Effects of Temperature on Anaerobic Lignin Degradation in Bioreactor Landfills

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Thesis submitted to the faculty of
the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Environmental Engineering

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December 06, 2008
Blacksburg, Virginia

Keywords: Lignin, Bioreactor Landfills

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Abstract

Bioreactor landfills have become a feasible alternative to the typical 'dry tomb' landfill. By recirculating leachate and/or adding additional liquid wastes, bioreactor landfills operate to rapidly degrade and transform organic wastes. The reactions within a bioreactor landfill create elevated temperatures. The intent of this study was to determine the effect of elevated temperature on the degradation of lignocellulose compounds. In order to observe the effects of temperature on lignin, small bioreactors were created in the laboratory. Several experiments were performed by the authors. Solubility of lignin based on temperature and time of thermal exposure were conducted. In addition, degradation studies were conducted based on biological treatment of lignin as well as a combination of biological and thermal treatment. Samples were collected at specified intervals to determine the amount of water soluble lignin (WSL), volatile fatty acids (VFAs), lignin monomers, and/or methane present. Lignin solubility increased as temperature rose in the thermal solubility experiments. The rate of solubility increased 15 times for office paper and 1.5 times for cardboard in the biological experiments when compared to the thermal treatment. The thermal and biological study indicates that as lignin is solubilized, it breaks down into lignin monomers, which can be converted easily by anaerobic bacteria into VFAs and subsequently, methane. These experiments indicate that temperature is crucial to the degradation of lignin compounds in a bioreactor landfill.

Acknowledgements

I would like to thank Dr. Novak who provided me with this opportunity to conduct research. Dr. Novak has developed an exceptional balance between directive and freedom that was helpful in making this research both productive and interesting for me to complete. I would also like to thank Dr. Goldsmith and Dr. Randall for serving on my committee. I would like to thank the Solid Waste Association of North America and the Environmental Research and Education Fund for their support of my education. I acknowledge the financial support I received from Waste Management, and would like to thank Mr. Gary Hater for continuing to support our work.

I would also like to thank Jody Smiley and Julie Petruska for their invaluable contributions to my experimental analysis and design. I am also very grateful to Betty Wingate for her support considering administrative matters. I am grateful to my colleague, Chris Wilson for his help and support in the lab. In addition, I would like to thank Clark Person for his encouragement and patience with me throughout this degree.

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I . Literature Review

Introduction

Municipal Solid Waste (MSW) disposal is a challenging issue in the United States. According to the most recent research by the Environmental Protection Agency (EPA), the average American creates 4.6 pounds of trash per day and recycles approximately 32.5% of the material. This resulted in a total of 251 million tons of solid waste in 2006, with only 82 million tons being recycled (USEPA 2007). Over 50% of MSW generated in the United States ends up in landfills.

The MSW in the average US landfill is composed of approximately 60% household waste and 40% commercial/public waste. A breakdown of the composition of MSW in 2006 is shown below:

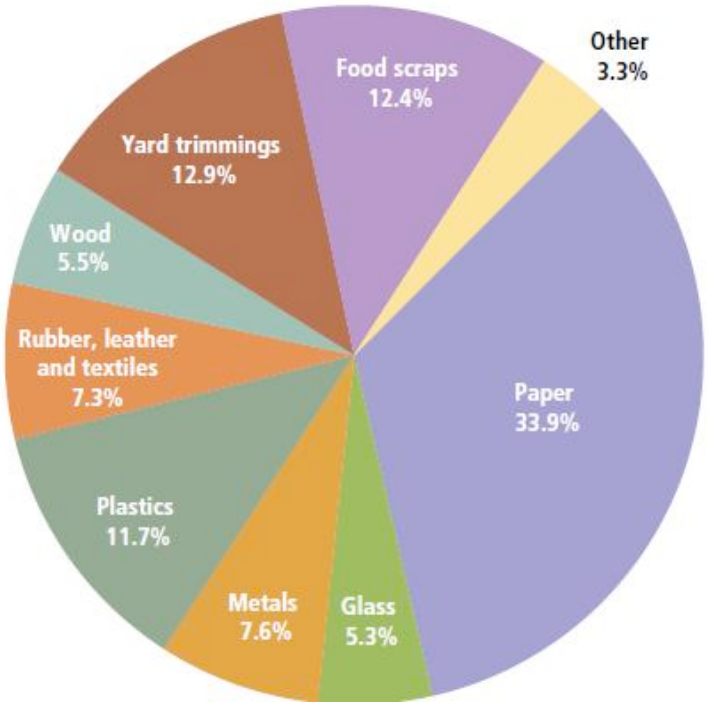

Figure 1-1: MSW Generation in 2006, by Material (before recycling)

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.

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 as an active biological process instead of as permanent storage. One of the processes developed and studied is a bioreactor landfill.

Bioreactor Landfills

The bioreactor landfill was developed in order to enhance the microbial degradation of organic wastes in a landfill. The Solid Waste Association of North America (SWANA) defines a bioreactor landfill as "any permitted Subtitle D landfill or landfill cell where liquid or air is injected in a controlled fashion into the waste mass in order to accelerate or enhance biostabilization of the waste". The operation of a landfill as a bioreactor can provide the following benefits (Bioreactors 2008):

- Increased decomposition and stabilization
- Lower mobility and toxicity due to shifting aerobic/anaerobic conditions
- Reduction of leachate disposal costs
- Increased landfill capacity
- Increased re-usable gas creation
- Reduced closure monitoring/care costs

A conventional landfill is frequently operated as a “dry tomb”, with leachate being collected and removed from the system. The main goal of a conventional landfill is to minimize moisture entering and retained within the system. This leads to decades of maintenance and operation due to low rates of decomposition (Benson, Barlaz et al. 2007). The main difference between a conventional landfill and a bioreactor landfill is

the need to control and monitor the bioreactor's leachate and aeration processes to maintain moisture levels as well as public health and safety (Barlaz and Reinhart 2003).

There are three main types of bioreactor landfills. Aerobic bioreactor landfills are designed to inject air into the landfill through horizontal/vertical well schemes to promote aerobic microbial activity. Anaerobic bioreactor landfills are designed to inject and recirculate liquids through the landfill to provide adequate moisture for the anaerobic microbial community. Hybrid bioreactor landfills are a sequential combination of the aerobic/anaerobic design (Bioreactors 2008).

The waste in a bioreactor landfill goes through five stages of decomposition. These five stages are:

1. Initial Adjustment
2. Transition
3. Acid Formation
4. Methane Fermentation
5. Maturation

Phase I, the initial adjustment phase, is when the accumulation of MSW and moisture begins. During this phase an acclimation period is often observed until a microbial community can be established. In Phase II, the transition phase, a transformation from an aerobic to an anaerobic environment occurs. In the third phase, the acid formation phase, high concentrations of volatile organic acids are observed due to the microbial conversion of biodegradable organic content in the solubilized solid waste. Phase IV, the methane fermentation phase, is characterized by the conversion of these volatile organic acids to methane and carbon dioxide via methanogenic bacteria. The final phase, known as the maturation phase, is when the landfill stabilizes due to limiting nutrients for the microbial community (Reinhart and Townsend 1998).

Bioreactor landfills are not widely accepted yet due to some of the unique hazards that are present. Some of these concerns are (Bagchi 2004):

- Additional liquid impacts on the liner system (weight, head, leakage)
- Lack of information on long-term settlement of the waste
- Leachate quality concerns
- Slope stability of moisture-laden waste

Microbial Degradation

As discussed in the previous section, MSW decomposition goes through many stages. Each of the phases is controlled by specific microbial functions. In the initial phase, oxygen is present and is utilized as an electron acceptor. In addition, the more easily degraded wastes serve as a carbon source for the microbial community resulting in the following typical reaction:

$$Degradable\ Waste + O_2 \rightarrow CO_2 + H_2O + Heat, Biomass, Acids, Residuals$$

As oxygen consumption is completed and the environment turns anaerobic, the landfill enters the next phase, the acid generation phase. This phase is characterized by the production and accumulation of volatile organic acids. In this anaerobic environment, nitrates, sulfates, etc. are now being utilized as electron acceptors. This continues until these acceptors are depleted or until conditions become limiting, entering the landfill in the next stage – methanogenesis (Irani 2005). In the methanogenesis stage, the volatile organic acids are fermented and carbon dioxide is used as an electron acceptor resulting in the following typical reactions:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Factors Affecting Biodegradation

There are several key factors that affect the microbial activity and population within a landfill. These factors include pH, moisture, temperature, waste composition, and operation of the facility (Reinhart and Townsend 1998). The pH level of a landfill fluctuates over time depending on the biochemical reactions and leachate quality. Accumulation of volatile fatty acids (VFAs) can cause a drop in pH. If the pH level drops to below five to six pH units, methanogenesis will be inhibited (Vavilin, Rytov et al. 2003).

Moisture is considered the most critical factor in biodegradation (Kim and Pohland 2003). Moisture is also the most easily maintained factor via leachate recirculation. In several case studies of active bioreactor landfills, cells with higher leachate circulation showed an increase in microbial population and rate of stabilization (Reinhart and Townsend 1998). Laboratory studies and field case studies indicate that moisture addition accelerates degradation of waste. A conventional landfill is typically 20% moisture, while a bioreactor landfill must maintain around 35-40% moisture content in order to be most efficient (Bagchi 2004).

During the acid formation and methane fermentation phases, temperatures within a bioreactor landfill can increase significantly due to biochemical reactions (Reinhart and Townsend 1998). The Yolo County Landfill, which has been in operation since 1994, sees temperatures in the cells ranging from 45° to 60° Celsius (Yazdani, Kieffer et al. 2006). Some bioreactors currently in operation do not see any significant temperature increase due to low moisture recirculation (Benson, Barlaz et al. 2007). Case studies have shown that landfill cells with lower internal temperatures produce lower quantities of methane gas (Reinhart and Townsend 1998).

Lignocellulose Compounds

As mentioned in the introduction, paper is one of the primary components of MSW. Paper is composed of three major compounds: cellulose, hemicelluloses, and lignin. These three constituents make up a lignocellulose. Cellulose is the most abundant biopolymer on earth. It is the basic constituent of a cell wall in any plant. Hemicellulose is the second most abundant biopolymer and mainly is utilized in plant material to chemically bind cellulose and lignin. Lignin, the third most abundant biopolymer, constitutes between 18-30% of the dry weight of wood products. Lignin provides the strength in a cell wall to protect the cell from attack (Senior 1990). A typical lignin structure is shown in Figure 1-2 (Glazer and Nikaido 1995). Due to these characteristics, lignin is thought to be the inhibiting agent in paper degradation (Stutzenberger and Kaufman 1970).

