Biochemical Lignin Related Processes in 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

Masters of Science
in
Environmental Engineering

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December 16th, 2005
Blacksburg, Virginia

Keywords: Lignin, Bioreactor Landfills, Paper, Soluble Lignin, Rumen, Cellulose/Lignin Ratio

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Abstract

The objective of this study was to determine how the key features of bioreactor landfills; increased temperature, moisture and microbial activity, affect the biological stability of the landfill material. In the first part of the study the solubilization and degradation of lignin in paper exposed to these bioreactor landfill conditions are explored. The solubility of the lignin in paper was observed at different temperatures and over 27 weeks at 55°C and the anaerobic bioconversion of office paper, cardboard and Kraft lignin was observed in bench-scale reactors over 8 weeks. As the temperature rose, lignin solubility increased exponentially. With extended thermal treatment, the dissolution of lignin continues at a constant rate. This rate increases 15 times for paper and 1.5 times for cardboard in the presence of rumen inoculum compared to un-inoculated systems. At around 6 weeks the inter-monomeric linkages between the solubilized lignin molecules began breaking down, releasing monomers. In cardboard and Kraft lignin, a significant amount of the monomers mineralize to CO_2 and CH_4 during this time period. The results indicate that small, but significant rates of lignin solubilization and anaerobic lignin degradation are likely to occur in bioreactor landfills due to both higher temperature and microbial activity.

In the second part of the study, field data from the Outer Loop Recycling and Disposal Facility in Louisville, Kentucky was evaluated to determine the effectiveness of an anaerobic-aerobic landfill bioreactor (AALB) vs. the control landfill that is managed as a traditional landfill. Moisture, temperature, elevation and the amount of time the MSW has spent in the landfills (age) were measured and compared to determine the factors that affect the biological stability of the landfill. The results showed that the MSW in the AALB is more biologically stable than the MSW in the control landfill, indicating that they are more degraded. Additionally, elevation or location of the MSW was the key factor in determining the extent of MSW stability within the AALB and temperature is the key factor in determining the biological stability of the MSW in the control landfill. Higher temperatures correlated with a more biologically stable waste. The cellulose to lignin ratio (C/L ratio) and biochemical methane potential (BMP) were the main biological stability parameters used.

Acknowledgments

My sincere gratitude goes out to Dr. Novak who provided me with this opportunity to explore my independent, creative and scientific thought process. The guidance he provides his students, coupled with his confidence in them allows them grow and develop as an engineer and researcher. I would also like to thank Dr. Goldsmith for his guidance especially with the Outer Loop data and Dr. Evanalyo for serving on my committee and providing me with various insights during the committee meetings. I acknowledge the financial support I received from Waste Management and I thank Mr. Gary Hater for continuing to support our work here at Virginia Tech.

I would also like to thank Dr. Knowlton for serving on my committee and introducing me to her helpful and generous Dairy Sciences research group. My sincere gratitude goes out to Cathy Parson who generously lent me equipment from her lab and provided me with guidance during the initial stages of my experimental set-up. Additionally I would like to thank Tzu-Hsuan Yang for teaching me how to collect rumen fluid from a cow and to Megan Taylor, Shelly Slemp and Aaron Cornman for providing me with encouragement each time I visited the cows.

I would especially like to thank Jody Smiley and Julie Petruska for their patience as I developed my laboratory skills and for their contributions to my experimental analysis and design. This thesis would have been impossible without their support. I am also very grateful to Betty Wingate and Sherry Bruke for their support considering administrative matters. I would also like to thank Dr. Gallagher for his help with the statistics used in this thesis. My fellow colleagues, Heather Rectanus, Chris Muller, Krista Rule, Chris Wilson, and Mert Muftugil were very supportive and provide me with much help and advice in the lab, I am very grateful to them. Additionally, I am very grateful to my lab mates Jong Min Kim, Olga Miroshnikova and Holly Hampton for creating a friendly, corporative and supportive work environment.

I would like give a special thank you to my family for their constant support and prayers; I could not have done it without them. Lastly, I would like to thank Mariano Velázquez for his encouragement, good humor and invaluable help.

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I Literature Review

Introduction

Municipal Solid Waste (MSW) generation in the United States has continued to grow (Figure I - 1).

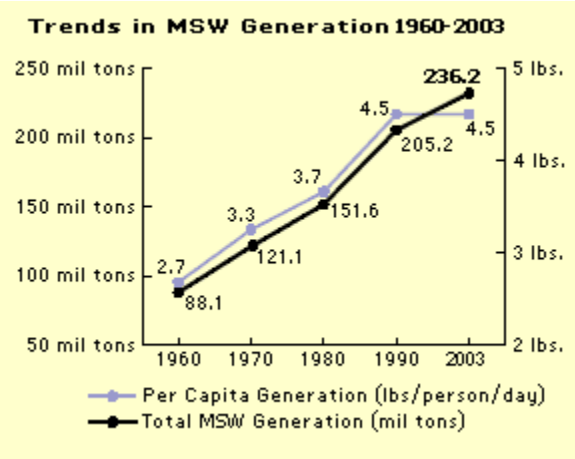


Figure I - 1: MSW generation in the US over the years (USEPA 2005)

Currently, in the United States, 56% of the MSW generated is disposed into landfills. The remaining is recovered, recycled or composted (30%) or burned at combustion facilities (14%) (USEPA 2005). Traditionally landfills were operated as waste containment facilities, designed to encapsulate and store MSW. This approach to waste disposal limits degradation of waste, especially carbon rich paper which accounts for 40% of a landfills contents (Barlaz et al. 1990). According to the USEPA, newspaper alone takes up more than 13% of the space in US landfills. With increasing amounts of waste generation, decreased amount of landfill space, increased landfilling costs and environmental concerns associated with landfills, there has been a push to operate landfills with a process based vs. a storage/containment approach.

About three decades ago, the idea of operating landfills as bioreactors was introduced. By increasing the moisture content and the flux of moisture through the landfill through leachate recycling and supplemental water addition, landfills become “bioreactors” processing facilities where waste is digested, versus the “dry tombs” landfills. With this approach, there is a rapid settlement of waste improving the yield of landfills by 15 to 30% (USEPA 2005). Additionally,

gas production rates are increased (Green 2000) and the overall leachate quality is improved, requiring less treatment and lower disposal costs (USEPA 2005).

Bioreactor Landfills

The aim of operating landfills as bioreactors is to accelerate the degradation of municipal waste and increase methane production in the short term during landfill gas (LFG) harvesting. This reduces fugitive methane release into the atmosphere after closure. The LFG production quality and quantity is directly related to the stability of the waste present in the landfills. The increased moisture/temperature conditions of bioreactors landfills induce the waste to settle. This permits a lager volume of waste to be disposed in a given area.

The design of bioreactor landfills must comply with the same RCRA regulations as a sanitary landfill, making the design of a bioreactor landfill a reworked copy of a sanitary landfill. However, because of the additional moisture, bioreactor landfills require carefully designed liners. Usually a double composite liner is recommended. Besides the liner, the drainage system above the liner is critical. The drainage system generally consists of highly permeable materials such as sand, gravel, or geosynthetic net. The drain must also be protected by a natural soil or geosynthetic filter to minimize clogging due to particulate in the leachate or biological growth. Since filter clogging results from factors like sedimentation, biological growth, chemical precipitation and/or biochemical precipitation which are difficult to control, proper filter selection to minimize clogging is important (Reinhart and Townsend 1998).

In order to gain benefits from leachate recycling, it is important that leachate does not excessively accumulate within the landfill and emerge from the toe of the landfill slopes. Therefore, it is important to have ex-situ storage of leachate especially during the early phases of the landfill and during storm events. The types of storage facilities differ and could include lined concrete tanks, elevated steel tanks, underground storage tanks and lined ponds. It is important that the construction material utilized withstand the corrosive nature of leachate (Reinhart and Townsend 1998).

In most modern landfill designs, gas collection is not practiced until after the landfill has closed, however bioreactor landfills can generate gas from the beginning of landfill operations. This

requires various horizontal pipes throughout the landfill to insure proper collection and management of methane. Since gas production is particularly enhanced near leachate reintroduction sites, horizontal leachate reintroduction pipes can also be used to extract gas. This aspect of the design also allows for landfill operators to benefit economically from the energy potential of the methane gas. One of the benefits of bioreactor landfills may be that they compress the time over which methane is generated, allowing most of the methane generated to be captured during active energy recovery vs. after closure (Reinhart and Townsend 1998).

A final and equally important aspect of bioreactor's landfill design is the final cap. Once the landfill reaches its design height, a final cap is needed to minimize infiltration of rainwater, dispersal of wastes, to accommodate subsidence, and to facilitate long-term maintenance. It is suggested that bioreactor landfills use an intermediate permeable cap and continue leachate reintroduction with horizontal pipes before a final cap is implemented. This will allow for the complete degradation of the wastes and optimize methane production. However, current regulations require a final cap once the landfill is no longer active. As confidence is gained in bioreactor landfills, these regulations are expected to change (Reinhart and Townsend 1998).

There are three different types of bioreactors landfill configurations: Aerobic: Air is injected into the waste mass using vertical or horizontal wells, to promote aerobic activity and waste stabilization. Anaerobic: Moisture is added in the form of re-circulated leachate and outside liquids. Hybrid or anaerobic-aerobic landfill bioreactor (AALB): where aerobic-anaerobic treatment is employed sequentially (Reinhart and Townsend 1998). Each type of bioreactor landfill is designed to increase microbial activity, allowing waste to decompose at a rapid rate.

Microbial Activity in Landfills

MSW within a landfill undergoes five stages of decomposition (Reinhart and Townsend). They are:

- Adjustment or acclimation
- Transition
- Acidogenesis
- Methanogenesis
- Maturation

These phases are not sharply defined within a landfill, the stages tend to overlap as lifts of MSW are added. Because of the heterogeneous nature of the MSW, pockets of microbial activity form, causing the different stages of decomposition to occur side-by-side within the landfill. Bioreactor landfills attempt to increase the rate of the first four phases. This is done by providing the waste with water and nutrient rich leachate during the transition and acidogenesis phase.

When the MSW in the bioreactor landfill is in the adjustment stage, the easily degradable waste such as food waste and other easily degradable organic matter serves as the carbon source for bacteria and the oxygen in the air serves as the electron acceptor resulting in the following reactions:

Degradable waste + oxygen => CO_2 + H_2O + heat + biomass + Acetic Acid + Residuals
$CO_2 + H_2O$ => H_2CO_3 (Carbonic Acid)

As oxygen is consumed, the waste gradually enters the transition phase where the complex organic matter is broken into simpler organic acids driving the MSW into the acidogenesis stage. During the acidogenesis phase volatile organic acids (VOAs) reach their peak concentration within the landfill. Water and leachate are added to the MSW when it is in the transition and acidogenesis stage. As the MSW enters an anaerobic environment the anaerobic bacteria utilize other electron acceptor like nitrates, sulfates and mixtures of other wastes to further degrade the MSW. The additional moisture provides an ideal environment for the anaerobes, and the bacteria in the leachate provide an extra seeding of bacteria to degrade the organic matter. Additionally, nitrates also serve as a sink for hydrogen as reduction to ammonia occurs within bioreactor landfills. The ammonia generated within landfills usually exceeds the microbial nutrient requirements, producing a leachate rich in ammonia requiring further treatment and stabilization (Kjeldsen et al. 2002). Through leachate quality monitoring and recirculation, a system analogous to an attached growth anaerobic process is developed within bioreactor landfills allowing microbial populations to develop and proliferate until the substrate is depleted or environmental conditions become limiting (Pohland and Kim 2000). As the alternative electron acceptors are used up, the methanogenic bacteria ferment the organic acids to methane and carbon dioxide. Methanogens start utilizing carbon dioxide as their terminal

electron acceptor and consume the high concentration of hydrogen ions that were produced during the acidogenesis phase to produce methane, resulting in the following reactions:

$4H_2 + CO_2 => CH_4 + 2H_2O$ $CH_3COOH => CH_4 + CO_2$

As the available carbohydrates are consumed the MSW enters the maturation phase and is then relatively inert (Christensen and Kjeldsen 1989; Gurijala and Suflita 1993; Shearer 2001; Hater et al. 2003).

Within the landfill, grass, leaves, and branches are the major contributors of refuse decomposing microbes to landfills, each contributes and average of 9.8, 6.3 and 6.5 Most Probable Number – $\log_{10}$ cells/dry g (MPN) respectively (Barlaz et al. 1997). A number of species belonging to the genus Clostridium have been found to be present in the leachate of 17 different landfills (Van Dyke and McCarthy 2002), this species is also common in the rumen of cow stomachs (Tajima et al. 2000).

As microbial activity increases within bioreactor landfills, the temperature rises. This correlation is shown in the Van't Hoff-Arrhenius equation (Metclaf and Eddy 1991):

$$k_t = k_{20} * \theta^{(T-20)}$$

Where:

k_t = degradation rate constant at a particular temperature

k_{20} = degradation rate constant at 20° C (0.23 is the typical value)

θ = constant of 1.056 for temperatures between 20 and 30° C

T = temperature for which k is desired.

With more microbial activity the temperature of the landfill naturally rises. The accumulation and distribution of moisture through leachate recirculation increases reaction rates within landfills, allowing for an increased rate of in situ waste conversion and leachate treatment. Likewise, methane generation occurs before landfill closure so the methane can be harvested and utilized for energy generation, instead of after closure where it is emitted as a green-house gas into the atmosphere.

The biochemical methane potential (BMP) is an important parameter that can be used to determine MSW stability. The BMP of a sample is the amount of methanogenic degradation still possible for a sample. A high BMP indicated that the waste is still active, containing an easily available carbon source while a low BMP indicates inertness and low carbon availability (Shearer 2001).

A traditional method to determine if the landfill has entered the maturation phase is the cellulose (C) to lignin (L) ratio. A lower C/L ratio indicates stability. Factors that contribute to the slow rate of paper degradation in landfills are moisture limitation, poor shredding, low cellulose/lignin ratio (high lignin content) and the lack of inoculum (Pohland and Kim 2000). As the landfill stabilizes and reaches the maturation phase, the cellulose is consumed by the anaerobes and their concentration is minimized. When compared to the relatively inert lignin, one can determine the extent of the cellulose degradation or MSW stability. However, recent research has shown that lignin does degrade under the high temperatures and moist conditions present in bioreactors landfills. A better understanding of lignin and the anaerobic conditions under which lignin degrades is crucial in determining the level of landfill gas production, settling rates and post closure monitoring requirements.

Lignin

Lignin is the most abundant naturally occurring source of aromatic compounds (Young and Frazer 1987) and the second most abundant organic material on earth after cellulose. It is a random three-dimensional network polymer consisting of phenylpropanoid units rich in methoxyl substituents (Young and Frazer 1987) linked together in random ways (Goring 1989) through ester, ether and C-C bonds. It is found in the cell wall of some cells of plants. Figure I - 2 shows a representative lignin structure. The insert highlights the phenylpropanoid structure and the methoxyl substitutes. The β-aryl-ether bond is the most common inter-monomeric linkage in lignin polymers (Adler 1977). It is impossible to define a typical structure since the deposition of the lignin compound occurs in a random manner.

Lignocellulose or lignin-carbohydrate complexes (LCCs) are the stable combination of cellulose, hemicellulose and lignin which compromise 89 to 98% of the dry weight of wood. The cellulose

component of lignocellulose provides flexibility and strength to the cell wall, the hemicellulose component chemically bonds the cellulose and lignin. The lignin, with its amorphous (Sarkanen 1963) and hydrophobic characteristics, protects the cell from attack by various chemical and enzymes. By forming LCCs, lignin restricts the biological availability of the hemicellulose and cellulose polysachrides (Young and Frazer 1987). This complex structure of lignin makes its degradation a rate-limiting step in the biospheric carbon cycle (Colberg 1988). Additionally, the lignin in paper is an inhibiting agent in paper degradation (Stutzenberger et al. 1970) in landfills.

