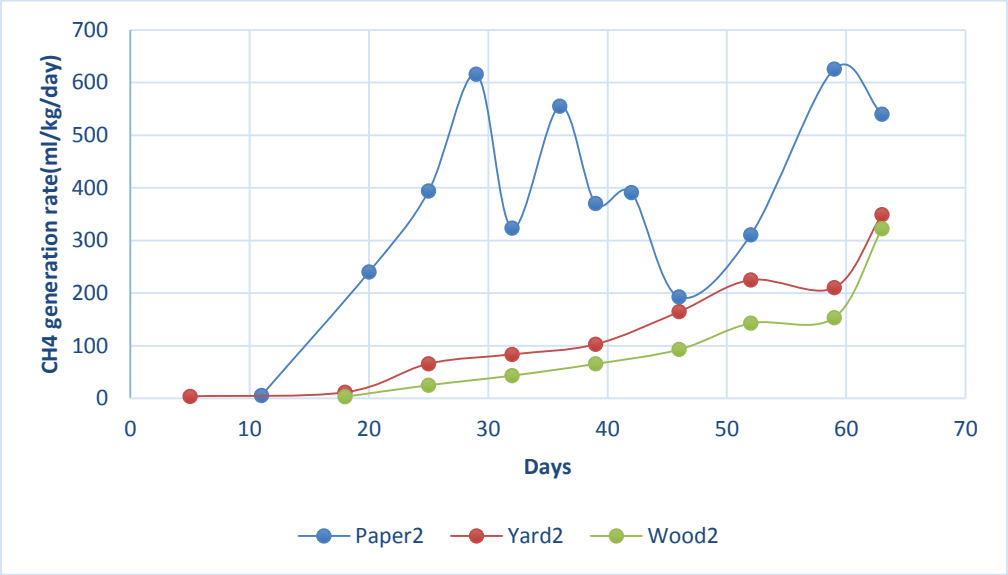

Figure 4-27 Methane generation rate (ml/kg/day) for Paper2, Yard2 and, Wood2

Figure 4-28 shows cumulative methane generation (ml/kg) over time for Paper1 and Paper2 for the first 63 days of reactor operation. Cumulative methane generation rate (ml/kg/day) for both Paper1 and Paper 2 increases over time. Cumulative methane generation for Paper 2 is higher than Paper1 .Paper 2 generates 18.34 (Liter/kg) in 63 days and Paper 1 generates10.86 Liter/kg in 63 days, which is about 1.7 times more than the amount of methane that Paper 1 produced in this period of time. The TAV5 microbe may be causing higher methane generation in Paper2. Reactor operation will be continued.

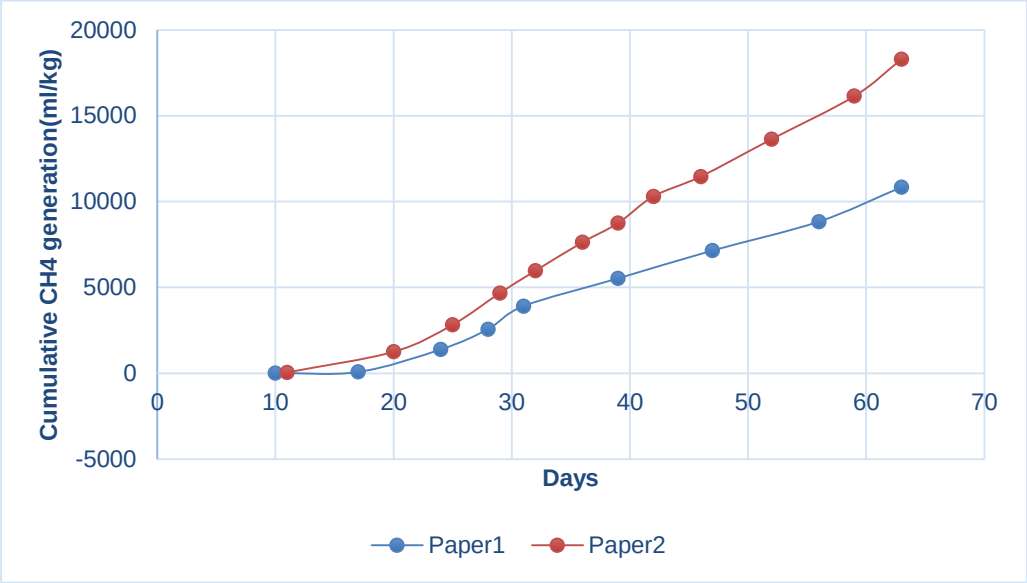

Figure 4.28 Cumulative Methane generation (ml/Kg) with time for Paper1 and Paper2

Figure 4-29 shows the methane generated rate (ml/kg/day) for Paper1 and Paper2. For Paper 1, the maximum methane generation rate occurs at day 24 and for Paper2 between days 25 and 29. The maximum generation rate is higher for Paper2 compared to Paper1.