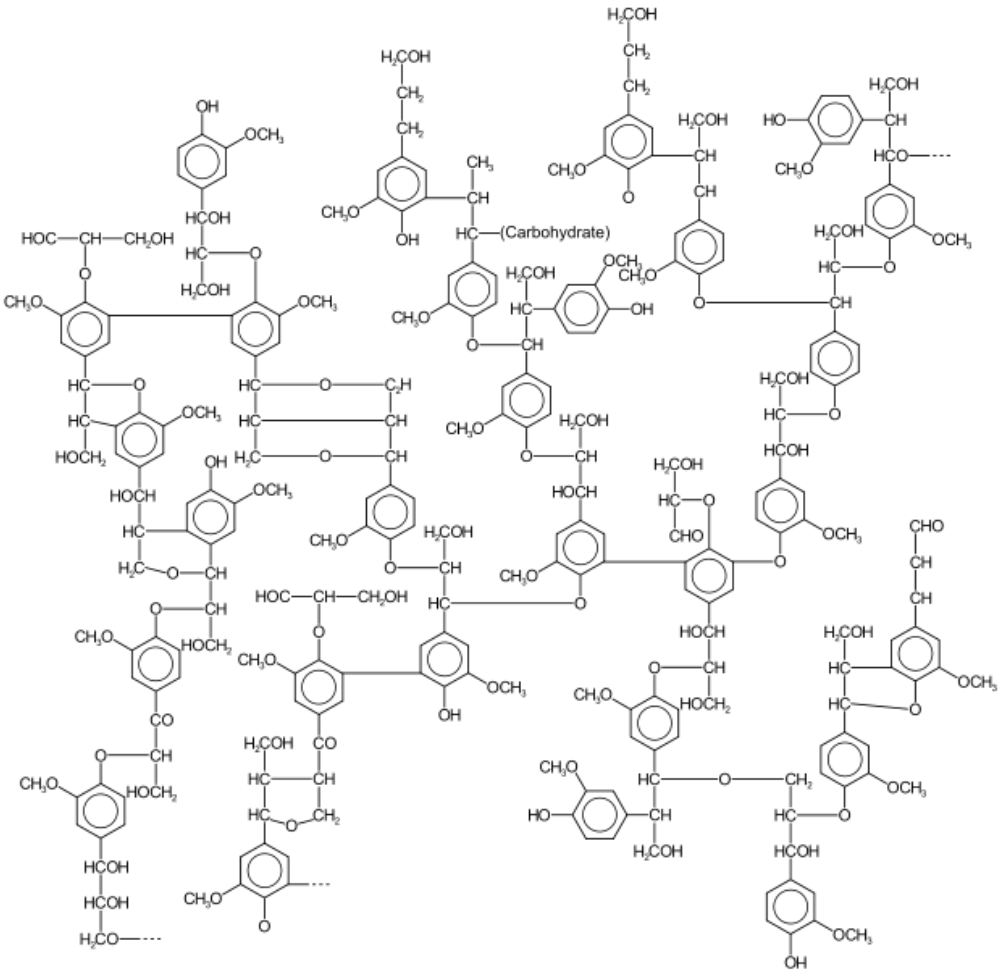

Figure 1-2: Representative Lignin Structure

Lignocellulose Degradation

Bioreactor landfills support an anaerobic environment. The anaerobic component of the carbon degradation cycle utilizes alternative electron acceptors to degrade complex carbons into alcohols and acids prior to the final degradation of these acids into carbon dioxide and methane. The conversion of lignocelluloses to methane is complex in an anaerobic environment. The biodegradation process requires many species of bacteria, such as acidogens and methanogens.

One way to study the degradation of lignin is to observe the intermediate degradation products. Lignin can be broken down into more simple structures called monomers. The most basic structure of lignin is the lignin monomer. Examples of lignin monomers include vanillin, syringic acid, benzoic acid, and ferulic acid and are shown in Figure 1-3. Research into this cycle is limited since previous studies identified that lignin does not degrade anaerobically (Irani 2005).

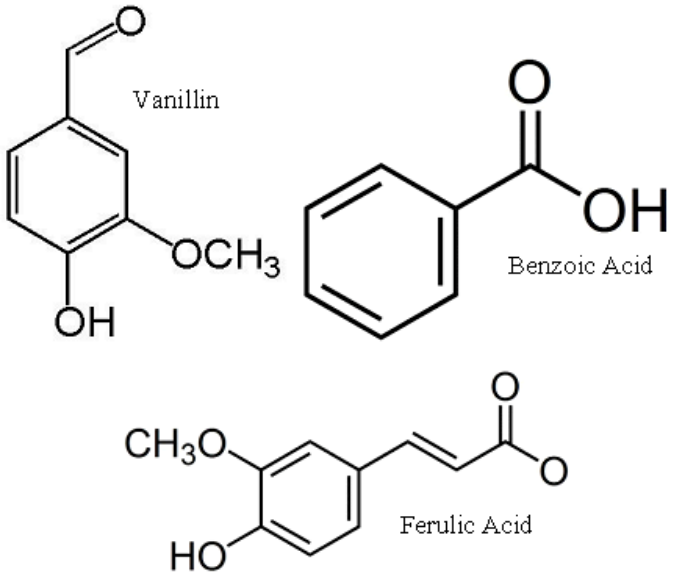

Figure 1-3: Vanillin, Benzoic Acid, and Ferulic Acid Structures

The anaerobic pathway for the degradation of these monomers involves breakdown of the structure by the removal of one or more functional groups (Irani 2005). This results in volatile fatty acids (VFAs) which then are easily degraded to carbon dioxide and methane (Young, Frazer et al. 1987). The pathway depicted in Figure 1-4 was proposed by Young and Frazer (et al, 1987) after extensive research which determined that anaerobically enriched cultures can solubilize lignin and degrade the oligomers and monomers produced in this process.

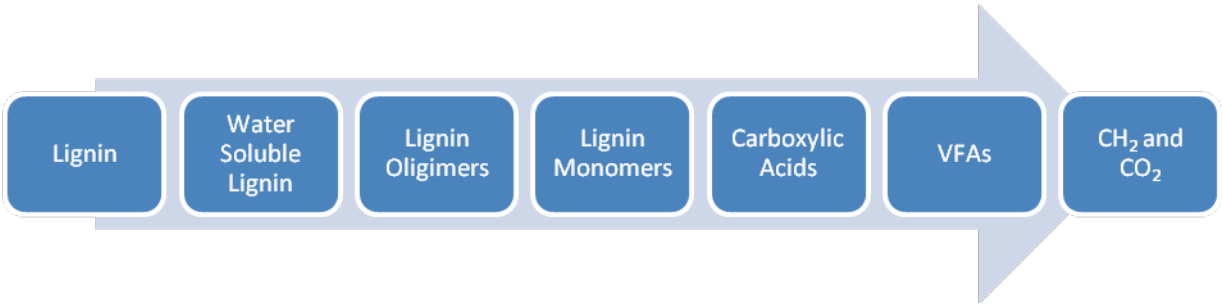

Figure 1-4: Schematic of Anaerobic Lignin Degradation Cycle

Therefore, by monitoring concentrations of lignin monomers, VFAs, and gas production, the lignin degradation cycle can be studied. Studies of this process are typically conducted using the microorganisms present in anaerobic sediments, rumen, or anaerobic sludge (Miroshnikova 2006).

Methane Generation

Landfills contain a large amount of carbon which can be degraded into methane and carbon dioxide. Since methane is a viable source of energy, there are a multitude of studies on the various landfill types and gas generation rates (Barlaz 2006). Large conventional landfills as well as bioreactor landfills can often generate enough methane to be energy self-sufficient or even enough to re-distribute. However, due to the lengthy operations of a conventional landfill, the methane is typically only recovered from year five to year 20. After year 20, the additional methane is either off-gassed or ignited. Conventional landfills can emit methane for up to 40 years post-closure (Micales and Skog 1997). Bioreactor landfills generate methane in the initial years of operation at a

higher rate; therefore, less fugitive emissions occur during the post-closure period. A comparison of bioreactor and conventional landfills with respect to gas generation is shown below (Bagchi 2004):

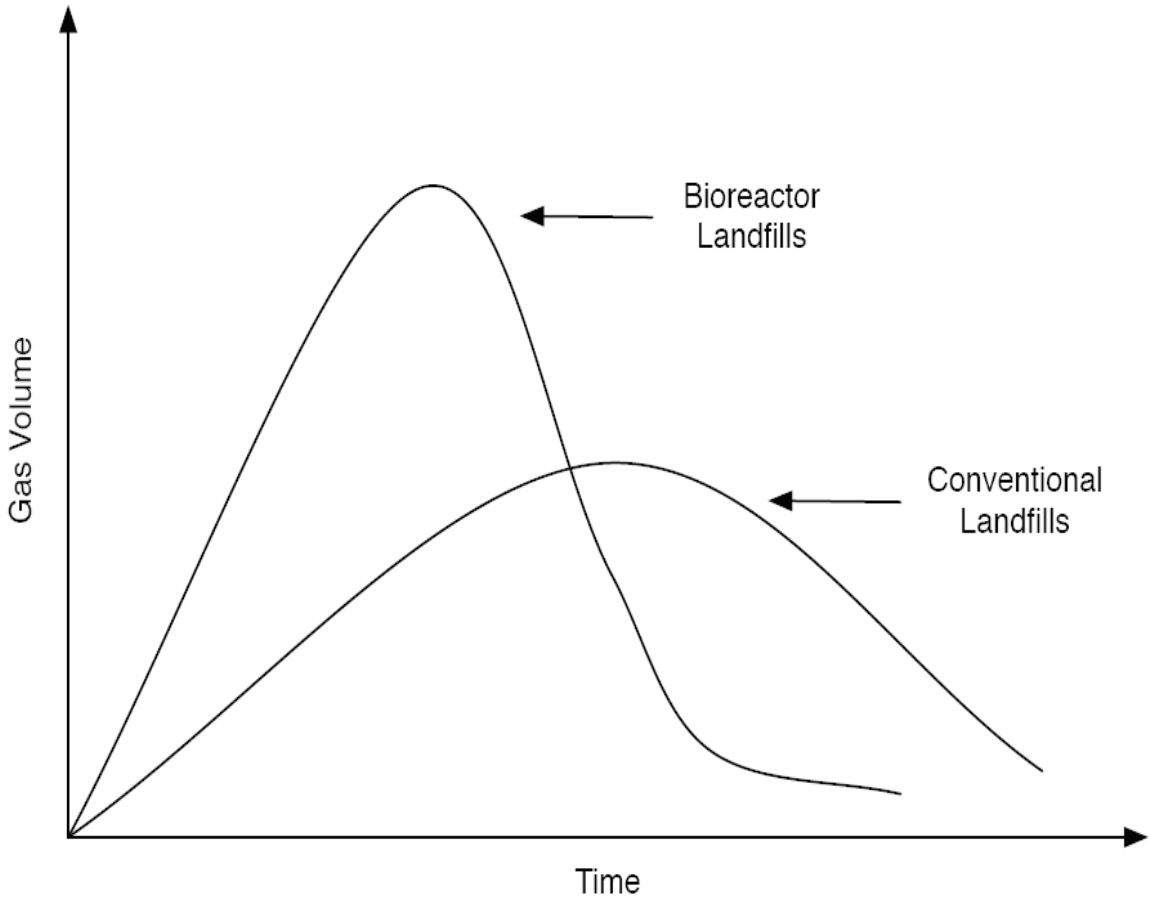

Figure 1-5: Comparison between bioreactor and conventional landfill gas production

Landfill Stability Parameters

There are many different parameters that are utilized to measure landfill stability. Typical parameters collected include cellulose/lignin (c/l) ratio, volatile solids content, and a biomethane potential (BMP). The assumption that lignin is recalcitrant is prevalent throughout industry. Therefore, using a c/l ratio to measure the degree of stability of a landfill has also been a common practice. This is because the c/l ratio will decrease as cellulose degrades, indicating a higher stability of the waste. However, this ratio relies on the assumption that lignin is not degrading.