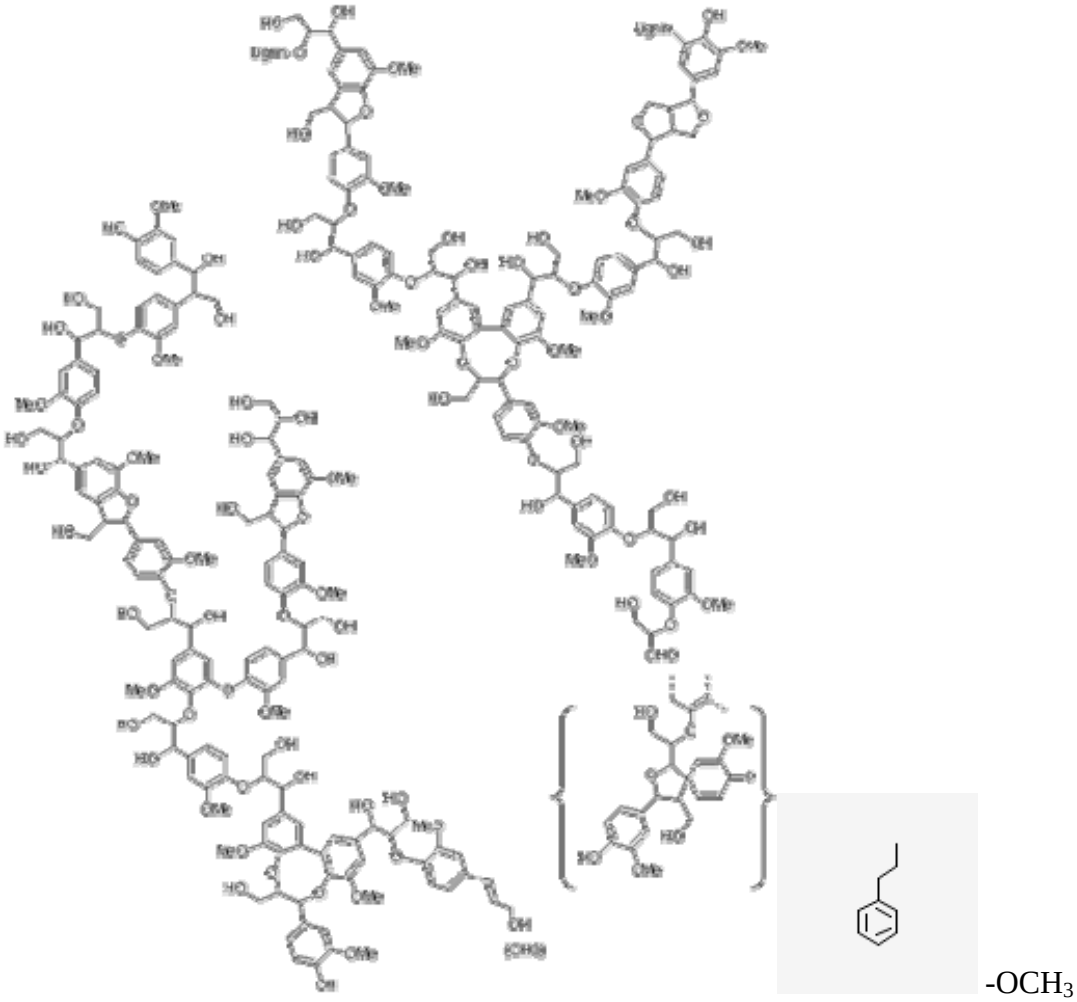


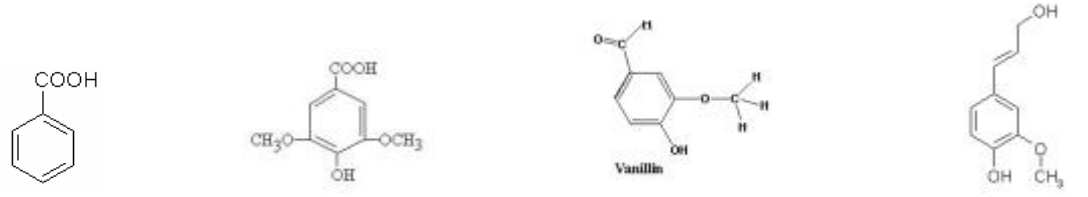
Figure I - 2: Representative lignin structure. (Organic Chemistry of Wood Components Labaratory 2005)

Overview of Lignin Degradation/Solubility

In the past years, much attention has been given to the aerobic metabolism of lignocellulose (Crawford 1981; Tien and Kirk 1983) and various complex carbons. However, the anaerobic component of the carbon cycle actively ferments and digests complex carbons into acids and alcohols and finally carbon dioxide and methane using alternative electron acceptors like nitrate, sulfate or carbonate. Well designed and monitored bioreactor landfills support active anaerobic environments permitting the anaerobic component of the carbon cycle to proceed. Previous studies showed that lignin degradation was not observed in anoxic sediments (Hackett 1977). This has led to the generally held belief that lignin does not degrade anaerobically. However, many studies since have shown that anaerobic lignin degradation does take place at a slow but significant rate. The earliest study in 1934 showed that corn stalk lignin partially degrades to CO_2 and CH_4 in anaerobic digestors (Boruff and Buswell 1934).

Currently there are two prevalent methods to study lignin degradation. The first is to develop synthetic model compounds and study their degradation pathway, and the second is to characterize intermediate lignin degradation products through chromatography and spectroscopy. The first approach assumes that the degradation mechanisms of the synthetic model are similar to the complex polymer. The second method requires an understanding of the lignin derivatives and their degradation pathway.

The most basic structure of lignin is the lignin monomer. Examples of lignin monomers include coniferyl alcohol, vanillin, syringic acid, cinnamic acid, syringaldehyde, benzoic acid, phenylacetic acid, protocatechuic acid, phenol, *p*-hydrobenzoic acid and ferulic acid



Benzoic Acid Syringic Acid Vanillin Coniferyl Alchol

Figure I - 3: Examples of Lignin Monomers Source: (Cook 2003-2005)

In 1934 Tarvin and Buswell were the first to prove that anaerobic metabolism of the aromatic ring is possible using benzoic acid as a model compound. Since then, many researchers have shown aromatic ring metabolism to CO_2 and CH_4 under anaerobic conditions. Additionally, the above lignin monomers have been shown to undergo complete methanogenic degradation using enriched culture from digester sludge (Healy 1979). The anaerobic pathway of the degradation of these monomers involves the simplification of the original structure by the removal of one or more substituent groups, forming phenol and benzoate as the key intermediates (Young and Frazer 1987). Additionally, it has been shown that water soluble phenolic compounds are released when lignin is metabolized by fungi and actinomycetes (Crawford 1981). In a study to model plant phenolic compounds, ferulic and syringic acid degradation under denitrifying, sulfidogenic and methanogenic conditions with bacteria from a wetland site, it was found that the lignin monomers ferulic and syringic acid degraded under all 3 reducing conditions (Phelps and Young 1997). These intermediates were reduced to organic acids and finally CO_2 and CH_4. It is generally accepted that lignin monomers can be readily metabolized and completely mineralized anaerobically.

Research showing the degradation of lignin oligomers is difficult because they are not readily obtained and the different bonds in lignin make it hard to represent all the oligomers (Young and Frazer). However Colberg and Young used alkaline heat treatment to solubilize [C^{14}-lignin] lignocellulose (Douglas fir) and then gel permeation chromatography (GPC) to separate the solubilized fraction into 3 major size fractions of molecular weight equal to 200, 600-700 and greater than 1000 (Colberg and Young 1985a). Each fraction was anaerobically inoculated with a microbial enrichment culture for 4 weeks. Radiolabeled CO_2 and CH_4 were released from each fraction. However, the lower size fraction (molecular weight 200) released the most gas while the fraction containing solubilized lignin of MW > 1000 released the least gas. Subsequent GPC analysis showed a shift in the profile indicating a lower overall molecular weight. This experiment led the authors to conclude that the molecular weight or size of the lignin fraction is inversely correlated with its degradation capacity (Colberg and Young 1985b). The above research also showed that the MW 600-700 fraction inhibited with bromoethanesulfonic acid (BESA), an analogue to Coenzyme M which is required for methane generation, released 10 different lignin monomers over a period of 2 years. Additionally, they found an accumulation of

aromatic and organic acid metabolites indicating that cleavage of the β-aryl-ether bond in polymeric lignin is possible (Colberg and Young 1985b).

The previous literature shows that soluble lignin monomers, dimers, and oligomers are readily degraded in anaerobic environments, and that the molecular weight of lignin present determines it bioavailability, with lower molecular weight fraction metabolizing easily. Additionally, it has been show that an important intermonomer linkage in lignin, the β-aryl-ether bond, can be cleaved completely with anaerobic enrichment cultures (Colberg and Young 1985b) and by rumen anaerobes (Chen et al. 1985).

In 1985 Bernner and Hodon showed that lignin in wood is mineralized to CO_2 and CH_4 with 18-74% of the total carbon was released into the soluble fraction. Most of the other research dealing with the degradation of the entire lignin structure has been performed using rumen bacteria as an inoculum.

Rumen Bacteria and the Degradation/Solublization of Lignin

Rumen microbes comprise strictly anaerobic and facultative anaerobic bacteria and protozoa. The most common isolated celluloytic microorganisms in the rumen are *Ruminococcus* and *Fibobacter*. While sequences of these bacteria have not been recovered in landfill leachate cloning studies (Van Dyke and McCarthy 2002; Burrell et al. 2004), a number of species of the genus Clostridium have been found to be present in the leachate of 17 different landfills (Van Dyke and McCarthy 2002).

The average pH of the rumen is 6.7 with an optimal growth temperature of 39°C (Akin 1980).
The presence of soluble lignin (Gaillard and Richards 1973) and the presence of aromatic compounds in the rumen indicates that plant cell walls, lignin and lignin precursors are being degraded (Borneman et al. 1986). Soluble lignin within the rumen can have a molecular weight as high as 15,000 (Kondo et al. 1994).

In 1980 Akins isolated a filamentous facultative anaerobic bacterium (Isolate 7-1) from the rumen fluid. This bacterium delignified Bermuda grass (6% lignin) showing that rumen

anaerobes can mediate the partial degradation of the lignin polymer present in plants. Despite its low numbers and slow growth rate within the rumen, Akins speculated it could exist because of its ability to use substrate that is unusable by other rumen bacteria (Akin 1980). In later research, Akins showed that vanillyl and syringyl components of lignin are the most resistant to decomposition; however, ferulic acid is readily solubilized, even in the absence of microbial activity (Akin and Benner 1988). The same study showed that a small percentage of lignin is mineralized to CO2 and CH4, and that most of the "losses" of lignin are represented by solubilization.

Additional studies have shown that the rumen anaerobic fungus *Neocallimastix patriciarum* solublizes 20-30% of the lignin in the sorghum rind. Since only 10% of the solubilized lignin could be distinguished as ester- and ether-linked hydroxycinnamic acids, it was assumed that the rest were LCCs (McSweeney et al. 1994). These reviewed studies show that the entire lignin structure can undergo structural changes and degrade when exposed to rumen microorganisms.

Additionally, the aerobic catabolism of lignin using rumen as the inocula, was studied using synthesized dimers dehydrodivanillin and veratrylglycerol-β-guaiacyl. Dehydrodivanillin catabolism generated various monomers including vanillic acid and veratrylglycerol-β-guaiacyl catabolized into guaiacoxyacit acid, phenoxyacetic acid, vanillin, guaicol and phenol (Chen et al. 1985; Chen et al. 1985). Various lignin compounds were synthesized and their ability and pathway of lignin degradation was studied using rumen microbes as the incubation (Kajikawa et al. 2000). While the smaller lignin molecules, non-phenolic benzyl ether diamers and penolic benzyl ether triamers, were easily degraded, the non-phenolic benzyl ether triamers were more resistant. This could have been because of the high redox potential of the p-etherfied phenylpropanoid structure. This study, like previous studies, showed that lignin can be partially degraded by cleaving several specific bonds among the linkages in LCCs. It also showed that high molecular weight lignins are more recalcitrant (Kajikawa et al. 2000).

Lignin Degradation in landfills

Researchers studying the significance of anaerobic microorganisms in the turnover of lignin derived carbon in the natural environment, would agree that this mechanism is underestimated in

the global carbon cycle (Colberg and Young 1982). The anaerobic conversion of lignocellulose to methane is a complex procedure involving many species of bacteria, including acidogens, methanogens, and acetogens. Additionally, methanogens are sensitive to environmental factors such as pH and a high content of un-ionized acids. Lechate recirculation aids in distributing the appropriate microbial community and maintaining the pH within bioreactor landfills; thereby, avoiding parcels of the heterogeneous landfill from becoming rate limiting. Hydrogen sink microorganisms may be involved in the metabolism of lignin compounds, since anaerobic biodegradation of aromatic compounds occurs in nitrate respiration and methanogenic fermentation (Heider and Fuchs 1997).

A recent study by Fox and Noike (2003) showed that wet oxidation treatment of newsprint solubilized lignin. However, lignin was not degraded anaerobically using anaerobic seed sludge. Wet oxidation, or the process at which organic material is oxidized with gaseous oxygen in water, was carried out at 170, 190 and 200° C for 1 hour (Fox and Noike 2004). Lignin solubility was shown to increase at higher temperatures by Tartakovsky et al 2003. In this study (Tartakovsky et al. 2003) no microorganisms were used and lignin is solubilized by pure thermal treatment.

Thermophilic (55° C) anaerobic enrichment cultures have shown that elevated temperatures enhance the rate of anaerobic degradation of lignin and lignified substrates to methane and low-aromatic compounds (Benner and Hodson 1985). The rates of degradation of high molecular weight synthetic lignin at 55° C was 10-15 fold higher than that at 25° C (Benner et al. 1984). These and other studies show that the moisture and high temperatures within landfills make the final anaerobic conversion of organic polymer containing waste, including lignin, to CO_2 and CH_4 possible.

A landfill specific study (Chen et al. 2004) to characterize the structural and compositional change of newspaper as a function of depth showed that residence time is not the only factor that affects the extent of MSW degradation in landfills. Microbial activity, which varies spatially and is affected by pH and moisture content, is key in determining the extent of degradation.

Additionally, this study shows that the lignin in the newsprint on the bottom layer of the landfill studied may have been anaerobically degraded via propyl side chain alteration.

The research presented shows that lignin can easily be solubilized and that the β-aryl-ether bond and other linkages in LCCs can be cleaved. The resulting lignin molecules of lower molecular weight become available for mineralization to carbon dioxide and methane. Additionally, the solublization of lignin makes it more bio-available to be degraded in aerobic and anaerobic environments.

As lignin is reduced to lower molecular weight compounds and degrades, the cellulose and hemicellulose component of the MSW is available for degradation. This accelerates the overall degradation of municipal waste, increasing the LFG generation rate and quality during active Gas-to-Energy operation (Attal et al. 1992). In bioreactor landfills, the LFG consists of 60/40 CH_4/CO_2. Because of the increased degradation, LFG is increased in the short term during harvesting and the time interval of LFG production is reduced. Thus, bioreactor landfills improve the economics of LFG utilization, and reduce the amount of fugitive methane release into the atmosphere.

Greenhouse Gas and Municipal Solid Waste

Anthropogenic activities represent approximately 60 percent of global methane emissions (2001). According to the USEPA's Inventory of US Greenhouse Gas Emissions and Sinks (1990-2003), landfills account for 24% of total U.S. CH_4 emissions. Additionally, municipal solid waste (MSW) landfills account for 94 percent of total CH_4 landfill emissions, and industrial landfills account for the rest.

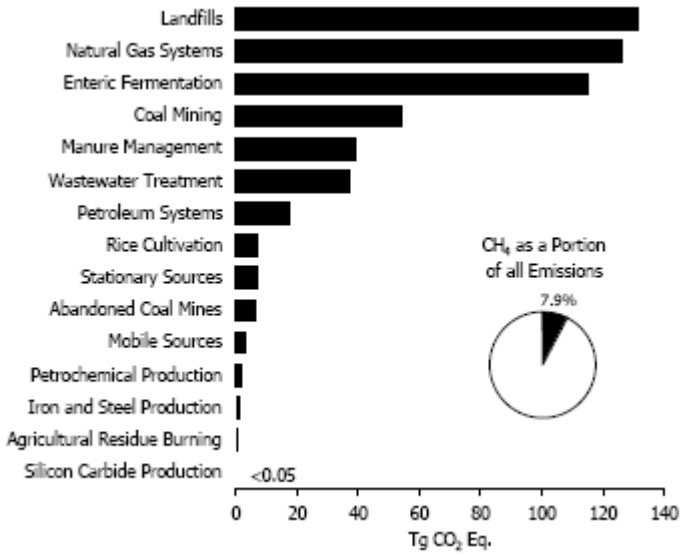


Figure I - 4: 2003 Sources of CH_4 (Inventory of US Greenhouse Gas Emissions and Sinks: 1990-2003)

The units in Figure I - 5 are presented in telegrams of CO_2 equivalent. This is measure by multiplying the gigagrams of gas by the IPCC developed global warming potential (GWP) as shown in the following equation.

$$\text{Tg } CO_2 \text{ Eq} = (\text{Gg of gas}) * (\text{GWP}) * \frac{\text{Tg}}{1{,}000 \text{ Gg}}.$$

LFG approximately contains equal amounts of CO_2 and CH_4, however CH_4 traps about 20 times more infrared energy than CO_2 on a volume basis, making it more significant with respect to atmospheric climate change. Methane (CH_4) is second to carbon dioxide as a contributor to climate change. Operating landfills as bioreactors, where the waste is actively decomposed decreases fugitive methane emissions into the atmosphere (Al-Yousfi A 1993). Understanding and applying the biochemistry of lignin degradation to landfills can increase the use of methane as an alternative energy source and limit the impact of green house gas emission, a major environmental problem.

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II Degradation of Lignin in Paper by Rumen Bacterium with Applications to Bioreactor Landfills

Abstract

The objective of this study was to determine how the key characteristics of bioreactor landfills; increased temperature, moisture and microbial activity, affect the biological stability of the organic fraction of the landfill material. In this paper the solubilization and degradation of lignin in paper exposed to these bioreactor landfill conditions are explored. The solubility of the lignin in paper was observed at different temperatures over 27 weeks at 55°C and the anaerobic bioconversion of office paper, cardboard and Kraft lignin was observed in bench-scale rumen microincubations over 8 weeks. Lignin solubility increased exponentially, as the temperature rose. The solubility of lignin continued at a constant rate with extended thermal treatment. This rate increased 15 times for paper and 1.5 times for cardboard in the presence of rumen inoculum, which was used as the experimental source of anaerobic microcosms, compared to un-inoculated systems. Around 6 weeks the inter-monomeric linkages between the solubilized lignin molecules began breaking down releasing monomers. A significant amount of the monomers mineralize to CO_2 and CH_4 during this time period in cardboard and Kraft lignin. The results indicate that small but significant rates of lignin solubilization and anaerobic lignin degradation are likely to occur in bioreactor landfills due to both higher temperature and microbial activity.

Introduction

It is estimated that paper accounts for 40% of a landfill's contents with newspaper alone taking up more than 13% of the space (USEPA 2005). Paper is composed primarily of lignin, cellulose and hemicellulose. Typical municipal solid waste (MSW) contains 40 to 50% cellulose, 10 to 15% lignin, and 12% hemicellulose (USEPA 2005). Lignocellulose or lignin-carbohydrate complexes are the stable combination of cellulose, hemicellulose and lignin which compromise 89 to 98% of the dry weight of wood. Lignin in the form of LCCs restricts the biological availability of the hemicellulose and cellulose polysachrides (Young and Frazer 1987). This complex structure of lignin makes its degrdedation a rate-limiting step in the biospheric carbon cycle (Colberg 1988). Additionally, the lignin in paper is an inhibiting agent in paper degradation within landfills (Stutzenberger et al. 1970). As a result, understanding the role of

lignin and its bioavailability is crucial in determining the gas production rates and biological stability of landfills.