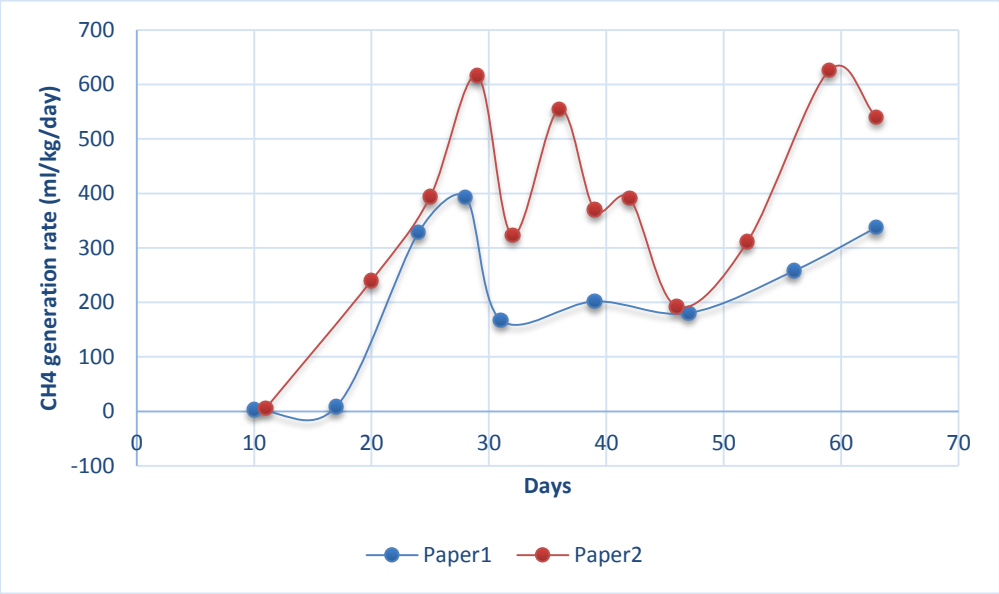

Figure 4-29 Methane generation rate (ml/kg/day) for Paper1 and Paper2

Figure 4-30 shows cumulative methane generation (ml/kg) for Yard1 and Yard2 over the first 63 days of reactor operation. Cumulative methane generation for Yard2 is higher than Yard1.Yard1 generates 4.2 (Liter/kg) Liter methane and Yard2 produces 7.34 (Liter/kg) methane, which is 1.75 times more than cumulative methane generated in Yard1. The TAV5 microbe may be causing higher methane generation in Yard2. Reactor operation will be continued.