Volatile solids (VS) percentage is another measurement of stability. The main advantage of using VS is the simplicity of the measurement. A landfill can be considered stable once the VS percentage is measured as approximately 10-20% of the total solids (Kelly, Shearer et al. 2006). However, the presence of plastics in MSW results in an uncertainty factor that could cause the waste to be mischaracterized.

BMP is another common stability measurement. BMP is a measurement of the potential methane still available in the MSW. The procedure for BMP is biological in nature and, therefore, has a high variability rate. BMP is often used as a supplement to other data, as it does not always correlate well with the physical measurements of stability (Kelly, Shearer et al. 2006)

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II. Manuscript

EFFECTS OF TEMPERATURE ON ANAEROBIC LIGNIN DEGRADATION IN BIOREACTOR LANDFILLS

Roberta J. Niemietz[1], John T. Novak[2], Ayesha Irani[3]

Abstract

Bioreactor landfills have become a feasible alternative to the typical 'dry tomb' landfill. By recirculating leachate and/or adding additional liquid wastes, bioreactor landfills operate to rapidly degrade and transform organic wastes. The reactions within a bioreactor landfill create elevated temperatures. The intent of this study was to determine the effect of elevated temperature on the degradation of lignocellulose compounds. In order to observe the effects of temperature on lignin, small bioreactors were created in the laboratory. Several experiments were performed by the authors. Solubility of lignin based on temperature and time of thermal exposure were conducted. In addition, degradation studies were conducted based on biological treatment of lignin as well as a combination of biological and thermal treatment. Samples were collected at specified intervals to determine the amount of water soluble lignin (WSL), volatile fatty acids (VFAs), lignin monomers, and/or methane present. Lignin solubility increased as temperature rose in the thermal solubility experiments. The rate of solubility increased 15 times for office paper and 1.5 times for cardboard in the biological experiments when compared to the thermal treatment. The thermal and biological study indicates that as lignin is solubilized, it breaks down into lignin monomers, which can be converted easily

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[3] Project Engineer, The Louis Berger Group, Inc., Ciudad del Saber, Edificio 116 Panama, airani@louisberger.com

by anaerobic bacteria into VFAs and subsequently, methane. These experiments indicate that temperature is crucial to the degradation of lignin compounds in a bioreactor landfill.

Introduction

Municipal Solid Waste (MSW) disposal is a challenging issue in the United States. According to the most recent research by the Environmental Protection Agency (EPA), the average American creates 4.6 pounds of trash per day and recycles approximately 32.5% of the material. This resulted in a total of 251 million tons of solid waste in 2006, with only 82 million tons being recycled (USEPA 2007). Over 50% of MSW generated in the US ends up in landfills. 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 as an active biological process instead of as permanent storage. One of the processes developed and studied is a bioreactor landfill.

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There are several key factors that affect the microbial activity and population within a landfill. These factors include pH, moisture, temperature, waste composition, and operation of the facility (Reinhart and Townsend 1998). The pH level of a landfill fluctuates over time depending on the biochemical reactions and leachate quality. Accumulation of volatile fatty acids (VFAs) can cause a drop in pH. If the pH level drops to 5-6 or below, methanogenesis will be inhibited (Vavilin, Rytov et al. 2003).

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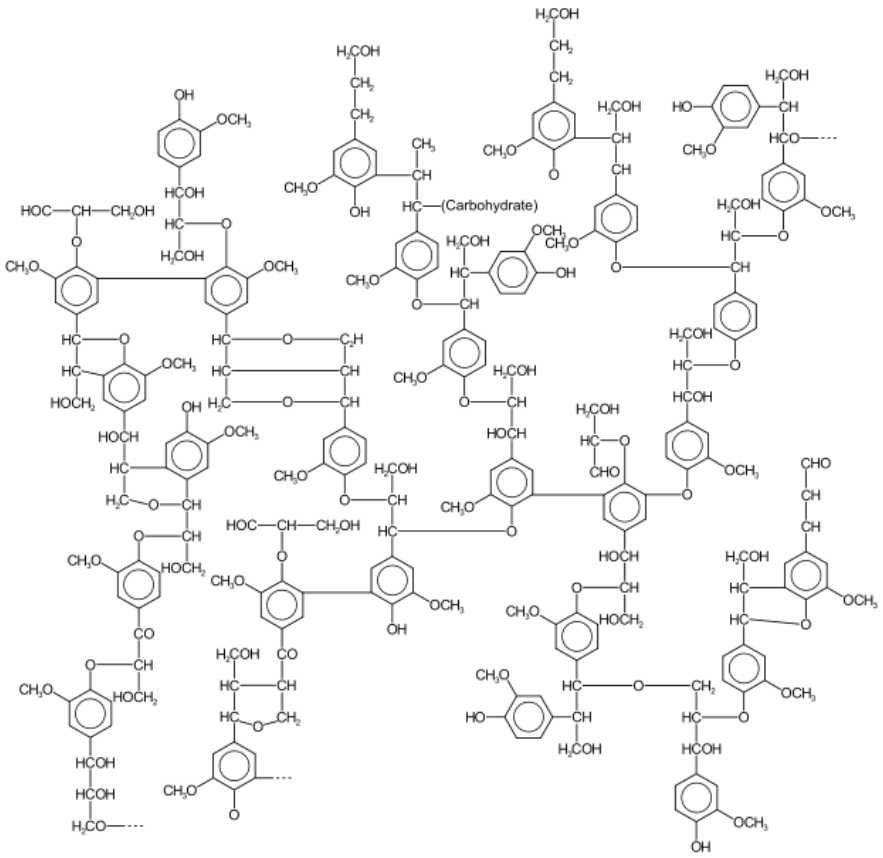

Figure 2-1: Representative Lignin Structure

One way to study the degradation of lignin is to observe the intermediate degradation products. Lignin can be broken down into more simple structures called monomers. Examples of lignin monomers include vanillin, syringic acid, benzoic acid, and ferulic acid and are shown in Figure 2-2. Research into this cycle is limited since previous studies identified that lignin does not degrade anaerobically.

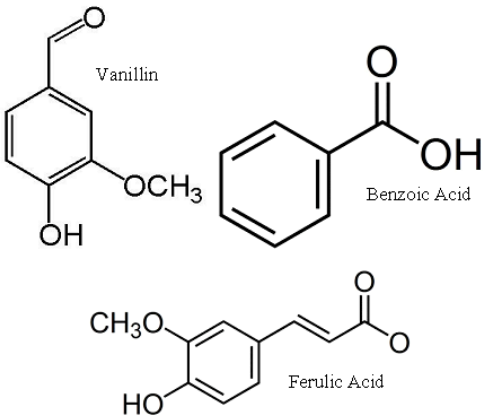

Figure 2-2: Vanillin, Benzoic Acid, and Ferulic Acid Structures

The anaerobic pathway of the degradation of these monomers involves breakdown of the structure by the removal of one or more groups. This results in volatile fatty acids (VFAs) which then are easily degraded to carbon dioxide and methane (Young, Frazer et al. 1987). The pathway depicted in Figure 2-3 was proposed by Young and Frazer (et al, 1987) after extensive research where it was determined that anaerobically enriched cultures can solubilize lignin and degrade the oligimers and monomers produced in this process.

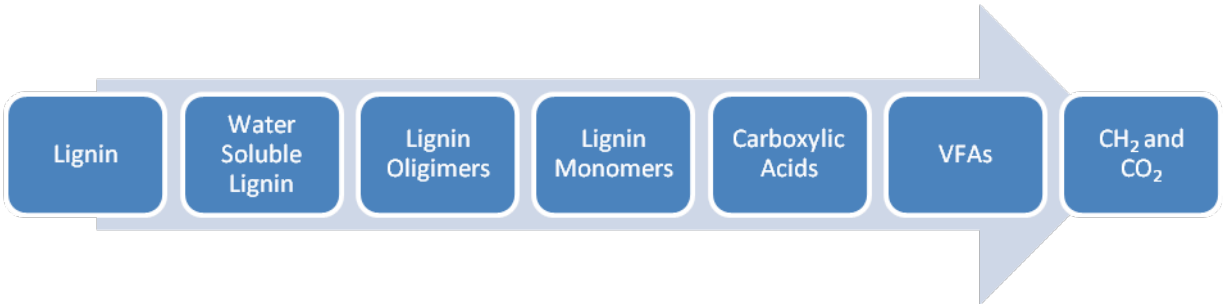

Figure 2-3: Schematic of Anaerobic Lignin Degradation Cycle

Therefore by monitoring concentrations of lignin monomers, VFAs, and gas production; the lignin degradation cycle can be studied. Studies of this process are typically conducted using the microorganisms present in anaerobic sediments, rumen, or anaerobic sludge (Miroshnikova 2006).

The objective of this study was to understand how one of the key features of bioreactor landfills; increased temperature, effects the solublization and degradation of lignin in paper products. An understanding of the rate, pathway and limiting steps of the solubilization and degradation of lignin in paper is crucial in determining the settling rates, gas generation, and post-closure monitoring requirements for bioreactor landfills.

Experimental Design

Three main experiments were conducted for the purposes of this research and are summarized in Table 2.1, below. Each experimental design is explained in detail in the following sections.