In nature lignin is found in the cell wall of some cells of plants. It is the most abundant naturally occurring source of aromatic compounds (Young and Frazer 1987) and the second most abundant organic material on earth after cellulose. It is a random three-dimensional network polymer consisting of phenylpropanoid units rich in methoxyl substituents (Young and Frazer 1987) and linked together randomly (Goring 1989) through ester, ether and C-C bonds. The β-aryl-ether bond is the most common inter monomeric linkage in lignin polymers (Adler 1977). Since the deposition of the lignin compound occurs in a random it is impossible to define a typical structure.

The biological stability of a landfill is often measured by the cellulose (C) to lignin (L) ratio (C/L) (Suflita et al. 1992). Since lignin is considered to be recalcitrant a lower C/L ratio indicates stability, demonstrating a decrease in the cellulose content of the landfill when compared to the constant lignin. Factors that contribute to the slow rate of paper degradation in landfills are moisture limitation, poor shredding, low cellulose/lignin ratio and the lack of inoculum (Pohland and Kim 2000). The idea of operating landfills as bioreactors was introduced to increase biological stability. By increasing the moisture content and the flux of moisture through the landfill by leachate recycling and supplemental water addition, landfills become biological processing facilities where waste is rapidly digested, versus dry-tombs landfills. There is a rapid settlement of waste improving the yield of landfills by 15 to 30% (USEPA 2005) with this approach. Additionally, increased moisture enhances microbial activity and gas production rates are increased (Christensen and Kjeldsen 1989; Green 2000). The temperature of the landfill naturally rises with more microbial activity.

It has been shown that the anaerobic conversion of lignocellulose to methane does occur and is a complex microbial process involving many species of bacteria, including acidogens, methanogens, and acetogens (Healy 1979; Akin 1980; Crawford 1981; Chen et al. 1985; Chen et al. 1985; Colberg and Young 1985a; Colberg and Young 1985b; Young and Frazer 1987; Akin and Benner 1988; Phelps and Young 1997). The high temperature and microbial activity within

a landfill are crucial for the anaerobic conversion of lignocellulose to methane and it has been shown that high temperature alone solublizes lignin (Tartakovsky et al. 2003).

It is generally accepted that lignin monomers can be readily metabolized and completely mineralized anaerobically (Healy 1979; Crawford 1981; Young and Frazer 1987; Phelps and Young 1997). Examples of lignin monomers include cinnamic acid, benzoic acid, phenol, and phenlyacetic acid. It has also been shown that lignin oligomers degrade anaerobically (Chen et al. 1985; Chen et al. 1985; Colberg and Young 1985a; Colberg and Young 1985b; Young and Frazer 1987; Kajikawa et al. 2000). Colberg and Young (1985b) showed that lignin of molecular weight 600-700 released 10 different lignin monomers over a period of 2 years when inhibited with bromoethanesulfonic acid (BESA), an analogue to Coenzyme M which is required for methane generation. Futhermore, it has been show that an important intermonomer linkage in lignin, the β-arylether bond, can be cleaved completely with anaerobic enrichment cultures (Colberg and Young 1985b) and by rumen anaerobes (Chen et al. 1985). Aditionally, these studies all indicate that molecular weight or size of the lignin fraction is inversely correlated with its degradation capacity.

In 1985 Bernner and Hodon showed that lignin in wood is mineralized to CO_2 and CH_4 with 18-74% of the total carbon released into the soluble fraction. Most of the other research showing the degradation of the entire lignin structure has been performed using rumen bacteria as an inoculum (Akin 1980; Akin and Benner 1988; McSweeney et al. 1994). Rumen microbes are comprised of strictly anaerobic and facultative anaerobic bacteria and protozoa. The most common isolated celluloytic microorganisms in the rumen are *Ruminococcus* and *Fibobacter*. While sequences of these bacteria have not been recovered in landfill leachate cloning studies (Van Dyke and McCarthy 2002; Burrell et al. 2004), a number of species of the genus Clostridium have been found to be present in the leachate of 17 different landfills (Van Dyke and McCarthy 2002). Grass, leaves, and branches are the major contributors of refuse decomposing microbes to landfills (Barlaz et al. 1997). A landfills specific study showed that the lignin in the newsprint on the bottom layer of the landfill examined may have been anaerobically degraded via propyl side chain alteration (Chen et al. 2004).

The objective of this study was to understand how the key features of bioreactor landfills; increased temperature, moisture and microbial activity, effect the solublization and degradation of lignin in paper. 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. Rumen bacteria are used as the experimental source of anaerobic microbes.

Methods and Materials

To determine the dependence of lignin solubility on temperature, microcosms with paper and water were incubated at different temperatures. To understand the optimal pathways and limiting steps in lignin hydrolysis and degradation, microcosms were incubated with a rumen inoculum and then analyzed for the presence of lignin monomers, VFAs and CO_2 and CH_4 gas production. Figure II - 7 shows the anaerobic phase of the carbon cycle that has been adapted to the simplified lignin mineralization pathway. Each step in this pathway will be compared to the data obtained to determine how lignin in paper is transformed and degraded. Since both cardboard and office paper contain cellulose, hemicellulose and other products in addition to lignin, the accumulation of VFAs, CO_2 and CH_4 gas in the cardboard and office paper microcosms cannot directly be attributed to lignin degradation. Instead, the accumulation of aromatic compounds in the BESA amended and non-amended microcosms were used to determine if the bonds between the lignin monomers were cleaved.

Paper/Carbon Source

Shredded cardboard, office paper, newsprint, magazine and phone book paper was supplied by Gary Hater of Waste Management, Inc. Paper samples were ground to a powder using a Thomas Intermediate Wiley Mill with a 10-mesh screen. Additionally, Kraft or alkali lignin (370959 Aldrich) with an average M_w ~ 28,000 and average M_n ~ 5,000 was used a synthetic model compound (Figure II - 1).

Nutrient Medium

The formula for the nutrient medium used was the same as that outlined by Goering and Van Soest (1970). The micromineral, buffer, and macromineral solution was mixed with resazurin, a

redox indicator dye. The media was incubated with the paper overnight at 35° C. On the day rumen was collected a reducing solution was prepared according to Goering and Van Soest (1970) was added to each serum bottle reactor while running CO_2 over the solution. Carbon dioxide was continuously passed over the solution in each bottle until reduced conditions were obtained as indicated by a change in the resazurin color from pink to clear, which occurs below -110 mV, in all the microcosms.

Inocula

Rumen fluid including ingesta was collected in a sealed pre-warmed thermos from a lactating cow at the Department of Dairy Sciences, Virginia Polytechnic and State University. The fluid and ingesta were blended in a Waring blender and the contents of the blender were filtered through four layers of cheese cloth. CO_2 was flowing over the rumen fluid at all times to maintain anaerobic conditions (Goering and Van Soest 1970). The filtered fluid was used as the inocula for the sacrificial microcosms.

Experimental Design

Temperature dependence of lignin solubility

Boston round septa bottles (250 ml) containing 2,000 mg of paper and 200 ml distilled water were incubated in triplicate at 20, 37, 45, 55 and 65 °C in a hot water bath shaker-incubator for 48 hours each. The samples were analyzed for soluble lignin concentration using the procedure described previously. Distilled water extracted with MTBE using the same procedure and dilutions of the samples was used as the blank.

Lignin solubility/degradation over time

Thermal Treatment

Boston round septa bottles (250 ml) containing 2,000 mg of paper on a dry weight basis and 200 ml distilled water (10,000 mg/L suspension of paper) were incubated at 55 °C in a hot water bath shaker-incubator. Samples were taken at different time intervals by agitating the bottles and pipetting 10 ml samples that included paper and water. This was carried out in an attempt to maintain the concentration of the paper in the bottles.

Biological Treatment (With Rumen)

Wheaton serum bottles (125 ml) were used as sacrificial batch reactors. Reactors were set-up using modifications to the procedure described by Goering and Van Soest (1970). On the day before rumen collection, 520 mg of the carbon source (paper or synthetic lignin) was added to each bottle with 40 ml of rumen media prepared as described above. The bottles were allowed to incubate overnight at 37°C. On the day of rumen collection, 2 ml of reducing solution was added to each bottle while running CO_2 over the solution. Once reduced conditions were reached, 10 ml of rumen fluid inoculum was added to each bottle resulting in a final suspension of 10,000 mg/L of paper. The bottles were closed with 22 mm septum stoppers (Bellco Glass) and crimped with 22 mm aluminum crimp seals (Wheaton). The stoppers used were gas impermeable after syringe sampling. The serum bottles reactors were then placed in a temperature controlled room at 37°C (optimal temperature for anaerobic microbial growth) for the desired amount of time (2, 4, 6, and 8 weeks). All samples were run in triplicate with one set of triplicate controls solely consisting of media and inocula. Additionally a set of samples amended with 20 mM BESA was incubated for 6 weeks under similar conditions. When testing for soluble lignin the control consisting of media and inocula was used as the blank, this allowed for the automatic subtraction of the solubilized lignin that was already present in the rumen fluid. Each 125 ml bottle consists of:

- 0.52 g sample
- 40 ml of rumen buffer prepared according to Goering and van Soest (1970)
- 2 ml reducing solution
- 10 ml rumen fluid

Resulting in a suspension of 10,000 mg/L paper

The gas quantity and composition was checked every two weeks.

Analysis

Determination of soluble lignin

Soluble lignin was measured using the method as developed by Orsa and Holmbom (1994). The contents of the sacrificial serum bottle reactors were centrifuged at 500g for 30 min. Then, the pH of the supernatant was adjusted to 3.5 and extracted with MTBE. MTBE effectively removes fatty and resin acids from the solution (Voss and Rapsomatiotis 1985). After MTBE extraction,

not only lignins, but some other dissolved and colloidal substances including polysaccharides existing as LCCs may remain in the solution. However, this is not more than 20% of the solution. The interference of these substances was not taken into consideration to keep the determination of lignin concentrations fast and convenient, (Orsa and Holmbom 1994). The water phase of the MTBE extractions was then diluted 1:4 and the ultraviolet absorption was measured at 280nm (Beckman DU 640 spectrophotometer). Kraft lignin in methanol:water (80:20) was used for quantitative calibration.

Determination of biogas composition and inhibition of methanogenesis

The volume of biogas produced by the biologically treated serum bottle reactors was measured by collecting the gas at 37°C. The additional headspace of the serum bottle (75ml) was added to the volume of the gas to determine the total volume of biogas produced. The biogas was sampled directly from the serum bottle using a leak-tight syringe (Supelco) and then analyzed for methane and carbon dioxide upon determining the volume. A Shimadzu model GC equipped with a thermal conductivity detector (TCD) was used to determine the concentration of methane and carbon dioxide. The volume of biogas produced by the control consisting solely of media and inocula was subtracted from the samples for final recording and analysis. The coenzyme M analog, BESA, was added at a 20 mM concentration to the selected biological treated assays to inhibit methanogenesis and encourage intermediate buildup.

Volatile acid analysis

The previously centrifuged samples were filtered through a 0.45 um filter and acidified with phosphoric acid at a 1:10 concentration. Volatile fatty acids (VFAs) were measured on a Shimadzu GC-14A. The VFAs accumulated in the blank were subtracted from the samples for final recording and analysis.

Extraction and GC-Mass Spectrometer Analysis of samples for aromatic compounds

The aromatic degradation products of Kraft lignin and the lignin present in office paper and cardboard were studied using Gas Chromatography mass spectrometry (GC-MS). The instrument was calibrated for 4-methyl phenol (Chem Service) a lignin monomer. Additional

aromatic lignin monomers were detected based on the retention time and ion match of the GC-MS internal library.

The supernatant of the previously centrifuged serum bottle reactor contents (see soluble lignin procedure) was used as the sample from where the aromatic compounds were extracted. The pH was reduced to approximately 2.5 using sulfuric acid and duplicate methylene chloride extractions at a 5X concentration were performed on the samples. The combined methylene chloride solution was analyzed by a HP6890 GC-MS using a J&W 1225532 Capillary Column at a temperature of 325°C and initial flow of 1.0ml/min. The phenol readings of the control were subtracted from the sample for final recording and analysis.

Data Presentation

All quantities including soluble lignin are presented as mg/L in solution. Soluble lignin data is also presented as the percentage of the total lignin present. The percentages of lignin in the different types of paper used are presented in Table 1. The lignin content of these paper samples were determined gravimetrically by ignition of acid-insoluble material, also know as Kalson lignin. The percentage of the total lignin solubilized is determined by:

$$\% \text{ of Total Lignin} = \frac{\text{Concentration of lignin in solution (mg/L)}}{10{,}000 \text{ mg/L} * \% \text{ of lignin in paper}} * 100$$

10,000 mg/L is the concentration of the paper suspension used consistently through all the experimental procedures.

Results and Discussion

Temperature dependence of lignin solubility

Soluble lignin increased exponentially as temperatures increased (Figure II – 2 and 3. Table II - 2). Up to 0.40% of the lignin in cardboard can be solublized at 60 °C in just 48 hours. This is double the amount as that which is solublized 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 aerobic 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 solubility over time

Thermal Treatment

In Figure II - 4 the increase of lignin solubility at 55 °C over a period of 27 weeks is shown for the different types of paper. All the different types of paper, cardboard, office paper, magazine, phone book and newspaper show a trend of increasing lignin solubility over time. The linear solubilization rates over time are shown in Table III - 3. These data indicate that prolonged exposure to high temperatures effectively solubilized the lignin in paper within landfills at a slow but significant rate.

Under ideal conditions, it would take 24 years to solubilize 25% of the lignin in office paper and cardboard at 55°C assuming that the lignin continues to solubilize at the same rate as occurred over the fits 27 weeks. This rate would be exponentially higher at a higher temperature and with additional microbial activity. Since the lignin is effectively removed from the solid paper at high temperature and moisture, it cannot be considered a constant denominator when determining the stability of a landfill using the C/L ratio. Another more constant denominator such as plastic should be used if the true level of degradation of the municipal solid waste (MSW) is to be determined.

Biological Treatment (With Rumen)

Previous experiments at the Virginia Polytechnic and State University labs showed that the gas production by rumen bacteria is higher at 37°C than at 55°C; indicating that rumen bacteria are more active at 37°C, although they can survive at 55°C. It has been shown that higher temperatures solubilize lignin at a faster rate; the microcosms exposed to rumen inoculum were maintained at 37°C to optimize degradation. This should improve our understanding of the pathways and limiting steps in the microbial hydrolysis and degradation of lignin.

Lignin was solubilized at a faster rate during the first 6 weeks at 37 °C in both office paper and cardboard in the presence of the rumen inoculum (Figures II – 5 and 6). The rate of lignin solublization of office paper is 0.003 mg/L per week with rumen vs. 0.0002 mg/L per week with only thermal treatment. This indicates that 25% of the lignin in office paper would solubilize in just 19 months vs. 24 years with only thermal treatment. The effect of the rumen inoculum on the rate of solublization of the lignin in the cardboard is not as pronounced, with the rate increasing to 0.0003 from 0.0002 mg/L per week. At this rate, 25% of the lignin in cardboard would solubilize in 16 years with biological treatment vs. 24 years with just thermal treatment. However, after the 6th week, the quantity of solubilized lignin decreases for all the samples. This indicates that the soluble lignin was being consumed or degraded.

Lignin degradation

The GC-MS was calibrated for 4-methyl phenol, a lignin monomer; however, structures similar to cynamic acid and phenylacetic acid, common aromatic compounds representative of lignin-derived monomers (Colberg and Young 1985b), were also present. Based on GC-MS library comparison of the major compounds, the chemicals found to accumulate were benzenepropanoic acid (aka: Hydrocynamic acid) and Benzeneacetic acid (aka: 2-phenylacetic acid) at higher amounts than the blanks in both the BESA amended and non-amended microcosms. The presence of hydrocynamic acid and 2-phenylacetic acid were based on a library comparison of the major compounds and their retention time. The structures and ion distribution of these compounds are shown in Figures II – 8 and 9.

Methanogenesis was inhibited with BESA to promote the build-up of intermediates and to understand the chemical degradation pathway of lignin. In general, the microcosms amended with BESA produced 20% less total gas than the ones in which methanogenesis was not suppressed. The BESA amended microcosms produced no methane gas. This indicates that BESA effectively inhibits methanogenesis. Additionally, the microcosms amended with BESA had 39% more VFAs accumulated than the microcosms that were not amended with BESA, indicating that the final methanogenic step of the carbon cycle had been effectively suppressed allowing the build-up of intermediates.

The pH of the rumen solution was approximately 6.5. After media and buffer addition; the pH was increased to 7.0. The pH of all the microcosms except that of the BESA amended office paper microcosms, naturally maintained a pH range of 6.8 to 7.1. However, the pH of the BESA amended office paper microcosms dropped as low at 6.4.

Kraft Lignin

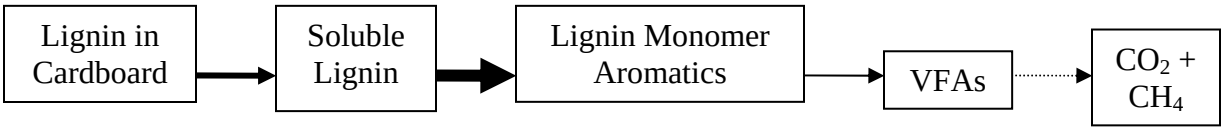
Figure II - 12 shows the accumulation of soluble lignin and 4-methyl phenol in the Kraft lignin microcosms at 6 and 8 weeks and at 6 weeks with BESA. When methanogenesis was suppressed by BESA, 4-methyl phenol a lignin monomer accumulated, indicating that the soluble lignin is breaking down into aromatic lignin monomers. Between 6 weeks and 8 weeks, without BESA inhibition, the amount of soluble lignin decreases from 14.36 mg/L to 11.75 mg/L. Since no 4-methly phenol accumulates at 8 weeks, it can be assumed that the 4-methyl phenol that accumulated at 6 weeks with BESA inhibition had been converted to VFAs. This assumption can be supported by the VFA and CO_2 and CH_4 data. Since the Kraft lignin microcosms do not contain any additional cellulose or hemicellulose. This data can be used to make conclusions on the fate of lignin.