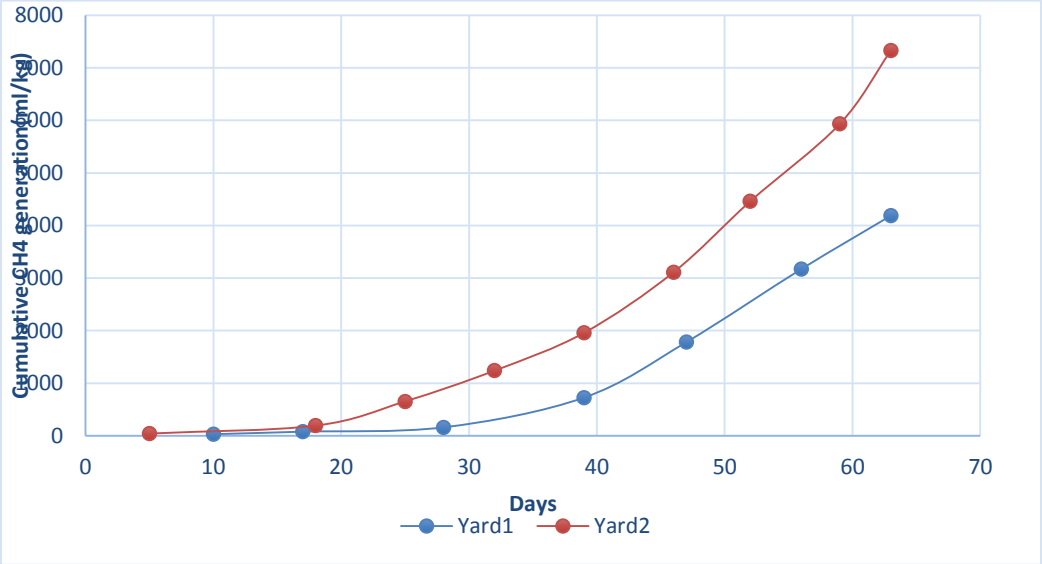

Figure 4-30 Cumulative methane generation (ml/kg) for Yard1 and Yard2

Figure 4-31 shows methane generation rate for Yard1 and Yard2 for the first 63 days of reactor operation. Methane generation rate for Yard1 increases after day 28. For Yard2 methane generation rate increases at day 25 and again at day 47. Methane generation rate is greater for Yard2 compared to Yard1.

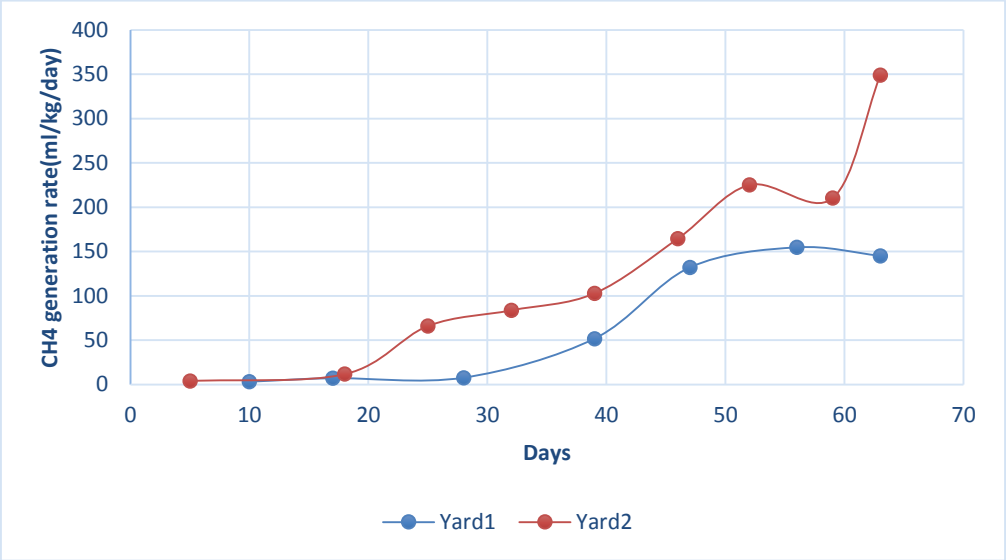

Figure 4-31 Methane generation rate (ml/kg/day) for Yard1 and Yard2

Figure 4-32 shows cumulative methane generation for Wood1 and Wood2. Cumulative methane generation increases in both reactors Wood1 and Wood 2 over time .Cumulative methane generated for Wood2 is 1.29 Liter/kg and for Wood1 is 0.750 Liter/kg, which is 1.72 times more than Wood2.