Table 2.1: Breakdown of Experimental Design

Experiment	Treatment type	Temperature(s) (˚C)	Duration
Lignin Solubility	Thermal	20°, 37°, 45°, 55°, 65°	Varying from 48 hours to 27 weeks
Lignin Degradation via Biological Treatment	Biological	37°	8 weeks
Lignin Degradation via Thermal/Biological Treatment	Thermal and Biological	Pre-treated: 37°, 50°, 60°, 65°, and 70° Incubated at 37°	Varying from 80 days to 1 year

Lignin Solubility

An experiment on the effect of temperature on lignin solubility was conducted. This work was completed by incubating septa bottles containing 2 grams (g) of paper and 200 milliliters (ml) of distilled water at 20°, 37°, 45°, 55° and 65 °C in a hot water bath shaker-incubator for 48 hours. The amount of water soluble lignin was then measured. The analytical methods are described in the Materials and Methods section of this report.

In addition to the above solubility experiment, small bioreactors containing approximately 2 g of a paper source and 200 mL of water were incubated at 55°C.

Samples were collected at regular intervals to determine lignin solubilizaion over a period of 27 weeks.

Lignin Degradation via Biological Treatment

Small sacrificial batch reactors were again utilized with approximately 520 mg of a Kraft lignin. In addition to the Kraft lignin, approximately 50 mL of rumen and rumen media were added to each reactor in a reduced environment. The reactors were sealed and incubated at 37°C for eight weeks. Periodic sampling was conducted every two weeks and samples were analyzed for the presence of water soluble lignin as well as lignin monomers.

Lignin Degradation via Thermal and Biological Treatment

In order to simulate the conditions in a bioreactor landfill, a second degradation experimental design was created using sacrificial reactors, paper substrate, and a nutrient solution. Sacrificial reactors were filled with approximately 0.5 g of paper substrate in 30 mL of nanopure water. A temperature of 37° C was chosen as the control temperature. Variable temperatures of 50°C, 60°C, 65°C, and 70°C were selected to compare lignin degradation in this experiment. These temperatures are within the typical range for a bioreactor landfill. Thermal pre-treatment of the reactors was conducted for a three month period.

Following thermal pre-treatment, the reactors were inoculated with a reduced media solution containing a microbial seed, nutrients, and resazurin dye. To accumulate more lignin degradation intermediates, the reactors were spiked with 2-bromoethanesulfonic acid (BESA) at a concentration of 10^{-3} Molar (M), which inhibits methanogenesis (Zinder, 1984). The reactors were then placed in incubation. A mesophilic incubation temperature of 37°C was chosen to reduce the temperature stress for the microbial seed, which was obtained from an anaerobic digester treating municipal wastewater sludge. Reactors were made in triplicate for each sampling period to provide representative

statistical analysis. In addition, seed control reactors, which contained the nutrient solution, but no paper, were used as negative controls to determine the seed effect on the results.

Materials and Methods

Paper Source

Lignin Solubility and Degradation via Biological Treatment

Shredded office paper, newsprint, magazines, phone books, and cardboard were supplied by Waste Management, Inc. Paper samples were milled to a powder. Additionally, Kraft or alkali lignin was used as a synthetic model compound.

Lignin Degradation via Thermal and Biological Treatment

In the main degradation experiment, a mixture of 75% shredded newsprint and 25% shredded cardboard was utilized as substrate for the reactors. This combination was chosen because newsprint and cardboard contain a high percentage of lignin from wood pulp as opposed to office paper (Barlaz, Eleazer et al. 1997). Newsprint has also been observed to be more recalcitrant than office paper (Clarkson and Xiao 2000).

Nutrient/Inocula

Lignin Degradation via Biological Treatment

The nutrient medium was utilized as outline by Goering and Van Soest (Goering and Soest 1970). The micromineral, buffer, and macromineral solution was mixed with resazurin and incubated with the lignin overnight at 35°C. On the day the rumen was collected a reducing solution was prepared and added to each reactor while running CO_2 over the solution. Carbon dioxide was continually passed over the solution until the resazurin dye indicated that reduced conditions had been obtained.

Lignin Degradation via Thermal and Biological Treatment

A nutrient solution, adopted from the solution utilized in Clarkson and Xiao (Clarkson and Xiao 2000), was added. The nutrient solution contains the macro- and micro-nutrients required to sustain the reactors for the duration of sampling period. These include sodium bicarbonate, ammonium chloride, potassium phosphate, calcium chloride, and iron chloride. Prior to inoculation, the nutrient solution was purged with nitrogen gas for one hour in order to reduce the amount of oxygen introduced into the reactor. BESA at 10^{-3} M was added to the solution for the inhibited reactors.

Sampling

For the pre-treated and innoculated reactors, gas was collected prior to sacrificing the reactor. Gas volume was measured and the percentage of methane was analyzed using a gas chromatograph (GC). The reactors were then opened and the supernatent was filtered and analyzed for total water soluble lignin (WSL), volatile fatty acids (VFAs), and selected lignin monomers. The methods utilized for each analysis are detailed below. The non-innoculated reactors were solely measured for water soluble lignin.

Water Soluble Lignin

The concentration of hydrolyzed lignin was measured as water soluble lignin (WSL) using the method developed by Orsa and Holmbom (Orsa and Holmbom 1994). Extraction of WSL was performed using methyl-tert-butyl-ether (MTBE). Once extracted, the total WSL lignin was measured using a spectrophotometer at a wavelength of 280 nanometers (nm). Lignin solubilization was measured as milligrams of water soluble lignin per liter (WSL, mg/L).

Monomers

Lignin Degradation via Biological Treatment

The lignin monomer, 4-methyl phenol, was measured utilizing GC Mass Spectrometry (GC-MS). Prior to analysis, the supernatant in the reactors was adjusted to a pH of approximately 2.5 using sulfuric acid and multiple methylene chloride extractions were performed at a 5X concentration.

Lignin Degradation via Thermal and Biological Treatment

Degradation products of lignin were selected based on their assumed concentrations. For the purposes of this research, benzoic acid, catechol, vanillin, syringic acid, and ferulic acid were quantified using an HP 1090 high performance liquid chromatograph (HPLC) equipped with an Alltech Econosphere C18 column at a flow rate of approximately one mL per minute. The samples were concentrated prior to analysis, and numerous blanks and standards were analyzed to maintain quality assurance.

Volatile Fatty Acids

The supernatant was filtered through a 0.45 um filter and acidified with phosphoric acid at a 1:10 concentration. VFAs were measured on a HP 5890A GC using a Supelco Nukol™ capillary column with flame ionization detector (FID). The VFAs accumulated in the seed control were subtracted from the samples for final recording and analysis.

Methane Generation

Gas production was monitored for each sampling period to determine the volume and percentage of methane present. In this study, methane generation was only partially inhibited in the BESA-amended reactors. To collect the sample, the accumulated pressurized biogas was released from the sacrificial reactors into one liter gas-tight Tedlar bags. The volume of gas was measured from the Tedlar bag with a syringe. The gas

composition was tested by the immediate injection of a sample from the headspace of the reactor into a Hewlett Packard (HP) 5890A GC equipped with an RTQ Plot Column. Gas standards were run for calibration.

Cellulose/Lignin

The cellulose and lignin analysis followed the procedures outlined in the American Society for Testing and Materials (ASTM). Approximately 300 mg milled sample was collected and hydrolyzed using sulfuric acid. Samples were then transferred diluted and autoclaved for one hour. The samples were subsequently filtered using standard TSS glass fiber filters. The volatile suspended solids remaining after hydrolysis was considered lignin. The filtrate was then neutralized and the cellulose quantified using a HPLC with a refractive index detector (RID).

Results and Discussion

Lignin Solubility

Total WSL was measured as described above. Soluble lignin increased exponentially as temperatures increased; see Figures 2-4 and 2-5 below. Up to 0.40% of the lignin in cardboard was solubilized at 60 °C in 48 hours. That is double the amount which was solubilized at 20 °C (0.20%). Therefore, increased temperatures within bioreactor landfills appear to be crucial to increasing the solubility of lignin. Besides supporting active anaerobic microbial environments, high temperatures within bioreactor landfill play a significant role in the physical transformation of the lignin structure. Previous research has shown that the molecular weight of lignin oligomers determines its bioavailability, with lower molecular weights having a higher degradation rate (Colberg and Young, 1985). Therefore, if the solubilization of lignin can change or decrease the overall lignin structure, it could also increase the degradation of lignin.