Figure II - 13 shows the accumulation of the various VFAs at 6 weeks with and without BESA and at 8 weeks without BESA. Only a very small amount of acetate was accumulated in the 6 week microcosms amended with BESA. In fact, Figure II - 14 depicts the gas production of the three different types of microcosms indicates little if any CO_2 production. This suggests that the lignin degradation pathway stopped at hydrolysis. The reason for this inhibition is unknown and needs to be investigated further. However, for the 6 and 8 week microcosms with no BESA, VFAs do accumulate. At 8 weeks there is no acetate accumulation, but there is a spike in the CO_2 production (Figure II - 14). This indicates that the acetate was consumed and produced CO_2. This data suggests that Kraft lignin can be completely mineralized at small but significant rates to CO_2 and CH_4 with rumen bacteria.

Additionally, both hydrocynamic acid and 2-phenylacetic acid accumulate in all of the three microcosms. The chromatogram for the 8 week Kraft lignin microcosm overlaid with the blank incubated for 8 weeks is shown in Figure II – 15. The figure shows the 4-methyl phenol peak that was calibrated for and the phenylacetic acid and hydrocynamic acid peaks that were examined for the presence or absences of these compounds. This figure shows that both phenylacetic acid and hydrocynamic acid were present at a higher level than the blank, further supporting the results that the lignin bonds in Kraft lignin are readily broken to release monomers.

Lignin in Cardboard

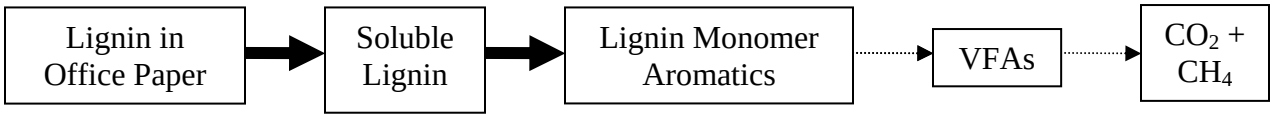
Figure II - 10 shows the accumulation of soluble lignin and 4-methyl phenol in the cardboard microcosms at 6 and 8 weeks and at 6 weeks with BESA. When methanogenesis is suppressed by BESA, 4-methyl phenol a lignin monomer is accumulates and this indicates that soluble lignin is breaking down to form aromatic monomers. Between 6 weeks and 8 weeks, without BESA inhibition, the amount of soluble lignin decreased from 5.59 mg/L to 4.98 mg/L and the amount of phenols increase to 4 mg/L. This indicates the bonds between the larger soluble lignin fractions are broken to release monomers such as 4-methyl phenol. Since the phenol accumulation in the 6 weeks BESA amended microcosm is higher than that of the 8 weeks un-amended microcosm, it can be theorized that the 4-methly phenol is further degraded to VFA's and possibly completely mineralized to CO_2 and CH_4. The theoretical pathway of the lignin in cardboard, based on these experiments, is shown below.



Additionally, hydrocynamic acid is accumulated in all of the three microcosms with 2-phenylacetic acid accumulating only in the 8 weeks un-amended microcosm. This further supports the results that the lignin inter-monomeric bonds in cardboard are readily broken to release monomers.

Lignin in Office Paper

Figure II - 11 shows the accumulation of soluble lignin and 4-methyl phenol in the office paper microcosms at 6 and 8 weeks and at 6 weeks with BESA. As mentioned earlier, the pH of the BESA amended office paper microcosms dropped to approximately 6.4. Additionally, research has shown that accumulation of VFAs causes pH dependent inhibition in the rumen (Van Kessel and Russell 1996). In the rumen, acetogens are poor competitors with methanogens, thus methanogens dominate the H_2 consuming process (Madigan et al. 2003). The drop in pH exceeds the buffer capacity of the media when methanogens are inhibited. While the other microcosms had enough buffer capacity to manage the hydrogen and VFA accumulation, the BESA amended office paper microcosms, with high amounts of easily degradable cellulose, had the most VFA accumulation causing the pH to drop. The inhibitory effect of low pH explains why 4-methyl phenol did not accumulate at a high rate in the BESA amended microcosm. However there is an increase in 4-methyl phenol and a decrease in soluble lignin between the 6 and 8 week non-amended cultures. Additionally, both hydrocynamic acid and 2-phenylacetic acid are accumulated in all of the three microcosms. This indicates that the lignin inter-monomeric bonds in office paper are broken to release monomers. However, with the given data, it is impossible to determine if the aromatic lignin monomers are further degraded into VFAs and completely mineralized to CO_2 and CH_4. The theoretical pathway of the lignin in office paper, based on these experiments, is shown below.



Note that the scale in Figure II - 11 is 7 times greater than that in Figure II - 10 for cardboard. Even though office paper has less lignin than cardboard (2.63% vs. 21.67%) higher concentrations of 4-methyl phenol accumulate and a higher percentage of total lignin is solubilized in office paper vs. cardboard (3.21% of the total lignin in office paper solubilized after 6 weeks of biological treatment vs. 0.26% of the total lignin in cardboard). Additionally, the office paper microcosms produced 22.4% more biogas than the cardboard microcosms, indicating more activity. Since office paper has a larger percent of easily degradable cellulose,

the organisms have an easily consumable carbon source making them stronger to attack the more difficult bonds between lignin monomers. Thus, lignin in office paper may be more readily hydrolysized to its aromatic monomers than the lignin in cardboard.

Conclusions and Applications

The inter-monomeric bonds between the lignin in paper can be broken to release the lignin monomers, phenol, hydrocynamic acid, and 2-phnylacetic acid. Since the β-aryl-ether bond is the most common inter monomeric linkage in lignin polymers (Adler 1977), it can be assumed that this bond is successfully broken by rumen bacteria. Furthermore, once solubilized and reduced to its monomers, small percentages of the lignin in paper can be completely mineralized to CO_2 and CH_4 by mixed rumen bacteria over a period of 6 to 8 weeks. Both the rumen and landfill environments have functionally parallel environments that demand the mineralization of organic materials like cellulose and lignin. Notably, clone libraries from rumen ecosystems, anoxic soils and landfill environments are all dominated by the *Clostridum* spp. Therefore, an understanding of lignin mineralization using a mixed rumen bacteria population can cast significant light on the optimal environment, rate, pathways and limiting steps of the process within landfills.

High temperatures and moisture are critical for the solublization of lignin to a lower molecular weight. Once lignin has been solubilized, it is broken into smaller molecular weight fractions and its bioavailability increases. The data point out that office paper, the carbon source with the least lignin had the highest rate of lignin inter-monomeric link breakage. Additionally, it was the most active microcosm producing the most gas and VFAs. This indicates that if the ratio of cellulose to lignin is higher, the bacteria are more active and hardy, allowing them to attack the tougher lignin molecule more efficiently. However, these same office paper microcosms when amended with BESA generates excess VFAs, thereby inhibiting the organisms responsible for lignin mineralization. Therefore, besides microbial activity, high temperatures and moisture, the loading rate of cellulose to lignin and acidity of the landfill conditions and recirculating leachate must be considered within bioreactor landfills to optimize the complete mineralization of the waste to CO_2 and CH_4.

As the 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 grossly 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 CO_2 and CH_4. This study provides 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. However, studies need to be developed that can quantify the rate of biological solublization of lignin and the molecular weight of the solubilized lignin so that the percentage of the solubilized lignin that is mineralized can be determined.

Currently, the biological stability of a landfill is often measured by the cellulose to lignin ratio (C/L). It has been shown 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 in a safe and environmental friendly way. The anaerobic degradation of lignin derived carbon affects settling and gas production rates. Additionally it is an important factor in determining post-closure monitoring requirements of bioreactor landfills.

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Tables and Figures

Table II - 1: Percentage of lignin in different papers

	Percentage of Lignin
Office Paper	2.63
Magazine	21.02
Cardboard	21.67
Phone Book	35.09
Newspaper	35.44

Table II - 2: Thermal Treatment – Exponential Solublization Rates of Lignin (Per °C)

	Exponential Solublization Rates (Per °C)
Kraft Lignin	0.037
Cardboard	0.014
Office Paper	0.0134

Table II - 3: Thermal Treatment – Linear Solublization Rates of Lignin ($Week^{-1}$)

	Linear Solublization Rates (Per Week)
Cardboard	0.0002
Office Paper	0.0002
Magazine	0.0001
Phone Book	0.0001
Newspaper	0.00008

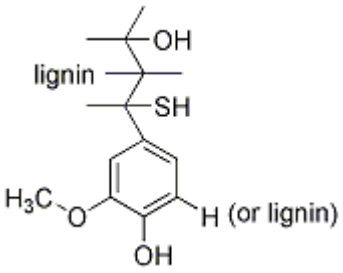

Figure II - 1: Kraft Lignin

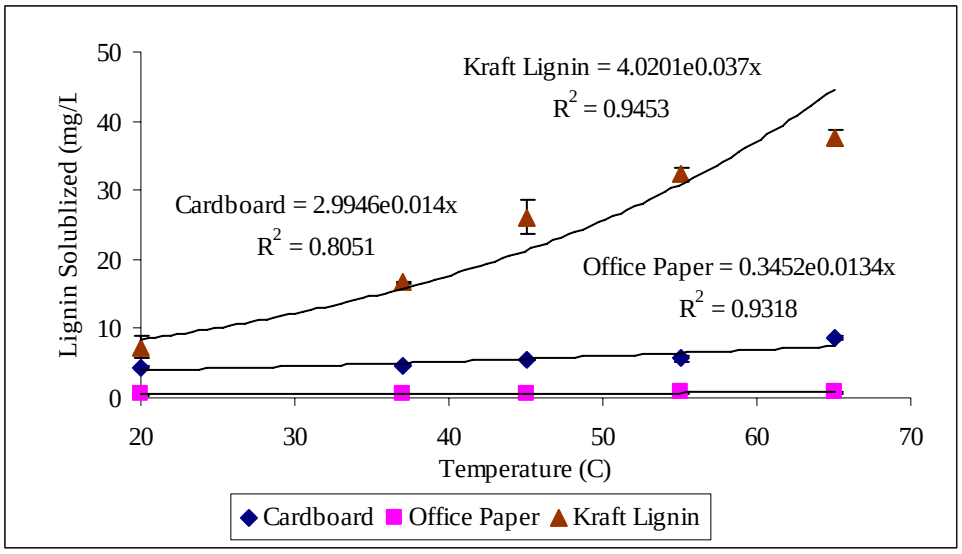

Figure II - 2: Temperature Dependence of Lignin Solubility (mg/L)

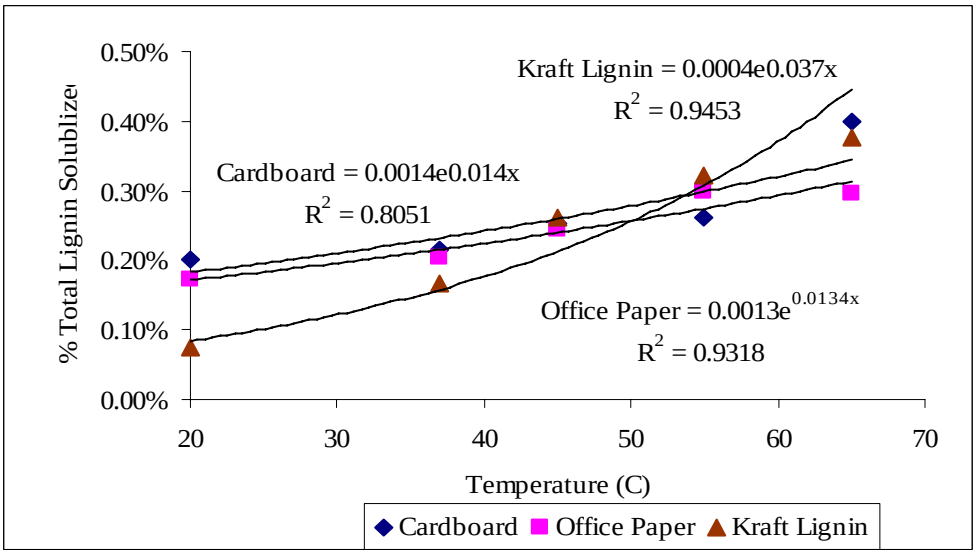

Figure II - 3: Temperature Dependence of Lignin Solubility (percentage of total lignin)

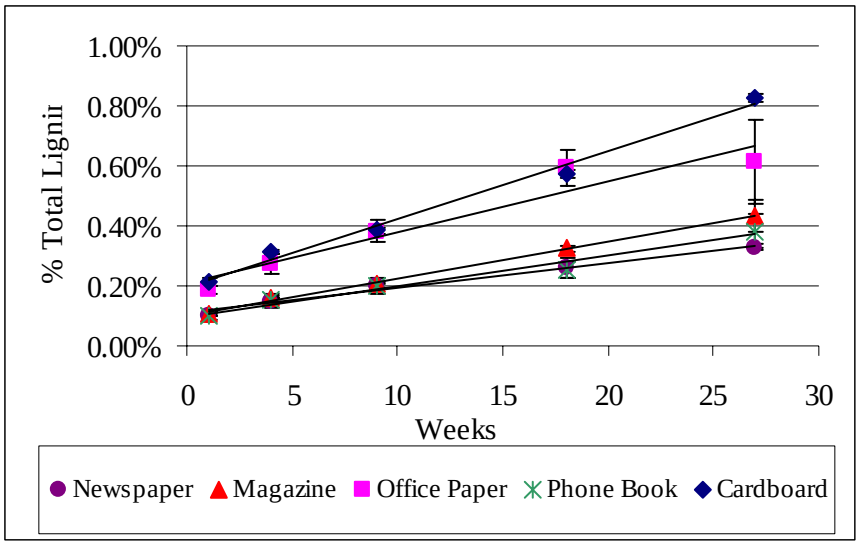

Figure II - 4: Thermal Treatment – Lignin solubility over 28 weeks at 55°C (percent of total lignin)

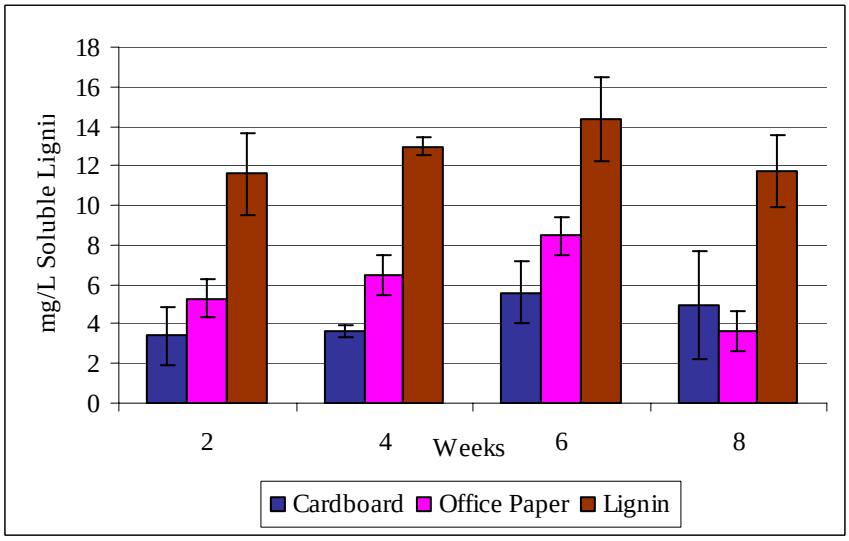

Figure II - 5: Biological Treatment – Lignin solubility over 8 weeks at 37°C with rumen inocula (mg/L)

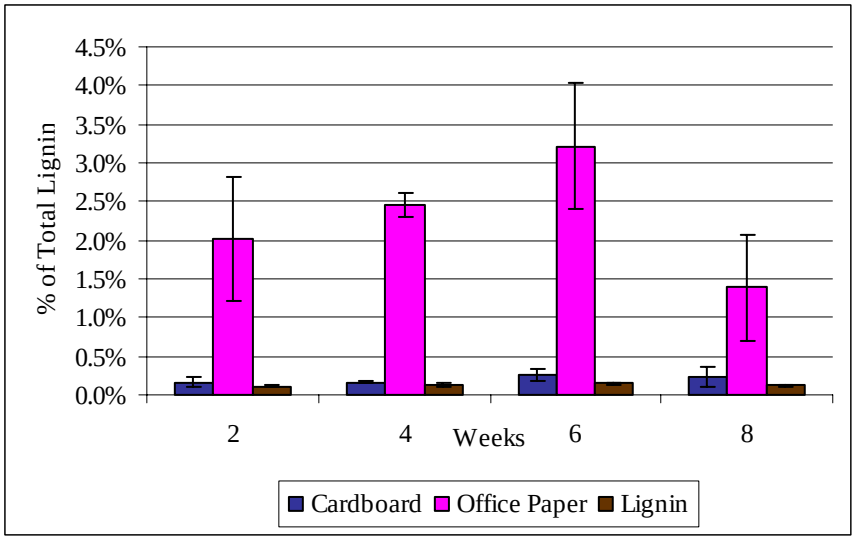

Figure II - 6: Biological Treatment – Lignin solubility over 8 weeks at 37°C with rumen inocula (percent of total lignin)