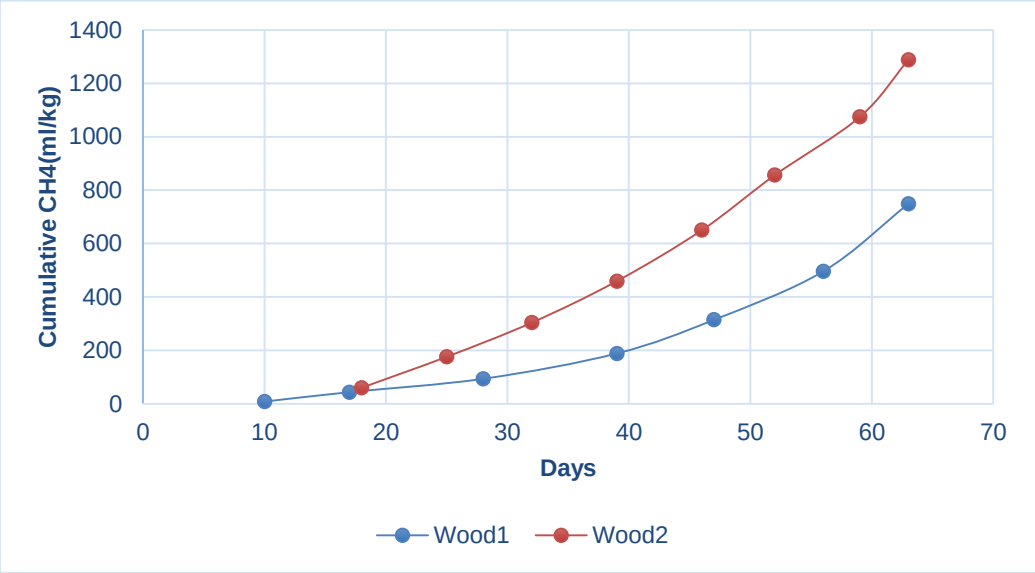

Figure 4-32 Cumulative methane generation with time for Wood1 and Wood2

Figure 4-33 shows methane generated rate (ml/kg/day) for Wood 1 and Wood2.

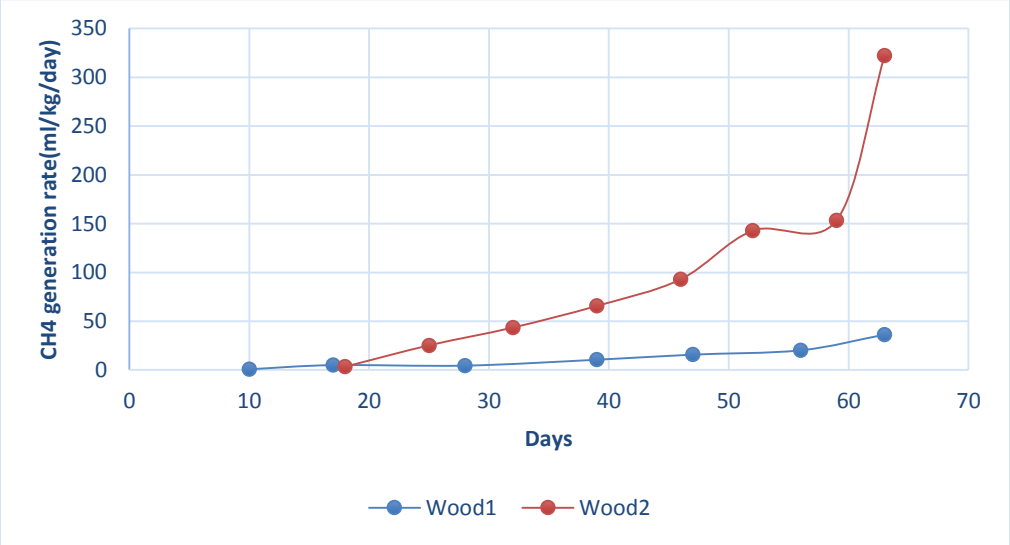

Figure 4-33 Methane generation rate for Wood 1and Wood2

Methane generation rate for both Wood1 and Wood2 increases over time, the rate of methane generation for Wood2 is higher than Wood1 which is obvious in above figure.

4.5 Summary of results

The amount of methane production for Paper1 is more than Yard 1and Wood 1 and the reason is that Paper has highest cellulose percent and higher surface area compared to wood. Table 4-4 shows the summery of cumulative methane generated for Paper 1, Yard1, and Wood1based on their age.

Table 4-4 cumulative methane generated for Paper1, Yard1, and Wood1

Reactors	Number of days	Cumulative CH_4 (Liter/kg)
Paper1	391	48.46
Yard 1	356	20.138
Wood1	315	14.58

Table 4-5 compares cumulative gas generated for Paper1, Yard1, and Wood1 in 315 days which is based on age of Wood1.

Table 4-5 cumulative methane generated for Paper1, Yard1, and Wood1 in 315 days

Reactors	Number of days	Cumulative CH_4 (Liter/kg)
Paper1	315	42.658
Yard1	315	19.767
Wood1	315	14.586

Table 4-6 shows total amount of methane generation for all reactors Paper 1,Paper2,Yard1 ,Yard2,Wood1 and Wood2.The mount of Cumulative methane generation for Paper 2 is 1.67

time the cumulative methane generation of Paper1 .For Yard 2 is1.75 times higher than Yard1,and for Wood2 is 1.72 times higher than Wood1.

Table 4-6 Total methane generation for Paper1, 2, Yard1, 2, Wood1, 2

Reactors	Number of days	Total CH_4 generated (ml/lb)	Total CH_4 generated (Liter/kg)
Paper1	63	4927	10.86
Paper2	63	8321	18.34
Yard1	63	1903.4	4.2
Yard2	63	3332	7.34
Wood1	63	340.56	0.750
Wood2	63	585.7	1.29

Table 4-7shows the start of methane generation day and percentage of initial methane and carbon dioxide for all 6 reactors.