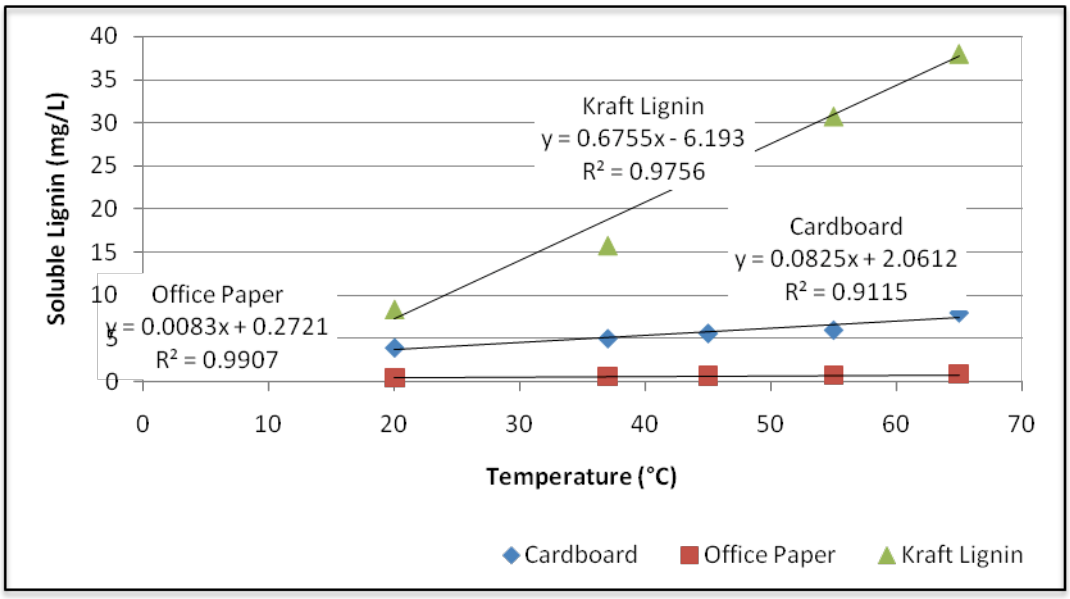

Figure 2-4: Lignin Solubility (mg/L) versus Temperature

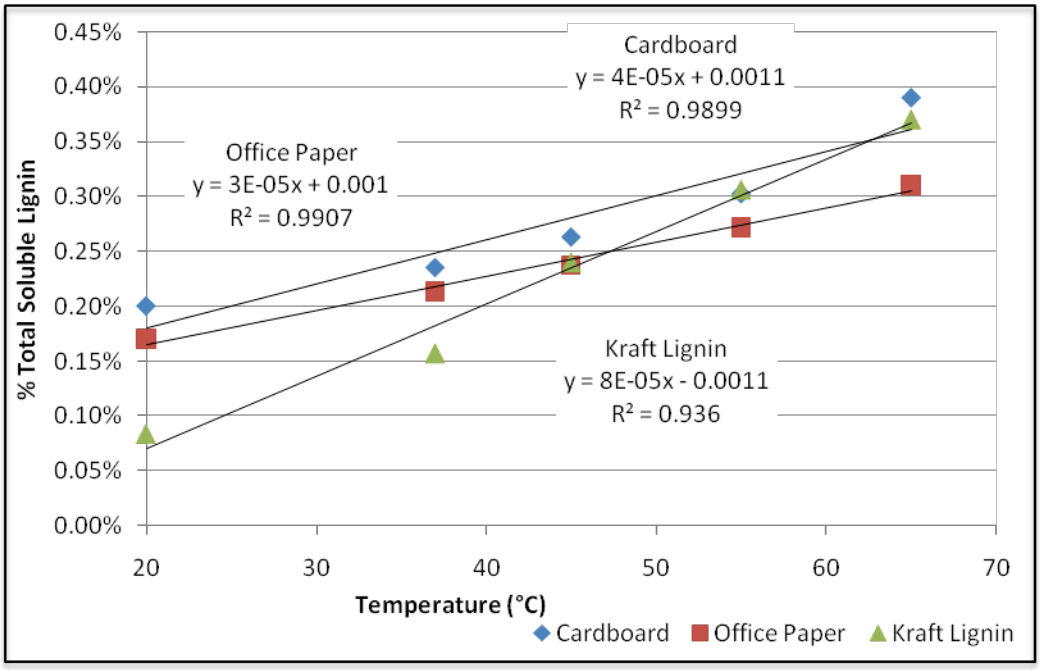

Figure 2-5: Lignin Solubilization (%) versus Temperature

In Figure 2-6 the increase of lignin solubility at 55°C over a period of 27 weeks is shown for the different types of paper. All the various types show a trend of increasing lignin solubility over time. The linear solubilization rates are identified in Table 2-2. This data

indicates that prolonged exposure to elevated temperatures effectively solubilized the lignin at a slow, but significant, rate.

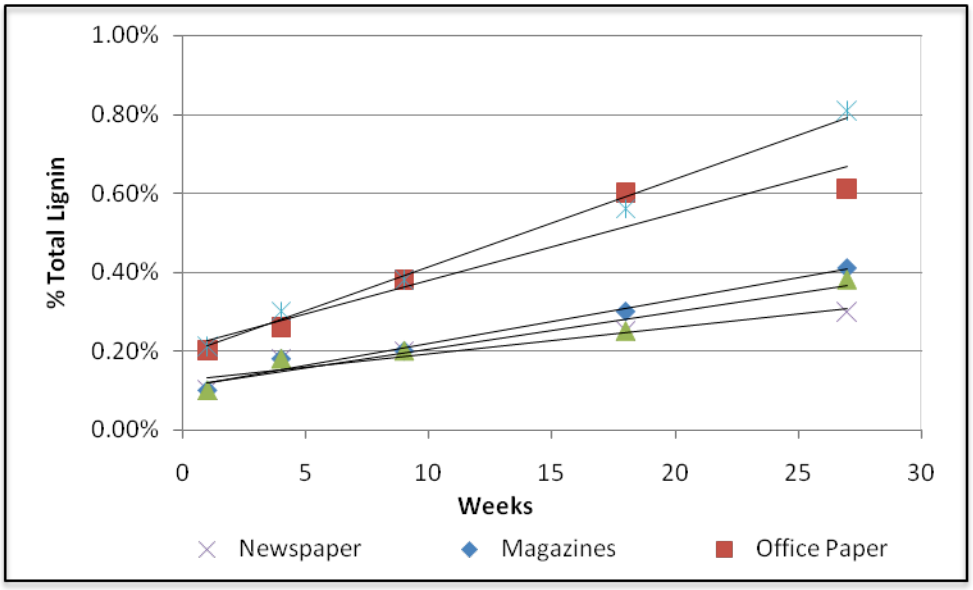

Figure 2-6: Lignin Solubility over 27 weeks at 55°C

Table 2-2: Solubilization Rates of Lignin (Thermal Treatment)

Substrate	Solubilization Rate ($week^{-1}$)
Cardboard	0.0002
Office Paper	0.0002
Magazine	0.0001
Phone Book	0.0001
Newspaper	0.00008

Lignin Degradation via Biological Treatment

Lignin degradation via biological treatment was the second experiment conducted. The previous experiment showed that the elevated temperatures would solubilize lignin at a greater rate. This experiment was conducted to determine if the soluble lignin could be produced and degraded via biological treatment. This research was conducted in sacrificial reactors with rumen bacteria at a temperature of 37°C over a period of eight

weeks. The reactors were sampled and analyzed for water soluble lignin and lignin monomers.

Water Soluble Lignin

Figure 2-7 shows that lignin was solubilized in the presence of microbes at a faster rate during the first six weeks versus the thermal treatment alone. The rate of lignin solubilization of office paper is 0.003 mg/L per week with biological treatment versus the 0.0002 mg/L per week of the thermal treatment experiment. This indicates that that 25% of the lignin in office paper would solubilize in 19 months with biological treatment versus 24 years with thermal treatment. After approximately six weeks the amount of solubilized lignin decreased, indicating that the soluble lignin was being consumed or degraded.

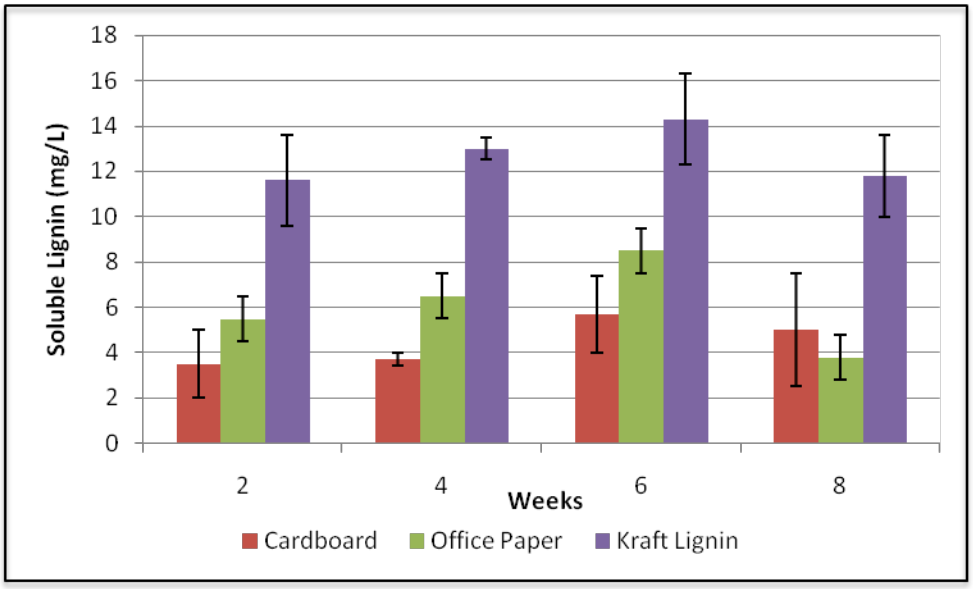

Figure 2-7: Lignin Solubility over 8 weeks (Biological Treatment)

Monomers

Figure 2-8 shows that the concentration of 4-methyl phenol, a lignin monomer, was observed after six weeks of incubation in the inhibited reactors. This indicates that after approximately six weeks of incubation with the rumen media, water soluble lignin is being degraded into its monomers.

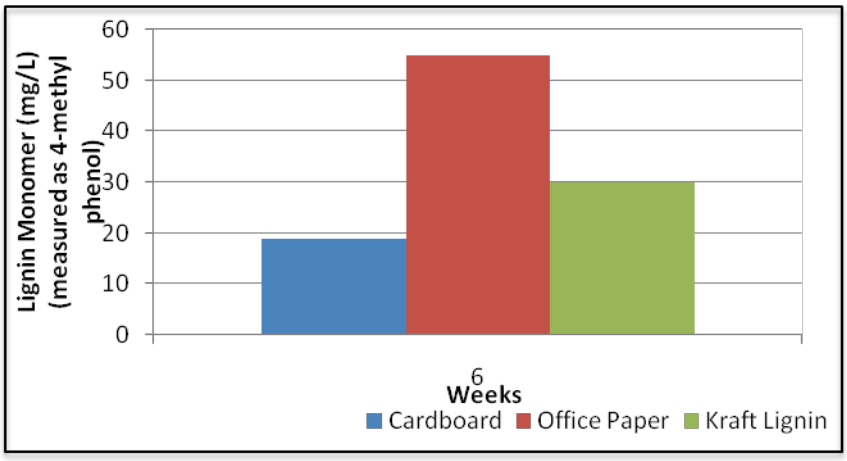

Figure 2-8: Lignin Monomers Present at 6 weeks (Biological Treatment)

Lignin Degradation via Thermal and Biological Treatment

The final experiment conducted, Lignin Degradation via Thermal and Biological Treatment, combined the two previous variables. As discussed previously, this experiment was conducted with an initial pretreatment period of three months at the elevated temperature. The reactors were then inoculated with a microbial seed and incubated up to a period of one year. The levels of WSL, lignin monomers, and VFAs were measured on the leachate. Methane concentration and volume was measured on the gas produced and the remaining solids were analyzed for lignin and total mass reduction.

Water Soluble Lignin and Lignin Monomers

Total WSL was measured as described above, and the data is summarized in Figure 2-9. Concentrations of WSL varied as time progressed. However, no significant difference was observed when the pre-treatment temperature reactors were compared. This was most likely due to the simultaneous production and degradation of WSL, which is shown in the lignin monomer production (Figure 2-10). In addition, the procedure utilized to measure WSL concentration has a high error value associated with it. According to Orsa (1994), up to 20% of the concentration can be attributed to dissolved or colloidal substances remaining in the water phase.