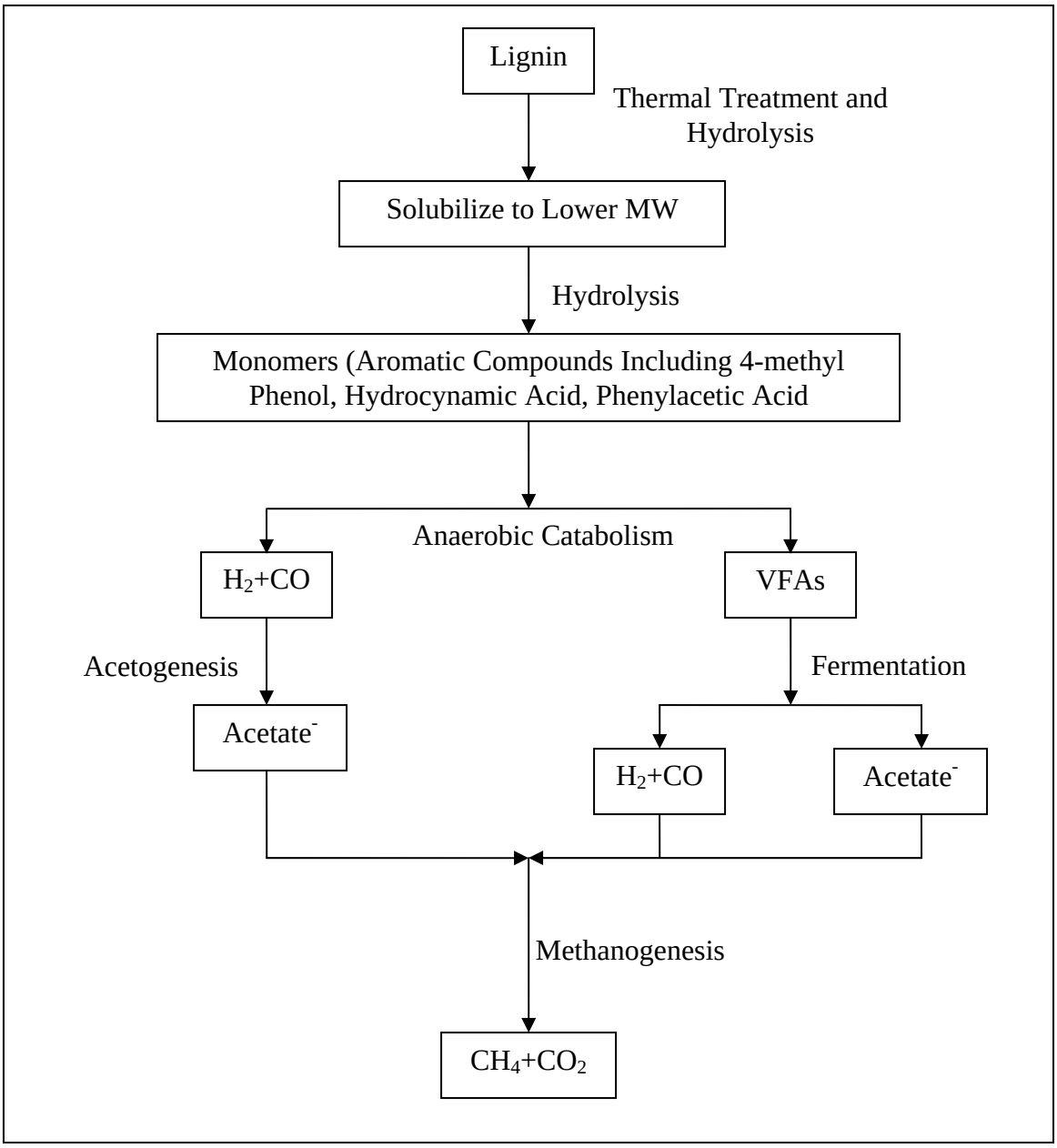

Figure II - 7: Anaerobic phase of carbon cycle adapted to lignin

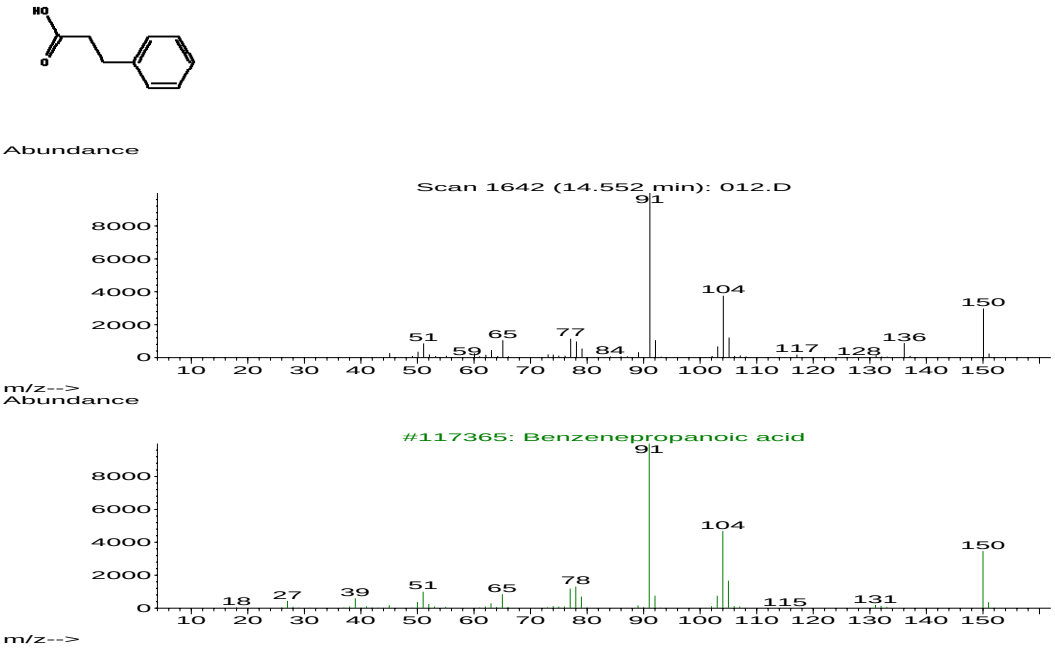

Figure II - 8: Ion distribution and structure for benzenepropanoic acid ($C_9H_{10}O_2$ hydrocinnamic acid) – lignin derived monomer.

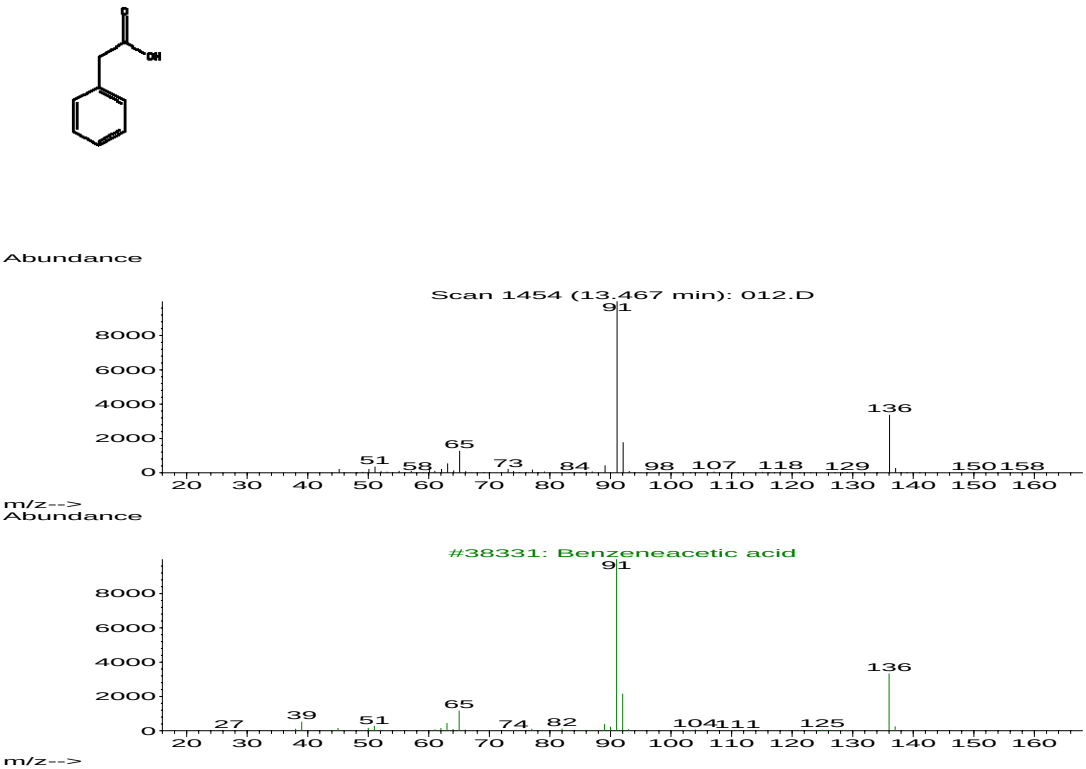

Figure II - 9: Ion distribution and structure for benzeneacetic acid ($C_8H_8O_2$ 2-phenylacetic acid) – lignin derived monomer.

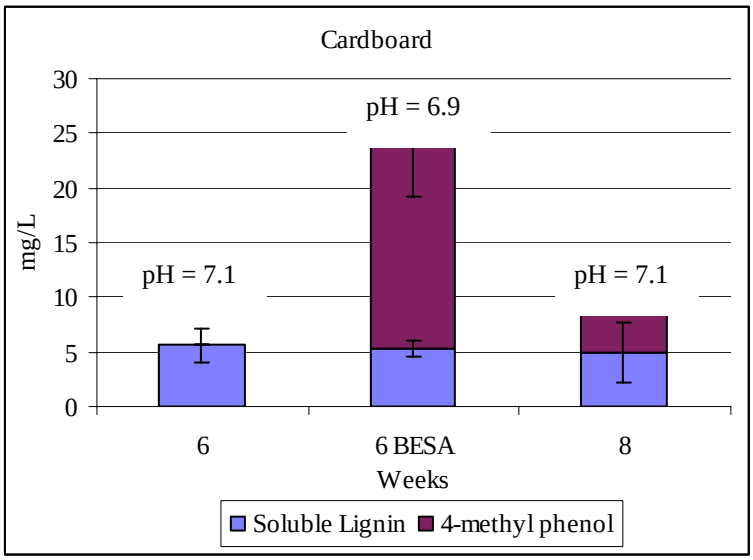

Figure II - 10: Soluble lignin and 4-methyl phenol accumulation at 6 weeks (BESA amended and un-amended) and 8 weeks (BESA un-amended) for cardboard**.**

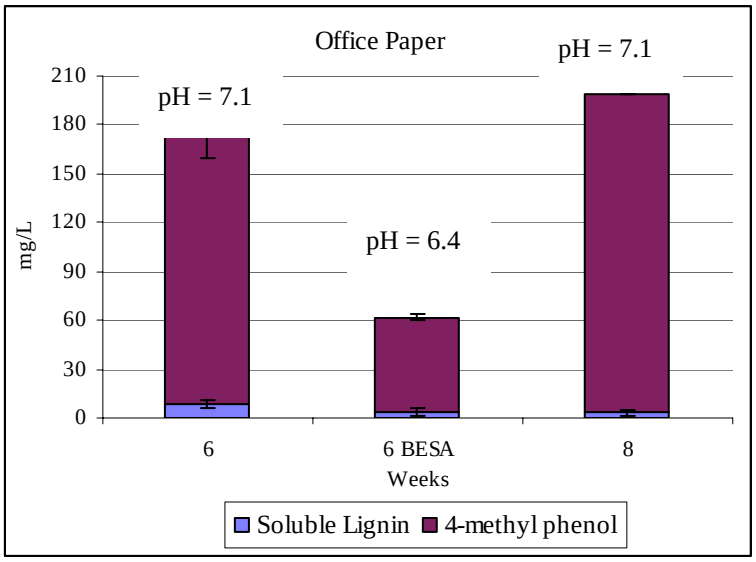

Figure II - 11: Soluble lignin and 4-methyl phenol accumulation at 6 weeks (BESA amended and un-amended) and 8 weeks (BESA un-amended) for office paper.

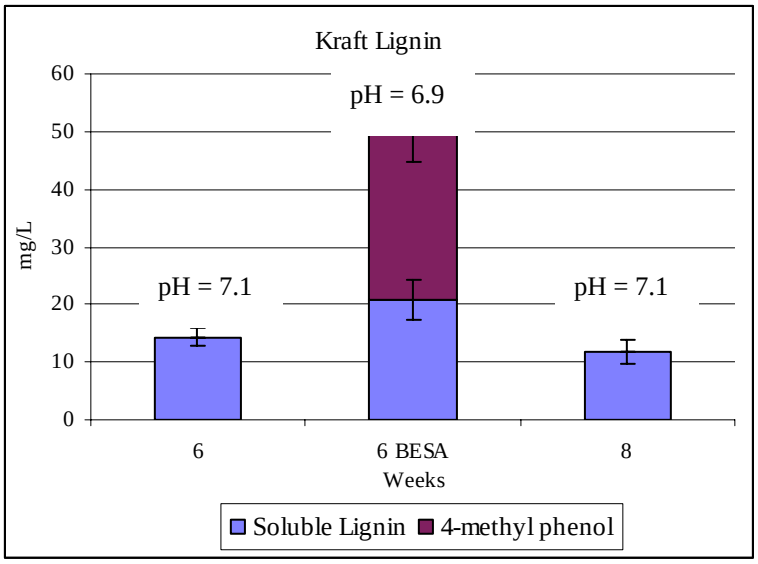

Figure II - 12: Soluble lignin and 4-methyl phenol accumulation at 6 weeks (BESA amended and un-amended) and 8 weeks (BESA un-amended) for Kraft lignin.

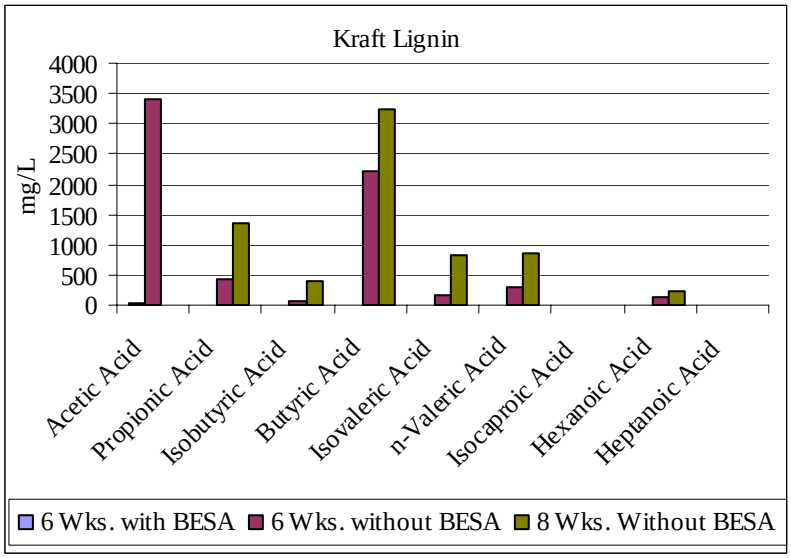

Figure II - 13: Volatile fatty acids accumulation at 6 weeks (BESA amended and un-amended) and 8 weeks (BESA un-amended) for Kraft lignin.

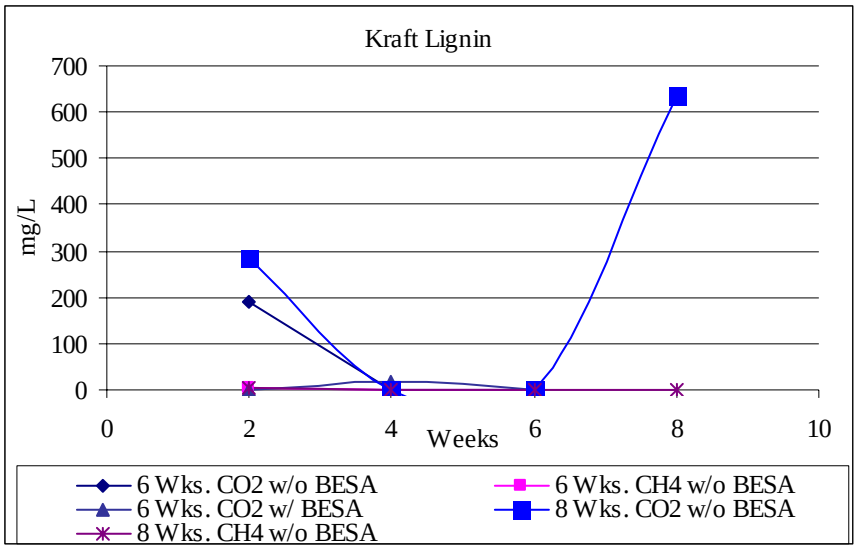

Figure II - 14: CO_2 and CH_4 production at 6 weeks (BESA amended and un-amended) and 8 weeks (BESA un-amended) for Kraft lignin.