Table 4-7 Start of methane generation and initial percentage of CH_4 and CO_2

Reactor	Starting day for generation CH_4	Initial CH_4 %	Initial CO_2%
Paper1	10	21.8	33.1
Paper2	11	7.9	55
Yard1	10	7.8	57.9
Yard2	5	6.7	57.3
Wood1	10	5.7	13.4
Wood2	18	15.1	16

Table 4-8 shows the methane generation percentage for Paper1, and Paper 2 for 63 days. Table 4-9 shows methane percentage for Yard1 and Yard2, and Table 4-10 compares methane

percentage for Wood1 and Wood2 for 63 days. The yellow color shows the maximum percentage of methane.

Table 4-8 Methane percentage comparison between Paper1 and Paper2

Day	CH_4 (paper2)	Days	CH_4 (paper1)
11	7.9	10	21.8
20	36	17	43.5
25	57.2	24	50.1
29	60.2	28	51.2
32	57	31	55.9
36	54.2	39	60
39	49.6	47	56.3
42	47.4	56	59.1
46	47.2	63	57.6
52	48.2		
59	50.6		
63	53.3		

Table 4-9 Methane percentage comparison between Yard1 and Yard2

Days	CH_4 (yard2)	Days	CH_4 (yard1)
5	6.7	10	7.8
18	21.4	17	12.5
25	27.4	28	19.1
32	33.3	39	35.1
39	40.7	47	50
46	45.2	56	54.3
52	51	63	56.4
59	55.1		
63	52		

Table 4-10 Methane percentage comparison between Wood1 and Wood 2

Days	CH_4 (Wood2)	Days	CH_4 (Wood1)
18	15.1	10	5.7
25	18.7	17	11.3
32	21.7	28	16.4
39	25.4	39	21.9
46	27.6	47	24.2
52	28.1	56	29
59	29.3	63	32.3
63	28.9		

Chapter 5

Conclusions and Recommendations

The main objective of this study was to determine the effects of microbe from termite gut TAV5 on municipal solid waste degradation and generation of landfill gas.

The results obtained from the current study can be summarized as follows:

- For both sets of reactors (Paper1, Yard1, and Wood1 seeded with digester microbes only, and reactors Paper2, Yard2, and Wood2 seeded with both TAV5 and digester microbes), pH was initially acidic, but stabilized between 7 and 8. The addition of the TAV5 microbes did not seem to impact reactor PH.

- For both sets of reactors (through day 63 of operation of the second set), the paper reactor had the highest rate of methane generation, as well as the largest cumulative volume of methane, followed by yard waste, and finally wood waste the reason is that paper has the highest amount of cellulose among yard and wood, and has higher surface area compared with wood. Previous studies that have shown that wood waste has the highest lignin content, which likely explains its lower methane volume.
- Through the first 63 days of reactor operation, for paper, yard waste, and wood waste, the reactors seeded with both TAV5 and digester microbes had higher rates of methane generation, as well as larger cumulative volumes of methane generated, compared to the reactors seeded with digester microbes only. Initial results thus indicate that TAV5 is increasing methane generation rate and quantity of methane generation. Reactor operation will be continued.

5.1 Recommendations for future studies

On the basis of results obtained in the current study, and to increase its reliability, the following recommendations are made for future study.

- Investigate lignin generation at 40°C, a typical landfill temperature. If the TAV5 microbe cannot grow at 40°C, other microbes or genetic modifications to TAV5 can be explored.
- To identify the optimum effect of mixed of sludge and microbes from termite gut TAV5, reactors can be seeded with different mixed culture and termite gut microbe TAV5 ratios such as, 45%, 50%, etc.
- To identify how much lignin has been degraded when using TAV5 microbes, HPLC tests should be conducted.
- Chemical pretreatment of waste samples can be done to accelerate rate of decomposition of the waste for lignin degradation.
- TAV5 genome was used because one paper published in January 2015 reported that microbe can degrade lignin. There are other types of genome in termite gut like TAV2 and TAV3 which both can degrade cellulose as well. To accelerate rate of decomposition and cellulose and hemicellulose degradation, a mixed of TAV2, TAV3, and TAV5 can be added to bioreactors.
- DNA extraction and polymerase chain reaction (PCR) sequencing can be conducted to identify the microbes present in the digester sludge, as well as in the reactors at the end of their operation.
- Operate a reactor with TAV5 microbe alone to see if any methane is generated.
- Identify the "other gases" formed during waste degradation using a GC.

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