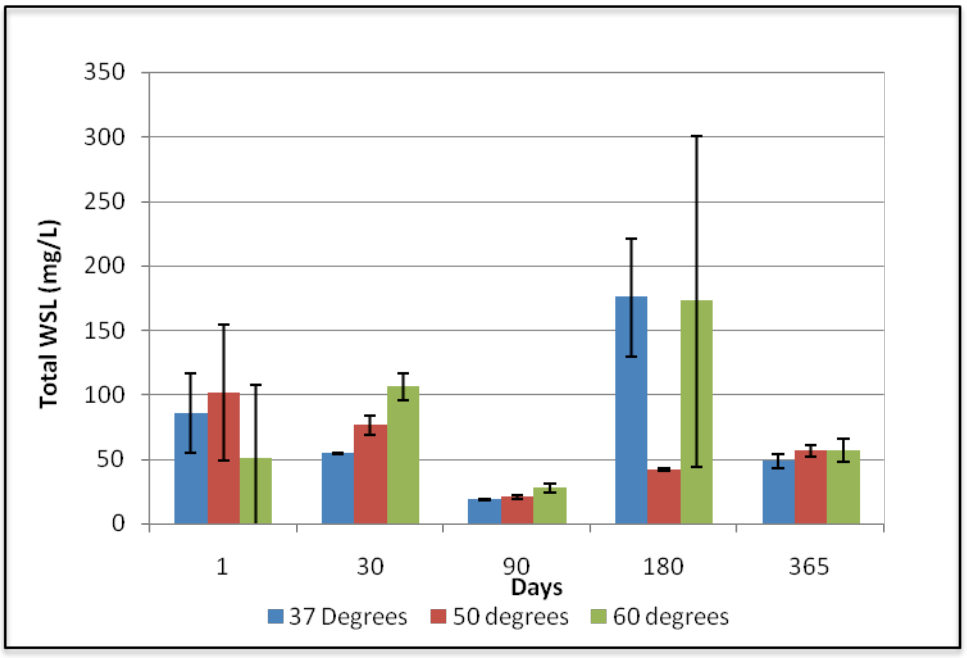

Figure 2-9: Total WSL Concentration versus Time

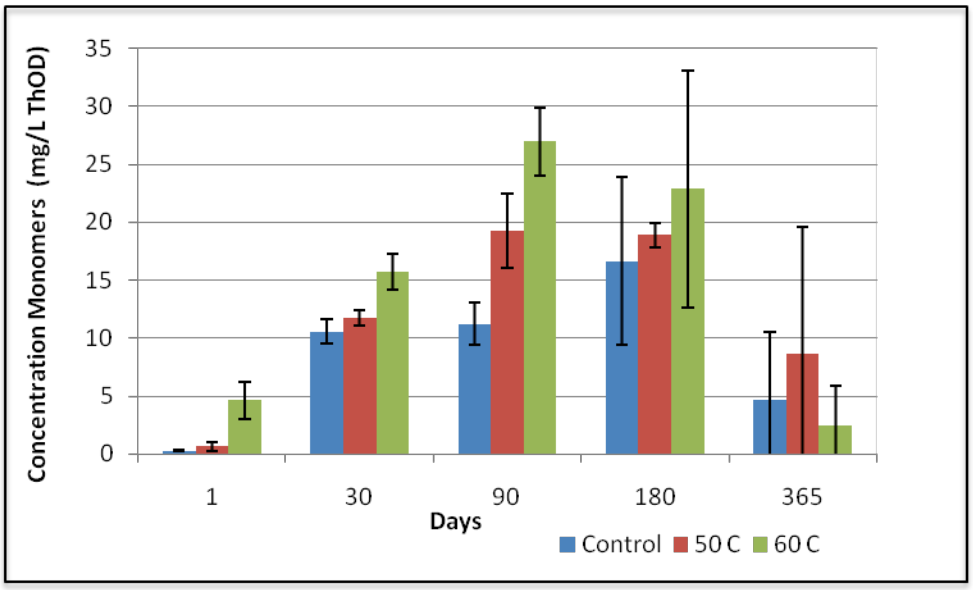

Figure 2-10: Total Monomers versus Time

To measure the amount of monomers released, catechol and vanillin, as well as benzoic, ferulic, and syringic acids were measured during each sampling event. Results are presented in Figure 2-10 as total monomers in mg/L of theoretical oxygen demand (ThOD). Concentrations of monomers were immediately present in the reactors

preheated at 60°C, indicating immediate breakdown of lignin into monomers in that reactor. Specifically at Day 1, which is just prior to introduction of the microbial seed, monomers are already present in the 60°C reactor. This initial presence indicates that lignin monomers are being generated in that set of reactors solely from the thermal pre-treatment. Over the incubation period, higher concentrations of monomers were found as temperature increased; indicating that elevated temperature increases the rate of degradation of lignin. Over time, these released monomers are degraded into LFG. As the LFG production rate increases, the concentrations of monomers present in the reactor decreases. This is observed in the latter sampling periods. Specifically, the sampling results from days 30 and 90 show an increasing concentration of lignin monomers present as the pretreatment temperature increased.

This experiment was conducted a second time at 60, 65, and 70°C. These results are shown in Figure 2-11. Similar to the first experiment, concentrations of monomers in the control and 60°C reactors were measured below 10 mg/l ThOD. However, concentrations of monomers in the 65 and 70°C reactors were observed to be significantly higher on day 1 than in other reactors. This supports the data collected in the initial solubility experiment. While all reactors contained concentrations of Catechol and Benzoic Acid, the 60-70°C reactors also contained elevated levels of Syringic Acid when compared to the other temperatures.

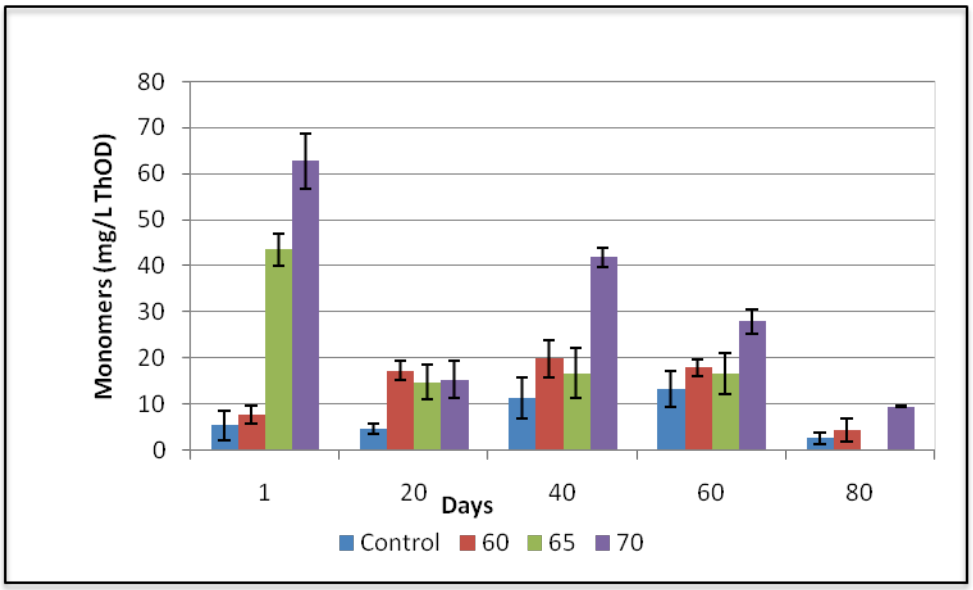

Figure 2-11: Total Monomers versus Time, additional temperatures

Volatile Fatty Acids

Acetic, butyric, heptanoic, hexanoic, isobutyric, isovaleric, isocaproic, valeric, and proprionic acids were measured at each sampling period. All nine fatty acids were observed with the dominating species being acetic and proprionic acids. Concentration trends appear similar in all reactors prior to Day 180. At Day 180, the 60° C reactor has minimal VFA accumulation (see Figures 2-12 and 2-13). This is likely due to the faster degradation rates being observed in the elevated temperature reactors. Higher rates of degradation may be occurring in the 60° C reactor because more readily degradable material is generated due to high temperature solubilization of lignin and cellulose. When this experiment was repeated at 60, 65, and 70°C it again showed that the elevated temperature reactors utilized the VFAs present before the control reactors

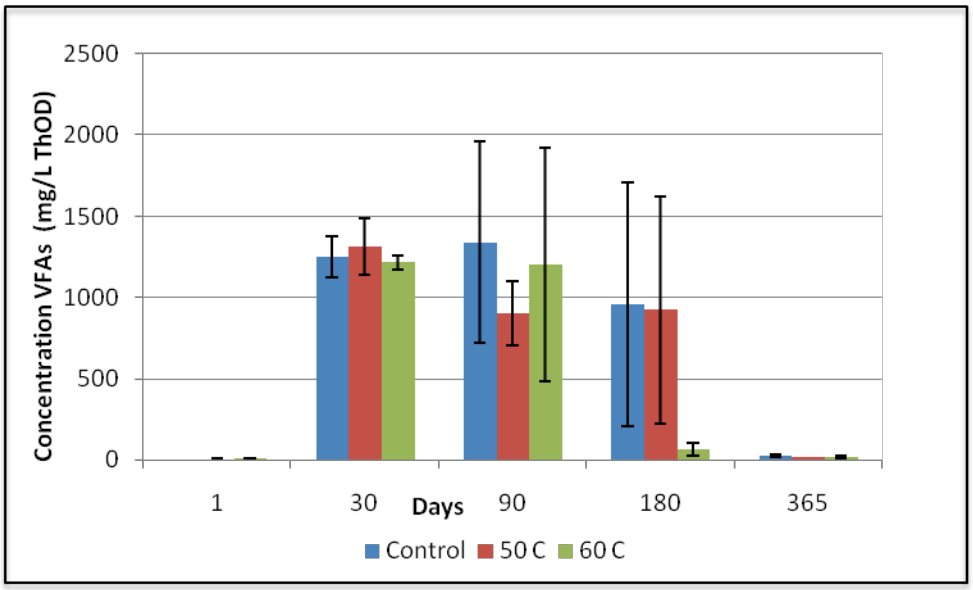

Figure 2-12: VFA Concentration versus Time

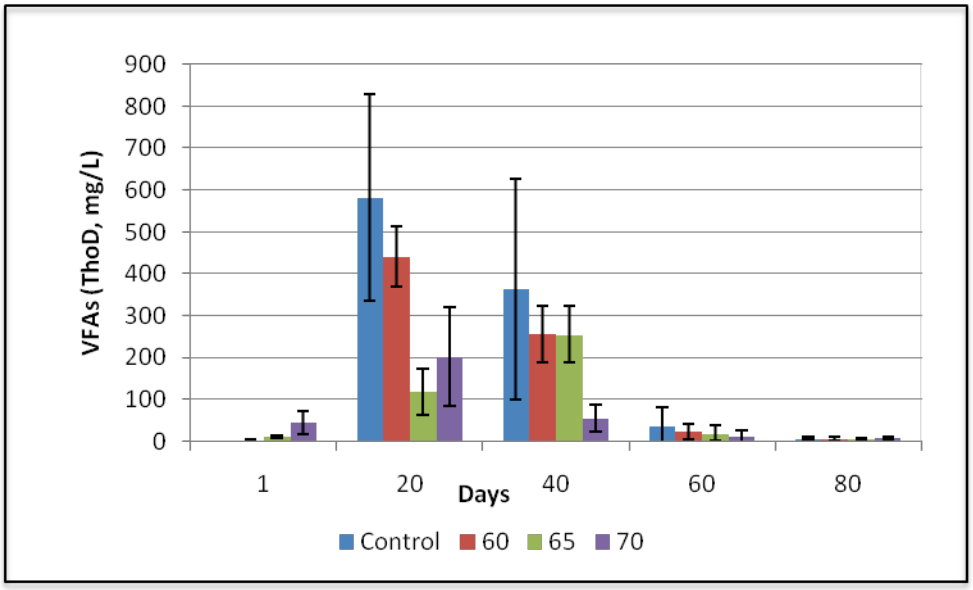

Figure 2-13: VFA Concentration versus Time, additional temperatures

Methane

Methane generation results are shown in Figures 2-14 and 2-15. Results from these reactors do not show a difference between the 37° C and 50° C pre-treated reactors. However, methane concentration and volume for the 60, 65 and 70° C reactors show a