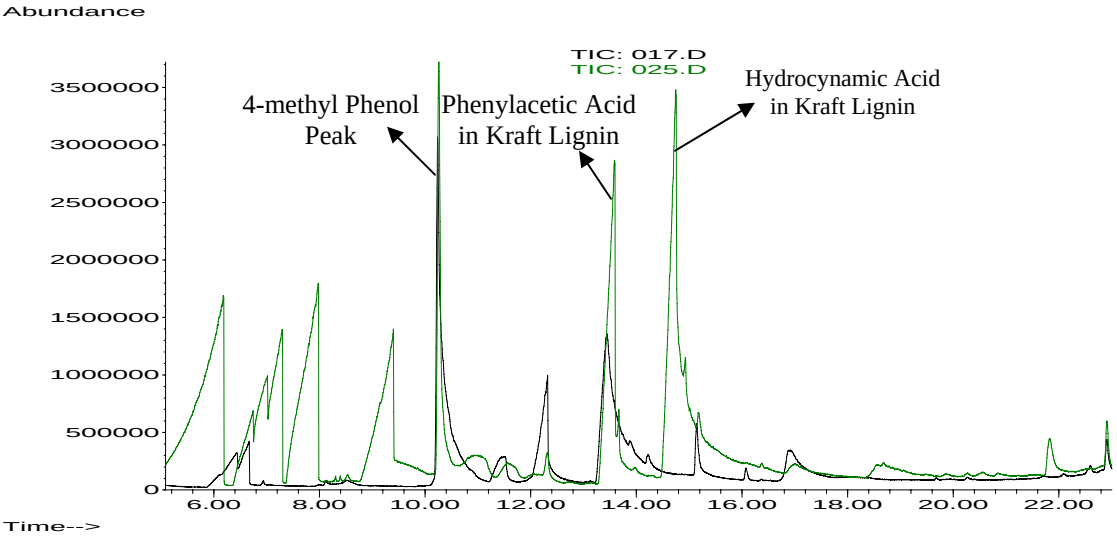

Figure II - 15: Chromatogram for 8 weeks Kraft lignin overlaid with 8 week blank. (Higher peaks are for Kraft lignin).

III Outer Loop Recycling and Disposal Facility a Case Study for Bioreactor Landfills

Abstract

Field data from the Outer Loop Recycling and Disposal Facility in Louisville, Kentucky were evaluated to determine the effectiveness of an anaerobic aerobic landfill bioreactor (AALB) vs. the control landfill that is managed as a traditional landfill. Moisture, temperature, elevation and the amount of time the MSW has spent in the landfills were compared to determine the factors that affect the biological stability of the landfill. The results showed that the MSW in the AALB is more biologically stable than the MSW in the control landfill, indicating that they are more degraded. Additionally, elevation or location of the MSW was the key factor in determining the extent of MSW stability within the AALB, and temperature is the key factor in determining the biological stability of the MSW in the control landfill. Higher temperatures correlated with a more biologically stable waste. The cellulose to lignin ratio (C/L ratio) and biochemical methane potential (BMP) were the main biological stability parameters used. Both parameters indicate that increased temperatures and increased microbial activity are the crucial aspects of bioreactor landfills that increase stabilization rates and the extent of degradation.

Introduction

Currently, fifty six percent of the MSW generated in the United States is disposed into landfills. The remaining is recovered, recycled or composted (30%) or burned at combustion facilities (15%) (USEPA 2005). The concept of operating landfills as bioreactors was introduced (Reinhart and Townsend 1998) about two decades ago. By increasing the moisture content and flux through the landfill by leachate recycling and supplemental water addition, landfills become bioreactor processing facilities where waste is rapidly digested, versus the dry-tombs of traditional landfills. With this approach, there is a rapid settlement of waste improving the capacity of landfills by 15% to 30% (USEPA 2005). Additionally, gas production rates are increased over a shorter duration (Green 2000) and the overall leachate quality is improved, resulting in lower treatment and disposal costs(USEPA 2005).

Bioreactor landfills attempt to speed up the timeframe of the first four phases of decomposition within a landfill. These phases are adjustment or acclimation, transition, acidogenesis and

methanogenesis. Decomposition is accelerated by providing the waste with air during the early acclimation stages and with water and nutrient rich leachate during the transition and acidogenesis phase. These phases are not sharply defined within a landfill and tend to overlap. Because of the heterogeneous nature of the MSW, pockets of higher and lower microbial activity form causing the different stages of decomposition to occur side-by-side within the landfill.

Waste Management, Inc. (WMI) operates and maintains a bioreactor landfill at the Outer Loop Landfill in Louisville, Kentucky. WMI, in collaboration with the Environmental Protection Agency (EPA) has commissioned a study to determine the benefits of operating landfills as bioreactors. The cells of the anaerobic/aerobic landfill bioreactor (AALB) at Outer Loop contain 15ft vertical sections of MSW. Each 15ft vertical section of MSW is called a lift. These shallow lifts allow waste to be homogenized as they enter the landfill. Water is added to each lift as it fills completely. A matrix of piping is installed in each lift as it is constructed.

The piping serves three functions in order:

- Providing air to the fresh MSW
- Providing water to the MSW after aeration
- Extracting landfill gas (methane and carbon dioxide) from the older MSW

Air is injected into the fresh, lower lift, for approximately 45 days. This initial aerobic condition permits the easily decomposable waste to rapidly degrade. This causes the MSW to compact, thus increasing the density. After aeration, moisture is added to the MSW allowing it to enter the transition stage and anaerobic conditions are established. Since the fermentable organic matter has been reduced in the aerobic phase, the acid generation phase is shortened and the decomposition rapidly enters the methanogenesis stage. In this phase, methane gas is collected for energy generation. As each new lift is added, the sequence is repeated. The piping that initially pumped air into the fresh MSW pumps water into the MSW after an additional lift is added. Finally, this same piping is used to collect landfill gas from the MSW in the methanogenesis stage.

Twelve acres of the Outer Loop landfill are operated as an AALB and an adjacent section is operated as a control landfill (CL). Figure III – 7 shows a map of the plane view of the AALB and the CL that are run adjoining each other. The CL is a dry tomb landfill; that is, no moisture or air is added to the MSW, however rain water is allowed to infiltrate into the MSW contained within. The MSW in the CL was sampled in duplicate during the years 2000, 2002, 2003 and 2005 and the AALB was sampled in duplicate during the years 2002, 2003 and 2005. The CL has been operating for 2.4 years before the operation of the AALB commenced. The MSW samples were analyzed in the North Carolina State University labs for cellulose, lignin, hemicellulose, volatile solids and biochemical methane potential (BMP).

In this study all the data generated since 2002 is analyzed to determine:

1. The effectiveness of the AALB vs. the CL in stabilizing the MSW.
2. The factors that affect MSW stabilization in each landfill. The factors that are investigated are moisture, temperature, MSW age and MSW elevation.

An understanding of the factors that effect biological stability in each different type of landfill is useful to landfill operators, allowing them to maximize the conditions within their particular landfill to optimize waste degradation, settlement and gas generation.

Biological Stability Parameters for MSW

The two most common methods to determine the biological stability or the near complete biological conversion of MSW to methane and carbon dioxide are the cellulose/lignin ratio (C/L ratio) and the bio-methane potential (BMP) of the waste (Barlaz et al. 1990). In this statistical study the C/L ratio and the BMP are used as the main parameters to determine MSW biological stability. Additional parameters used to determine the biological stability of the MSW are the concentration of the MSW's cellulose, hemicellulose, lignin and volatile solids (Kelly et al. 2006). These parameters are analyzed to provide support information

As the landfill stabilizes and reaches the maturation phase, it is expected that cellulose and hemicellulose will be degraded; in fact, it is estimated that cellulose and hemicellulose account for 91 percent of the methane potential of MSW (Barlaz et al. 1990). However, the transformation of lignin is much slower; therefore, the C/L ratio is used to determine the extent

of MSW degradation with the lignin used as an internal standard. Although, recent research has shown that lignin degrades under the high temperatures and moisture conditions present in bioreactor landfills (Chen et al. 2004), the cellulose to lignin ratio is still a very useful parameter to determine landfill stability. A lower C/L ratio indicates the material is biologically stable. Based on the samples provided, fresh MSW has an average C/L ratio of 2.51±0.92. For this study: C/L ratio = (%Cellulose + %Hemicellulose) ÷ %Lignin

The BMP, an anaerobic equivalent to the BOD test performed in the wastewater industry, measures the amount of material in the MSW that may be readily converted to methane (Kelly et al. 2006). A high BMP indicates that the waste is still active, containing an easily available carbon source while a low BMP indicates inertness and low carbon availability. The BMP was measured using the method outlined by Dr. Barlaz at NSCU and explained by Shearer. Based on the samples provided, fresh MSW has a BMP of 64.30±20.14 mL/g. BMP measurements are recorded as ml of methane per g of MSW.

Methods and Materials

Sampling Methods

The MSW used in this analysis was collected using a drill rig equipped with 3' bucket augers. The initial elevation above sea-level of the landfill area to be drilled was measured as the base elevation. Approximately 10 feet of the initial drilled material was discarded because it is mostly soil and not MSW. MSW samples were then collected at approximately every 10 foot of the vertical section of the landfill. The temperature of the fresh samples was measured and recorded and then the samples from each section were mixed thoroughly and then transferred at ambient temperature in contractor grade plastic bags and plastic containers to the NCSU laboratories for analysis. The bucket augers stopped sampling approximately 10 feet above the landfill liner. Samples came from two sections of the landfill, the AALB and the CL. Each section was marked off into a numbered grid, and then numbers were randomly selected to determine the sampling location. A point activity analysis as shown in Figure III – 3 was maintained for each sampling location.

Statistical Methods

All statistical methods were run with an alpha = 5%. To determine the effectiveness of the AALB vs. the CL in stabilizing the MSW the difference of the means of the stability parameter were tested using the non-parametric Wilcoxon t-test. A non-parametric test was used because the data is not normally distributed (See Table III - 1). Additionally, box plots which display the median, spread, skew, and outliers of the all the data since 2000 for the CL and 2002 for the AALB are presented. This gives a visual representation of the distribution of data for each landfill, the AALB and the CL.

Linear regressions were used to determine the factors that affect MSW stabilization in each landfill, the AALB and CL. Linear regressions show the relationship between a dependent variable (the Y variable which will be the different landfill biological stability parameters) and the independent variables (the X variable which are age, elevation, temperature, and moisture), to determine how the dependent variable (stability parameters) can be predicted from the independent variable (age, elevation, temperature, and moisture). The data for the AALB and the CL are analyzed individually so that an understanding of which factors affect biological stability in each landfill can be determined.

Additionally, the age of the two landfills separately are categorized by years and the difference of the means of the stability parameters within the years were tested using the Wilcoxon t-test with the Bonferroni correction factor. Likewise, the elevations of the two landfills separately are categorized by depths and the differences of the means for each elevation are evaluated using the same statistical methods. This provides an additional analysis to the linear regressions in determining the affects of age and elevation on the biological stability for each different type of landfill. A visual representation of the distribution of the data for each elevation and age category is presented using a box plot. Geostatistic (contour plots) are used to visualize the concentration of C/L ratio and BMP for a given elevation.

Results and Discussion

Effectiveness of the AALB vs. the CL in stabilizing the MSW

To determine the effectiveness of the AALB vs. the CL in stabilizing the MSW, the hypothesis that the MSW within the AALB is more biologically stable than the MSW within the CL was tested using the following null and alternative hypothesis.

Ho: Mean BMP AALB >= Control	and	Mean C/L Ratio AALB >= Control
Ha: Mean BMP AALB < Control		Mean C/L Ratio AALB < Control

The Wilcoxon rank sum test showed that the mean of the C/L ratio in the AALB is less than that of the CL and that the mean of the BMP in the AALB is less than that of the CL. Thus we can reject the null hypothesis and conclude that in the MSW in the AALB is more biologically stable than in the CL. Additionally, the volatile solids, cellulose and hemicellulose are statistically lower in the AALB than the CL. Although the mean lignin concentration in the AALB is lower than that of the CL, it is not at a statistically significant level. The box plot of Figure III - 1 showing the distribution of the data for each landfill, the AALB (Bio) and CL, and for the fresh MSW clearly indicates that the C/L ratio (mean of 1.59) in the AALB is lower than the C/L ratio in the CL (mean 2.18). Figure III - 2 confirms the same for the BMP (mean AALB = 48.8 and mean Control = 72.2).

The effectiveness of the AALB over the CL is more pronounced when the age of the MSW in each landfill is considered. The average age or amount of time the MSW has been in the landfill for the AALB is 19 months (1.58 years) with a maximum age of 3.83 years and the average age of the MSW in the CL is 26.8 months (2.23 years) with a maximum age of 6.25 years. This indicates that the MSW in the AALB has achieved a higher degree of biological stability over a shorter period of time. This suggests that the treatment the MSW in the AALB received is effective in stabilizing the MSW. To determine which factors affect MSW biological stability in each landfill, the MSW age, elevation, temperature and moisture have been investigated in more detail.

Factors that affect MSW stabilization in each landfill

MSW Age

An understanding of the time needed for MSW to stabilize is important in determining post-closure landfill monitoring and the gas generation potential of the MSW. Furthermore, an investigation of the biological stability of the MSW based on the amount of time it has been in the landfill can cast a light on the effectiveness of the AALB vs. the CL. Age, as used in this analysis, is the number of years the MSW has been in the landfill. The age of each MSW sample was determined based on the sampling location and elevation of the MSW at the time of sampling. Each sampling location has a point activity analysis which tracks the elevation of the MSW for different points in time. An example of a point activity analysis is given in Figure III - 3. With data on the sampling date and the elevation at sampling time, the age of the MSW can be determined from the point activity analysis.

The data for each landfill, the AALB and the CL, are investigated separately to determine which landfill effectively stabilized MSW over time. Figure III - 4 shows a box plot of the distribution of the C/L Ratio and the BMP data for the different MSW ages categorized by age in years. The AALB data is presented on the left and the CL data on the right. The y scale for each parameter is identical for both types of landfills, allowing a visual comparison between landfills. A visual interpretation of the box plots indicate that there is no apparent trend in the CL, that is the MSW does not stabilize over time but is instead very variable with a broad distribution. However, the MSW within the AALB shows a decreasing trend with age coupled with a decrease in the spread of the data as the MSW matures. This indicates that with time one can expect the MSW within the AALB to consistently stabilize. On the other hand, data for the CL indicates that no biological stabilization is occurring over the time the MSW is contained within. These results indicate that the AALB will need less post-closure monitoring as the CL since the waste more effectively stabilized over time in the AALB vs. the CL.

However, a Wilcoxon t-test with a Bonferroni correction factor does not show that the means of the C/L ratio or BMP are lower at a statistically significant level as the MSW matures in the AALB. Additionally, the linear regression indicates that the MSW age (independent variable) cannot be used to predict the biological stability (dependent variable – C/L ratio and BMP) of the

MWS in the AALB. These results could be because of various reasons including the fact that the time period of sampling is not sufficient and because of the sample spread within each year.

Elevation

In the AALB each 15 ft. vertical lift of MSW is homogenized and fitted with a matrix of piping, thus it can be assumed that elevation plays an important role in the AALB with MSW at a given elevation experiencing the same sequence of treatment: air, followed by water, and final gas removal. Therefore, it can be hypothesized that waste at a given elevation will experience the similar phase of decomposition, making elevation, depth, or piping activity (air and water addition or gas removal) an important parameter in determining the stability of the MSW. For this analysis, the elevation of the waste has been divided into three depths, 400 ft., 450 ft., and 500 ft. A higher elevation implies more recently added MSW.

Figure III - 5 shows box plots of the C/L ratio and the BMP of the MSW in the AALB and the CL for the different elevations. The AALB data is presented on the left and the CL data on the right. The y scale for each parameter is identical for both types of landfills, allowing a visual comparison between landfills. The box plots clearly indicate that the waste at a lower elevation is more stable in the AALB. Additionally, a Wilcoxn t-test with a Bonferroni correction comparing the means of the C/L ratio for each elevation category in the AALB shows that the mean decrease at a statistically significant level from 500 ft. to 450 ft. and from 450 ft. to 500 ft. The decrease of the BMP is only significant between 500 ft. to 450 ft. without a significant decrease between 450 ft. to 400 ft. Considering both these results together, one can conclude that the easily degradable waste like food waste etc. is efficiently decomposed thus lowering both the BMP and the C/L ratio significantly between 500 ft. and 450 ft. However, at lower elevations, as the waste enters methanogenesis the more recalcitrant waste, such as paper, is degraded lowering only the C/L ratio.

The effects of elevation on MSW stability in the AALB are further amplified by the linear regressions which show that the elevation of the MSW (independent variable) can predict the biological stability (dependent variable – C/L ratio and BMP) of the MSW in the AALB. Specifically, the C/L ratio will increase by 0.013 and the BMP will increase by 0.60 units for each foot of increase in elevation. Table III - 2 shows the degree to which each stability

parameter including volatile solids, cellulose and hemicellulose raise as elevation increases in the landfill (decreases with depth). Figure III - 6 shows scatter plots providing a visual representation of the relationship between elevation and the stability parameters. All the stability parameters, except for lignin, show a trend of increasing as elevation increases. These data indicates that the elevation of the MSW in the AALB is the only significant factor in determining the level of biological stability of the waste. However, as Figure III – 5 indicates the CL has no trend of increased biological stability with decreased elevation. This implies that the behavior and level of stabilization of the MSW in the CL is unpredictable based on age or elevation.

The results for the effects of elevation on the MSW in the AALB are logical when considering its design which consists of 15 ft. lifts. Each lift contains a matrix of pipes that either provides the MSW with air or water or removes methane from the MSW. Thus, each 15 ft. lift of MSW is guided through the different decomposition phases. Assuming that the piping is laid flat at each elevation, and that each elevation received the same treatment at a given time, two contour plots were developed in the statistical program R to visualize the distribution of the C/L Ratio and the BMP between the AALB and the CL. Data at elevation 460 ft. above sea level was available for most of the sampling locations; therefore, this elevation was used. Figure III - 7 is a map of the boring locations in the area of Outer Loop landfill that was studied. The area to the left of the line is the AALB and to the right is the CL. The contour plot of the C/L ratio is shown in Figure III - 8. This plot shows that the C/L ratio is higher in the control landfill than in the AALB at 460ft. The same is clearly noticeable in Figure III - 9 which shows that the BMP concentration is lower in the AALB than in the control landfill. Contour maps such as these can be very useful to MSW practitioners, providing an understanding of the level of microbiological stability at each lift of the landfills development. The contour plots contain data only for the years 2000, 2002 and 2003 for the control side and the years 2002 and 2003 for the AALB side.

Improved sampling and monitoring that keeps track of the level of treatment MSW at each elevation has received will provide a better understanding of the effectiveness of the AALB design. However, current data clearly indicates that the AALB stabilizes MSW at a faster and more reliable rate than the CL.

Temperature

Air and moisture addition to the AALB causes an increase in the microbial activity of the MSW; this increase in microbial activity naturally causes the temperature to rise. The Wilcoxon t-test comparing the mean temperature of the AALB vs. that of the CL shows that the mean temperature of the AALB (112°F) is significantly higher than that of the control (105°F). Even though the temperature of the AALB is significantly higher than that of the CL, linear regressions on the data collected from the samples in the AALB indicates that the temperature (independent variable) cannot be used to predict the biological stability (dependent variable – C/L ratio and BMP) of the MSW within the AALB. That is, the given data did not indicate that temperature played a role in increasing the biological stability of the waste in the AALB.

However, linear regressions on the data collected from the samples in the CL indicate that the temperature (independent variable) can be used to predict the biological stability (dependent variable – C/L ratio and BMP) of the MSW within the CL. Specifically, the C/L ratio will decrease by 0.008 and the BMP will decrease by 0.41 units for each °F rise in temperature. That is, as temperature rises in the CL, the MSW becomes more stable. This is true for all the other stability parameters excluding lignin. Table III - 3 shows the degree to which each stability parameter including volatile solids, cellulose and hemicellulose raise as elevation increases in the landfill (decreases with depth). Figure III - 10 shows scatter plots providing a visual representation of the relationship between elevation and the stability parameters. All the stability parameters, except for lignin, show a trend of decreasing as temperature increases.

These results indicate that when no other treatment is provided to the MSW, as is the case with the CL, elevated temperatures play a crucial role in stabilizing the waste. However, within the AALB where the MSW receives treatment, the temperature of the MSW is not as crucial in stabilizing the waste.

Moisture

As stated earlier, a crucial aspect of a bioreactor landfill is moisture addition. The Wilcoxon t-test comparing the mean moisture of the AALB vs. that of the CL shows that the mean moisture of the AALB (42.6%) is significantly higher than that of the CL (35.4%). Moisture is considered the main factor that enhances degradation within a landfill(Barlaz et al. 1990); however, the data

for the Outer Loop facility indicates no affects of moisture on the biological stability of the waste. That is linear regressions on the data collected from both landfills indicates that the moisture (independent variable) cannot be used to predict the biological stability (dependent variable – C/L ratio and BMP) of the MSW.

These results could be attributed to many reasons. For the AALB, samplers reported that in some cases as the bucket auger removed the MSW sample water poured out of them. This indicates that the lab measurement of the moisture ((wet sample weight-dry sample weight)/wet sample weight) is an inaccurate measure of the actual moisture present in the AALB. A better method of measuring the moisture present in the landfill is necessary to determine the true impact of moisture on biological stability. Additionally, previous research by Kelly et al. (2006) showed that excessive moisture within a landfill could dissipate heat, negating the effects of moisture coupled with high temperatures. This could be a possible reason why the excessive moisture within the AALB did not affect the biological stability of that landfill. Improved moisture monitoring methods are necessary to understand the optimum moisture level within bioreactor landfills.

Typically traditional landfills have a moisture content of approximately 20%, the value of the control (35.4%) is higher than this average. This is because rainfall is allowed to infiltrate into the MSW of the CL at the Outer Loop facility. A more controlled CL which does not permit rainfall infiltration would have been more effective in truly determining the affects of moisture in traditional landfills vs. bioreactor landfills.

Conclusions

The MSW within the AALB is more stable than the MSW within the CL; additionally the AALB is more efficient in stabilizing the MSW than the CL. This was indicated by all the stability parameters (C/L ratio, BMP, volatile solids, cellulose, and hemicellulose) except lignin, which were lower in the AALB than the CL. Studies over longer time periods are necessary to determine the fate of lignin in bioreactor landfills.

The MSW in the AALB shows a consistent trend of stabilization with age; however, there is no stabilization trend with age within the CL. Considering this, the AALB will require less post

closure monitoring than the CL. Besides age, the amount of treatment the MSW within the AALB receives determines its level of stabilization. This is demonstrated by the regression performed on the elevation of the MSW versus the biological stability parameters. This regression showed that elevation or location of the MSW relative to the surface is the only factor that can predict the level of biological stability within the AALB. Considering this, better sampling and monitoring procedures are necessary to factor in the treatment the MSW has received relative to its biological stability.

The temperature of the AALB is higher that that of the CL. However, temperature does not play a role in the stabilization of the MSW within the AALB, but higher temperatures in the CL increases the rate of stabilization of MSW within that landfill. Moisture was not a factor affecting the biological stability in either landfill. Better techniques to measure the actual moisture present in the landfill are necessary to determine the role it plays in MSW stabilization.

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Tables and Figures

Table III - 1: Shapiro Wilks test p-values used to determine normality.

	Ratio	BMP
Control p-value	2.45E-05	2.64E-04
AALB p-value	6.02E-06	9.63E-07

Table III - 2: Increase in stability parameters per foot of elevation in AALB (decrease with depth).

	C/L Ratio	BMP	VS	Cellulose	Hemicellulose
Increase per foot of depth	0.013145	0.60031	0.16801	0.18613	0.033574
p-value	1.24E-06	3.48E-08	3.62E-04	1.91E-08	1.40E-05

Table III - 3: Decrease in stability parameters per °F rise in temperature in control landfill

	C/L Ratio	BMP	VS	Cellulose	Hemicellulose
Decrease °F rise in temperature	-0.008348	-0.40999	-0.23326	-0.1342	-0.0302005
p-value	5.14E-02	1.88E-02	2.07E-03	1.09E-02	5.21E-03

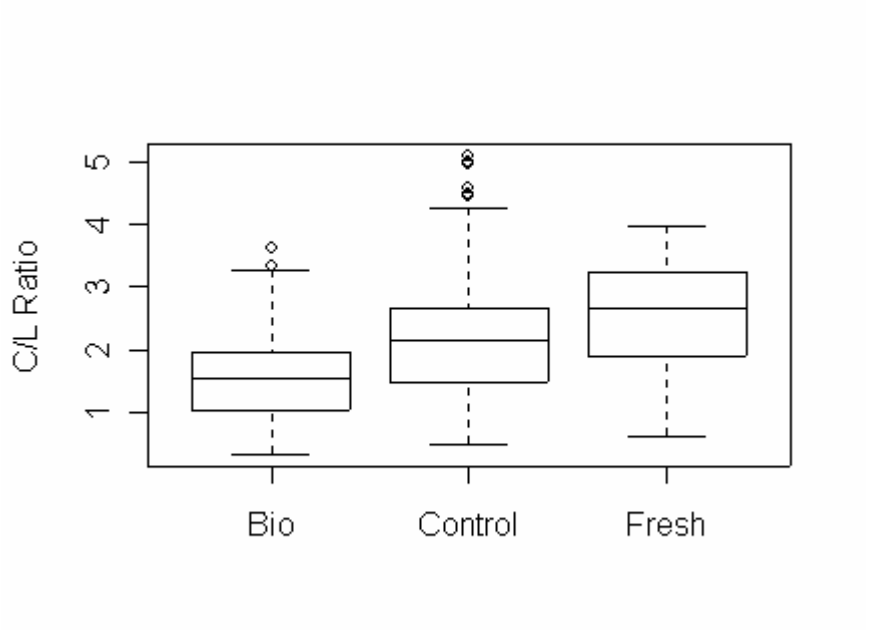

Figure III - 1: Box plot of the C/L Ratio for different types of MSW

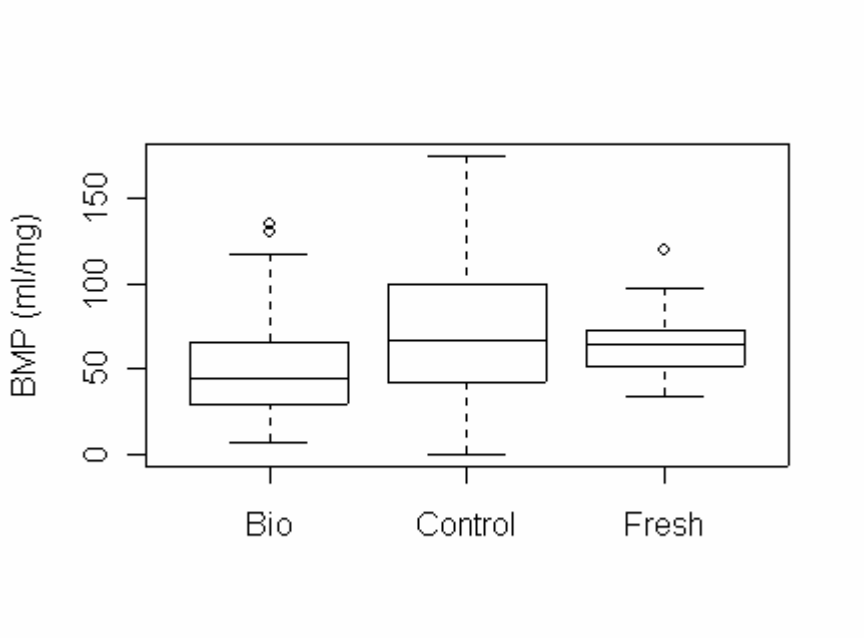

Figure III - 2: Box plot of the BMP of different types of MSW

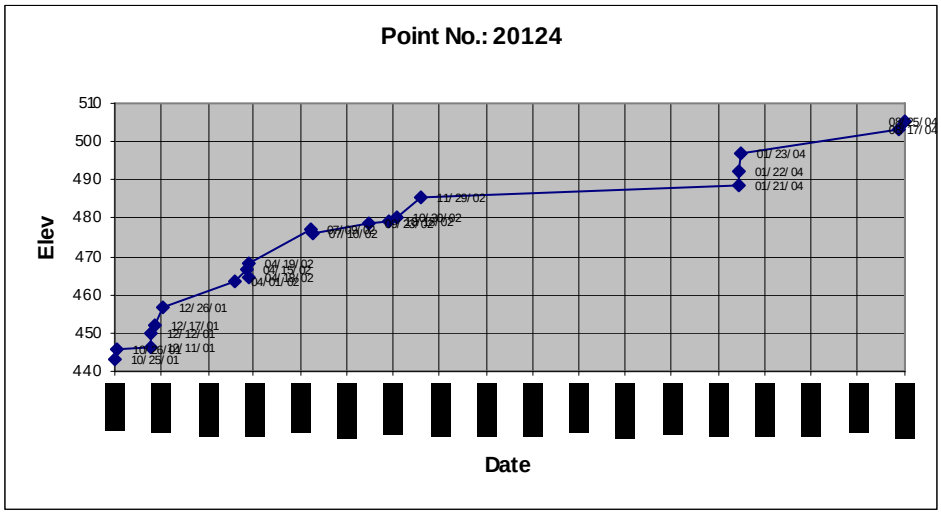

Figure III - 3: Example of a point activity analysis.

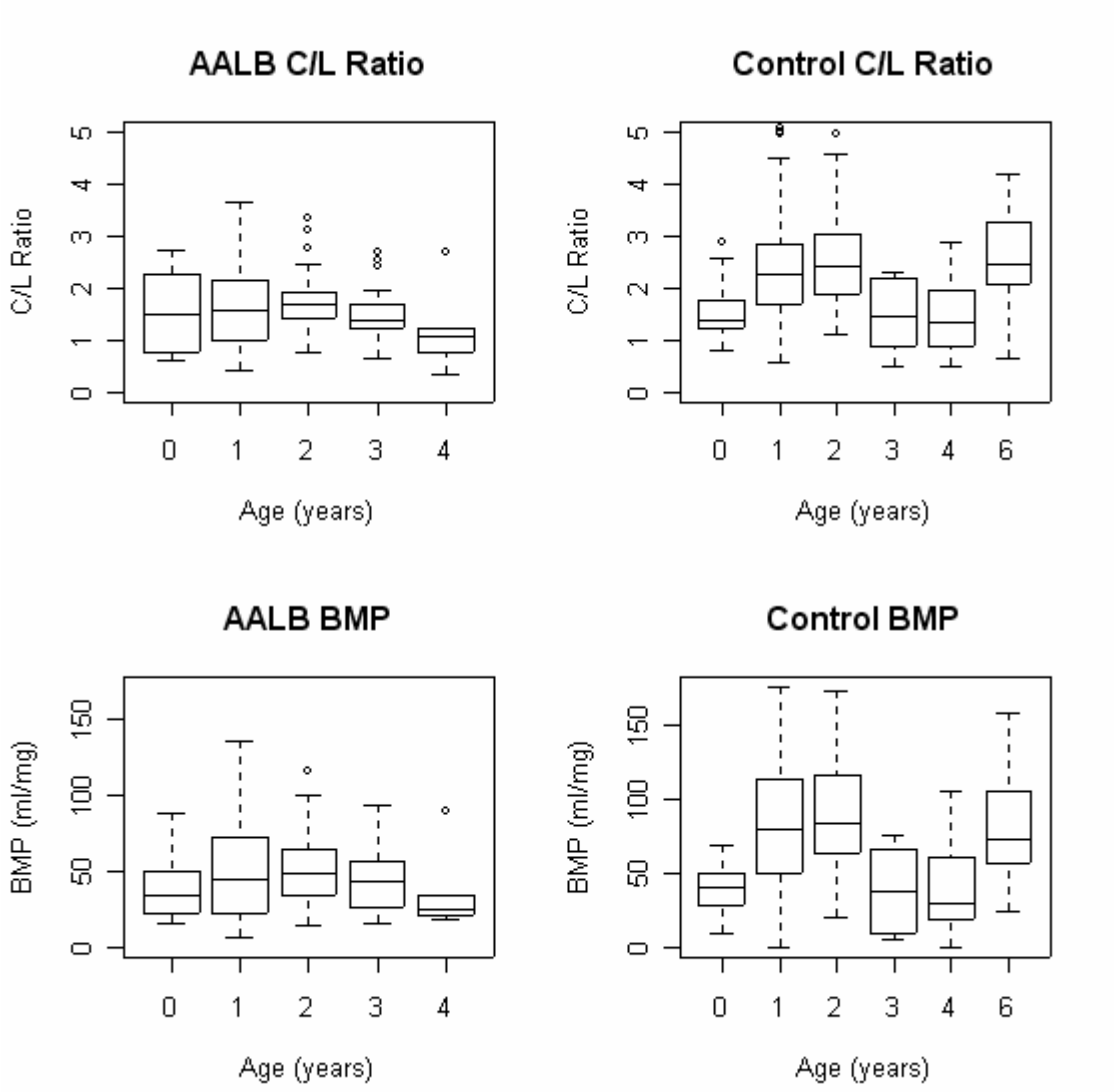

Figure III - 4: Box plot of the C/L Ratio and BMP over the age of the MSW (AALB and Control Landfill)

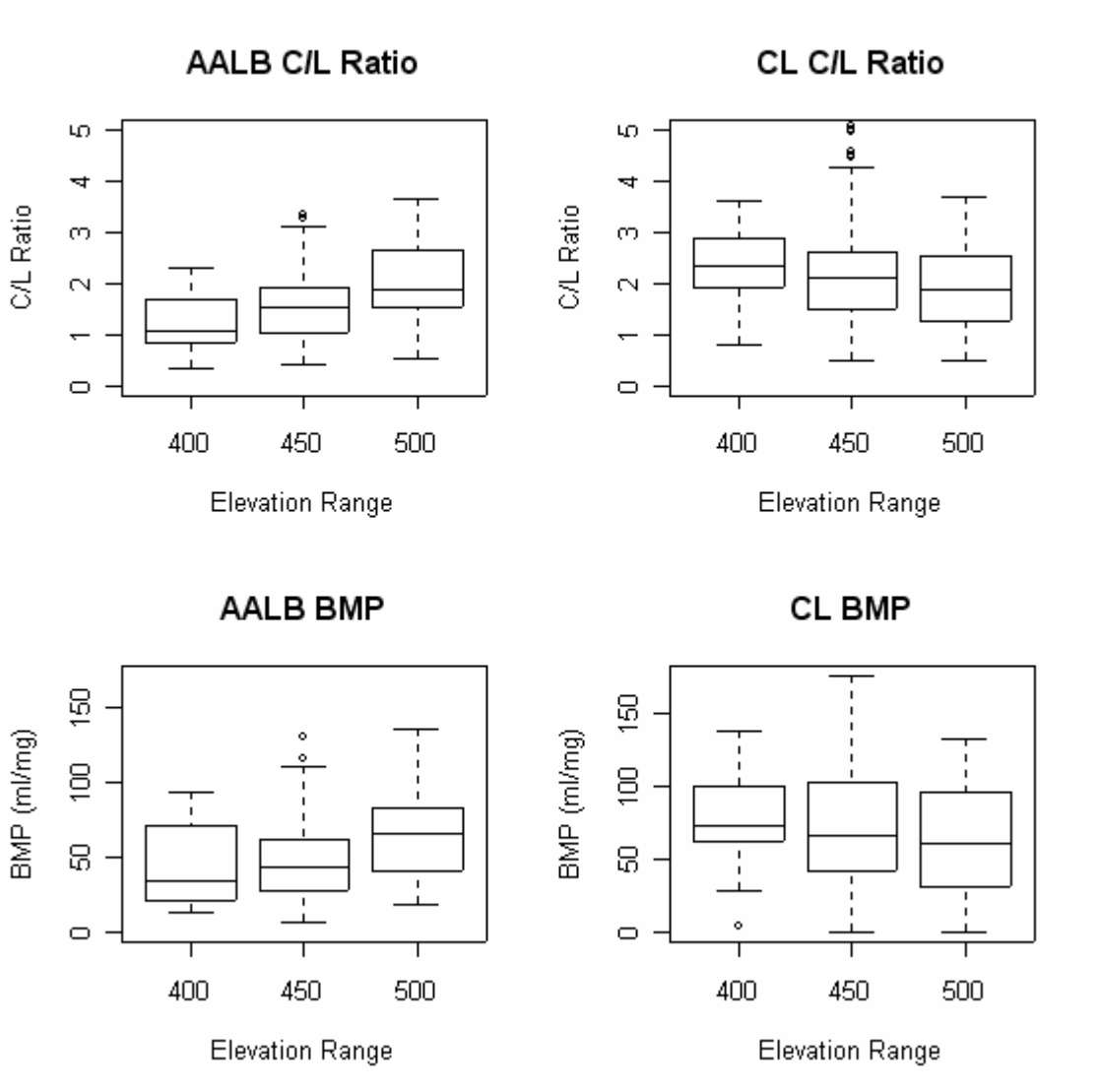

Figure III - 5: Box plot of the C/L Ratio and BMP over the different elevations in the landfill