significant increase over the other pre-treated temperatures and control. This indicates that the elevated temperature has affected the degradation of the substrate and increased the LFG production. Specifically, the long term experiment 60°C reactor shows approximately 25% more methane production versus the control reactor (Figure 2-14).

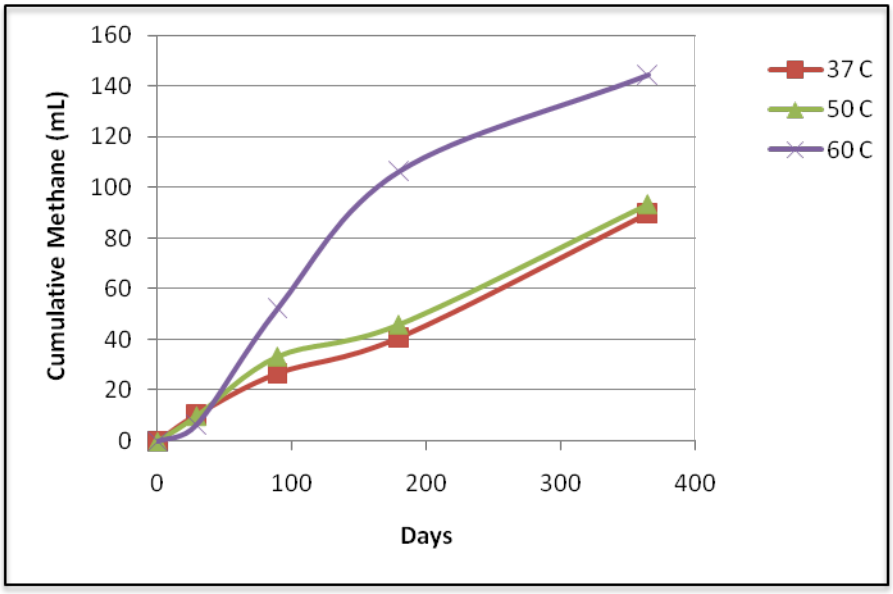

Figure 2-14: Cumulative Methane Volume

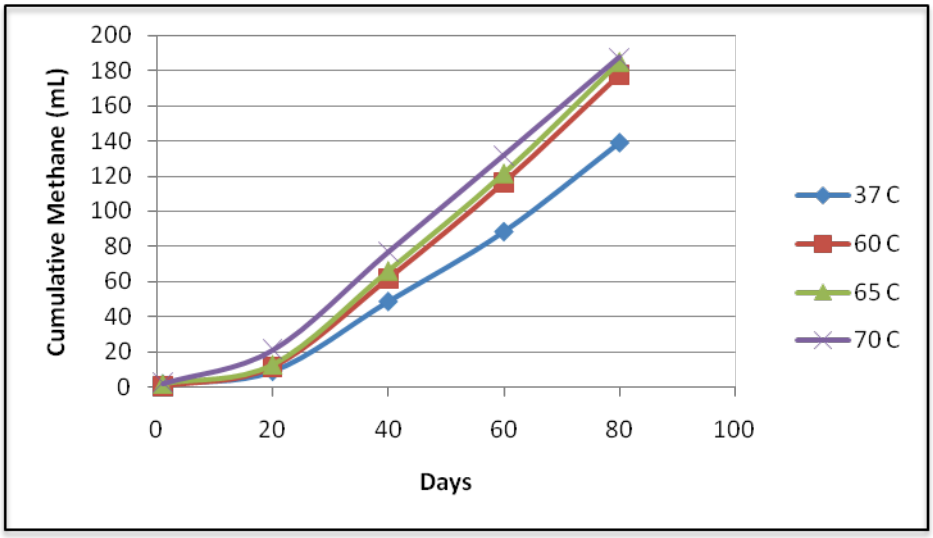

Figure 2-15: Methane Volume over Time, additional temperatures

Overall Degradation Pathway

One goal of this research was to observe the completed lignin degradation pathway. Figures 2-16 and 2-17 show the observed production of WSL, lignin monomers, VFAs and methane over time. These graphs were generated by normalizing the concentrations and plotting the results versus the sampling time. Figure 2-16 shows the results for the control reactor. In this graph you can see there is no clear definition between the production periods. This is because the production and degradation of WSL, lignin monomers, and VFAs are occurring slowly. Figure 2-17 shows the results for the 60°C reactor. In this graph, the peak production is more defined. The peaks for lignin monomers and VFAs are occurring at similar place due to the simultaneous production/degradation that is occurring at this temperature. As mentioned before, lignin monomers were produced via thermal treatment alone in this reactor, therefore VFA production was initiated quickly in the 60°C reactor.

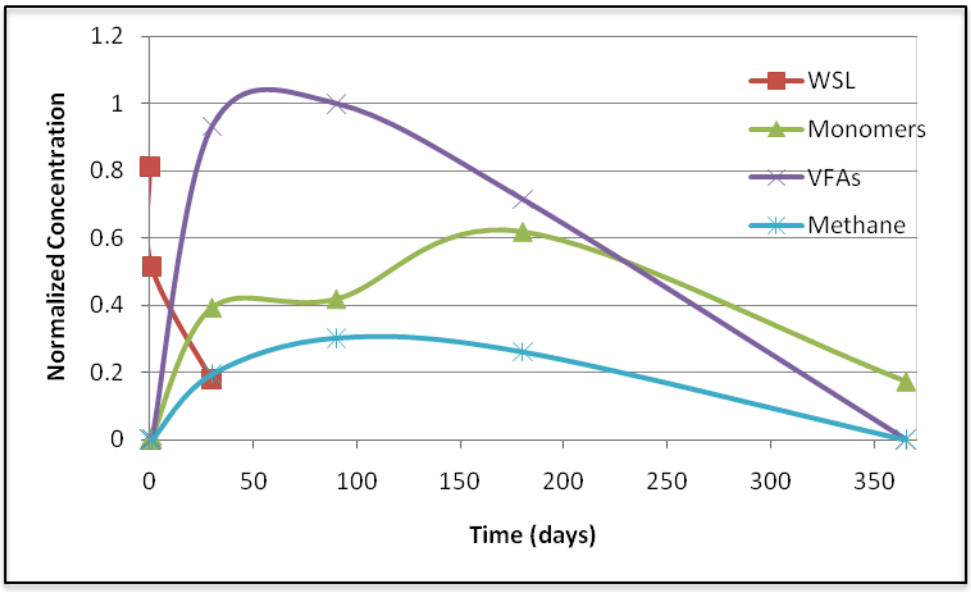

Figure 2-16: Overall Response Pathway for Control Reactors

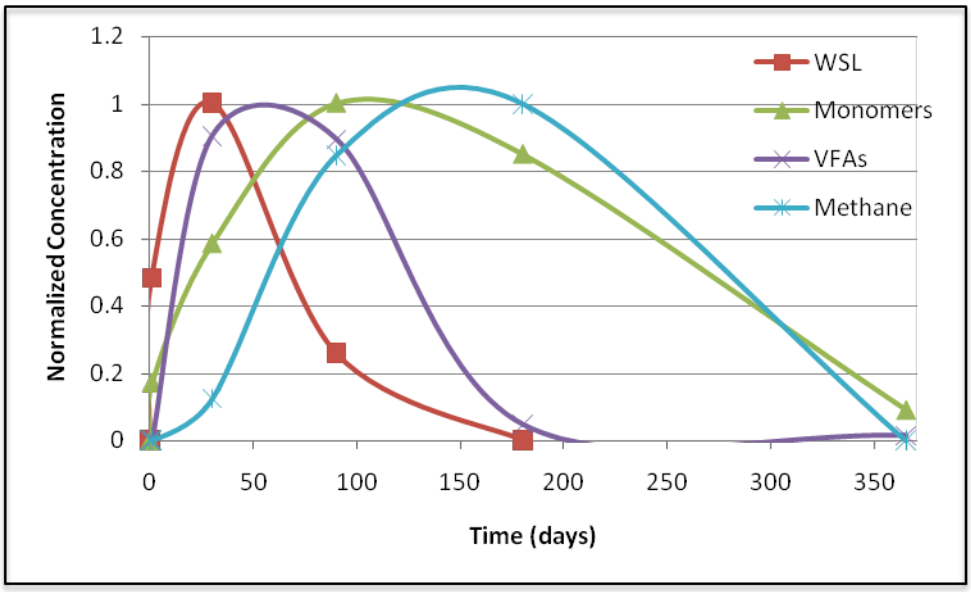

Figure 2-17: Overall Response Pathway for 60°C Reactors

Cellulose/Lignin

The paper products utilized for this experiment were measured as approximately 25% lignin. With an initial total mass of 500 mg, each reactor initially contained approximately 125 mg of lignin. Figure 2-18 shows the total mass of lignin in the reactors over time for the 37° and 60° C reactors. Figure 2-19 shows the total mass reduction over time for the same two reactors. This data supports the conclusion that lignin is degrading in the reactors. However, the treatments do not appear to change the overall amount of lignin degradation. The decrease in total mass (Figure 2-19) during the thermal pre-treatment period, -90 to 1 on the X-axis, indicates that temperature is affecting the rate of degradation, but not the overall total degradation.

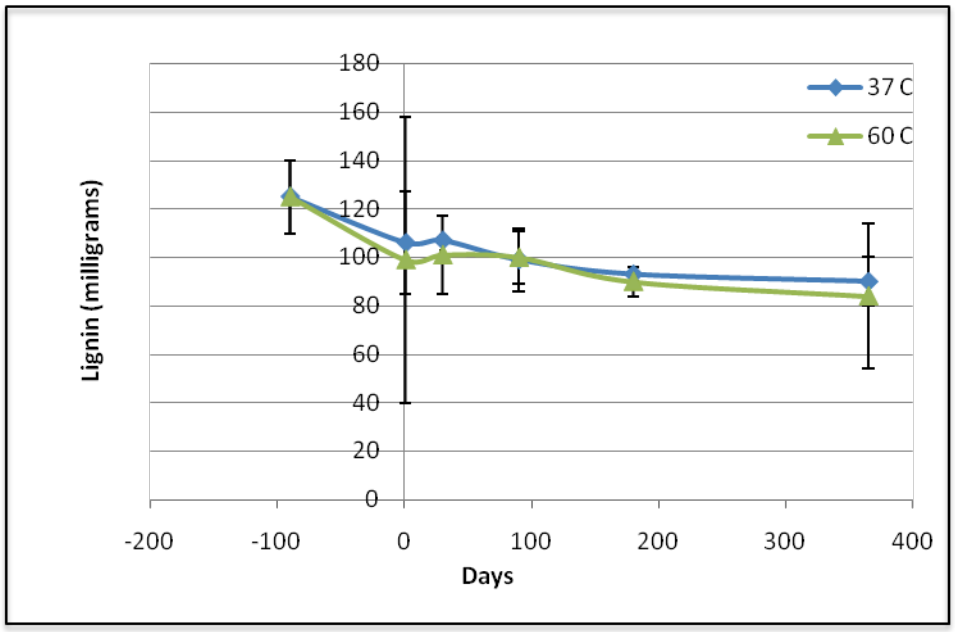

Figure 2-18: Lignin Mass versus Time

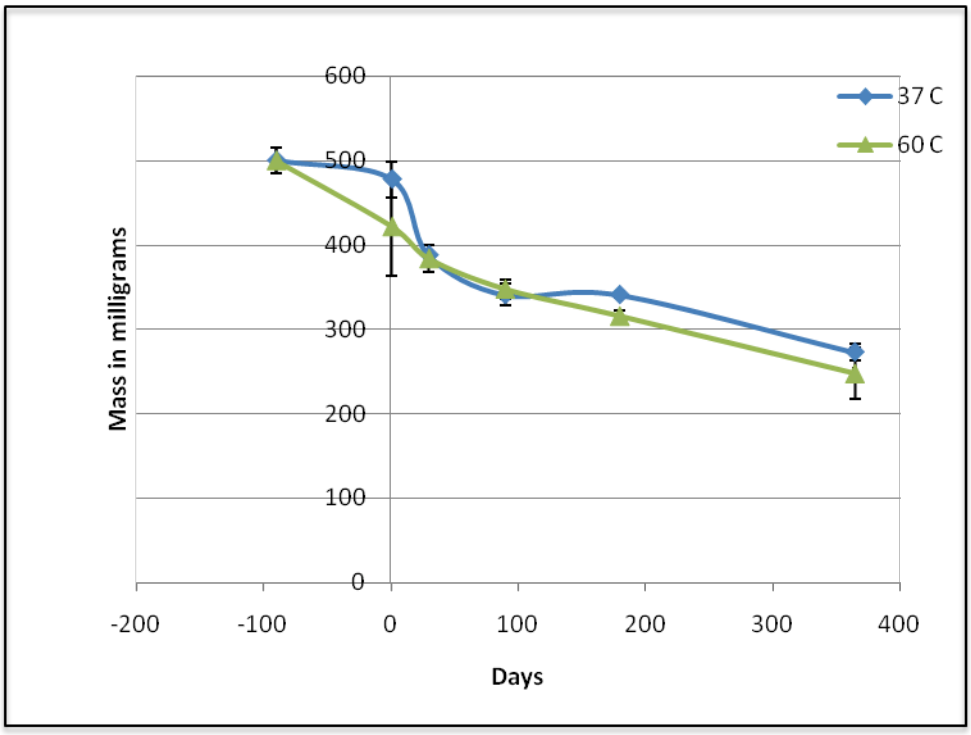

Figure 2-19: Total Mass versus Time

Summary and Conclusions

Lignin Solubility

Soluble lignin increased exponentially as temperatures increased. Up to 0.40% of the lignin in cardboard can be solubilized at 60 °C in just 48 hours. This is double the amount as that which is solubilized at 20 °C (0.20%). Considering this, increased temperatures within bioreactor landfills appear to be crucial to increasing the bioavailability of lignin. The resulting solubilized lignin molecules are of lower molecular weight that the intact lignin structure and more readily available for mineralization to carbon-dioxide and methane. Additionally, the solublization of lignin makes it more bio-available in anaerobic environments. Besides supporting active anaerobic microbial environments, high temperatures within bioreactor landfill play a significant role in the physical transformation of the lignin structure.

Lignin Degradation via Biological Treatment

The inter-monomeric bonds of lignin can be broken down to release monomers via a biological treatment of a mixed rumen bacteria. Furthermore, once solubilized to its monomers, small percentages of lignin can be completely mineralized to LFG over a period of six to eight weeks. Both rumen and landfill environmental have functionally parallel environments that demand the mineralization of organic materials such as cellulose and lignin. Notably, clone libraries from rumen ecosystems, anoxic soils, and landfills are all dominated by the same bacteria species. Therefore, an understanding of lignin mineralization using rumen can illuminate the process within a landfill as well.

Lignin Degradation via Thermal and Biological Treatment

By simulating a bioreactor landfill during the acetogenesis and methanogensis stages, the degradation of lignin compounds from total lignins to LFG was observed. As the lignin present in the reactors solubilized and degraded, increased concentrations of lignin

monomers, VFAs, and LFG were observed. The degradation pathway is best observed in the subsequent production and degradation of WSL to lignin monomers as shown in Figure 2-20, which was created from data points included in Figures 2-9 and 2-10. This graph shows that as WSL was being degraded, the concentrations of lignin monomers were increasing, thus completing that portion of the degradation pathway.

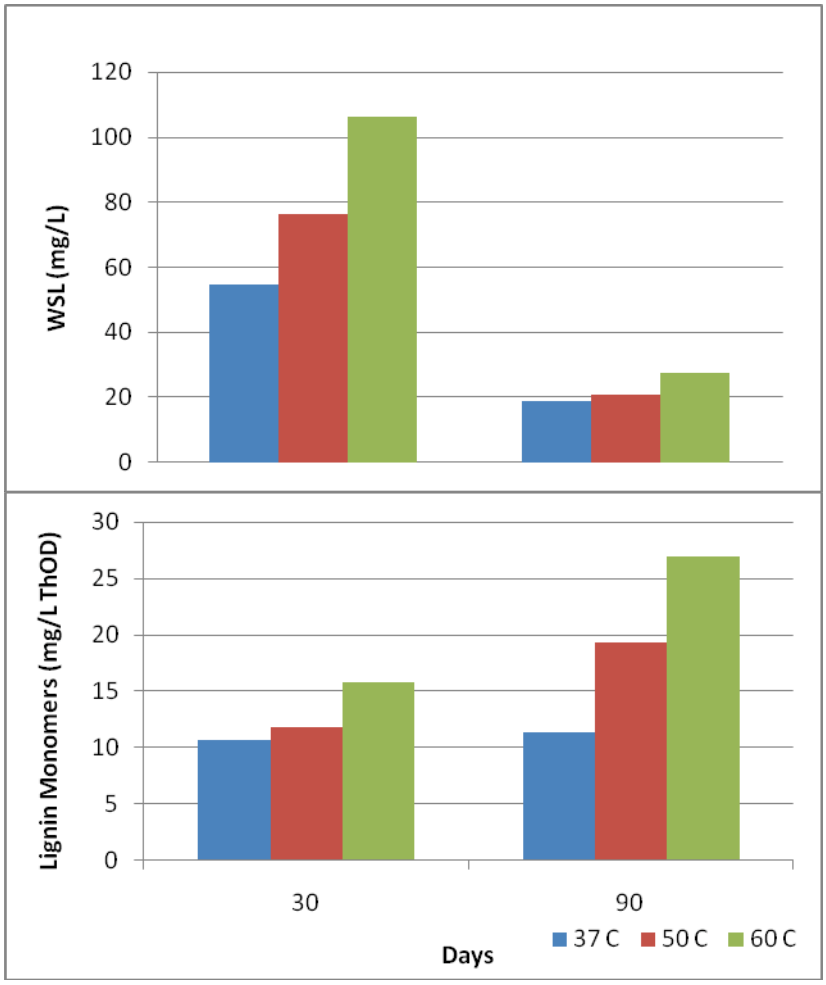

Figure 2-20: WSL and Lignin Monomer Concentrations from Day 30 and 90

The concentrations of lignin monomers and methane, as well as total LFG volume, were significantly higher in the reactors pretreated at elevated temperatures. These reactors released up to seven times more monomers than the control reactors on Day 1 of the experiment with only thermal treatment. With the increased release of monomers, the methane production in the elevated temperature reactors also increased. By analyzing the

step-by-step anaerobic lignin degradation cycle, this study shows that high temperatures and moisture are critical in degrading lignin.

High temperatures and moisture are critical for the solublization of lignin. Once lignin has been solubilized, it is broken into smaller molecular weight fractions and its bioavailability increases. As bioreactor technology evolves, it is necessary to have a solid understanding of the settling and gas generation rates of these landfills. If lignin degradation is not considered, these factors can be miscalculated resulting in the mismanagement of the bioreactor landfill technology. Further studies are necessary to determine the exact percentage of, and time frame over which, lignin can be degraded to its monomers and mineralized to LFG. These studies provide an introductory understanding of the rates at which lignin is solubilized with microbial activity, it also provides rates at which lignin is solubilized with thermal treatment alone.

Currently, the biological stability of a landfill is often measured by the cellulose to lignin ratio (C/L). These studies show that the lignin in paper can degrade in bioreactor landfill environments at a slow, but significant rate. Considering this, a different denominator like plastic, that is more stable than lignin, must be used to determine that biological stability of landfills. Additionally, leachate analysis for the presence of soluble lignin and lignin monomers could be crucial in understanding the effectiveness and rate of lignin degradation in bioreactor landfills.

The significance of anaerobic microorganisms in the turnover of lignin derived carbon in the natural environment cannot be underestimated if bioreactor landfill technology is to be utilized. The anaerobic degradation of lignin derived carbon affects settling, gas production rates and carbon sequestration estimates.

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