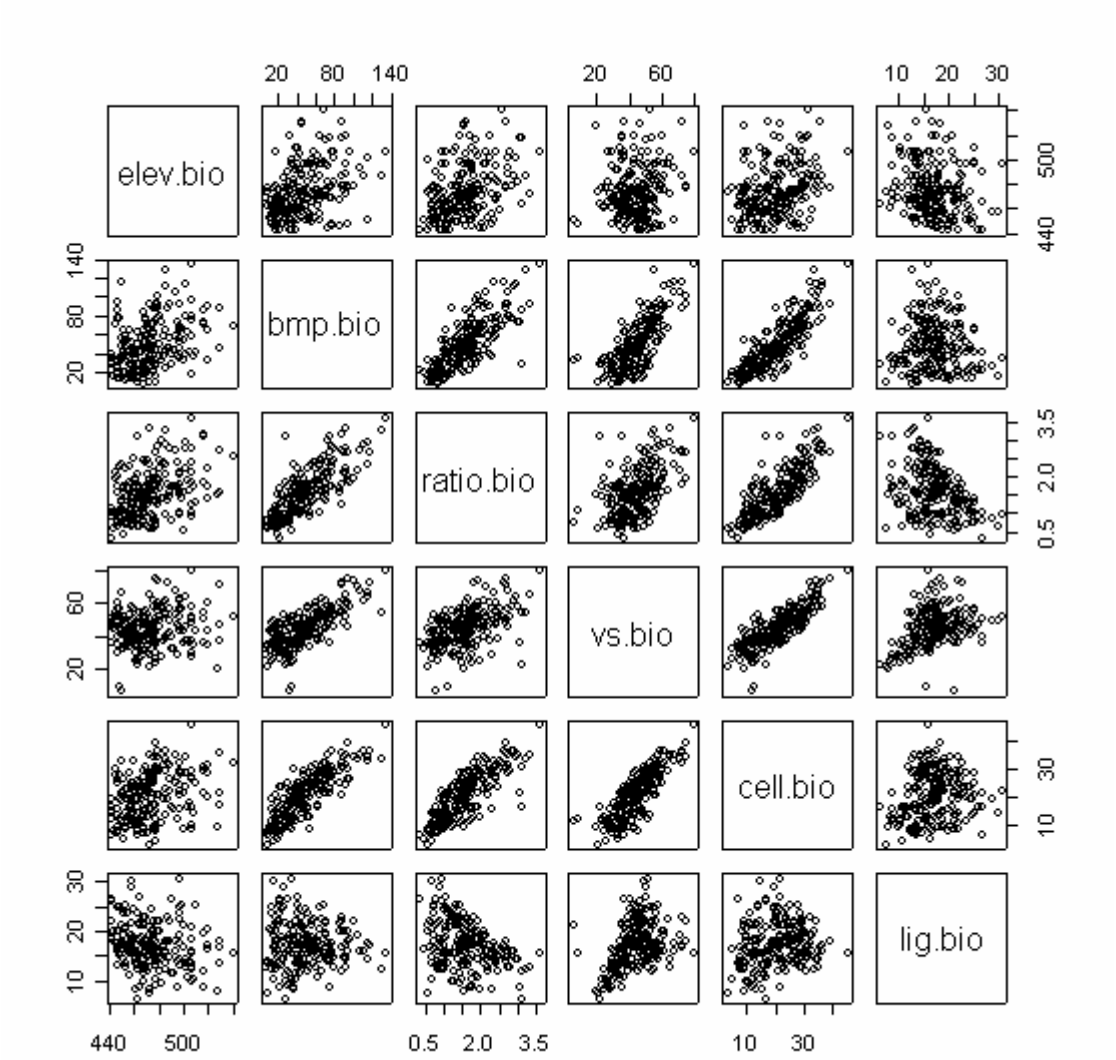

Figure III - 6: Scatter plot of elevation vs. stability parameters in AALB. Elevation is given in feet.

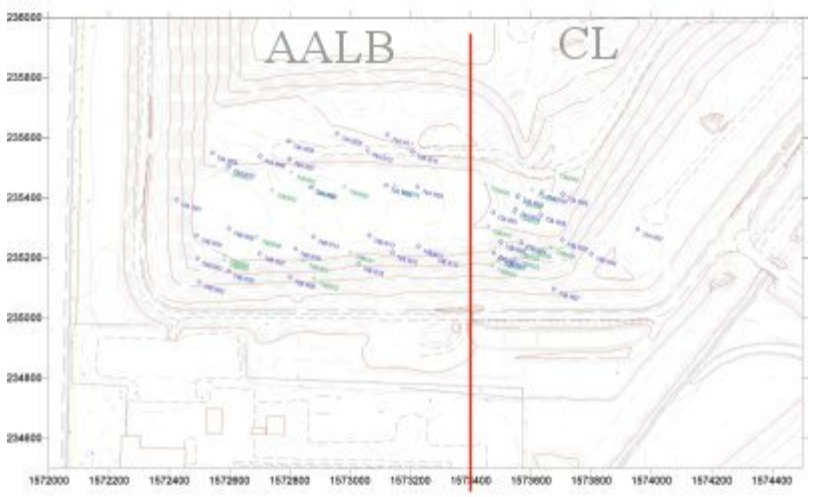

Figure III - 7: Boring locations and plane view of the AALB and the CL at Outer Loop.

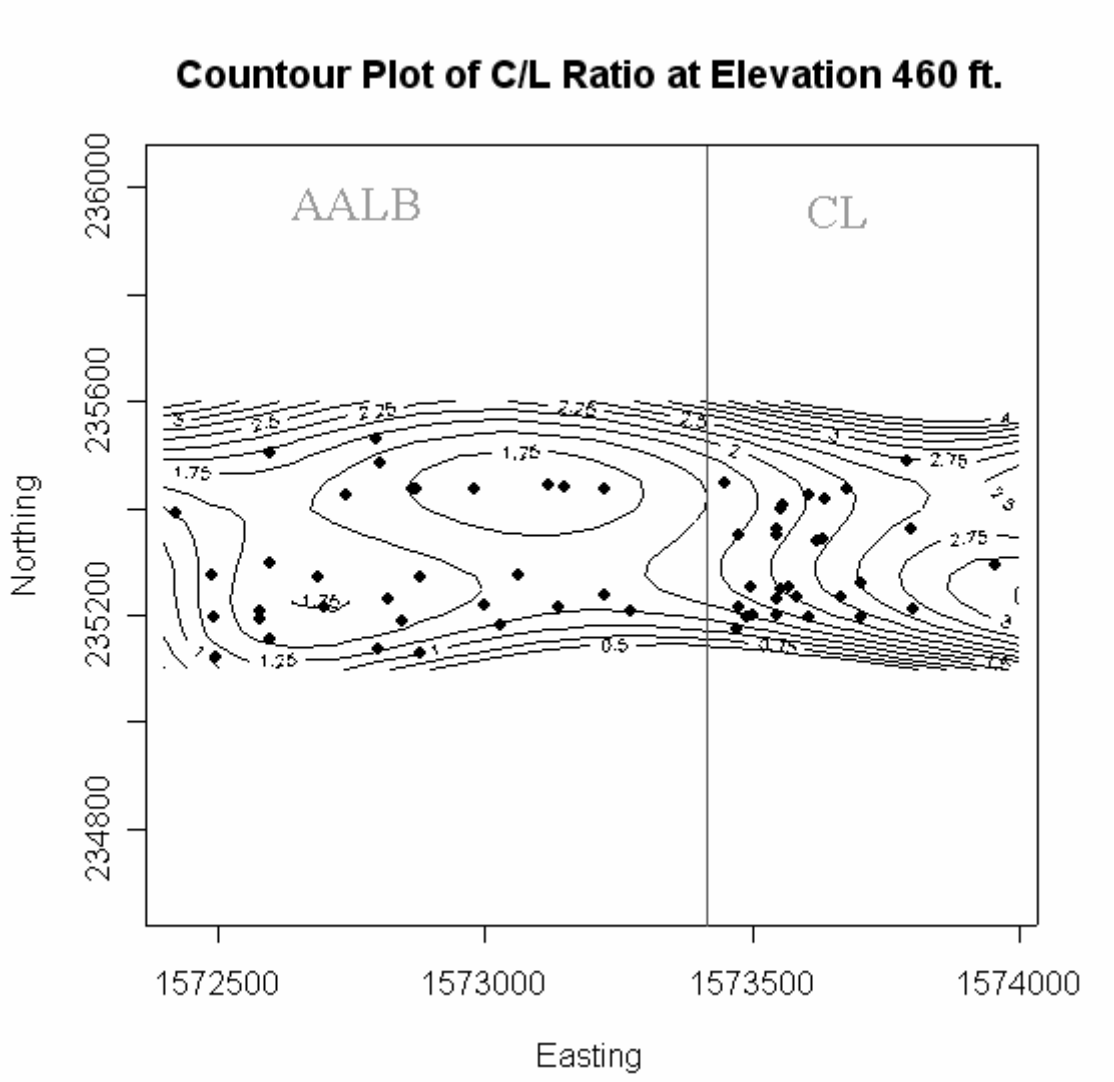

Figure III - 8: C/L ratio at 460 ft.

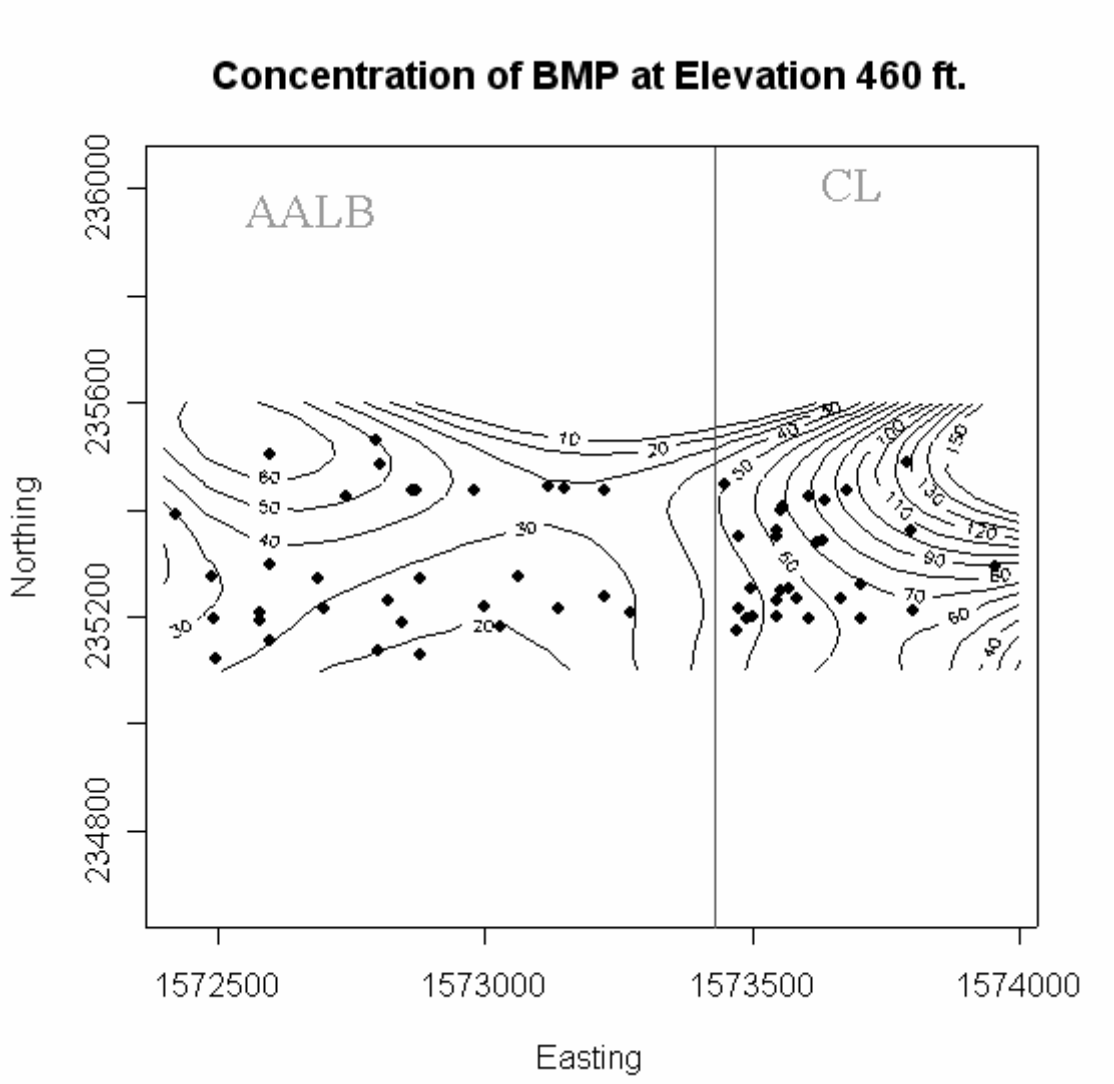

Figure III - 9: BMP concentration at 460ft.

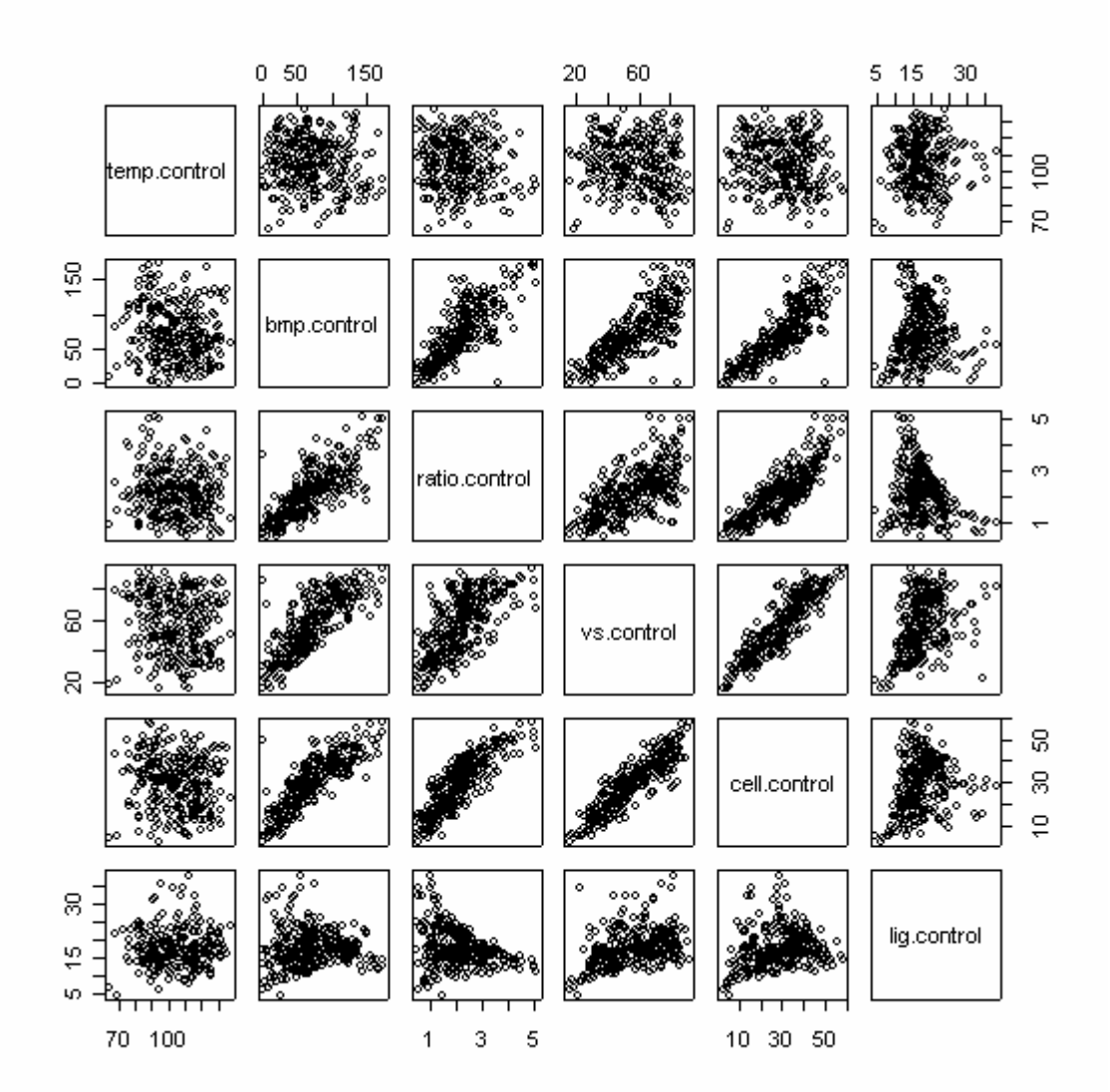

Figure III - 10: Scatter plot of temperature vs. stability parameters in control landfill -- temperature in °F.